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16. WELTKONGRESS FUR BUIATRIK

13 A 17 DE AGOSTO/1990
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TOMO I

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No hay Propheta en su tierra

THE ROLE OF THE VETERINARY PROFESSION AND IT'S ADAPTATION TO THE CHALLENGES OF AMELIORATING BOVINE PRODUCTIVITY IN THE SOUTHERN HEMISPHERE ON THE DAWN OF THE THIRD MILLENIUM.

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Mr. President, Ladies and Gentlemen, Dear Colleagues,

I am deeply honoured and very proud to have been chosen by the Scientific Committee to present the Festive Opening Lecture, thus enabling me to return for a second time to visit this breath taking country, so pregnant with enormous potential electrifying the very air we breath.

If I have to single out the one predominant trait of character to describe my very good friend and Co-Vice-President of the World Association for Buiatrics, the President and our Host at this XVIth World Buiatrics Congress, Dr. René Dubois, it would be his ever present never to be daunted optimism. It is this highly infective optimism which had made me light-heartedly accept the Herculean Task of trying to present this All-embracing subject of:

"The Role of the Veterinary Profession and it's Adaptation to the Challenges of Ameliorating Bovine Productivity in the Southern Hemisphere on the Dawn of the Third Millennium", and to do so in less than one hour. I cannot do it justice, it demands at least a 2-3 days Symposium and many specialists and my one deep regret is that I shall disappoint René and his optimistic expectations.

I have to first rectify a misnomer. Southern Hemisphere means areas South of the Equator. This would leave out all of Mexico, Central America and even parts of Brasil and Ecuador, as well as over half of Africa, the Arab countries, India the Indo-Chinese peninsula and the Philippines.

We will therefore deal with the areas situated more or less South of Latitude 30° North. Geophysically these countries have nothing in common ranging from desertic, limitrophe arid areas, temperate, sub-tropical to tropical zones. This means totally different veterinary and management problems.

The common trait between most of these areas are: under-production and an uncontrolled population growth accompanied by a gradual depopulation of farming areas and the creation of unmanageable megalopoleis. (The economically stable countries Australia, New Zealand, South Africa and the rich oil producing Arab States are outside the scope of our theme, though situated within the geographical area concerned).

First a few numbers and facts:

TABLE 1. Cattle Population (in Thousands) (6).

	Cattle	Dairy Cattle	Buffaloes	Human Population
WORLD	1,263,584	222,003	136,926	5,114,000
Africa	181,190	26,104	2,600	610,000
America: South	257,199	28,535	1,100	285,000
Central	52,589	2,687	9	58,000
Mexico	31,200	6,400		85,000
Canada	12,050	1,437		26,000
U.S.A.	98,994	10,243		246,000
Latin Am. Total	340,988	37,622	1,109	928,000

TABLE 1. Cattle Population (in Thousands) (6) (Cont.)

	Cattle	Do. Cattle	Buffaloes	Human Population
Asia Total	384,057	54,218	132,531	2,993,000
(Indian Penins)	(234,765)	(36,203)	(89,020)	(1,067,000)
Europe Total	124,780	45,922	367	497,000
East	37,073	14,800	261	141,000
West	87,707	31,122	105	356,000
Oceania	32,122	4,456		26,000
USSR	120,593	42,000	320	286,000
Western World (Incl. S. Africa and Japan)	297,370	49,608	105	877,000

These bare numbers do not mean much, the real situation looks as follows:

TABLE 2. The real World Situation.

	World	Western World	%
Population	5,114,000,000	877,000,000	17.15
Cattle	1,263,584,000	297,370,000	23.53
Dairy Cattle	222,003,000	49,608,000	22.35
Milk Production (tons)	458,023,000	265,653,340	58
Meat Production (bovine) (tons)	46,072,000	24,418,200	53

This means that 1) Africa with 14% of the World's Bovine population produces but 7% of the World's meat, Asia with 29% of the World's cattle produces but 6, I repeat 6% of the World's meat, and South America with 20% produces 14% of the World's meat. In milk production the conditions are even worse. 2) Africa milking 11% of the World's dairy cows produces but 3% of it's milk, Asia milking 23% of the World's dairy cows produces but 9% of it's milk, and South America with 11% produces but 6% of the World's milk (Figs. 1,2,3,4).

The per capita daily protein consumption looks as follows:

TABLE 3. Per Capita availability of total protein and animal protein (grams per day).

	Year	Total Protein	Total Animal Protein	of which: Meat	Milk	Fish	Eggs
World	1971-73	65.1	21.5	10.0	6.6	3.3	1.6
	1981-83	68.3	23.1	10.9	6.8	3.7	1.7
Developing countries	1971-73	52.7	9.2	4.1	2.5	2.1	1.5
	1981-83	57.6	11.3	5.1	3.1	2.3	0.8
Developed countries	1971-73	96.0	52.3	24.6	16.8	6.8	4.1
	1981-83	99.2	56.8	27.3	17.4	7.7	4.4

Source: FAO Committee on Commodity Problems, Intergovernmental Group on meat, ME 87/4, December 1986.

Please notice the amount of milk consumption in the developing countries having the World's highest birth rate (and a much lower life expectancy and average life span).

TABLE 4. World Meat Consumption (all meats incl. Pork and Poultry).

	Year	Total ('000 tons)	Per Capita (kg)
World.....	1971-73	103,752	27.0
	1980-82	133,958	29.6
Developing.....	1971-73	28,988	10.5
countries.....	1980-82	45,112	13.5
Developed.....	1971-73	74,767	68.3
countries.....	1980-82	88,846	75.4

Source: FAO Bulletin of Agricultural Economic Statistics, ME 87/4.12, 1986.

Developed countries, with but 17.15% of the World's population consume two thirds of all meat produced in the World. For developing countries (DVC) the averages vary enormously, Argentinians consume about 100kg of meat a year, with Uruguay and Paraguay not far behind, in South Asia, protein of animal origin consumption is but 8 grams per day.

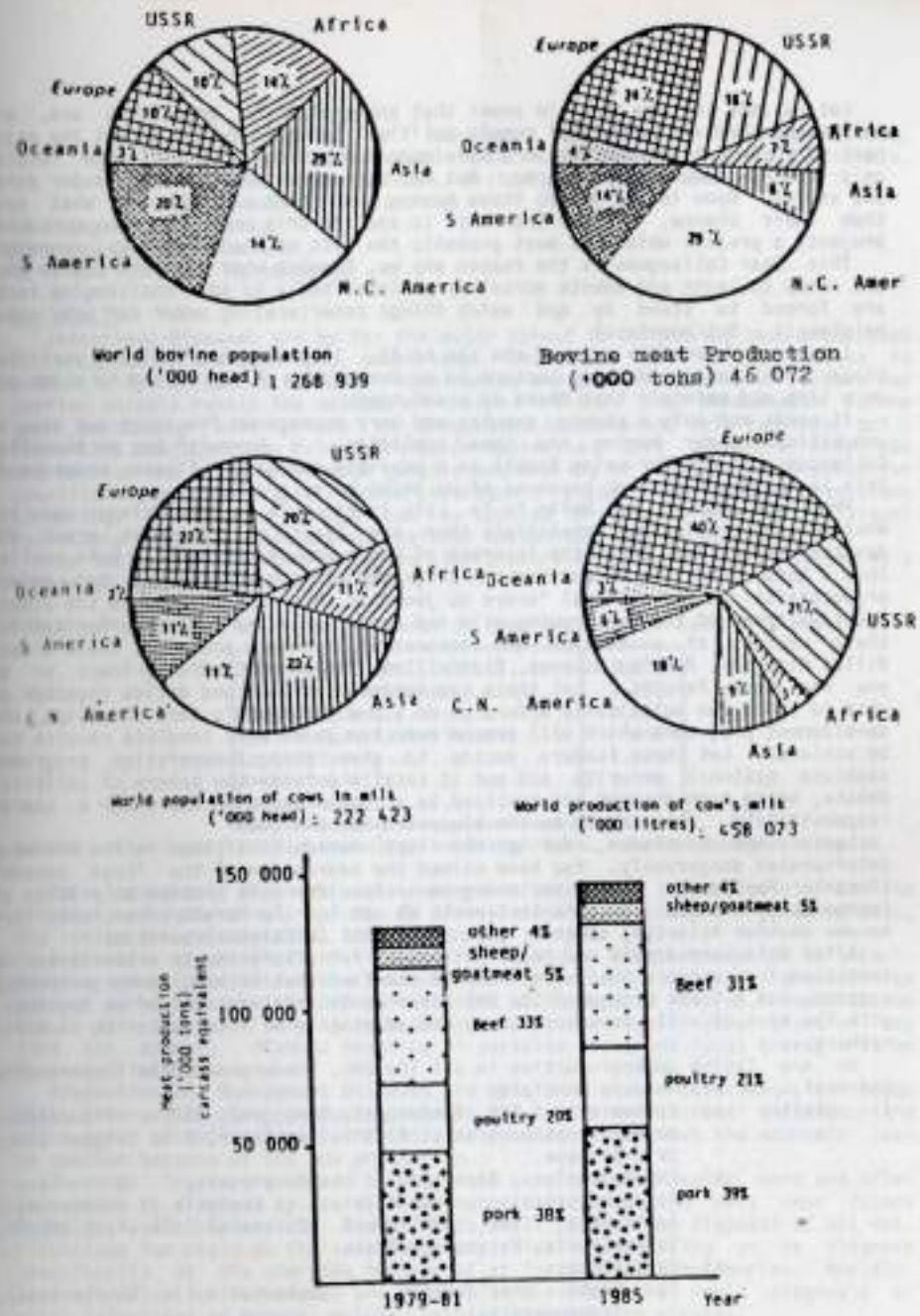
In spite of a yearly increase of food production worldwide, by a yearly average of 2% in the West and about 3% in the DVC, this hardly keeps pace with the population expansion and we now have over 800 million people consuming daily food rations inferior to the critical minimal limit.

To date, somehow, agricultural production and livestock increases have been able to maintain the low per capita consumption in DVC. Till 1980 there was an almost parallel increase in human and livestock populations (FAO 1980), this Balance has now been broken. As Professor Jasiorowski said (24): "It cannot continue. This vicious circle must be broken but to make this possible two changes are necessary: a reduction in the rate of increase of the human population and an increase in livestock productivity per animal". There is nothing we, as Buiatricians, can do about the population explosion. Every single religion opposes induced human abortion and most preach against any form of birth control.Except for a State imposed limitation of births (Communist China), only common-sense and a Worldwide awakening to the plight and suffering of hundreds of millions starving and maimed children may in the future reduce the birthrate to economically sound levels, thus giving them a chance.

On the other hand there is a lot we, as a profession and as specialists could do in the field of the increase of animal productivity in general and bovine productivity per animal in particular. But let us be realistic. Changing the low genetic potential of the bovine populations in the DVC, increase in Latin America (L.A.) for instance agricultural production by 46% through itensification and irrigation and increase the production of existing arable land by 73% (as determined by the FAO) (7), demand both time and financing on a huge scale. Only Governments can do it.

Except for a few halfhearted attempts no democratically elected government in any of the 90 DVC has done it,with the result that an ever increasing percentage of their populations are literally starving, Why? You can say anything you want about politicians, but believe me they are not stupid, and they have top advisers. It took me some time to figure it out.

While in opposition the main purpose of every party is to be elected to reign. They will make promises on the one hand and attack every mistake of the party in power. Once in power themselves, their top priority will be to remain in power at the next election in 4 or 5 years time. That is their raison d'être. They must therefore show results, things they can point to as achievements. By definition these must be short term development projects. Changing the agricultural production pattern of a country, upgrading the low genetic potential of their livestock industries, mainly that of the bovines, demand enormous investments and a lot of time, at least 10-15 years.



Source: FAO Production Yearbook, 1985
Fig. 1. World meat production, 1979-81 to 1985

Let us face it, any party in power that knows what the priorities are, and believe me they all know, will commit political suicide if they invest the major part of their 4-5 year budget in a Development Programme that will show results only during some future regime. But let us even assume that one leader dares and starts. Upon losing power, those having taken over and knowing what gave them their chance, will be the first to abolish this money and time consuming project, a project which was most probably the butt of their political campaign.

This Dear Colleagues is the reason why we, knowing what must and can be done and ready to learn and devote ourselves wholeheartedly to this challenging task, are forced to stand by and watch things deteriorating under our very eyes. Helpless!! - But hopeless?

I have very recently reached the age of 65. I am now an officially certified Viejo. This Festive Opening Lecture is my Swan Song. I can afford to climb out on a limb and maintain that there is a solution.

It needs not only a strong, popular and very courageous President but also an opposition leader having the same qualities. I herewith beg my Brasilian Colleague's pardon for using Brasil as a possible example. Please understand, this is for demonstration pruposes of my point only.

President Collor de Mello is in. Luis Inacio Lula da Silva almost made it. Whatever their political convictions they are first and foremost proud and devoted Brasilians with the interest of their country at heart. Both realise that a total long term reconstruction programme of both agriculture and animal productivity is the national "ordre du jour" if they want to reverse the almost suicidal trend of the evergrowing wild and uncontrolled so called urbanisation, these centers of starvation and sickness of both body and mind, these slums, Villas Miserias, Pueblos Jovenes, Bidonvilles, Poblaciones, Shanty-Towns or as you say here Favelas. Let these two leaders sit down and decide together on what no party can politically afford to do alone during it's term of office, the development programme which will demand over ten years till tangible results can be achieved. Let these leaders decide to give this recuperation programme absolute national priority and put it totally outside the sphere of political debate, being both planned and realized by a common decision and under a shared responsibility. This should be the blueprint for all DVC.

Ladies and Gentlemen, during the last decade conditions in the DVC have deteriorated dangerously. You have coined the expression of the "Lost Decade" (Decenio Perdido). I am imploring you: Consider this problem as a State of Emergency in all DVC. On this Continent, do not let the Dorado of ancient lore become another Atlantis, another "Lost Continent" (Continente perdido).

After this very deeply and heartfelt appeal for a solution to a heartbreaking condition, I am certain that we all accept the fact that without heavy government support and a total change of the DVC Governments attitude, all of us together, with the best of will, devotion, study and adaptation will be able to achieve nothing.

We are facing underproduction in all the DVC. Underproduction in both milk and meat. The reasons are manifold:

1) Health:
   a) Epidemics: F.M.D. Rinderpest, Contagious Bovine Pleuropneumonia, Trypanosomiasis, Rift Valley Fever, Blue Tongue, Lumpy Skin Disease.
   b) Communicable Diseases: Leptospirosis, Q Fever, Trichomoniasis, Brucellosis, Leukosis, Hemorrhagic Septicemia, IBR, Malignant Catarrhal Fever, BVD/MD, Tuberculosis, Paratuberculosis.
   c) Parasitoses:
      I) Blood: Anaplasmosis, Babesiosis, Theileriosis, Trypanosomiasis.
      II) Other: Echinococcosis/Hydatidosis, Cysticercosis, Dermatophilosis etc.

   d) Production or Management Diseases: Crowding Disease, Neonatal Diarrhoea, Mastitis, Shipping Fever, Acetonemia, Milk Fever, Ketosis, Avitaminoses etc. etc.
   e) Subclinical Diseases:
2) Impaired Fertility.
3) Low Genetic Potential.
4) Nutrition and Management.

1 - HEALTH

Infectious Diseases are by far the major threat in DVC, having been eradicated or contained in the developed countries: and cause the largest losses to productivity (Figs. 5,6,7,8). Vaccination and the eradication of vectors and carrier animals remain the methods of choice. Within the foreseeable future gene technology will permit the production of cheap and safe vaccines, but I leave this subject to my friend Professor Espinasse. I'll therefore only add and mention the transfer through genetic engineering of isolated genes coded for specific disease resistance, these transgenic animals to produce resistant offspring. This same method can be applied in order to transfer individual resistance or genes which enhance defence mechanisms against pathogens (3).

The following table will give the 1986 picture of economic losses caused by a number of common diseases (Table 5).

TABLE 5. Estimates of worldwide costs of some diseases of cattle ($ million).

Shipping Fever.....	3,000
Neonatal Diarrhoea.....	1,750
Blue-Tongue.....	3,000
Bovine Enz. Leukosis.....	900
Leptospirosis.....	4,500
Brucellosis.....	3,500
Mastitis.....	35,000

Source: The impact of Biotechnology on Animal Care. Techn. Managemt. Grp.86.

Parasitological Diseases cause enormous economic losses mainly in the tropical and subtropical regions. In Africa alone, and excluding Trypanosomiasis, the losses are calculated at 2.5 billion US$ per year (5).

Australia spends 53.3% of it's entire veterinary drug consumption on anthelmintics and ectoparasitics, New Zealand 60.5% (20). The total loss of productivity through parasitoses in the DVC is estimated at between 10-20% (1). Immunisation has to date succeeded only against dictyocaulus, here again gene transfer may find the answer. Planned breeding of parasite resistant local breeds will be discussed later.

Production and Management Diseases are mainly "rich men's diseases", though Milk Fever hits low producing animals who suffer from mineral disbalance and often types of Avitaminoses. Mastitis is just as common, but the economic loss is smaller because of the low production.

Enormous losses are caused by undefined subclinical diseases, hard and often impossible to diagnose by the clinical veterinarian. In the very near future Monoclonal Antibodies and Rapid Tests will be at the disposal of all vet. clinicians for rapid on the spot diagnoses, finally permitting us to diagnose specifically at the cow-side instead of at far central laboratories. Nucleic-acid hybridization techniques are already in use for the rapid diagnosis of viral infections in humans, mainly those of slow growing viruses.

Health is our domain, our basic specialisation. We have learned to treat the various diseases, to operate, reset broken bones, examine the individual animal, use the microscope and the home laboratory to diagnose parasitological and some bacterial diseases. Our schools have created good clinicians. But our tradition bound faculties have shown a total lack of adaptation an irresponsible

Fig 5a THE FMD SITUATION IN THE WORLD 1985 - 87

Fig 6 TWO MAJOR THEILERIOSIS

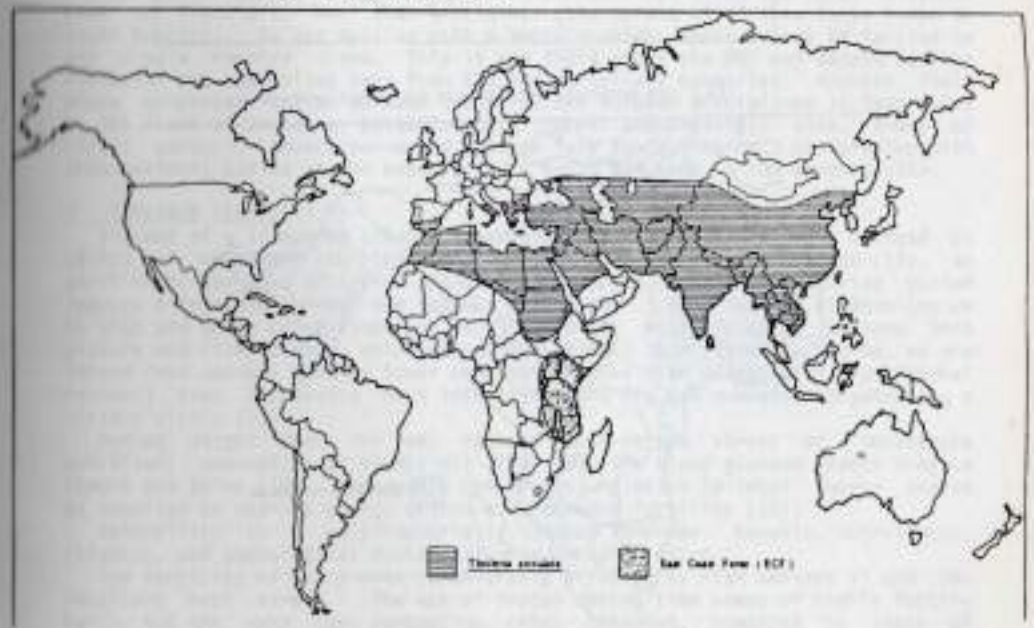


Fig 2 TSETSE INFESTED AREAS AND CATTLE DISTRIBUTION IN AFRICA

Fig 3 THE RINDERPEST SITUATION IN THE WORLD 1945 - 80

blindness to the changes occurring Worldwide. In the Western World and in those intensive high producing herds based on exotic breeds, mainly Holstein, in the DVC, cows produce almost up to their genetic potential, both for milk and daily weight gain of offspring, and demand a production adapted nutrition, highly digestible feeds with a high protein content. They also require a novel class of veterinary specialist, versed in herd medicine, Management, production and nutrition, demanding a totally different veterinary formation. We are not dealing with the industrialized countries here, so I advise those interested, to read W.R. Pritchard's "Future Directions for Veterinary Medicine", (Ed. Pew, National Veterinary Education Program, Inst. of Policy, Sciences etc. Duke University, 2016 Campus Drive, Durham, N. Carolina 27706, U.S.A 1988) and O.M. Radiostits "Personal Preparation for Change in Bovine practice - The Bovine Practitioner in 2000" (Proceedings of the 21st Annual Convention of the American Ass. of Bovine Practitioners, 1988, Alberta, Eric Williams Editor, 1226 N. Lincoln Stillwater, OK 74075).

In the DVC the problem is even worse. The entire educational system is based on the Western approach, teaching staff having mostly been formed there. With very few exceptions, graduates have not received the knowledge nor the tools to tackle the problems facing the DVC and the specific problems of the various countries concerned. I shall return to this subject. But first back to the eradication and the organized fight against the various large scale epidemics. The USA has managed to eradicate the "cattle tick" Boophilus annulatus, through dipping methods, and the Screwworm. FMD has been eliminated from North and Central America. Rinderpest has been contained, Tuberculosis eradicated from many countries. But recently we evidence a breakdown in control, due to lack of funds, transportation and an attitude maintaining that Governmental Veterinary Services are not necessary anymore. With the result that we have had an enormous spread of both Rinderpest, African Swine Fever, the resurgence of Rift Valley Fever with terrible havoc both animal and human in Egypt and the Sudan, the spread to Egypt of Lumpy Skin Disease all come to remind us that Diseases know no frontiers, not even Continents (the spread of African Swine Fever to South America). We are dealing with a World Problem, which cannot be tackled by any single country alone. This is one field where the DVC may expect serious financial and specialist help from the industrialized countries. Another field where concerted action between countries and between disciplines is imperative is the field of Zoonoses, bacteriological, viral and parasitic ones, those of direct contact, insect borne and through food contamination. Cooperation with international bodies can be expected here too. But back to low productivity.

2 - IMPAIRED FERTILITY

Instead of a 12 months calving interval we have a 16-18 one and instead of 18-22% of cows dry in the herd we face a 35-40% dry cow average (13). In addition to the loss of calves and milk thus caused, a long intercalving period imposes a rotation through the seasons of the year. Thus instead of enabling us to plan and guide conceptions and calvings to the most suitable seasons both pasture and climate wise, which may differ greatly from region to region, we are forced into seasons showing lower conception rates (for climatic or nutritional reasons) thus increasing herd infertility and dry cow numbers, perpetuating a vicious circle (13).

During weight loss for any reason, post-partum stress or inadequate nutrition, conception is almost nil (Fig. 10), the blood glucose levels drop to 22mgr% and below (14). Glucogenic precursors, relative to total energy should be supplied to improve energy status and increase fertility (13).

Infertility is a multifactorially induced Syndrome. Genetic, nutritional, climatic, and pathological factors playing deciding roles.

The fertility of dairy cows is adversely affected by high ambient t° and the resultant heat stress. The use of frozen spring time semen of highly fertile bulls, and the very low conception rates obtained, compared to those of temperate seasons proves it (13).

Recent studies show (8):

1) that a significant, 23% reduction in blood flow occurred during hyperthermia in the ovaries of all animals, and a 37% one in the undifferentiated uterine wall of both non-pregnant and early pregnant animals. The reduction in ovarian blood flow during heat stress was most prominent in early gestation, the effect subsiding with advancing gestation.
2) Plasma Progesterone levels were significantly reduced in all animals during heat stress, the reduction being related to the degree of body t° increase. During heat stress, the peripheral progesterone levels were correlated with ovarian blood flow.

There is evidence to show that an ambient t° of above 40°C induces hyperactivity in spermatozoa, reducing their survival rate significantly; thus insemination of heat stressed cows carried out several hours before ovulation have a low chance of proving fertile. The survival rate of fertilized ova in a high t° uterine millieu (above 39.7°C) has still to be elucidated (13).

During heat stress, detectable oestrus is shorter, silent heat is frequent, seriously impairing conceptions in general and through A.I. specifically. The effect of suckling on progesterone levels, lowering them significantly is well documented (Fig. 9).

Hot Climate adversely influences production both directly - by reducing ingestion and indirectly by negatively affecting reproduction as shown.

The digestive process in ruminants, creates heat. Non-producing dairy cows (dry cows and heifers) fed but for body and foetus maintenance, are able to radiate that heat even under adverse climatic conditions. Cows ingesting additional fodder for milk production, can get rid of excess heat in temperate climates.

Above certain ambient temperatures (t°), radiation of excess heat becomes impossible and the body t° of cows rises by up to 1.5 and even 2° Celsius in extreme cases (40.5°-41°C).

Exposure to direct solar radiation through lack of shadow during the hot hours (as measured by us with normal and black globe thermometers (13)), can increase environmental heat stress by up to 10°C.

Whereas in Europe, dairy cows react adversely to t° above 23°C, Holsteins in hot U.S. regions (Arizona, Nevada) and Israel, do so only at t° above 28-29°C. Through the systematic culling of low producing and infertile cows for selection purposes, we automatically eliminated all cows adversely reacting to heat stress by lowering production and fertility. We thus indirectly selected for heat resistant animals for over 60 years, as has been done in the hot areas in the U.S. (Arizona, California, Nevada etc.).

The fact that their offspring show the same adaptation capacity permits us to assume that heat resistance is an acquired hereditary trait.

The defence of milk producing cows to heat stress and the ensuing rise in body t° is to limit DM intake, thus lowering milk production (High energy containing, easily digestible rations must therefore be provided during the hot season).

Humidity as well as high atmospheric pressure increase heat stress. Regions showing a THI (Temperature Humidity Index) of 75 and above, show a consistently lower milk production average, whereas cows in even very hot dryer areas, blessed with cool nights permitting heat discharge and recuperation, are capable of producing top records of well above 10,000kgs.

Tropical areas and sub-tropical ones with a high THI are totally unsuitable for intensive milk production, mainly based on exotic breeds. The body t° increase resulting from the incapacity to radiate excess heat, reduces ingestion, lowers production and almost nullifies conception rates.

The treatment of infertility is one of the subjects we have been trained for, mainly in the individual cow.The enormous and complex subject of environmentally

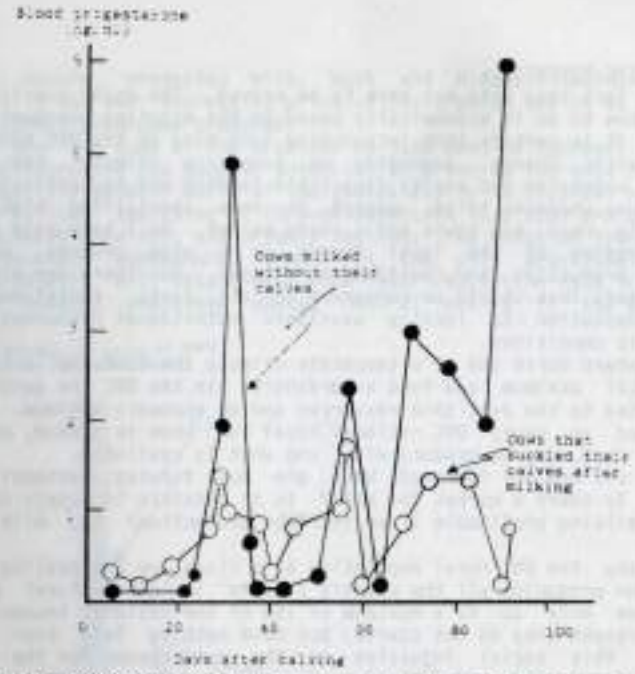


Fig. 9: Blood progesterone concentrations in Holstein cows during the first 100 days of lactation, milked without or with calf suckling after milking (Velazco et al 1982b).

induced infertility, is not normally a subject taught even in the DVC most directly concerned.

It is imperative that bovine practitioners in the DVC be trained to differentiate between infertility caused by A: factors which can be treated or acted against (eradication, vacciantion A.I.), like pathological ones: a) infective: Trichomoniasis, Brucellosis, Viral Abortion, IPV, Epivag in Africa, Granular Vaginitis, Akabane, etc. and b) non infective pathology: uterine and vaginal infections, anoestrus, ovarian cysts with or without nymphomania, Repeat Breeding. c) defiency induced infertility: Manganese, beta carotene and Vitamin A, Vit. D, Vit. E, Calcium, Phosphorus, Magnesium, Zinc, Copper, Iodine, Selenium; and excesses: Manganese (Abortion and cysts), Fluoride (anoestrus and anovulation) Calcium, Phosphorus (retarded uterine involution), d) Excesses of: crucifères and their antithyroidal activities, excesses of trifolium and it's antigonadotropic activity, and phytooestrogenes, etc. e) toxicologically induced infertility: moulds, mycotoxines, etc. f) Mistakes in nutrition (false supplementation, disbalanced diet), g) faulty heat detection in A.I. served herds.

and B: factors which cannot be treated: the lower fertility of the Bos indicus breeds, seasonal heat, climate, draught causing undernourishment, etc.

The ability to diagnose these multiple causes of infertility is an art that has to be taught and then learned and developed through continuous application in the field. In many cases the pinpointing of the specific factors involved, demands the use of records that in the DVC each clinician has to learn to apply, and the lack of laboratory backup is a severe handicap he has to work under. Here too the development of rapid test kits, activated by car batteries will be of enormous help.

3 - LOW GENETIC POTENTIAL:

This is a fact that does not have to be proved. The major question is how to improve it, how to do it economically based on the existing manpower and local resources. It is certain that introducing into many of the DVC Western technologies and exotic breeds, depending on temperate climate, top management, specialized expensive and easily digestible feeding and mechanization, has been a total failure wherever tried, except in very specialized high production units, mainly near big towns and a ready market. As I have said here in 1985 (13) the upgrading of the local breeds is a slow process, necessitating unavailable production and performance records. But there are many traits of the local breeds that should be conserved at all cost: resistance to local diseases, adaptation to locally available nutritional resources, and to the local climatic conditions.

In the Western World and it's temperate climate the tendency is to produce to the biological maximum and feed accordingly. In the DVC the genetic aptitude must be adapted to the available resources and an economic optimum. What has to be determined on every DVC national level is: what is needed, which type of animal do we need, can we produce with, and what is available.

But first who are our farmers? What are our farming systems? Pastoral? Sedentary? Is there a market for milk? Is it possible to supply the milk? Is twice a day milking profitable if we increase production? Can milk be cooled and stored?

If we take the DVC rural population as a class, we are dealing with 40% of the population producing all the country's needs in agricultural produce but bringing home only 10 to a maximum of 15% of the national income. Except in periods of draught they do not starve, but have nothing left over from their hard toil. This social injustice is the main reason for the rural exodus lured by the large cities, and the hope for a better income. This changes the most stable, the most traditional, the most dependable part of each national population into a beggars proletariat, and governments stand by and let this happen instead of raising their standard of living by increasing their means of production and ensuring a dependable market for their produce. What is the use of producing milk if the average worker is unable to pay for it? Why in that case increase meat production? No farmer will invest in order to improve production unless he gets a rapid and just return for his investment. In many countries traditional constraints have to be overcome, farmers whose animals are often their only banking system are hard to persuade to cull say 20% of their heads of cattle because of overgrazing, thus lowering not only their credit but also their social standing. Most of the so called advisers sent to these countries with a lot of goodwill, are totally unaware of many of these traditional constraints and cause more harm than good. But local veterinarians and extension workers are ideally suited to approach these farmers and achieve the necessary goals.

The next cardinal question to be answered before we decide how to change the genetic potential of our cattle population remains the same as asked by me in 1985: Is there a market for milk? What does the Government do to create and support such a market? Is a glass of milk available to every child every day? If not, why isn't that a national priority in countries with such an enormous birth rate?

What is the present use of bovines? Are they used as draught animals in addition to their normal functions? This is a cardinal question when we deal with Southern Hemisphere countries. If so we need slower maturation in order to create larger animals and rumens with a greater fermentation capacity. If double purpose, doble proposito, we must maintain a balance in the genetic aptitude to both milk and meat production. At present we need 200 head of cattle in African DVC to produce 1 ton of meat, compared to 5 head in the Western countries.

In many DVC the farming backbone cultivates about 5 hectars of land and owns about 1-5 heads of cattle. They are needed as draught animals, supplying

important manure, producing milk, meat and hides. Their main economical constraint is the ever increasing price of fuel, which has to be catered for in any development programme proposed.

These are but some of the problems we face when we approach the complicated problem of improving the genetic potential in order to increase productivity.

As shown, the genetic merit of indigenous cattle in tropical and sub tropical countries is low. Improving it through selection is a slow process, and demands infrastructures like A.I. and progeny testing. The solution found and applied by the Southern Hemisphere farmers is the only practicable one: Doble Proposito animals. A.I. obtaining a much lower conception rate (Fig. 10), for the many reasons explained above, natural mating is the only solution of choice.

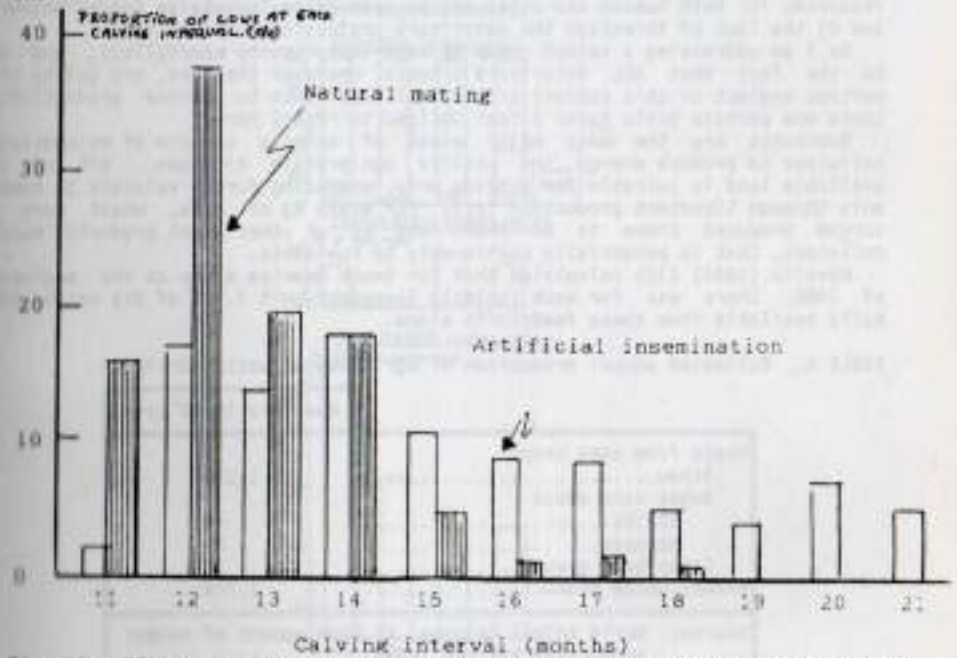


Fig. 10: Effect of mating system (AI versus natural mating) on calving interval in a dual purpose herd (Friesian x Creole) in which calves were reared by restricted suckling (Naidoo et al 1981).

Even climate adapted imported bulls cannot resist nor survive under local conditions. A two step plan is necessary. It is possible to import semen of proven sires cheaply. For the regions concerned only high climate adapted sires should be selected: California, Nevada, Arizona, Israel. We need large frames and the sires MUST be progeny tested for growth rate of offspring. Because we have to maintain the meat producing hereditary traits. In specialised government or regional centres, preselected dams of the local breed are inseminated. The male bulls thus produced, will serve as mating bulls running with the herds. New genes can thus be introduced through the imported semen in each generation, maintaining heterosis and avoiding inbreeding (17). As Preston also states this gives the possibility to evaluate beef traits on the bulls themselves (performance testing) instead of progeny so hard to carry out in the DVC and without A.I. The introduction of these mating sires into village centers etc. necessitates a close veterinary supervision of both the bulls and of the local herds in

order to minimize the risk of infection. Based upon the amounts of milk produced, 50-75% of exotic blood should be maintained, and a mean production of 2,500 and even above will be achieved based on the locally available resources with but a minimal supplementation, which should never exceed a maximum of 30%. Let us not forget that the heritability of beef traits (growth and feed conversion rates) is much higher than for milk production ones.

4 - NUTRITION AND MANAGEMENT:

This subject is exceptionally complicated because a) of the great geophysical differences between the DVC (arid and semiarid zones, where water and lack of natural pasture is the main concern to sub-and tropical ones), b) the resources natural and by-products available, c) the "competition" for certain key resources for both humans and other animal production industries (pork, poultry) and d) the lack of formation the veterinary profession suffers from.

As I am addressing a select group of veterinary bovine specialists, and due to the fact that ALL veterinary schools, wherever they are, are guilty of a serious neglect of this subject of cardinal importance to bovine productivity, there are certain basic facts I feel obliged to repeat here.

Ruminants are the only major class of animals capable of metabolizing cellulose to produce energy and utilize non-protein nitrogen. 60% of the available land is suitable for grazing only, producing forage valuable to humans only through livestock production (11). For every kg of rice, wheat corn or sorgum produced there is at least one kg of other plant products, mostly cellulose, that is potentially usable only by ruminants.

Kossila (1984) (10) calculated that for South America alone at the beginning of 1980, there was for each (animal) livestock-unit 4.7kg of dry matter (DM) daily available from these feedstuffs alone.

TABLE 6. Estimated annual production of agricultural waste worldwide.

	Quantity in 10^6 tons
Waste from some crops	
Straw.....	1,358
Sugar cane waste	
Stalks.....	44
Bagasse.....	59
Sugar beet greens.....	19
Animal waste products.....	1,759

Source: World Animal Science, A2 Development of animal production systems, 1984.

Available for bovine consumption are 40% of all root and tuber crops, 60% of all cereal crops, 85% of all oilseed crops, and 90% of all sugar crops (16). Banana, cassava, citrus fruit and coffee residues in Latin America, Asia and Africa alone equal 124 million tons.

The efficiency of conversion of plant protein and energy to animal protein is of 22-25% for milk (eggs and poultry) and for ruminant meat only 5%. In order to produce animal protein more efficiently, certain amounts of grain and protein (mainly bypass protein i.g. fishmeal), are needed. During recent years an increase in meat consumption in North East Asia has occurred, China doubling it's consumption by 10 million tons/year. This entire increase is due to pork and poultry meat! Humans need the grain too and all these compete for the grain produced locally and/or imported. The argument you will repeatedly encounter maintains that it is wasteful to feed grain to animals while people starve. It is important that you realize that this argument is fallacious. The question should NOT be how much protein is fed to animals, but how much Human Edible Protein (HEP) is fed. For milk production an input of but 15.9% HEP gives a

return of 181% (an eleven fold increase) for bovine meat production, an input of but 4.9% HEP, yields 109% (a twentyfour fold increase) the ruminants performing much better than the pork (86%) and poultry (75%) (Fig. 11) (4,19).

There is no doubt therefore that from a national-economy point of view and in spite of the fact that poultry and pork production is a rapid process, the fourty and 44% of HEP necessary for it's production constitute a serious drain on human grain supply and therefore influence it's price. The advantage of bovines is enormous not only because of their far superior conversion rate to HEP both for milk and meat but also because over 90% of their energy and protein is derived from otherwise unusable pasture and the enormous residues which would otherwise become a severe ecological problem and because their digestive process produces wastes to be used as fuel in large quantities, quite apart from their contribution as energy saving draught animals.

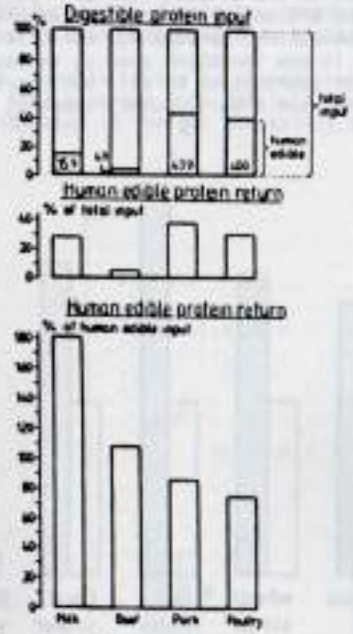


Fig. 11: Human edible Protein input and return (Bywater & Baldwyn, 1980).

The main feed resource in DVC are large grazing areas (from intensive to dry or semi-dry rangelands) (21). Their imporovement is relatively easy through better management avoiding overgrazing and introducing richer perennial grasses (mainly leguminous ones). In arid and semi-arid areas we have the problem of abundant pasture during the rainy season and poor woody feed during the dry one. This season is the problematic one. Due to the nomadic nature dictated by the poor pasture, it is almost impossible to ensile or produce hay there. Creating "fodder banks" by leaving large areas ungrazed is possible, but there is a big loss of both energy and palatability of the standing once green grasses, and a very real danger of fires. The natural ability of zebu cattle to deposit fat during the period of abundance should be strengthened through selection (21).

Our strategy as defined before is to go for the 50% exotic x local cross. This means that suckling calves are necessary in order to induce milk letdown. Whether the cows are milked once or twice daily, all newbornes till the age of one month should suckle twice a day partly before and after milking, as milk is

it's only source of nutrients. After this month it should suckle once a day after the morning's milking. The trials of Paredes in Venezuela (1981) (15) have shown that restricting the time the calf suckles the dam to 15-30 minutes after each twice a day milking, instead of remaining with the dam on pasture during most of the day, increased milk production. In cases where markets for milk are available and prices received satisfactory, the percentage of exotic blood in the cross can be increased to 75%, the suckling calf is then unnecessary for milk let-down. The remainder of the calf's diet is normally the same as that of the dam, and in order to achieve superior growth rates should be supplemented. Without going into details it is certain that inadequate protein and insufficient energy supply during the critical pre & Post weaning period lead to stunted growth, explaining the small breed size of many of the traditionally kept herds (21).

The basis of nutrition in the tropical DVC are pasture and crop residues, i.e. carbohydrates, and NPN either in the form of urea or ammonia both forming the basis of rumen fermentation, producing Volatile Fatty Acids (VFA) and microbial protein. It is now incumbent upon us to maximise rumen fermentation, by increasing the rate of degradation of cell wall carbohydrates. This can be achieved through steaming, and alkali (mainly ammonia) treatment (Fig. 12).

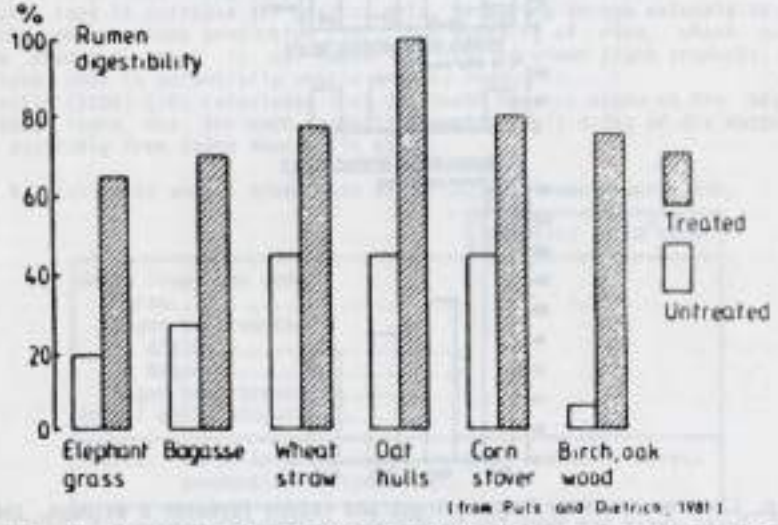


Fig. 12: Increase of rumen digestibility by steaming and extraction.

The supplementation necessary is dictated by the production functions to be achieved, namely, growth, work, reproduction and lactation. Glucogenic energy, Amino Acids, VFA energy and Long Chain Fatty Acids, (LCFA) all can be steered, on condition that the parameters of normal rumen function and the values and parameters for each region and regime are known and well analyzed. The clinical veterinarian has to become a specialist in nutrition in order to advise, warn and guide. He can for instance increase VFA energy by advising the increase of certain feeds, increase rumen degradability by supplementing with bypass protein and/or alkali treatment (18). There is a whole group of supplements which act as "catalytic agents" (ammonia, cottenseed res. fish meal, molasses with or without urea (in blocks or liquid), highly digestible forage etc.). The DM content of the ration in "catalytic agents" should never pass 30% at the utmost.

What should mainly be remembered here is that bypass protein increasing feed intake always stimulates milk production, but a careful balance of the nutrients (mainly in fat and proteins) must be maintained in order to avoid body reserve mobilization (Table 8).

This can be prevented by bypass starch, permitting higher propionate production (e.g. by feeding Monensin) increasing energy efficiency and body-weight increase (18). We need a minimum level of rumen ammonia in order to degrade the fibrous substrate. This can be monitored through addition of urea. In tropical areas the molasses/urea block is ideal. When treating straw, corn stovers etc. veterinarians should always keep in mind that higher temperatures approaching 90°C cause a toxic reaction of ammonia with sugars producing methyl imidazole compounds inducing severe nervous disorders (bovine hysteria). It is excreted through milk to suckling calves. An excess of molasses above 20% of DM intake depresses milk production by depressing propionate (It is NOT the sugar that causes this depresion as sugar cane residues containing much higher amounts can be fed up to 50% (18).

There is a definite genetic trend to channel nutrients to serve specific function. The same feed intake served both Holstein and a Holstein Zebu cross was converted to milk production in the one and mainly to liveweight gain in the other (Fig. 13).

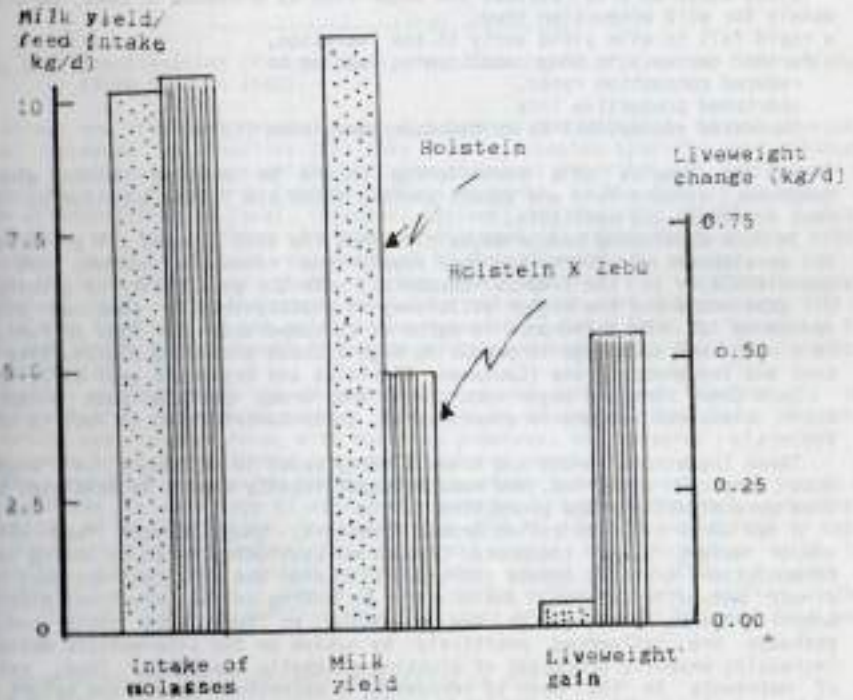


Fig. 13: Partitioning of nutrients (feed intake was almost identical) was almost exclusively towards milk in purebred Holstein but was divided equally between milk and body tissue in crossbred Holstein X Zebu animals (J. Ugarte and T.R. Preston, unpublished data).

TABLE 7. Some selected examples of the effects of supplements rich in bypass protein (BP) on the liveweight gain and supplement conversion rate (kg gain/kg supplement) of cattle fed different basal diets all supplemented with adequate levels of fermentable nitrogen (from Preston and Leng 1986).

Bypass protein source	Basal diet	Growth rate (g/d)		Supplement conversion
		-BP	+BP	
Fish meal.....	Molasses.....	370	1,000	0.7
Fish meal.....	Rice straw**....	100	400	0.17
Cottonseed.....	Dry pasture.....	-320	+20	1.9

* Amount of supplement (kg)/kg of additional liveweight gain compared with the unsupplemented diet.
** Ammoniated by urea-ensiling method.

Diets based on crop residues and sugar-rich by products in cows partitioning mainly for milk production show:
a rapid fall in milk yield early in the lactation,
a further decrease in body conditioning leading to
reduced conception rates,
shortened productive life
increased susceptibility to diseases/parasitism (18).

In the tropics this partitioning should be avoided unless glucogenic compounds, dietary fats and bypass protein (both the latter supplied by cotton-seed residues) are available.

In most developing countries,mainly since the OPEC imposed oil price squeeze, the development of alternative fuel supply from renewable sources has become unavoidable. In the tropical countries, with the possibility of growing crops all year round and the higher efficiency of photosynthesis, combined with the narrowing of the difference in value of carbohydrates for food or fuel, there is a long term advantage in producing high biomass producing plants, like sugar cane and leguminous trees (Leucaena, Gliricida and Erythrina spp) (17)(Table 8).

Both sugar cane and Leguminous shrubs and trees perform much better than their classical expensive counterparts, their contribution as fuel is shown in Fig. 14.

These leguminous shrubs and trees already exist in abundance as "weeds" in most tropical countries, and research made locally should be initiated to make them more digestible and productive.

A few words about so called Growth Promoters: they increase feed efficincy and/or weight gain. Ionophores (Monensin, Lasalocid) do so by acting on rumen fermentation, Anabolic Agents (Zeranol, Estradiol and it's combinations) through direct metabolic action, Antibiotics by acting on the intestinal microflora. Anabolic Agents do not act on the digestion of feed, but their consequent pathways are influenced positively by action on the intermediate metabolism, increasing anabolic processes or blocking catabolic ones (22). Thus, retention of nutrients in the body is increased, N excretion lowered and weight gained from one kg of DM increased. They can be subdivided as follows: Stilbenes (now forbidden), Natural Compounds (Estradiol 17 beta, Testosterone, Progesterone) Non-Stilbene Xenobiotics (Zeranol, Trenbolone acetate, Melengesterol acetate), Growth Hormone and associated compounds (G.H., G.H. releaser, Somatostatin and Somatomedine).

TABLE 8. Biomass production from temperate and tropical crops (Cereal grain/soybean vs sugar cane/tree legumes).

	Energy crops		Protein crops	
	Sorghum	Sugar cane	Soybean	Gliricidia
Harvests per year.....	2.5	1	3	5
Annual yield (tonnes DM/ha) Biomass.....	20	34	9	25
"Soluble" carbohydrate.....	10	13		
Protein.....			2	4

Gliricidia sepium (a tree legume native to the tropics).

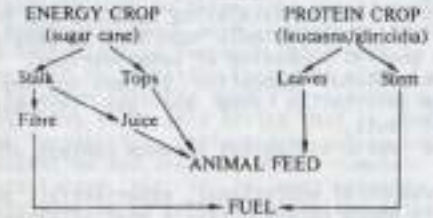


Fig. 14: Fractionation of sugar cane and a tree legume for animal feed and fuel (from Preston 1980).

Animal and treatment related factors determine the magnitude of the physiological response to anabolics (26) they are: a) species specific, b) influenced by sex (hormones), c) age related (stages of sexual maturity), d) influenced by composition of the ration (mainly protein content), e) the dose is dependent on mode of administration (oral, injected, implant) and the site, f) blood levels, dependent on the number of times administered, g) administration time before slaughter (because of peak response) and h) type of releasing device.

In the near future we shall witness the creation of additional growth promoters like neuro-hormonal stimulators and recombinant DNA products. It is one of the functions of the buiatrician responsible for a herd, to determine the type and timing of the Growth Promoters to be used based upon the feed resources available (12).

Another subject where the veterinarian has to get more involved is the supplementation of trace elements. This wont be so easy because heavy financial interests are involved here, with marketed premixes, the contents of which is most often totally unsuitable for specific local needs.

The standard values for the blood composition for each race, age, period of the year and for each type of management must be determined for each veterinary practice, in healthy cattle, in order to be able to pinpoint variations in cases of underproduction and infertility, to permit early supplementation with the necessary electrolytes and/or correct the ration composition. A number of transparencies will give a sample of what can be determined and applied. They were studied and analyzed from many thousands of bovines in Paraguay last year (2).

THE ADAPTATION OF THE VETERINARY PROFESSION IN THE DVC:

Many DVC have a dangerously insufficient number of veterinarians, and too many of these are white collar office dwellers, mainly in Africa. In many other countries, mainly in Latin America we have many, some say too many Vet. schools. This is not bad in itself, but owing to tasks these schools must fulfill in the future, their very numbers put an enormous strain on both top level scientific faculty and the increased financial requirements for the formation of the

specialists needed. The already mentioned Pritchard Committee Report on the "Future Directions for Veterinary Medicine", which should become mandatory reading for all Faculty members worldwide, sums up as follows:

Summary of recommended future directions for veterinary medicine:

1. Change the focus of the veterinary medical profession from animal disease to animal health in all its dimensions.
2. Abandon the unrealistic concept of the universal veterinarian who can minister to the health needs of all creatures great and small.
3. Restructure veterinary practice to better serve the needs of society and the veterinary profession in the future.
4. Make research a higher priority for individual veterinarians, the veterinary medical profession, and for veterinary medical colleges.
5. Establish a more rational system of funding for veterinary medical research
6. Improve the quality of veterinary services delivered to all species of animals in response to the escalating expectations of the public as to the health care of all of the animals important to people.
7. Strengthen the general education of veterinarians.
8. Focus the professional education process and the practice of veterinary medicine on the ability to find and use information rather than the accumulation of facts.
9. Strengthen the basic biological science content of the veterinary medical curriculum.
10. Make the achievement of educational, experiential, and cultural, racial and ethnic diversity among veterinarians a goal of veterinary education.
11. Reorient clinical veterinary education to enable a student to elect in-depth instruction and clinical experience with a practice theme (class of animals or a single species), rather than require all students to obtain clinical experience with numerous species.
12. Change the emphasis in the veterinary curriculum from almost total concentration on clinical practice to include important public sector needs for veterinarians.
13. Move toward a national perspective or strategy of veterinary medical education.

The limitations of both top specialists and finances prevailing in state financed Vet. schools on the one hand and the necessity for specialization needed in order to lead the growth process in animal productivity, search for inexpensive solutions and conduct the manifold research projects needed, can be solved only through restructuration of under-graduate and graduate education. For the creation of Buiatricians for instance, specialists should be encouraged to transfer to schools already strong in this field, undergraduates choosing it would naturally prefer to study there. Others should lead specially gifted students towards a career of biological research, necessary because it becomes ever more apparent that the entire field of biotechnology is becoming the domain of private companies, because government support of research, mainly in agriculture is receiving almost no development funds (To illustrate this point, in 1940 the US invested 40% of it's entire research budget in argricultural research, by 1973 only 1.8%!! In most other countries conditions are much worse). The moment key research passes into private hands, their interest being financial gain, it will not be devoted to solving problems of the poor countries, unable to afford the final products. Their priorities must remain income oriented. They are offering outstanding inducements to the best

scientists, who tend to accept. That wouldn't be so bad but for their avidity to apply for patents thus closing the doors on any ongoing research in the same field in other poorer countries. The Industrialized countries use 63% of all veterinary drugs (20) while the disease charts show their high frequency in the DVC. Professor Jasiorowski brings a shocking example (21) of a leading bio-tech company which refused to cooperate with WHO in developing a cheap anti-malaria vaccine, because it demanded high profits. Another company had taken over the project expecting to make their profit from tourists visiting the DVC, not from the inhabitants who most need the Vaccine. This happened in humans, can we expect a better situation with drugs for livestock? The patenting craze of only potentially useful products hits DVC most severely, mainly if it's applicability is in the South, not only stopping the production of the vaccine but preventing others from producing it. This demands a total revision of the patent laws worldwide in the field of medicine and agriculture. Recognition of the investments in private research must be taken into consideration but an equitable way must be devised to cover this ever more important issue. Another important aspect is that bio-technological research which will be of interest to Southern Hemisphere countries with no lucrative income in view, wont even be started. Bio-technological research into the deadly Trypanosomiasis Zoonosis should have been started long ago, but it is only Africa that is concerned so... The Western World feeding high production rations does not as yet need bio-tech research into the genetic adaptation and creation of a rumenal microflora capable of decomposing ligno-cellulose but it is most urgently needed in the DVC. Bio-technology is already applied to produce lysine and methionine, both essential amino-acids. Other targets for gene-transfer would be for certain protein hormones to enhance the productivity or fertility of bovines, or transfers to alter the metabolism in needed directions, or for using new techniques where conventional breeding methods have failed (3). All needed urgently in DVC.

The governments of the DVC must realize that it is imperative to invest in their proper local research and finance it either directly or with the help of IFAD or OECD. The time is ripe for DVC to develop their own research and development projects in bio-technology, and form their own scientists. It must also be realized that the blind adaptation of imported high specialized technologies in agriculture has done more harm than good and local solutions must be found locally. The veterinary profession is ideally suited to become ever more deeply involved in both the research and application in the field of enhancing livestock productivity.

There is no doubt that the role of the veterinarian has to become much broader, and must in each country be formed according to the existing constraints on animal productivity, the veterinary curriculum to be adjusted accordingly.

In the short time at my disposal for this World embracing subject, I was only able to point to a few chosen focal points, leaving by the wayside Embryo Transfer, Nucleus herds, new delayed action and release devices for drugs to point out but a few. I have tried to point the way for improving the education and the function of the veterinarian, enlarging his already wide facetted art. He is most admirably suited to enlarge his sphere of knowledge and apply it. He is willing and capable to work as the pivot with multi-disciplinary teams of specialists, but formation centers must be created to give him the necessary scientific background to extend his expertise. Herd and environmentally induced Management Problems must become his major fields of additional activity, nutrition his specialty, herd infertility his expertise.

All these are the very fields most heavily curtailing bovine productivity. But with all that let us not forget for one minute that the conditio sine qua non for this target of ameliorated bovine productivity is HEALTH. Eradication, vaccination, quarantines etc. are the domain of Veterinary Services, but the clinician is the reliable early warning system, and as far as the various parasitoses and their control are concerned, they are in his hands and they alone cause a loss of 10-20% of bovine productivity.

The Buiatrician is like the conductor of a large orchestra, all instruments must be faultlessly tuned and function together harmoniously in order to produce the perfectly pitched Raphsody to Bovine Productivity.

Ladies and Gentlemen, Thank you for your patience.

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PROCEDIMIENTO DEL SEMINARIO TALLER

sobre

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Table 2: ELECTROLYTES CONCENTRATIONS AND HEMATOCRIT IN THE BLOOD OF DIFFERENT RACES OF CATTLE IN PARAGUAY (x ± SD)

	PARAMETER					
RACE	Ca (mg/dl)	Mg (mg/dl)	P (mg/dl)	Cu (mg/dlx10^{-1})	Ca/P	Hct
Nelore	8.7±1.3	2.02±0.33	4.3±1.1	9.0±1.1	2.12	30.0±0.2
Holland-Mestizo	9.2±0.1	1.94±0.18	4.9±0.1	9.4±0.1	1.88	31.5±0.3
Criollo-Mestizo	9.5±1.1	2.0±0.20	4.8±1.5	9.4±1.2	1.98	32.9±0.1
Cebu-Mestizo	9.5±0.6	1.96±0.12	4.5±0.8	9.17±0.70	2.11	35.3±4.7
St. Gertrudis	8.8±0.2	1.90±0.2	3.8±0.1	10.2±0.1	2.32	36.0±0.4
Pardo-Suiza	8.8±0.5	2.01±0.07	5.0±0.5	6.6±0.1	1.76	35.1±1.2
Hollando	9.9±0.6	1.95±0.12	6.5±0.6	9.9±0.1	1.52	34.2±3.7
Brahman-Cebu	9.2±0.1	2.00±0.15	4.6±0.1	9.4±0.1	2.00	36.8±0.2
Others	9.1±0.2	1.99±0.05	4.3±0.1	9.0±0.1	2.12	34.5±0.8

Table 1: BLOOD COMPOSITION OF DIFFERENT RACES OF HEALTHY CATTLE IN PARAGUAY (x ± SD)

Table 3 Effect of Farm Size and Management on Blood Composition from Beef Cattle ($\bar{x}$±SD±SEM)

Parameter	G R O U P			
	I	II	III	IV
AST (U/l)	49.9±14.2±0.7	58.4±21.4±1.4	43.6±18.9±0.6	50.3±20.6±0.5
ALT (U/l)	16.4± 5.7±0.3	16.3± 6.6±0.4	16.0± 6.7±0.2	17.7± 9.7±0.3
TP (gr/l)	73.8±10.4±0.3	74.8± 6.0±0.3	68.4± 8.9±0.2	69.3±11.1±0.3
Alb (gr/l)	26.0± 7.0±0.2	26.8± 7.2±0.3	28.1± 5.9±0.2	29.8± 6.8±0.2
Glob (gr/l)	52.6±12.3±0.5	49.0±13.0±0.9	43.5±10.3±0.5	39.5±11.3±0.6
Alb/Glob	0.50	0.57	0.63	0.76
Gluc (mg/dl)	62.2±13.3±0.7	64.6±16.6±1.1	70.4±14.7±0.5	85.6±29.1±0.8
Urea (mg/dl)	30.1±11.0±0.3	30.3± 8.4±0.4	26.9± 9.4±0.3	28.5±11.8±0.3
Bilir (ug/l)	3.6± 1.4±0.1	3.8± 2.1±0.2	4.2± 2.8±0.1	4.0± 1.9±0.1
Ca (mg/dl)	9.0± 1.1±0.03	9.2± 1.0±0.04	9.4± 1.1±0.03	9.2± 1.3±0.03
Mg (mg/dl)	2.0± 0.3±0.01	2.0± 0.3±0.01	1.9± 0.3±0.01	2.0± 0.3±0.01
P (mg/dl)	4.9± 1.4±0.04	4.6± 1.1±0.05	3.7± 1.2±0.03	3.9± 1.5±0.04
Cu (ug/dl X 10^{-2})	9.4± 1.6±0.05	9.4± 1.4±0.06	9.3± 1.3±0.01	9.5± 1.1±0.02
Ca/P	2.0± 0.7±0.02	2.1± 0.6±0.03	2.9± 1.3±0.03	2.8± 1.6±0.04
Hct (%)	32.0± 4.5±0.1	31.6± 4.8±0.2	36.3± 4.2±0.1	37.0± 4.3±0.1

Table 4 Effect of the Time During the Year on Blood Composition from Beef Cattle Kept Under Different Management

MONTH	GROUP	PARAMETER							
		AST u/l	ALT u/l	TP gr/l	Alb gr/l	Glob gr/l	A/G	Gluc. mg/dl	Urea mg/dl	Bilir. mg/dl
June	I	44.3±3.1	18.1±0.8	72.0±0.8	25.2±0.5	47.9±2.0	0.53	71.2±2.8	29.6±0.7	4.6±0.3
	II	5.9±3.1	19.1±0.9	68.9±1.8	27.6±0.5	44.7±3.1	0.62	70.3±2.0	28.9±0.8	4.3±0.3
	III	43.1±1.0	19.2±0.4	66.9±0.8	30.1±0.4	36.7±0.8	0.82	87.5±1.9	29.8±0.7	6.2±0.4
	IV	50.9±2.3	25.8±0.9	71.3±0.9	30.1±0.5	40.9±2.6	0.74	79.6±1.4	26.5±1.0	3.6±0.2
August	I	47.7±1.2	19.6±0.8	72.7±1.3	31.8±0.9	40.9±	0.78	66.8±2.9	35.1±1.2	3.7±0.2
	II	50.9±4.0	19.7±0.8	71.8±1.2	31.4±1.5	40.4±	0.78	64.7±1.2	33.7±1.0	3.7±0.3
	III	33.2±1.3	27.4±1.4	58.4±1.2	34.7±0.4	24.6±1.5	1.39	74.4±2.9	40.4±1.3	3.4±0.2
	IV	54.2±1.2	22.7±0.6	70.5±0.6	32.3±0.4	34.0±0.8	0.95	107 ±2.2	31.4±0.5	3.8±0.1

Table 6 LEVELS OF ENZYMES , PROTEINS, METABOLITES AND ELECTROLYTES IN BLOOD OF MALE AND FEMALE PARAGUAYAN CATTLE (x±SD±SEM)

PARAMETER	MALE	FEMALE
AST (U/l)	48.4 ± 11.1 ± 0.9	50.5 ± 11.5 ± 0.6
ALT (U/l)	16.8 ± 5.1 ± 0.41	17.4 ± 4.7 ± 0.2
Total Proteins (gr/l)	69.4 ± 4.2 ± 0.4	72.4 ± 3.1 ± 0.2
ALBUMIN (gr/l)	27.7 ± 3.8 ± 0.3	28.2 ± 7.1 ± 0.13
GLOBULIN (gr/l)	41.7 ± 5.3 ± 0.8	44.2 ± 9.6 ± 0.34
A/G	0.66	0.64
GLUCOSE (mg/dl)	77.7 ± 13.6 ± 1.0	77.5 ± 12.5 ± 0.5
UREA (mg/dl)	28.9 ± 5.3 ± 0.34	28.6 ± 5.5 ± 0.3
BILIRUBIN (mg/l)	3.89 ± 0.93 ± 0.07	4.04 ± 1.22 ± 0.05
CALCIUM (mg/dl)	9.2 ± 0.6 ± 0.1	9.3 ± 0.6 ± 0.1
PHOSPHORUS (mg/dl)	4.6 ± 0.7 ± 0.1	4.0 ± 0.7 ± 0.1 *
Ca/P	1.92	2.33 *
MAGNESIUM (mg/dl)	1.98 ± 0.30 ± 0.09	1.97 ± 0.14 ± 0.05
COPPER (mg/dl x 10^{-2})	9.35 ± 0.65 ± 0.04	9.39 ± 0.70 ± 0.02
Hematocrit (%)	35.8 ± 2.3 ± 0.1	36.4 ± 2.1 ± 0.1

n = 900 - 3000

* significantly different at $P < 0.05$

Table 5 Effect of Time During the Year on Blood Electrolytes from Beef Cattle Kept Under Different Management

MONTH	GROUP	PARAMETER				
		Ca (mg/dl)	Mg (mg/dl)	P (mg/dl)	Cu (mg/dlX10^{-2})	Ca/P
June	I	8.9 ± 0.1	2.2 ± 0.03	4.8 ± 0.1	9.6 ± 0.1	1.87
	II	9.3 ± 0.1	2.1 ± 0.03	5.2 ± 0.1	9.9 ± 0.1	1.77
	III	9.1 ± 0.1	2.0 ± 0.02	2.8 ± 0.1	8.8 ± 0.1	3.2
	IV	10.0 ± 0.1	2.2 ± 0.02	3.4 ± 0.1	8.8 ± 0.1	3.0
August	I	9.8 ± 0.1	2.0 ± 0.02	3.8 ± 0.1	9.4 ± 0.1	2.6
	II	10.1 ± 0.2	2.0 ± 0.04	3.6 ± 0.1	9.2 ± 0.1	2.8
	III	8.7 ± 0.1	2.0 ± 0.02	3.5 ± 0.2	9.2 ± 0.1	2.5
	IV	8.7 ± 0.1	1.9 ± 0.01	3.6 ± 0.1	9.4 ± 0.1	2.4

TABLE 7 BLOOD LEVELS OF INORGANIC PHOSPHORUS, TOTAL PROTEINS, GLOBULINS AND GLUCOSE IN PARAGUAYAN CATTLE AT DIFFERENT AGES (X ± SD± SEM)

		AGE (YEARS)					
PARAMETER		1	1-2	2-3	3-4	4-5	5-6
Phosphorus (mg/dl)	X	5.00	4.5 *	4.0	4.0	3.8	3.7
	SD	1.50	1.5	1.2	1.4	1.3	1.2
	SEM	0.04	0.1	0.1	0.1	0.10	0.1
Total Proteins (gr/dl)	X	66.1	68.0	70.9	73.4	72.8	74.3
	SD	10.2	10.8	11.3	9.5	10.9	11.1
	SEM	0.3	0.5	0.5	0.5	0.6	0.5
Globulins (gr/l)	X	41.7	43.2	48.4	47.7	51.8	51.3
	SD	12.7	14.1	12.1	12.8	13.7	14.2
	SEM	0.7	1.0	0.9	1.0	1.2	1.1
Glucose (mg/dl)	X	84.4	77.0	77.9	76.0	74.0	73.7
	SD	29.4	24.2	22.8	25.0	24.1	22.9
	SEM	1.1	1.3	1.2	1.4	1.4	1.3

(N = 150 - 1000)

BLOOD COMPOSITION OF PARAGUAYAN BEEF CATTLE WITH AND WITHOUT BLUETONGUE

(x ± sem)

PARAMETER	Bluetongue -	Bluetongue +
AST (U/l)	50.6 ± 0.5	51.3 ± 0.7
ALT (U/l)	17.1 ± 0.2	17.6 ± 0.3
TP (gr/l)	70.1 ± 0.2	73.4 ± 0.3
Alb (gr/l)	28.5 ± 0.1	27.5± 0.2
Glob (gr/l)	41.6 ± 0.4	45.9 ± 0.8
A / G	0.69	0.60 *
Gluc (mg/dl)	79.5 ± 0.6	74.8 ± 0.9
Urea (mg/dl)	29.2 ± 0.2	27.7 ± 0.3
Bilir (mg/l)	4.2 ± 0.1	3.9 ± 0.1
Ca (mg/dl)	9.2 ± 0.1	9.4 ± 0.1
Mg (mg/dl)	1.96 ± 0.05	2.00 ± 0.08
P (mg/dl)	4.2 ± 0.1	3.9 ± 0.1
Cu (mg/dl x 10^{-2})	9.4 ± 0.1	9.3 ± 0.1
Ca/P	2.19	2.41
Hct (%)	35.0 ± 0.1	34.9 ± 0.1

n = (Bluetongue - over 2,0000; Bluetongue + over 800)

* $P < 0.05$

BLOOD COMPOSITON IN CATTLE WITH AND WITHOUT LEUCOSIS
(X̄ ± SD ± SEM)

PARAMETER	LEUCOSIS	
	negative	possitive
Calcium (mg/dl)	9.3 ± 1.2 ± 0.02	9.1 ± 1.08 ± 0.1
Phosphorus (mg/dl)	4.17 ± 1.48 ± 0.02	4.35 ± 1.41 ± 0.1
Ca/P	2.23	2.09
Magnesium (mg/dl)	1.97 ± 0.29 ± 0.01	1.94 ± 0.25 ± 0.03
Total Proteins (gr/l)	70.6 ± 6.6 ± 0.19	75.8 ± 6.7 ± 1.1
Albumin (gr/l)	27.8 ± 3.4 ± 0.12	27.7 ± 3.3 ± 0.72
Globulins (gr/l)	42.8 ± 6.1 ± 0.38	48.1 ± 7.4 ± 0.46
A/G	0.65	0.58
Glucose (mg/dl)	77.8 ± 12.7 ± 0.5	75.2 ±16.2 ± 3.5
Urea (mg/dl)	28.6 ± 5.4 ± 0.18	28.2 ± 5.2 ± 1.1
Bilirubin (mg/dl)	4.06 ± 1.15 ± 0.01	3.70 ± 1.1 ± 0.24
Iodine	3.61 ± 2.20 ± 0.01	3.42 ± 2.09 ± 0.22
AST (U/l)	50.7 ± 11.5 ± 0.2	49.2 ±10.2 ± 1.1
ALT (U/l)	17.2 ± 4.7 ± 0.18	14.3 ± 3.8 ± 0.8

N = 100 - 3000

EFFECT OF TICKS NUMBER ON THE BLOOD COMPOSITION OF CATTLE

PARAMETER	NO TICKS	LOW <10	MIDDLE (11 - 50)	HIGH (> 50)	TOTAL
	Mean	Mean	Mean	Mean	Mean
Ca (mg/dl)	9.24	9.24	9.24	9.24	9.24
P (mg/dl)	4.62	4.04	4.06	4.03	4.19
Mg (mg/dl)	2.00	1.98	1.95	2.00	1.98
I	3.41	4.17	3.98	4.95	3.63
Cu (mg/dl)	0.09	0.09	0.09	0.09	0.09
PT (gr/l)	70.31	70.49	71.73	74.23	70.94
Alb - (gr/l)	28.30	28.01	28.01	28.63	28.11
Glob (gr/l)	44.70	46.29	49.77	51.23	46.87
Urea (mg/dl)	28.39	28.94	29.02	28.77	28.81
Glic (mg/dl)	75.35	78.93	76.35	77.40	77.50
GOT (U/l)	50.22	50.62	47.83	52.89	50.06
GPT (U/l)	17.17	17.61	16.89	15.86	17.28
Bil (mg/l)	4.09	4.09	3.82	3.56	4.01
Hct	35.8	35.2	34.2	33.5	35.01
A/G	0.63	0.61	0.56	0.56	0.60

EFFECT OF AGE AND NUMBER OF TICKS ON THE BLOOD LEVEL OF TOTAL PROTEINS

AGE (MONTHS)	NO TICKS	TICKS 1- 10	TICKS 11-50	TICKS > 50	TOTAL
	Mean	Mean	Mean	Mean	Mean
0 - 12	66.01	65.39	64.61	64.17	65.39
13 - 24	67.45	68.06	67.28	68.49	67.98
25 - 36	69.91	70.53	71.12	79.41	70.87
37 - 48	74.09	73.27	73.18	72.42	73.44
49 - 60	72.83	72.18	73.54	76.60	72.84
61 - 72	73.63	74.30	75.01	74.74	74.36
73 - 84	76.95	71.63	73.57	78.42	73.65
84	74.63	73.37	74.75	76.60	74.35
Total	70.31	70.49	71.73	74.23	70.94

BLOOD COMPOSITION IN CATTLE WITH AND WITHOUT ENDOPARASITES

($\bar{x}$ ± SD ± SEM)

PARAMETER	ENDOPARASITOSIS	
	negative	possitive
Calcium (mg/dl)	9.3 ± 0.7 ± 0.02	9.2 ± 0.6 ± 0.02
Phosphorus (mg/dl)	4.07 ± 0.73 ± 0.03	4.39 ± 0.74 ± 0.03
Ca/P	2.29	2.10
Magnesium (mg/dl)	1.99 ± 0.15 ± 0.01	1.96 ± 0.15 ± 0.01
Total Proteins (gr/l)	7.7 ± 5.6 ± 0.02	69.9 ± 5.5 ± 0.2
Albumin (gr/l)	28.4 ± 3.5 ± 0.14	27.6 ± 3.8 ± 0.2
Globulins (gr/l)	43.3 ± 6.1 ± 0.17	42.3 ± 6.7 ± 0.23
A/G	0.66	0.65
Glucose (mg/dl)	75.7 ± 12.1 ± 0.3	78.6 ± 13.7 ± 0.8
Urea (mg/dl)	28.8 ± 5.6 ± 0.2	29.0 ± 5.4 ± 0.3
Bilirubin (mg/l)	4.06 ± 1.1 ± 0.05	3.89 ± 1.3 ± 0.07
Iodine	3.53 ± 1.1 ± 0.04	3.82 ± 1.18 ± 0.05
AST (U/l)	50.1 ± 11.6 ± 0.5	49.2 ± 10.6 ± 0.6
ALT (U/l)	17.2 ± 4.7 ± 0.2	17.1 ± 4.7 ± -.28

N = 1200 - 2500

PERSPECTIVES D'AVENIR DANS LE TRAITEMENT DES MALADIES RESPIRATOIRES BOVINES

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1. INTRODUCTION

Malgré la diversité de l'arsenal thérapeutique dont dispose le Praticien, les maladies respiratoires des bovins engendrent encore des pertes considérables pour l'économie agricole. Ces pathologies représentent en effet le problème sanitaire numéro un chez les bovins de type viandeux. Rares sont les veaux qui n'ont nécessité aucune intervention thérapeutique respiratoire pendant leur période de croissance somatique. D'autre part, l'importance des séquelles irréversibles, le taux élevé de mortalité et le coût des interventions thérapeutiques expliquent l'énorme impact économique de ces maladies respiratoires.

Plusieurs explications peuvent justifier cette situation. D'une part, les bovins, et surtout ceux de type viandeux, sont défavorisés sur le plan de leur fonction pulmonaire (46). Cette dernière est particulièrement vulnérable chez les jeunes animaux, en raison de l'immaturité fonctionnelle de leur système respiratoire (44). D'autre part, la pathogénie des lésions pulmonaires est encore loin d'être définitivement élucidée chez les bovins et de nombreuses recherches multidisciplinaires sont plus que jamais nécessaires. Enfin, les dommages lésionnels et fonctionnels provoqués au niveau pulmonaire sont dus non seulement à l'action directe des agents infectieux mais encore et surtout aux réactions de l'organisme vis-à-vis de ces agresseurs (9).

Malheureusement, la modulation de ces réactions n'est pas simple en raison du grand nombre de médiateurs incriminés, de leurs nombreuses interactions, de la complexité de leurs mécanismes d'action ainsi que de la difficulté d'une action thérapeutique suffisamment sélective.

Néanmoins, l'amélioration des techniques d'investigation de la fonction pulmonaire et la disponibilité d'antagonistes spécifiques vis-à-vis des médiateurs chimiques ont permis d'éclaircir quelque peu la pathogénie du syndrome de détresse respiratoire aiguë (SDRA) et d'envisager différentes stratégies qui devraient améliorer l'efficience de sa thérapeutique. La prévention hygiénique et médicale des maladies respiratoires, bien qu'elle constitue un élément déterminant dans la maîtrise de ces pathologies, ne fait pas l'objet de cette revue.

2. REACTION INFLAMMATOIRE PULMONAIRE

La séquence des évènements qui conduisent à l'inflammation du poumon est encore peu connue. De plus, elle varie en fonction de nombreux facteurs comme le type d'agent infectieux, l'espèce animale, le statut immunitaire de l'individu, etc... Il semble cependant que, parmi tous les mécanismes incriminés, quatre jouent un

rôle important (73). En bref, soit le lipopolysaccharide endotoxinique (LPS) active le complément, avec comme conséquences successives la libération du peptide C5a, la stimulation des neutrophiles et des macrophages alvéolaires, la production de métabolites de l'acide arachidonique, du platelet activating factor (PAF), des autacoïdes, des radicaux oxygène, des enzymes protéolytiques, des cytokines et d'autres médiateurs (58). Soit le LPS peut stimuler directement les neutrophiles (92). Soit le LPS agresse directement l'endothélium (32). Soit le LPS induit la libération par les macrophages du facteur de nécrose tumorale (TNF) ou cachectine, qui est l'une des cytokines les plus actives dans la réaction inflammatoire pulmonaire (83).

Ces différents mécanismes conduisent principalement à l'apparition de lésions au niveau des cellules endothéliales pulmonaires, de dysfonctionnement au niveau des sécrétions pulmonaires, de la perméabilité alvéolo-capillaire et de la contractilité des muscles lisses respiratoires, ainsi que d'autres anomalies au niveau de l'homéostasie de l'organisme.

Lorsque le système respiratoire est agressé de façon modérée par des agents pathogènes, différents mécanismes vont tenter d'en limiter l'impact fonctionnel, selon le principe du feed-back négatif.

L'hypoxémie et l'hypercapnie stimulent les centres respiratoires afin d'accroître la ventilation alvéolaire (7).

Le tonus des muscles respiratoires est augmenté afin d'en accroître l'efficacité (55). La vasoconstriction hypoxique empêche le sang de s'engouffrer dans les zones pulmonaires mal ventilées afin de corriger les inadéquations du rapport ventilation/perfusion (91).

La clearance muco-ciliaire est augmentée par une modification de la secrétion de mucus et du mouvement des cils (38). Il en est de même au niveau des macrophages alvéolaires (70). Ces mécanismes de feed-back négatif associés à d'autres non décrits dans cette revue (47) vont donc compenser les effets néfastes causés par les agents pathogènes afin de restaurer progressivement l'homéostasie du système respiratoire.

Lorsque l'agression du système respiratoire est trop violente, trop virulente ou trop massive, les réactions de l'organisme sont telles qu'elles ont tendance à aggraver le déficit fonctionnel, selon le mécanisme du feed-back positif.

L'hypoxie tissulaire est responsable d'une augmentation du métabolisme anaérobique, avec comme conséquence l'apparition d'une acidose métabolique qui aggrave l'acidose respiratoire due à l'hypercapnie. Or, une grave acidose peut être responsable d'un dysfonctionnement des centres respiratoires et d'une diminution de la clearance muco-ciliaire, avec les conséquences qu'on imagine sur la fonction pulmonaire (90).

L'afflux de cellules sanguines au niveau pulmonaire peut y induire l'apparition en quantité excessive de différentes substances comme des radicaux oxygène, des médiateurs de l'inflammation, des enzymes protéolytiques, etc ... (18, 43, 63). Les radicaux oxygène, qui ont une action positive grâce à leur effet bactéricide, peuvent aussi induire des lésions graves du parenchyme pulmonaire. Certains de ces médiateurs peuvent avoir une action très néfaste au niveau des muscles lisses pulmonaires et au niveau de la perméabilité capillaire, et ce d'autant plus que les

fonctions de clearance de l'endothélium vasculaire pulmonaire sont déficientes. De même, les enzymes protéolytiques peuvent induire des lésions d'emphysème et détruire des neuropeptides comme le vasoactive intestinal polypeptide (VIP). Or, ces derniers semblent être des neuro-transmetteurs indispensables au bon fonctionnement de la voie inhibitrice non adrénergique et non cholinergique (39). Il est en effet révélateur de constater qu'une pasteurellose expérimentale est beaucoup moins grave chez des veaux préalablement dépouillés de leurs globules blancs (81).

La fonction de clearance par l'endothélium vasculaire pulmonaire des amines et autres substances vasoactives peut être inhibée par une pathologie pulmonaire, ce qui a tendance à aggraver les dysfonctionnements dus à ces substances (22).

Les processus inflammatoires peuvent inhiber la vasoconstriction hypoxique, physiologique et donc bénéfique (48, 69), et la remplacer par une vasoconstriction "aveugle" due à des médiateurs comme l'histamine, la sérotonine, les dérivés de l'acide arachidonique, le PAF, etc ... L'hypertension qui en résulte peut être responsable d'une perturbation de la dynamique des fluides pulmonaires et d'une augmentation du travail du coeur droit (91).

De même, l'augmentation brutale du travail des muscles inspiratoires peut induire une fatigue diaphragmatique avec comme conséquence une insuffisance ventilatoire et donc une aggravation de l'état clinique (59).

Tous ces mécanismes de feed-back positif peuvent donc aggraver de façon exponentielle l'hypoxémie, l'hypercapnie et l'acidose qui en résulte. Dans ces circonstances, une déficience respiratoire devient rapidement fatale si une intervention thérapeutique appropriée n'arrive pas à mettre fin à ce cercle vicieux infernal. Entre ces deux situations extrêmes, guérison spontanée grâce aux feed-back négatifs d'une part et évolution fatale à cause des feed-back positifs d'autre part, il existe de très fréquentes situations intermédiaires qui évoluent le plus souvent vers l'établissement de lésions pulmonaires irréversibles et donc préjudiciables aux performances zootechniques de ces bovins.

3. AGENTS ANTI-MICROBIENS

Nul ne conteste l'indispensabilité de l'antibiothérapie dans les pathologies respiratoires bovines. Le facteur limitant de son efficience ne semble pas être la qualité de l'agent antibactérien. En effet, de nombreuses substances pharmacologiques se sont montrées très actives vis-à-vis des germes responsables des pathologies respiratoires bovines (28, 45, 64). Les éventuels problèmes d'efficacité semblent plutôt résulter du non respect de deux autres règles d'or de l'antibiothérapie, en l'occurrence la précocité et la durée de l'intervention. Ceci est principalement du aux conditions de surveillance et de stabulation auxquels sont soumis les bovins de type viandeux.

4. INHIBITEURS DE L'ACTIVITE DE LA CYCLOOXYGENASE

Parmi les produits de la cyclooxygénase, les prostaglandines PGD2, PGF2α et TXA2 sont principalement responsables de la contraction des muscles lisses pulmonaires (17). L'étude de l'intérêt de l'utilisation des antiinflammatoires non stéroïdiens (AINS) dans le traitement des pathologies pulmonaires a donné des résultats pour le moins contradictoires (17, 78, 82). Plusieurs explications peuvent y être apportées. D'une part, en bloquant la synthèse des produits de la cyclooxygénase, les AINS induisent une augmentation de la synthèse des produits de la lipoxygénase (voir ci-dessous). D'autre part, parmi les prostaglandines inhibées par les AINS, certaines exercent une action favorable sur la fonction pulmonaire (PGE1, PGE2, PGI2) (17).

C'est pourquoi il semblerait plus approprié d'utiliser des inhibiteurs plus spécifiques des écosanoïdes. Parmi ceux-ci, les antagonistes spécifiques des thromboxanes semblent lever quelques espoirs thérapeutiques (41, 68).

5. INHIBITEURS DE L'ACTIVITE DE LA LIPOXYGENASE

Parmi les leucotriènes synthétisées à partir de l'acide arachidonique, LTC4, LTD4 et LTE4 semblent jouer un rôle dans l'inflammation pulmonaire et les anomalies lésionnelles et fonctionnelles qui en résultent (3). Ces phénomènes semblent être atténués par l'utilisation d'inhibiteurs spécifiques (12).

6. ANTAGONISTES DU PAF

Le PAF semble être responsable d'un certain nombre d'anomalies fonctionnelles pulmonaires lors de SDRA (11, 34). L'utilisation d'antagonistes spécifiques semble capable d'en moduler les conséquences pathologiques (34, 35, 79).

7. GLUCOCORTICOIDES

L'intérêt thérapeutique des corticostéroïdes dans le SDRA a fait l'objet de nombreuses polémiques. Leur capacité à inhiber les métabolites de l'acide arachidonique dépend de nombreux facteurs (74). D'autre part, leur action défavorable vis-à-vis des mécanismes de défense contre les microorganismes limitent leur intérêt curatif lorsque ces derniers sont responsables du déclenchement de la pathologie respiratoire. Enfin, des études récentes ont démontré l'inefficacité à terme des stéroïdes dans le traitement du SDRA d'origine infectieuse (6, 49).

8. ANTI-OXYDANTS

Les radicaux libres dérivés de l'oxygène sont parmi les principaux médiateurs des pathologies respiratoires aiguës (86). Leur inhibition revêt donc un intérêt thérapeutique potentiel sous la forme d'anti-oxydants enzymatiques comme les

catalase, superoxyde dismutase et gluthation peroxydase (51, 57, 77), d'anti-oxydants non enzymatiques comme les vitamines A, C et E et la taurine (50, 52) ou de chélateurs du fer comme la desferioxamine (30).

9. INHIBITEURS DES ENZYMES PROTEOLYTIQUES

Les protéases comme les élastases et la thrombine sont responsables de certaines lésions observées lors de pathologies pulmonaires (42, 75). Leur inhibition pourrait diminuer leur nocivité tissulaire, comme cela a été démontré in vitro (87).

10. ANTI-HISTAMINIQUES

Bien que la stimulation des récepteurs H1 induise une contraction des muscles lisses respiratoires (26) et que l'histamine soit libérée au cours du SDRA (61), l'intérêt des anti-histaminiques semble limité sur le plan du traitement des maladies respiratoires bovines (27).

11. INHIBITEURS DE LA SEROTONINE

L'action pathogène de ce médiateur au niveau du système respiratoire des bovins a été décrite à la fois in vitro (62) et in vivo (15, 16). Au vu des résultats préliminaires obtenus en laboratoire et sur le terrain, le blocage des récepteurs S2 semble être une composante très prometteuse dans le traitement du SDRA.

12. MODULATEURS DU SYSTEME NEURO-VEGETATIF

Les systèmes adrénergiques (α et β) et cholinergiques jouent un rôle indubitable dans l'inflammation pulmonaire. Cependant, la modulation de ces effets lors de pathologies respiratoires bovines est loin d'être évidente (14, 27, 29, 88). Néanmoins, l'administration de substances β2-agonistes ou anticholinergiques par nébulisation semble améliorer leur efficacité (60, 76) tout en compliquant leur utilisation sur le terrain.

13. ANTAGONISTES DES PEPTIDES

De nombreux peptides semblent jouer un rôle dans la genèse des lésions pulmonaires. Il s'agit entre autre du complément et des peptides associés (40, 85), des cytokines comme le TNF (83), de l'interleukine 1 (25) et l'interleukine 2 (19), des peptides chémotactiques comme le formyl-méthionine-containing tripeptide (10), des peptides opioïdes (33), de la substance P (71), de la neurokinine A (4), etc ... L'utilisation d'antagonistes ou d'anticorps spécifiques vis-à-vis de ces substances représentera peut-être une voie thérapeutique nouvelle comme le suggèrent certaines études récentes (5, 21). D'autre part, l'utilisation à des fins

thérapeutiques de certaines cytokines comme l'interféron fait l'objet de rapports contradictoires (67).

14. BLOQUEURS DES CANAUX A CALCIUM

De nombreux processus inflammatoires nécessitent la médiation du calcium en tant que second messager (37). C'est ce qui explique l'effet bénéfique du verapamil lors de SDRA expérimental chez le mouton (2, 66). D'autre part, des antagonistes de la calmoduline comme le calmidazolium pourraient également présenter un intérêt thérapeutique (80).

15. INHIBITEURS DES NEUTROPHILES ET MACROPHAGES

L'observation qu'une déplétion en neutrophiles réduit l'intensité des dégâts pulmonaires lors de SDRA (81) a attiré l'attention sur des substances capables d'inhiber le fonctionnement de ces cellules sanguines. Il en résulte un intérêt potentiel de l'utilisation de substances comme le dapsone (56) ou un inhibiteur de la protéine-kinase C (84), dans la prévention de la libération, au niveau pulmonaire, de métabolites toxiques par les neutrophiles et les macrophages.

16. VASODILATATEURS PULMONAIRES

De nombreuses substances exercent une action dilatatrice sur les muscles lisses pulmonaires via une augmentation de l'AMP cyclique (20). Il s'agit de substances aussi diverses que le nitroprusside de sodium (93), la papavérine (54), la théophylline (24), la pentoxifylline (36, 53, 89), l'adénosine (1, 13) et le VIP (23, 65, 72). L'intérêt thérapeutique dans le SDRA de ces trois dernières substances semble être confirmé par les premiers résultats expérimentaux (13, 72, 89), même si la compréhension de leur mécanisme d'action nécessite encore de nombreuses recherches.

17. DIVERS

D'autres stratégies curatives comme les mucolytiques, les diurétiques, l'oxygénothérapie, etc ... ont été récemment analysées chez les bovins (8). Il en résulte qu'il existe peu d'évidence quant à leur intérêt thérapeutique dans cette espèce.

18. CONCLUSIONS

Il ressort de cette synthèse que les progrès récents réalisés dans la physiologie, la physiopathologie et la pharmacologie respiratoires n'ont guère simplifié notre compréhension des maladies respiratoires. Il en résulte une plus grande complexité dans l'approche thérapeutique de ces pathologies. Cependant, il semble évident que (1) toute une série de substances pharmacologiques encore utilisées sur le terrain n'ont plus leur place dans l'arsenal thérapeutique du praticien, (2) de

nouvelles molécules semblent douées d'une efficacité très prometteuse à la condition d'une utilisation appropriée, et (3) il existe un réel espoir de réduire la mortalité et les lésions irréversibles dues aux pathologies respiratoires bovines. Cependant ces progrès dans le domaine thérapeutique n'auront un impact réel sur l'économie de ce type de production que s'ils sont accompagnés de mesures appropriées dans d'autres domaines comme la prévention hygiénique et médicale.

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20. RESUME

Le but de cette synthèse est de passer en revue les stratégies thérapeutiques dont pourrait disposer le praticien dans le futur afin d'améliorer l'efficience du traitement des maladies respiratoires bovines.

21. ABSTRACT

The purpose of this review is to analyse how the practitioner could ameliorate in the future the efficiency of the treatment of bovine respiratory diseases.

22. ZUSAMMENFASSUNG

Der Zielpunkt dieses Übersichtsreferats ist die verschiedenen Arzneimittel zu übersehen, die für den praktischen Tierarzt in der Zukunft nützlich sein könnte um die Wirksamkeit der Behandlung von den Atmungskrankheiten der Rinder zu erhöhen.

BIOTECHNOLOGIES ET BUIATRIE

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1.INTRODUCTION

Biotechnologie est un mot issu de deux termes grecs : "bios" signifiant vie, à l'origine du mot biologie et "technologie" déjà employé par Cicéron et Plutarque. Il s'agit d'une science interdisciplinaire, systémique, qui fait appel à la chimie, à la biochimie, au génie enzymatique, au génie chimique ou industriel, à la microbiologie, à l'ingénierie génétique, au génie microbiologique, aux mathématiques, à l'informatique, etc...
Dans le domaine de la pathologie et des productions bovines les biotechnologies les plus fécondes peuvent être regroupées sous quatre thématiques : anticorps monoclonaux, hybridation moléculaire, génie génétique, manipulation des embryons. L'ampleur de ces différents domaines justifie que nous ne nous intéressions qu'à certains d'entre eux. Notre choix concerne les deux derniers dont les applications sont davantage en rapport avec les productions qu'avec la pathologie bovine.

2. GENIE GENETIQUE ET BUIATRIE

2.1 DEFINITION

La technologie de l'ADN recombinant permet la production de protéines dans un organisme hôte génétiquement modifié. Ces protéines peuvent être des enzymes, des récepteurs, des hormones, des peptides régulateurs, des facteurs de croissance, des immunomodulateurs, etc...

2.2 TECHNIQUES (7)

Les étapes suivantes (Fig.1) sont nécessaires pour la production d'une protéine par une bactérie comme Escherichia Coli :

2.2.1 Isolement du gène codant pour la protéine

Après identification du gène celui-ci peut être synthétisé par voie chimique à partir d'un assemblage de nucléotides. Il est possible aussi de faire appel aux enzymes de restriction pour disséquer le gène recherché dans l'ADN chromosomique de l'organisme producteur habituel de la protéine. Plus communément est utilisé l'ADN complémentaire (ADNc) de l'ARN messager (ARNm) codant pour la protéine après intervention de la transcriptase inverse qui en assure la copie.

2.2.2 Incorporation du gène dans un vecteur.

Les vecteurs les plus régulièrement utilisés sont des plasmides formations d'ADN circulaire, bicaténaire et extrachromosomiques, capables de se reliquer de façon autonome. Les plasmides peuvent être ouverts par les enzymes de restriction ce qui permet l'insertion du gène.

2.2.3 Transfert du vecteur dans la cellule réceptrice

La souche de colibacille K12 est la bactérie la plus souvent utilisée dans la technologie de l'ADN recombinant. Le plasmide et la souche d'E. Coli K12 sont mis en présence dans un milieu enrichi en calcium

libre ce qui perméabilise la membrane du colibacille et permet la pénétration du plasmide. D'autres cellules réceptrices peuvent être utilisées : virus (baculovirus), levures, cellules animales ou végétales. Certaines offrent l'avantage de fournir une protéine immédiatement active ce qui n'est pas toujours le cas avec les bactéries.

2.2.4 Expression du gène

Etant donné que toutes les cellules n'incorporent pas forcément le plasmide vecteur il est nécessaire d'identifier celles qui l'ont effectivement incorporé. Pour les bactéries il est commode de marquer le plasmide en le couplant avec un autre gène codant par exemple pour la résistance à un antibiotique.Par la suite on reconnaîtra les colonies détentrices du gène par leur possibilité de culture sur un milieu contenant l'antibiotique vis-à-vis duquel elles ont acquis une résistance. Après fermentation la protéine recherchée est soit disponible dans le milieu de culture, ou bien elle est obtenue par extraction-purification après lyse de la masse cellulaire.

2.2.5 Optimisation de la production

Il s'agit de l'étape clef du processus de production industrielle dont dépendra le rendement économique de l'opération.

2.3 APPLICATIONS

Les applications de la technologie de l'ADN recombinant en buiatrie sont multiples :
* production de quantités industrielles de matières premières par fermentation et à bas prix de revient : antibiotiques, acides aminés, vitamines,
* production de protéines par ailleurs difficiles à obtenir dans d'excellentes conditions de coût : antigènes viraux ou bactériens base de la préparation de vaccins, hormones type somatotropine, interferons type interferon recombinant bovin alpha I[1],
* production de microorganismes dont les caractéristiques ont été modifiées à des fins vaccinales (vaccins recombinants) ou zootechniques (probiotiques).

2.4 QUELQUES EXEMPLES D'APPLICATIONS

2.4.1 L'hormone somatotrope bovine (BST)

La sécrétion hypophysaire de BST est régulée par deux facteurs hypothalamiques d'une part la somatoliberine qui la stimule et d'autre part la somatostatine qui l'inhibe. La somatotropine agit sur la galactopoïèse (ou la croissance chez le jeune animal) par l'intermédiaire des somatomédines qui sont synthétisées par le foie dont les récepteurs spécifiques existent entre autres dans le tissus mammaires. Il est intéressant de noter que le potentiel des vaches laitières est directement lié à leur taux plasmatique de BST circulante.
Injectée à la dose de 500 mg par vache, par voie sous-cutanée, tous les 14 jours, de récents essais menés en France ont montré une augmentation moyenne de la productivité laitière de 2,3 à 4,7 kg de lait standard à 4 % de matière grasse par vache et par jour. Cette augmentation de la production laitière est systématique et toujours statistiquement significative bien qu'elle apparaissent inférieure à la moyenne de 5,7 kg par animal et par jour rapportée dans la littérature mais correspondant souvent à des injections journalières (30). Cette rémanence de 15 jours mériterait d'être accrue pour des utilisations très prolongées au cours de la lactation. En revanche

elle apparaît adéquate pour un emploi tactique visant à valoriser les opportunités techniques et économiques pouvant s'offrir aux éleveurs : en effet cela semble être actuellement la vocation majeure de l'utilisation de la somatotropine chez la vache laitière en régimes de quota.
Toujours dans les essais français la réponse à la BST est assez nettement supérieure :
* chez certains individus qui mériteraient d'être identifiés par des critères objectifs à définir, il en découlerait des indications sélectives permettant d'assurer de meilleurs résultats zootechniques et économiques,
* chez les primipares, à titre d'exemple 4,6 kg de lait par animal et par jour pour l'ensemble des animaux traités, au lieu de 3,4 kg ce qui prouve que ces animaux n'ont pas souffert de sous-alimentation et de l'effet de concurrence de leurs besoins de croissance. Ces données suggèrent qu'il est permis d'amortir plus vite les frais de sélection et d'élevage mais imposent d'être plus vigilant sur la qualité de l'alimentation sous peine de pénalités en raison de la compétition avec les besoins de croissance et du plus faible appétit de ces génisses,
* chez les multipares à production moyenne, les vaches les plus productrices n'étant pas les meilleures répondeurs. Ces constatations fournissent un moyen d'augmenter l'homogénéité des troupeaux et d'uniformiser les rations,
* chez les sujets en début de lactation plutôt qu'à la fin de celle-ci. Dans ces conditions s'il est déconseillé d'intervenir pendant les trois premiers mois de lactation afin de profiter de la consommation volontaire maximum et d'assurer la réussite de la fécondation, il convient de ne pas trop différer le traitement même si une certaine amélioration des taux compense en partie la moindre stimulation de la production lactée.
Pendant la durée de l'utilisation de la BST la composition du lait n'est pas statistiquement modifiée. Il pourrait se manifester certaines tendances à l'augmentation des proportions des matières grasses, des protéines totales, de la caséine, de la lactalbumine, du calcium et du phosphore alors que les concentrations en acides gras à chaîne moyenne ou courte sont susceptibles de diminuer en faveur des acides gras à longue chaîne (4). Comme cela été vérifié en France chez des animaux bien alimentés, après la période critique qui suit le vêlage, la BST procure une augmentation de la productivité laitière qui n'entraîne pas d'altération de la qualité du lait en particulier de sa valeur fromagère : par exemple pour le camembert fromage français typique les qualités gustatives (arôme, saveur, fermeté) et les rendements fromagers ne sont pas modifiés (30).
L'administration de BST à des vaches laitières ne s'accompagne d'une augmentation des acides gras libres cirulants qu'en début de traitement ou chez des animaux en période de déficit de leur balance énergétique. Les risques de surcharge graisseuse du foie et d'altérations de l'état corporel ne concernent que les animaux sous alimentés (3). Au cours des traitements avec la somatotropine l'efficacité alimentaire est régulièrement et sensiblement améliorée souvent de près de 8 à 10 % à la fois à cause de la dilution des frais fixes d'entretien chez les vaches plus productrives et en raison de l'effet sur l'homéorrhèse qui privilégie le partage des nutriments en faveur de la sécrétion lactée au détriment des réserves.
Tant en France qu'à l'étranger (3, 30) les performances de reproduction des sujets recevant de la BST évaluées par le nombre d'inséminations nécessaires pour l'obtention de la fécondation ou par l'intervalle entre 2 vêlages successifs sont modérément altérées.

Cette régression n'est manifeste que la première année de traitement. Il apparaît que l'élévation de la productivité laitière et le risque concomitant de déficit énergétique suffisent pour une bonne part à expliquer le retard de fécondation. On sait en effet que l'intervalle entre vêlages s'accroît de 1 jour pour toute augmentation de production de 100 kg. L'utilisation de la BST n'a par ailleurs aucun effet sur les taux de kystes ovariens et de rétentions placentaires, sur la facilité au vêlage et sur les performances zootechniques des descendants.
La BST n'augmente pas la fréquence des cétoses cliniques, des cétoses subcliniques (rôle protecteur du phénomène d'homéorhèse) ou des fièvres vitulaires (rôle protecteur d'une légère augmentation de la calcémie et de l'augmentation du turn-over du calcium). La BST pourrait favoriser les indigestions par surcharge ou avec météorisation mais n'a pas d'effet sur la fréquence des boiteries.
La conséquence de l'administration de BST à des vaches laitières sur la santé de la glande mammaire font toujours l'objet d'investigations. Au cours des premiers essais cliniques aucune anomalie n'avait été signalée. Par la suite un taux plus élevé de mammites a été relevé chez les animaux traités à des doses fortes et ayant le plus d'antécédents de mammites avant traitement. Le comptage des cellules somatiques dans le lait destiné à approcher les mammites subcliniques a fourni selon les essais, soit des résultats normaux, soit des taux plus élevés chez les sujets traités. L'influence de la BST sur la durée d'évolution des mammites cliniques est variable selon les observations (nulle, négative ou positive). Les travaux de BURVENICH (10) se rapportant à la mammite colibacillaire montrent que la somatotropine modifie favorablement l'activité fonctionnelle des polynucléaires neutrophiles circulants (augmentation de la production des radicaux oxygénés), facilite la restauration qualitative et quantitative de la production laitière.

2.4.2 L'interferon recombinant bovin alpha1[1]

Il existe 3 familles d'interferon qui se distinguent par la nature de la cellule sécrétrice mais aussi par l'agent inducteur :
* l'interferon alpha ou leucocytaire est produit par les macrophages et les lymphocytes non B et non T. Il existe une vingtaine de sous-types dont la différence porte sur la répartition des acides aminés qui les composent.
* l'interferon beta ou fibroblastique glycoprotéine est produit par les fibroblastes et les cellules endothéliales en réponse à une stimulation virale ou antigénique. Deux sous-types ont été identifiés composés de 166 acides aminés, ils ont en commun le tiers de leurs molécules avec les interferons alpha,
* l'interferon gamma est élaboré par les lymphocytes T, composés de 146 acides aminés, il est le plus cytotoxique et possède l'activité antitumorale la plus puissante. Il est proche des lymphokines du type interleukine 2.
Les propriétés antivirales r Bo IFN alpha 11 ont été démontrées in vitro et in vivo. Sur différents supports cellulaires et en comparaison avec d'autres types d'interferons bovins ou humains, le r Bo IFN alpha 11 réduit la multiplication des principaux virus impliqués dans les bronchopneumonies infectieuses enzootiques (BPIE) des jeunes bovins : virus de la maladie des muqueuses, virus parainfluenza 3, virus respiratoire syncitial et virus de la rhinotrachéite infectieuse bovine. Tous ces virus ne sont pas également sensibles, leur sensibilité semble décroître dans l'ordre de citation ci-dessus. In vivo l'activité antivirale spécifique de r Bo IFN alpha $_1$1 a surtout été étudiée dans un modèle expérimental de

maladies respiratoires associant le virus de la rhinotrachéite infectieuse bovine à Pasteurella haemolytica A1 inoculés en aérosol à 4 jours d'intervalle (2, 12). La protection conférée par l'interféron est effective tant par voie intranasale que par voie intramusculaire. L'absence de diminution significative de l'excrétion virale dans le mucus nasal associée à la mise en évidence d'une réduction de titre dans les produits d'aspiration transtrachéale suggère plusieurs mécanismes d'action possibles pour l'interferon : soit un effet central sur l'ensemble du système immunitaire, soit un effet local au niveau de la cible pulmonaire. Etant donné la relative résistance du virus de la rhinotrachéite infectieuse bovine à l'interferon, la protection clinique conférée par le r Bo IFN alpha 11 pourrait aussi résulter de l'effet immunomodulateur entrevu plus haut (5).
Les indications du r Bo IFN alpha 11 en buiatrie sont essentiellement la prévention rapprochée des bronchopneumonies infectieuses enzootiques des jeunes bovins. Pour les bovins de races à viande, sevrés, nés dans l'année, ayant effectué une saison de pâturage et rassemblés pour l'engraissement, les essais sur modèles expérimentaux, les essais terrain nord-américains (20) et les essais français (15) montrent l'efficacité clinique d'une injection de r Bo IFN alpha 11 de 5 mg par voie intra-musculaire une seule fois avant l'exposition au risque ou dès l'identification des premiers malades dans le groupe. Pour les veaux de boucherie et pour les veaux d'élevage les essais cliniques effectués en Suisse (21) sont également en faveur de l'utilisation d'une dose de 5 mg de r Bo IFN alpha 11 administrée une seule fois par voie intra-musculaire au moment de la mise en place des animaux dans les ateliers de production.
L'existence d'une homologie de séquence de près de 70 % entre la trophoblastine bovine ou bovine trophoblastine protéine A (b TP-1) et les interferons alpha de classe II permet d'envisager une application du r Bo IFN alpha j1 dans la maîtrise de la reproduction. En effet, la trophoblastine représente un signal embryonnaire qui secrété entre le 15ème et le 17ème jour de gestation contribue à prévenir la lutéolyse et à conserver la sécrétion de progestérone par l'ovaire. L'utilisation de l'interferon à cette phase de la gestation pourrait donc concourir à la prévention des avortements embryonnaires. Dans cette voie des essais réalisés avec le r Bo IFN alpha 11 par PLANTE et coll (23) ont montré que l'administration de la molécule à des bovins tant par voie intra-utérine que par voie intra-musculaire augmente l'intervalle entre deux oestrus et prolonge la durée de vie du corps jaune (Fig.2).

2.4.3 Probiotiques

Les probiotiques sont des microorganismes : bactéries, levures ou champignons utilisés en général seuls, parfois en mélanges binaires (lactobacilles et streptocoques) ou même complexes, dont l'activité et/ou les produits issus de leur métabolisme ou de leur structure sont favorables au bon fonctionnement de l'écosystème microbien digestif. Ils sont utilisés chez le jeune pour améliorer l'état sanitaire surtout chez les sujets ayant subi un stress ou pour prévenir ses effets, chez les animaux en croissance pour améliorer les performances zootechniques, chez les animaux en gestation pour améliorer l'état sanitaire et la survie de leur progéniture.
Différentes préparations commerciales d'extraits de rumen contenant des microorganismes ou des enzymes ont été proposées pour augmenter les performances et préserver la santé des bovins. Aucune n'est vraiment efficace. L'emploi de levures vivantes (Saccharomyces cerevisae) comme probiotiques destinés au rumen s'est largement

développé au cours de ces dernières années avec des résultats intéressants : 6 à 8 % de plus pour la production laitière avec augmentation de la synthèse des protéines et des matières grasses. Une plus grande activité microbienne à l'égard des constituants pariétaux peut être obtenue en introduisant des gènes pour la dégradation de la lignine et/ou pour la production de cellulases dans l'ADN de bactéries cellulolytiques ou même non cellulolytiques. Certains transferts ont été réalisés mais aucun n'a permis d'améliorer le fonctionnement du rumen, les bactéries manipulées ne parvenant pas à se maintenir dans le milieu (17).
Dans l'intestin grêle l'efficacité thérapeutique et/ou zootechnique des probiotiques reposerait sur trois propriétés essentielles : effet de barrière vis-à-vis de certaines bactéries, amélioration de la capacité digestive, effet immunostimulant. Aucun argument expérimental irréfutable ne permet actuellement de penser que les probiotiques soient capables de s'établir dans l'intestin, d'adhérer aux entérocytes et de ce fait de protéger l'animal contre les bactéries nuisibles. Les deux autres hypothèses formulées semblent en revanche dignes d'intérêt : les probiotiques agiraient en accroissant la capacité digestive de l'intestin grêle de l'hôte, mais la nature des mécanismes nous échappe encore. Ils agiraient aussi ou indépendamment sur le système immunitaire en améliorant l'état de santé de l'hôte et par là même le rendement de sa machinerie digestive ou seulement son appétit ici encore par un mécanisme inconnu (25).
Dans le domaine nouveau et plein de promesses des probiotiques l'insuffisance des connaissances sur leur mode d'action ralentit considérablement les améliorations d'efficacité pouvant découler de la technologie de l'ADN recombinant. Il importe aussi de savoir si cette efficacité résulte de leur présence ou de leurs produits. Dans ce dernier cas glycoprotéines, enzymes et autres oligosaccharides pourraient être issus des biotechnologies. Dans tous les cas le transfert des gènes de manière à stimuler l'activité des souches productrices sera à la base de tout progrès significatif (19).

3. MANIPULATIONS DES EMBRYONS ET BUIATRIE

3.1 DEFINITIONS

L'amélioration des performances du cheptel bovin a largement bénéficié des progrès en matière de reproduction.La production d'embryons (superovulation des donneuses, fécondation, collecte des embryons 6 à 8 jours après, évaluation de leur qualité) et la transplantation embryonnaire (synchronisation des receveuses, sélection du jour du transfert, transfert proprement dit) ne sont plus à proprement parler des techniques nouvelles. Pratiquées en ferme depuis une dizaine d'années ces techniques ont été particulièrement prolifiques puisqu'elles ont généré bon nombre de nouvelles possibilités qui en dérivent et qui possèdent en commun la "manipulation" de l'embryon : fécondation in vitro, sexage, duplication, clonage et transfert de gènes.

3.2 FECONDATION IN VITRO

Le premier bébé éprouvette Louise BROWN est né en 1978, le premier veau né après une fécondation in vitro en 1981 et jusqu'en 1987 la naissance de moins d'une dizaine de veaux par fécondation in vitro avait été rapportée. Les raisons de cette situation proviennent des actes différents qui doivent être entrepris dans l'une et l'autre espèce. Chez la femme la technique ne se rapporte qu'à la fécondation in vitro proprement dite, c'est-à-dire la mise en présence d'ovocytes sur le point d'ovuler avec du sperme fraîchement éjaculé. En outre,

l'embryon est remis dans l'utérus de la mère dans les meilleurs délais et en moyenne dans les 24 h suivant la fécondation. Chez les bovins l'intérêt est de prélever des ovocytes à un moment quelconque du cycle sexuel et de les féconder avec du sperme congelé. Les ovocytes doivent d'abord être cultivés (maturation ovocytaire in vitro), après avoir assuré la capacitation des spermatozoïdes décongelés la fécondation a lieu suivie d'une culture des embryons en éprouvette jusqu'à un stade de développement où ils sont transplantés (7-8 jours). L'ensemble constitue la fécondation in vitro in toto (Fig. 3). Jusque-là les rendements sont très faibles et une performance est d'obtenir un embryon au stade blastocyte (8 jours) par ovaire. Dans les conditions de fécondation in vitro in toto THIBIER (28) avance des taux de 10 % de morulas et de 6 % de blastocytes. Ces taux auraient été récemment améliorés. De plus il semble que les critères d'évaluation de la qualité des embryons congelés in vitro ne sont pas exactement semblables à ceux des embryons obtenus par fécondation in vivo. Aussi, certains s'orienteraient actuellement vers le recours à des hôtes intermédiaires comme la brebis pour assurer le développement de l'embryon fécondé in vitro avec un pourcentage de succès qui peut être supérieur.
La fécondation in vitro en permettant la production simultanée d'un grand nombre de veaux demi-frères ou demi-soeurs à partir de la même vache dont les ovocytes sont fécondés par le sperme de plusieurs taureaux offre des conditions de progrès réelles pour le testage. Des test d'évaluation de la semence des taureaux peuvent aussi avoir pour base la fécondation in vitro. A court terme l'éleveur peut faire appel à la fécondation in vitro pour produire à partir d'une population homogène et de valeur génétique moyenne (femelles destinées à l'abattoir) des embryons issus de ces femelles et de semence provenant de taureaux présentant telles ou telles caractéristiques particulières pour alimenter une filière d'animaux de boucherie. A moyen terme la fécondation in vitro ne s'imposera que si ces performances sont supérieures à celles des méthodes conventionnelles de transplantation embryonnaire et si elle s'intègre dans la chaîne des manipulations de l'embryon.

3.3 SEXAGE DE L'EMBRYON

Le but du sexage est de modifier le sex-ratio (nombre de naissances de mâles pour 100 naissances de femelles, soit 104 à 106 chez les bovins) par la détermination rapide et peu coûteuse (moins de 100 dollars) du sexe des embryons avant leur transfert chez des receveuses. En effet jusqu'à présent en élevage laitier, les veaux mâles sont un "mal inévitable" et les efforts de sélection se heurtent à la barrière de 50 % de mâles pour 50 % de femelles. De même en élevage à viande la réciproque est presque vraie les carcasses de mâles étant les plus recherchées. La détermination du sexe des embryons de bovins n'est envisageable qu'à l'âge où s'effectue habituellement le transfert soit 7-8 jours, âge lui-même conditionné par le fait qu'il s'agit du stade optimum pour la congélation de l'embryon à -196°C.
Les premières techniques de sexage de l'embryon reposent sur l'analyse chromosomique (recherche de la différence de taille entre les chromosomes X et Y sur les cellules en division d'un demi embryon de 7-8 jours ou sur les biopsies de 20 cellules, méthode lourde mais fiable à 100 % et qui reste la référence pour éprouver les autres techniques), sur la détection immunologique d'une molécule de membrane l'antigène H-Y considéré comme spécifique des cellules du mâle, sur la mise en évidence d'activités enzymatiques codées par des gènes localisés sur le chromosome X doublement actives chez les femelles XX (glucose 6 phosphate deshydrogenase, hypoxanthine phosphoriboxyl

transferase). Des techniques plus récentes sont basées sur l'utilisation de sondes moléculaires. Suite aux travaux de BISHOP et coll (6) des sondes nucléiques spécifiques de séquences associées exclusivement au chromosome Y du taureau ont été préparées et appliquées à des échantillons de 10 à 20 cellules embryonnaires. Aux USA par exemple sur une centaine d'embryons de bovins 76 % ont été sexés, 44 % de gestations ont été obtenus à partir de ces embryons sexés et congelés avec une fiabilité de 100 % dans la détermination du sexe (14). Des sondes non radioactives marquées par la biotine et révélées par des moyens immunocytochimiques appliqués sur un produit de biopsie de 15 à 25 cellules trophoblastiques dont le rôle n'est pas vital pour l'embryon sont en cours de développement. L'utilisation de la technique de la "Polymerase Chain Reaction" permettant l'amplification de séquences uniques plus d'un million de fois en quelques heures à partir de demi embryon de 70 cellules fourni déjà des résultats encourageants et laisse augurer la possibilité de travailler à l'avenir sur un nombre très restreint de cellules (11).
Il convient de souligner que le sexage de l'embryon ne constitue qu'une étape intermédiaire dans le choix et la connaissance du sexe du produit à naître. La situation changera radicalement lorsque paraîtront sur le marché des techniques permettant le sexage des spermatozoïdes. Le sexage de l'embryon ne consiste en effet qu'à caractériser le sexe et non pas dans l'état actuel de nos connaissances à l'orienter. Il n'en est pas de même dans le cas du sexage de la semence puisque ce dernier porte sur les gamètes précédant la fécondation. Il est probable toutefois que le sexage du produit de conception après traitement approprié de la semence ne sera pas de 100 %, modifier le sex-ratio de 50 à 70-80 % constitue déjà une gageure et représente un intérêt considérable. On pourra même appliquer alors la combinaison du sexage de la semence à celui de l'embryon...

3.4 DUPLICATION DE L'EMBRYON (Fig. 4)

L'intervention permet de produire des jumeaux identiques donc de doubler la production d'embryons et d'augmenter ainsi le nombre de veaux produits par donneuse. Au cours des premiers essais la présence de la zone pellucide semblait indispensable pour le premier stade de développement, la difficulté était surtout de placer les demi-embryons supplémentaires dans la membrane pellucide d'un embryon dégénéré ou d'un ovule non fécondé. Par la suite différentes études ont montré que la zone pellucide n'était pas indispensable au développement d'un embryon de 6-7 jours et qu'il suffisait de couper l'embryon en deux à l'aide d'un microscalpel en métal ou d'une micro-aiguille de verre fixée à un micromanipulateur. LEIBO et RALL (18) en utilisant cette technique sur 422 embryons ont obtenu 842 demi-embryons transférable et 441 gestations, soit un taux de réussite de 52 % peu différent de celui des transferts d'embryons entiers. Dans ces conditions le rendement de l'opération classique de transplantation peut être augmenté de près de 70 %. Cependant il serait illusoire d'espérer ainsi un quasi doublement du nombre d'embryons utilisables par collecte qui passerait alors de 5 à 9 ou 10 car tous les embryons transférables ne peuvent pas subir une bissection, seuls les très beaux y résistent. Malgré le conseil de MORIE et coll (26) de procéder à la bissection de préférence après décongélation de l'embryon ici encore la nécessité de la protection de la membrane pellucide est remise en cause et actuellement la congélation des demi-embryons demeure à l'étude.

3.5 CLONAGE DE L'EMBRYON

Le clonage ou multiplication d'un individu par reproduction asexuée représente le moyen idéal de conserver et de propager un phenotype exceptionnel. Il constitue une étape plus élaborée que la bissection de l'embryon.La transplantation nucléaire qui consiste immédiatement après la fécondation à remplacer un ou les deux pronucléus de l'ovocyte par d'autres est susceptible d'application en buiatrie (Fig. 5). On peut imaginer après fécondation in vitro remplacer le pronucleus de la femelle par le pronucleus d'un second spermatozoïde. Si les deux spermatozoïdes sont du même mâle on a croisé un mâle avec lui-même, s'ils sont de pères différents c'est un croisement de mâles entre eux. De la même manière on doit pouvoir effectuer un croisement entre femelles en fécondant un ovocyte par un autre ovocyte en remplaçant le pronucleus du mâle par celui d'une femelle (22). De tels procédés permettent d'envisager la production de clones d'embryons, il n'apparaît guère possible pour l'instant de produire des clones, copies fidèles des adultes parentaux. En effet, si l'on reprend encore une fois l'ovocyte précédent après la fécondation et qu'on lui enlève les deux pronucléus pour les remplacer par le noyau de cellules de diploïdes d'un adulte on devrait obtenir une copie conforme de cet individu. Aucune cellule d'adulte n'a encore fourni de noyaux cellulaires capables de pronouvoir le développement d'un nouvel organisme (9). Au plan zootechnique cela signifie qu'il faudra conserver par congélation les embryons clonés jusqu'à ce que quelques uns d'entr'eux, tranférés et nés puissent exprimer leur potentiel afin de s'assurer de l'intérêt économique de ceux-ci. Cela nécessitera un délai de quelques années mais peut s'avouer avantageux. Les clones ont un handicap ils ne permettront pas le progrès génétique. En revanche ils ont potentiellement un avantage : celui de diffuser le progrès car une fois quelques éléments du clone testés on peut les reproduire au moins théoriquement en un nombre d'exemplaires illimité. On imagine aisément les répercussions que ces techniques peuvent avoir sur l'homogénéité des produits livrés à la production ou à la consommation. Leur intérêt dans le cadre de la production d'animaux de boucherie apparaît évident. Enfin les clones constituent un modèle de choix pour la mise en oeuvre des techniques futures de transfert de gènes.

3.6 TRANSFERT DE GENES

3.6.1 Définitions

L'animal transgénique ou transgène a son génome modifié non pas par les méthodes classiques de la génétique mais par une manipulation de celui-ci à l'aide des techniques de l'ingénierie génétique. En effet il est possible d'introduire un gène étranger dans les cellules de l'embryon dans les tous premiers stades du développement, ce gène peut s'exprimer et conférer à son hôte un nouveau caractère génétique. Cette opération s'appelle transgénèse.

3.6.2 Techniques

La production d'animaux transgéniques suppose l'accomplissement des étapes suivantes :

* isolement (qui pourrait être facilité par le sequençage du génome bovin), multiplication du gène à transférer, apposition des séquences contrôles,
* insertion du gène étranger dans l'embryon par micro-injection dans le pronucleus (Fig. 6), par utilisation d'un vecteur du gène type retrovirus ou mieux chez les bovins par injection de cellules

embryonnaires non différenciées véhiculant le gène à insérer (8) (Fig. 7). Ces cellules réintroduites dans un blastocyste se multiplient, elles participent à la formation des organes de l'individu en développement qui devient alors un animal chimère et mosaïque. De telles lignées cellulaires contenant le gène suite par exemple à une transfection par un retrovirus permettraient de contourner la méthode de micro-injection si peu adaptée à la transgenèse chez les gros animaux. A signaler encore deux nouvelles méthodes de transfert : l'injection de fragments de chromosomes dans le pronucleus et l'utilisation des spermatozoïdes comme véhicule de la nouvelle information génétique,

* clonage de l'embryon, contrôle de l'incorporation du gène et de sa position dans une région du génome où il pourra s'exprimer, transfert des embryons chez des receveuses,
* obtention de son expression dans les tissus et dans des conditions appropriées chez le transgène,
* contrôle de l'héritabilité du gène.

3.6.3 Applications

Chez les bovins, en dehors du transfert réussi d'un gène du papillomavirus sans par ailleurs d'expression, la transgenèse est surtout porteuse d'immenses espoirs dans les domaines ci-dessous :

* amélioration des performances de croissance et de l'efficacité alimentaire à l'aide des gènes de l'hormone de croissance et des somatonédines,
* amélioration de la qualité du lait à l'aide du gène de la caséine (qualité fromagère) de la desaturase (modification de la répartition des acides gras saturés et insaturés) ou de la lactoferrine à activité antimicrobienne (lait destiné aux enfants ou lait diététique),
* production par la mamelle de molécules à propriétés pharmacologiques (facteur IX non contaminé par le virus du SIDA),
* amélioration de la résistance aux maladies : résistance aux mammites à staphylocoques à l'aide du gène de la lysostaphine léthale pour cette bactérie.

Les animaux domestiques ayant des caractéristiques génétiques nouvelles n'arriveront probablement pas de manière massive dans les élevages avant la fin du siècle. Une étude sur l'impact des biotechnologies dans l'agriculture de la Communauté Economique Européenne (CEE) jusqu'en l'an 2005 montre que tant pour les bovins viande que pour les bovins laitiers les premiers transgènes ne seront introduits en élevage que vers 1997-1998 avec une probabilité aux alentours de 2 (1 = certain, 3 = non certain). En l'an 2000 il n'y aura que 10 % des éleveurs qui utiliseront ce type d'individu contre 25 % en 2005, avec l'espoir d'une augmentation des performances zootechniques de 20 % environ (1).

3.7 PRODUCTION DE CHIMERES (Fig. 8)

Une chimère est un organisme viable composé de différentes populations cellulaires issues de plusieurs oeufs fécondés ou de l'union de plus de deux gamètes à distinguer des mosaïques constituées aussi de deux types de cellules génétiquement différentes mais provenant d'un même zygote. Les deux termes sont confondus par abus de langage. Diverses micromanipulations ont abouti à la naissance de moutons, de chèvres et de bovins chimériques (24), des chimères moutons-chèvres sont même nées. Un inconvénient majeur de cette technique est que la proportion de chaque génotype dans les différents tissus est très variable et change avec l'âge. Les veaux obtenus ainsi sont un mélange au hasard des différents génotypes des embryons utilisés. Cependant le jour où

l'on sera capable de produire des chimères de composition souhaitées avec précision et répétabilité de nombreuses applications en découleront. On pourrait produire par exemple des bovins chimériques qui auraient la mamelle de la vache laitière et le développement musculaire d'une vache de race à viande.

4. LES PROBLEMES POSES PAR LES BIOTECHNOLOGIES EN BUIATRIE

Ils sont de différentes nature et l'inventaire proposé ne saurait être exhaustif, il s'agit simplement d'illustrer les principaux aspects des controverses soulevées sans pouvoir toujours apporter de réponse aux questions posées.

4.1 PROBLEMES ETHIQUES

Ils interpellent à la fois le grand public, le vétérinaire et les chercheurs.
Pour le grand public la préoccupation essentielle se rapporte au bien être de l'animal :
* l'utilisation de la BST par exemple ne parait pas avoir de conséquences néfastes sur le bien être des vaches laitières. Les résultats obtenus (augmentation de la productivité) ne pourraient assurément pas être enregistrés si de telles substances perturbaient les équilibres biologiques en violant les limites physiologiques et s'accompagnaient de troubles pathologiques (psychiques ou organiques). En effet après l'administration de BST s'établit une coordination plus précise des métabolismes pour permettre une répartition des nutriments plus favorable à la synthèse du lait, phénomène déjà préparé et réalisé par la sélection des vaches laitières à haute production (diminution de la capacité de l'insuline à stimuler la lipogenèse dans les tissus adipeux et à inhiber la néoglucogenèse hépatique). Les ajustements de l'homéorhésie encore appelée "homeostasie de développement" répondent à des étapes particulières des cycles biologiques (croissance, gestation, lactation) en allant au delà des régulations homéostasiques de routine sans dépasser pour autant les limites physiologiques et concernent non seulement le métabolisme mais encore l'hémodynamique (augmentation du débit cardiaque, du flux sanguin mammaire) concourant ainsi à faciliter la production laitière,
* la mise en oeuvre des techniques de manipulation des embryons en particulier transgenèse et chimérisme incite à la prudence. L'insertion de nouveaux gènes dans un embryon a souvent des effets léthaux. L'embryon peut mourir suite à des anomalies de son développement ou donner naissance à un individu porteur de défauts congénitaux ou infécond.Ces tares ne sont parfois évidentes que tardivement sans qu'il soit possible de les prévoir. Il semble que de tels incidents puissent résulter de l'impossibilité de contrôler le site d'insertion du gène transféré dans le génome du receveur. De plus les observations issues d'une espèce ne sont pas immédiatement transposables dans une autre : les souris et les lapins transfectés avec le gène de l'hormone de croissance humaine ne montrent pas la pathologie rencontrée chez le porc (apathie, troubles de la vision, arthropathies, etc...) (16).
Pour le vétérinaire la traditionnelle distinction entre la thérapeutique curative et l'intervention préventive se complique singulièrement quand il s'agit d'administrer ou de prescrire des substances d'aide à la production animale c'est-à-dire des produits dont l'objectif n'est ni de soigner l'animal ni de l'empêcher de tomber malade mais d'améliorer des performances. Une assimilation superficielle de la responsabilité sociale du vétérinaire à celle du médecin peut amener le premier à considérer l'utilisation de la BST par exemple comme une entorse à l'éthique professionnelle voire un

acte de soumission à la primauté économique. Il peut être encouragé dans cette attitude par la règlementation actuelle en matière d'anabolisant dans la CEE : les stéroïdes sexuels sont interdits en élevage pour des indications autres que thérapeutiques (13).
Pour le chercheur, les manipulations du génome, la création de nouveaux types d'individus, la multiplication des clones auxquels est attaché pour le grand public une image négative de standardisation aveugle et de science fiction peuvent déranger ses options philosophiques et/ou religieuses.

4.2 PROBLEMES DE SECURITE

Ils s'entendent à deux niveaux : sécurité pour l'environnement et sécurité pour le consommateur.
La diffusion dans la nature d'organismes nouveaux, qu'il s'agisse de virus, de bactéries, de plantes ou d'animaux nécessite une évaluation préalable du rapport coût/bénéfice de l'opération et une surveillance ultérieure de leur comportement à la lumière de précédentes expériences aux conséquences désastreuses (virus de la myxomatose par exemple). En dehors des vaccins recombinants, en buiatrie ces précautions s'adressent éventuellement aux probiotiques mais surtout aux animaux dont le génome a été modifié, en particulier par des vecteurs rétroviraux. S'il s'agit de rétrovirus dont les gènes nécessaires à leur replication ont été délétés le risque d'activation d'oncogènes cellulaires est très réduit (27). Par ailleurs la technique des transgènes ne fait que reproduire en des temps records le processus de mutation base de la sélection naturelle spontanée. Il conviendra toutefois de veiller à ce que l'acquisition de nouveaux caractères génétiques n'aboutisse pas à un appauvrissement du génome de l'espèce ou de la race concernée.
Si l'innocuité des produits alimentaires (viande, lait), issus des animaux transgéniques doit être démontrée celle des produits issus des animaux traités avec des molécules issues du génie génétique comme la BST a été établie. On a déjà vu que la composition du lait des vaches traitées à la BST n'était pas modifiée, de même que son aptitude à la fabrication des fromages. On sait de plus que la BST est dégradée par les enzymes protéolytiques digestives, qu'elle n'a aucune activité biologique chez l'homme et que les taux de somatomédine C (IGF_1) dans le lait des animaux traités avec la BST ne peuvent avoir aucun effet néfaste chez les consommateurs y compris des jeunes enfants (29).

4.3 PROBLEMES SOCIO-ECONOMIQUES

Le progrès en agriculture a toujours profité à ceux qui ont su le saisir pour diminuer leurs coûts ou augmenter leurs profits. Ceux-là dans des périodes difficiles ont pu faire face plus facilement aux problèmes que les producteurs moins attentifs à l'évolution des techniques. En d'autres termes, le recours aux biotechnologies a une forte probabilité d'accélérer le processus d'intensification de l'élevage et d'accroître la pression exercée sur les producteurs traditionnels. Ainsi dans la CEE a-t-il été envisagé la possibilité de demander une étude d'impact socio-économique aux firmes voulant mettre sur le marché des produits dérivés des biotechnologies. La seule parade pour les biotechnologistes est d'oeuvrer par eux-mêmes pour empêcher la déformation de l'information par les protectionnistes et les associations de consommateurs. La même rigueur que celle qui est censée régir la mise au point de nouvelles techniques d'élevage devrait s'appliquer à l'étude des moyens à mettre en oeuvre pour entraîner l'adhésion du grand public car il n'est pas sûr que la société autorise la pleine expression du potentiel des biotechnologies.

5. CONCLUSIONS

La buiatrie est un secteur d'excellence pour les biotechnologies. Ses deux volets production et pathologie sont également concernés par ce déferlement de nouvelles méthodes. Le génie génétique laisse déjà entrevoir de spectaculaires moyens d'amélioration des productions. Les manipulations de l'embryon vulgarisées avec la transplantation et la congélation des embryons sont prêtes pour de nouveaux bonds avec le clonage et le transfert de gènes. Ainsi risquent d'apparaître des animaux plus performants, plus résistants vis-à-vis des pathologies classiques, fournissant des produits mieux adaptés aux souhaits nutritionnels et diététiques des consommateurs. Ces mêmes consommateurs se référant à leur culture scientifique, à leurs croyances religieuses, à leurs penchants philosophiques et à leurs convictions politiques auront-ils assez de sagesse pour accepter le meilleur de ces nouvelles technologies ?

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7. RESUME

Seules les biotechnologies ayant davantage d'applications en matière de productions que de pathologie bovine sont exposées : génie génétique et manipulations de l'embryon. Le génie génétique avec l'hormone somatotrope bovine recombinante, l'interferon bovin recombinant alpha 1 et les probiotiques apportent des aides à la production du lait et à la production de la viande, au contrôle de certaines dominantes pathologiques et bientôt à la maîtrise de la reproduction. Les manipulations de l'embryon préparées par la transplantation et la congélation des embryons comportent la

fécondation in vitro, le sexage, la duplication, le clonage et le transfert des gènes aboutissant à des animaux transgéniques, chimères ou mosaïques. Ces techniques sont génératrices dans l'opinion publique et chez les professionnels de l'élevage de problèmes relatifs à l'éthique, à la sécurité et à l'équilibre socio-économique qui sont exposés et discutés.

SUMMARY
Only biotechnologies with wider applications to production than cattle diseases are presented : genetic engineering and embryo manipulation. Genetic engineering with recombinant bovine somatotropin, α1-recombinant bovine interferon and probiotics boost milk and meat yields and help to control some dominant pathogens as well as soon offering help in controlling reproduction. Embryo manipulations, proceeded by transfer and freezing of the embryos, include in-vitro fertilization, sexing, duplication,cloning and gene transfer resulting in transgenic animals, chimeras or mosaics. Amongst the general public and in people involved in livestock husbandry, these methods raise questions relating to ethics, safety and a socio-economic balance and these issues are described and discussed.

RESUMEN
Se exponen solamente las biotecnologias que tienen mas aplicaciones en materia de producciones que de patologia bovina : ingenieria genetica y manipulaciones del embrio. La engenieria genetica con la hormona somatotropica bovina recombinante, el interferon bovino recombinante α1 y los probioticos aportan ayudas à la produccion de carne, al control de ciertas dominantes patologicas y, en breve, al dominio de la reproduccion. Las manipulaciones del embrion preparadas por la transplantacion y la congelacion de los embriones incluyen la fecundacion in vitro, el sexaje, la duplicacion, la clonacion y el traslado de genes que desembocan en animales transgenicos, quimeras o mosaicos. Estas tecnicas generan en la opinion publica y en los profesionales ganaderos problemas de etica, de seguridad y de equilibrio socio-economico, problemas que se exponen y debaten.

Figure 1 : Production de protéines par les bactéries recombinantes

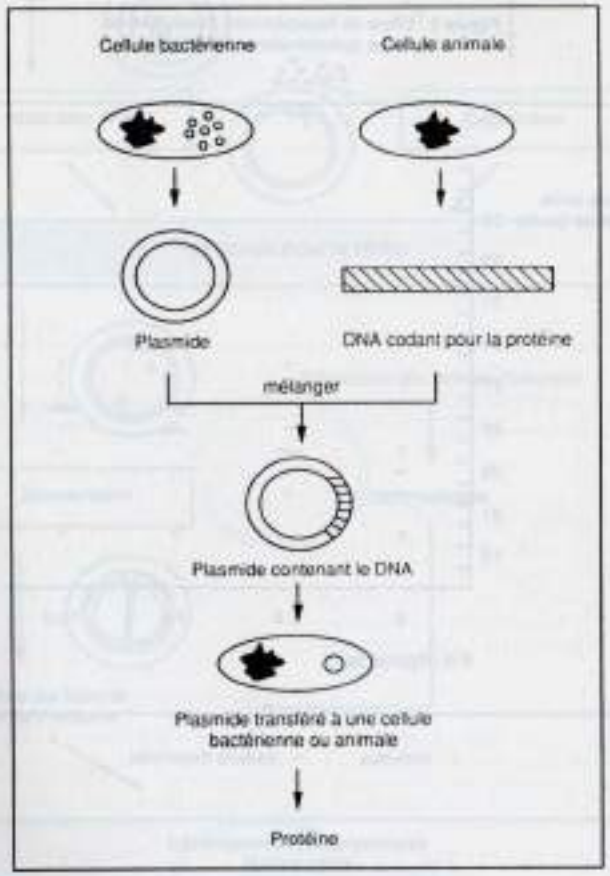
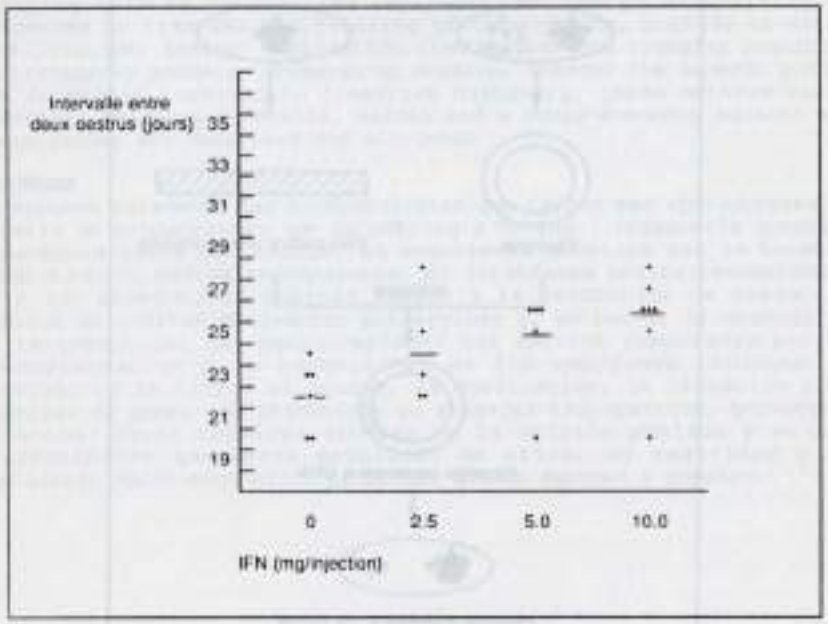


Figure 2 : Effets de l'injection intra musculaire de différentes concentrations d'interféron



+ individus — valeurs moyennes

Figure 3 : Les différentes étapes de la fécondation in vitro

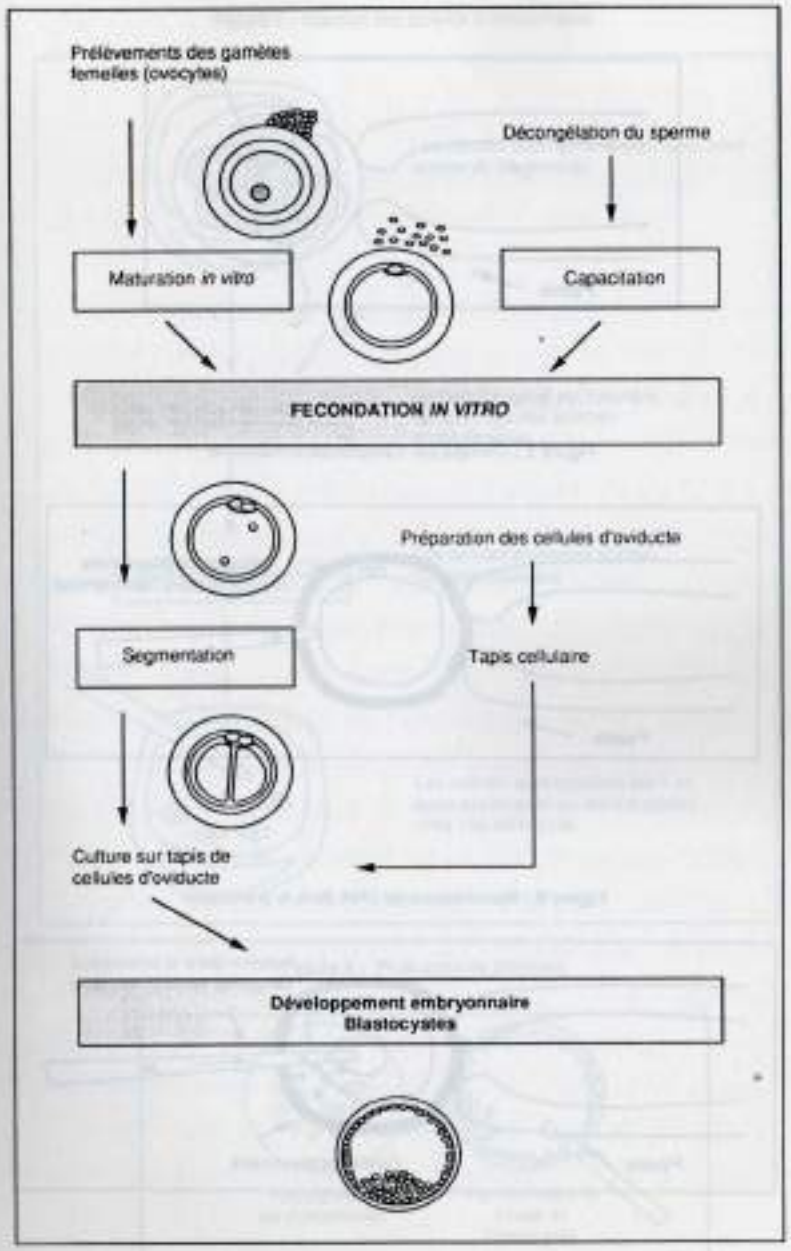


Figure 4 : Bissection de l'embryon

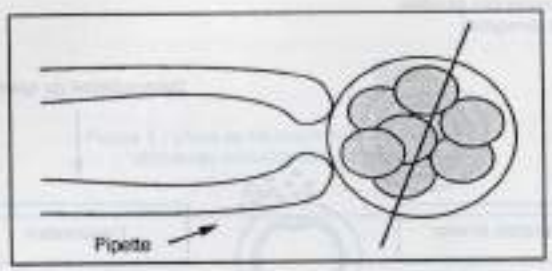


Figure 5 : Clonage par transplantation nucléaire

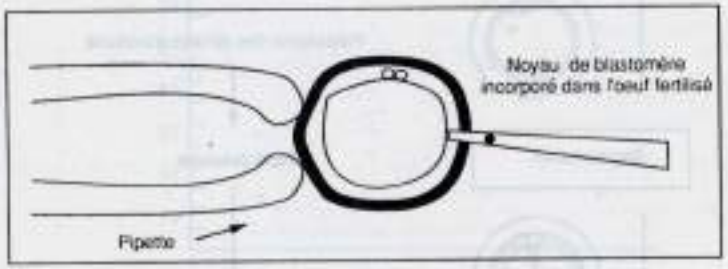


Figure 6 : Microinjection de DNA dans le pronucléus

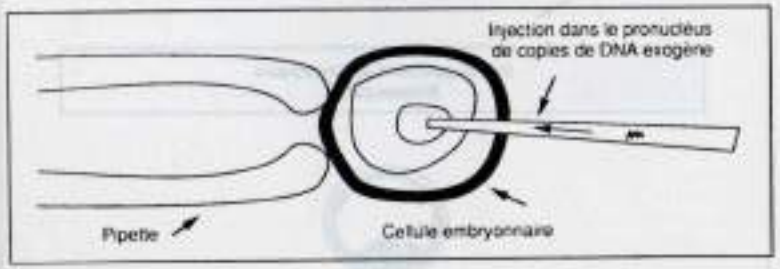


Figure 7 : Injection des cellules embryonnaires

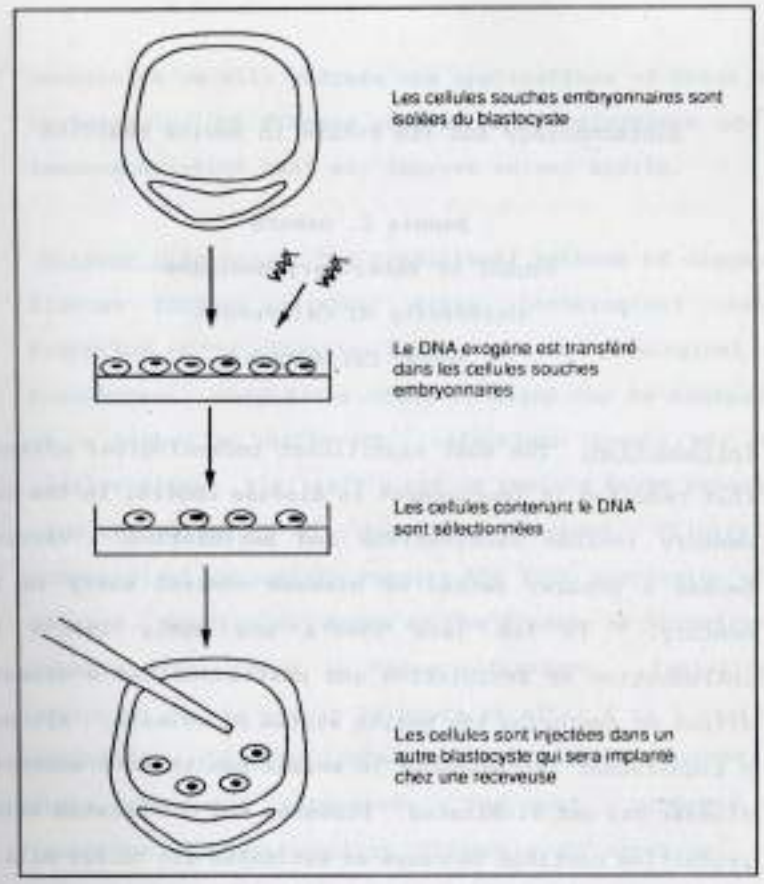
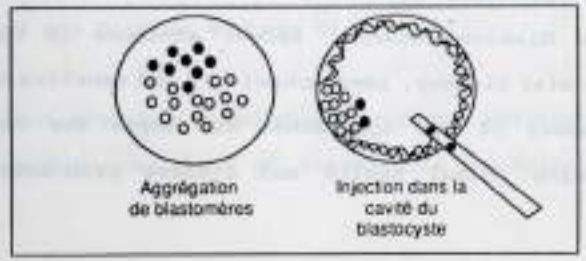


Figure 8 : Production de chimères



Biotechnology and Its Future in Bovine Medicine

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Introduction: The most significant technological advances that resulted in improvement in disease control in the last century include vaccinations and antibiotics. Vaccines became a popular method of disease control early in the century. In the late 1940's and early 1950's the introduction of antibiotics and pesticides had a dramatic effect on improving the health status of animals.[1] Although a significant improvements in animal health care occurred; disease was not eliminated. Diseases and the related animal production continue to cause an estimated $15 to $16 billion dollars a year loss in the United States alone. New methods and approaches are needed to significantly improve upon these disease losses. Recent advances in the areas of molecular biology, immunochemistry and genetics have brought a number of new approaches and hopes for significantly reducing animal health and disease problems. In this

manuscript we will address the applications of these newer technologies to disease diagnosis, vaccinations and the immunomodulators that may improve animal health.

Disease Diagnosis: The traditional methods of diagnosing disease include clinical signs, pathological lesions, isolation of the causative agent, and/or serological test procedures.[2] Many times clinical signs may be nonspecific as a number of different infectious agents may cause similar signs. Similarly a set of lesions maybe associated with the pathology of a number of diseases. Clinical and pathological parameters require the full expression of the disease. Subclinical forms of the disease or infection are usually over looked in these situations. Isolation of organisms can be within 24 hours or after 4 to 6 months of culturing. In many instances, the methods currently in place are labor intensive. The most commonly used technique for confirmatory diagnosis is serology. This procedure indicates that an animal has been exposed to an agent, however it is not an indication that the agent is the cause of the disease at that time.

The recent advances in molecular biology and biotechnology are impacting the area of diagnostics in both human and

veterinary medicine. Specifically, the use of radiolabeled or enzymatically labeled markers makes it possible to identify with great sensitivity molecular markers in tissues, in solutions or on solid matrices. These markers can be attached to nucleic acids or to antibodies that are used to identify genetic material or subunit antigens on microorganisms or for specific chemicals. Cloning of genes provides a means of rapidly propagating large quantities of specific nucleic acids which can be used as standardized reagents for diagnostic procedures. Similarly, monoclonal antibodies are highly specific reagents which are directed to specific epitopes on microbial agents or to specific chemical structures associated with toxic principles.

Detection Systems: Major advances in detection systems have had a significant inpact on rapid diagnostic technology. The principle detection systems in use today, are the radioactive /nonradioactive and direct/indirect detection systems. These detection systems consist of signal generating molecules which are linked to ligand-specific biomolecular probes such as nucleic acids or antibodies. The enzyme/ radiolabeled molecules offer the means for evaluating the result of an assay. Most detection systems

are multipurpose, that is they can be used on more than one probe. The specificity of the response lies with the nucleic acid or antibody probe.

Molecular Biochemistry and Diagnosis: Modern molecular biochemistry is proving helpful in providing more rapid and sensitive diagnostic techniques to detect infections in domestic animals. These techniques do not rely on the isolation and identification of a live replicating microorganisms, but on the detection of highly specific molecular subunits of the inciting agent in tissues. These subunits may be either gene sequences specific for the microbe or specific protein epitopes on the protein components of the microorganism.

Biochemical structures: Polyacrylimide gels (PAGE) have made it possible to separate genomic and subunit proteins by molecular weight and charge. The migration distance in a gel is related to molecular weight and possible secondary structure of the genomic segments. Genomic segments may be obtained from microorganisms by enzymatic digestion by restriction endonucleases or in the case of reoviruses by genomic dissociation at the time the viruses are released from the nucleocapsid. This technique represents a means

of fingerprinting; however it has proven very useful for determining biotypic characteristic of certain viruses and bacteria. For instance, the technique has been used to demonstrate reassorting gene segments in bluetongue viruses and to differentiate reproductive from respiratory strains of bovine herpes viruses.'' This procedure is also used to identify plasmids that may transmit virulence or antibiotic resistance factors in bacteria.' PAGE is also used to separate microbial proteins. Again, the subunit proteins are separated based upon molecular weight, conformation and charge. This procedure permits one to determine the number of subunits associated with the microbe and their functional activities.'

Molecular Cloning of Microbial Genomic Material: Cloning of genetic material provides a means of propagating large quantities of a single gene that can be used for diagnostic purposes. Individual gene sequences can be isolated on PAGE cut out of the gel, eluted, copied and then spliced into a bacterial plasmid.' The plasmid is then exposed to bacteria. The bacteria with the plasmid incorporated into it undergoes replication. As the replication occurs, there is also replication of the foreign genes. Since bacteria replicate rapidly, it is possible to obtain large quantities

of the isolated gene as well. The copied foreign gene can then be extracted from the plasmid and used for diagnostic purposes.

Genetic Probes as Diagnostic Tools: Cloned cDNA copies of individual genome segments are exact replicas of the original genomic segment from which they had been cloned. Genetic strands with complementary nucleotide sequences will hybridize to gene strands with similar nucleotide sequences. Hybridization can occur between complimentary DNA and/or RNA genomic materials.'' Hybridization conditions for DNA/DNA and DNA/RNA genetic hybridizations on solid supports have been standardized.

Molecular Genetic Diagnostic Tests: The cDNA probes can be used in four diagnostic tests, northern and Southern Blots, dot blot hybridization and in situ hybridization. Northern blots can be used to identify RNA in cDNA/RNA hybridization assays. The most common use is for RNA viruses. Southern blots can be used for DNA in viruses or bacteria. Northern and Southern blots are performed following the placing of the test sample on PAGE and then electrophoretically transferring the genetic material to a solid matrix. The cDNA labeled probe is administered to

the matrix and if the complementary nucleic acids are present, hybridization occurs and a signal appears on the matrix. Dot blots are performed by extracting the nucleic acids from tissues and applying directly to the solid matrix. The labeled probe is then administered and if the complimentary nucleic acid sequences are present hybridization will occur. In situ hybridization utilizes the labeled probe by applying it directly to tissues. The probe will seek out complimentary nucleic acid sequences and hybridize to it with in cells or tissues on glass slides.

A relatively new procedure known as the polymerase chain reaction (PCR) offers great promise for expediting the time required for a diagnosis.[7] The principle of the procedure is to use an oligonucleotide primer from a constant region of a gene. Extracted nucleic acid from tissue is mixed with the oligonucleotide primer. The primer will anneal to complimentary nucleic acid of the microbe and then the bound nucleic acids are placed in a thermocycler along with a polymerase enzyme. The thermocycler will alternatively heat to 90C and then cool to 30C. This difference in temperature is important for separating the annealed nucleic acids and to create new copies of the gene through the action of the polymerase. The result is

a rapid amplification of the specific nucleic acid which can then be used for diagnostic purposes.[8] A single virus can be identified in a sample of blood by using this amplification system.[9]

Molecular Diagnosis of Specific Microbial Antigens: Another approach to modern molecular diagnosis involves the immunological identification of specific epitopes on proteins.

Protein Dot Blot Assay: The assay involves dotting 1-2 ul of monoclonal antibodies onto nitrocellulose strips. After air drying and blocking with gelatin, supernatants from microbial cultures are incubated with the dots on the strips. The monoclonal antibody originally bound to the NC paper forms complexes with the specific protein in the test culture supernatant. A rabbit polyclonal antiserum to the test enriched supernatant is added followed by a biotin-conjugated affinity-purified goat IgG antirabbit IgG. The presence of bound goat antirabbit is detected by an avidin-peroxidase conjugate with appropriate substrate. A positive color reaction is an indication of monoclonal specific antigens in the test culture supernatant.

In situ Hybridization Assay: Indirect immunoperoxidase procedures using both polyclonal and monoclonal antibodies have been successfully applied to detect microbial proteins in formalin fixed tissues. After fixing in formalin or by rapid freezing, tissue sections are washed, blocking steps applied, and the tissues are incubated with antimicrobial specific antibodies.

Immunoblotting Diagnostic Test: Identification of microbial subunit proteins using a western blotting procedure has been successful. Extraction of microbial proteins are then applied to a PAGE which results in separation of the subunit proteins. The proteins are then electrophoretically transferred (western blotted) to NC paper. The strips of NC paper containing the separated microbial proteins are blocked with gelatin and incubated with polyclonal antiserum to the microbial agent. Biotinylated affinity-purified anti-immunoglobulin and avidin/peroxidase conjugate are added to the reaction. The microbial specific proteins on the NC strip are visualized for a color reaction of the substrate. Monoclonal antibodies against microbial-specific epitopes may detect the subunit proteins on western blots or by immunoprecipitation.

Vaccination: Vaccination is one of the more important means of controlling disease. The term vaccine is derived from the fact that Dr. Edward Jenner noticed that milkmaids did not develop smallpox, mainly because many developed a mild pox-like skin disease from the cattle they were milking. These cattle suffered from the condition cowpox." The term "vaccine" is derived from the Latin word for cow. Vaccines may consist of either killed or live organisms, usually the live organisms have been attenuated so that they do not cause disease. Killed organisms have to be given in relatively large quantities to assure that sufficient antigenic mass is present to stimulate a good immune response. On the other hand modified live viruses can be given in small quantities, however, these viruses do have the potential for replicating in the host, thereby causing a mild to moderate infection. The amplification associated with this viral replication provides sufficient antigenic mass to stimulate a good immune response.

Although vaccines have proven to be quite successful there are a number of disadvantages associated with their use." These disadvantages include the fact that if there are live organisms present within the vaccine, a mild or sometimes a severe disease can result following the administration of

the vaccine. Secondly, animals vaccinated with whole organisms develop a full range of immune responses so it is difficult to utilize a serologic test to differentiate the vaccinated animals from those that recover from a clinical disease. Third, in certain instances some of the modified live virus vaccines will actually lead to persistent and latent infections. These latent viruses can periodically be shed and infect other animals, which in turn may develop clinical disease. Fourth, it is also possible that some of the viruses can be transmitted through semen, embryos, and eggs. Modified live viruses may also during the course of their viremia be picked up by insects and transmitted to other susceptible animals in an area. Fifth, in some instances the administration of these vaccines to pregnant animals will lead to congenital infections and in some instances death of the developing fetuses. Sixth, many of the vaccines, particularly some of the killed ones, do not have the complete array of antigens associated with the different serotypes or strains of a virus and as such they do not provide widespread protection. Seventh, modified live vaccines are not stable enough for use in areas where refrigeration is required to properly store these specimens.

The new approaches used in biotechnology have provided some

innovative vaccines. It is now possible to develop subunit vaccines. These vaccines are made possible by the fact that recombinant DNA/RNA technology permits large scale production of the immunogenic surface proteins. It is also possible to synthesize short polypeptides that represent the immunogenic region of the antigenic portions of molecules. The vaccina virus recombinant system may incorporate into vaccina virus, genes coding for the immunogenic proteins of other pathogens. In some instances it is possible to delete genes in either viruses or bacteria that remove the virulent properties of these particular microorganisms.

Subunit Vaccines: An example of one virus on which many of the new technologies have been utilized in vaccine development is that associated with vesicular stomatitis virus. This virus is a member of the Rhabdovirus family and it causes infection in cattle, pigs, and horses. The usual clinical signs are associated with vesicular lesions on the tongue and oral mucosa. In the United States there are two types of virus, New Jersey and Indiana, which cause infection.

The VSV virus is a single negative stranded RNA that codes for five messenger RNAs and the corresponding five proteins,

which have been identified as G, L, M, N, and NS. The surface of the virus consists of many spikes containing the G glycoprotein. The G glycoprotein spikes are responsible for attachment to the virus to cells, which is an important first step in the initiation of the infection. The M protein is the matrix or membrane protein. The other proteins, L, N, and NS are closely associated with the genonic RNA and as such form the internal nucleocapsid.

In order to make an effective subunit vaccine it is important to identify the major surface proteins of the virus. It is these proteins to which the appropriate neutralizing or inactivating antibody responses are usually directed. With VSV the key protein is the G glycoprotein to which antibodies completely blocked the ability of this virus to cause infection. It is possible to purify this subunit protein and immunize cattle with this glycoprotein in Freund's adjuvant. By immunizing cattle it is possible to prevent infection at the time of challenge with infective virus. This clearly demonstrates that the G glycoprotein is the key antigen in initiating an effective immune response which would prevent subsequent infections. It is possible to develop vaccines for a limited number of animals utilizing this technique, however, it is costly and labor

intensive. Therefore other approaches utilizing biotechnology have been developed for this particular virus. The use of recombinant DNA technology has made it possible to mass produce the G glycoprotein in large quantities.

Synthetic Peptide Vaccines: In order to develop effective synthetic peptide vaccines, it is important to determine amino acid sequence of the antigenic proteins. There is then a need to link together these amino acids to form a peptide chain. Amino acid sequence of the protein is coded by the DNA and RNA of the organism. Chemical and enzymatic techniques have been devised for determining the nucleotide sequence of DNA or RNA. By knowing the structure of the nucleotide coding for a protein, the genetic code can then be deciphered to derive the amino acid sequence of the protein. The sequence of amino acids and their local molecular attractions determine the secondary structure of the protein, and the relationship of the structure determines the final folding characteristics of the tertiary structure of a protein.

Computer programs are now available which provide a graphic display of tertiary structure of a protein once its crystalline structure is known. Immunogenic sites are

the vaccinia virus as a vector."[7] Genes from other viruses, such as vesicular stomatitis virus, rabies, rotavirus and rinderpest can be inserted into the genome of the vaccina virus."[8] When the vaccina vector is inoculated into an animal, the foreign gene is expressed as the virus replicates in the host cells. If the foreign gene is used as an immunogenic protein, the host develops antibodies against that protein and the animal becomes immune to both the vaccina virus and the virus from which the foreign gene was taken.

In order to utilize the vaccina virus as a vector it is necessary to create a plasmid which carries a chimeric gene containing a translocated vaccinia virus promoter region which is linked to the coding segment of a foreign gene. The chimeric gene is then incorporated into the vaccina virus genome by homologous recombination in tissue culture cells that have been transfected with the plasmid and infected with the wild type vaccina virus. Although nonessential regions of the vaccina virus genome can be used as the site of gene insertion, the thymidine kinase (TK) gene locus provides some advantages, because the recombinants are then TK-, which distinguishes them fron the wild-type TK+ virus. The TK- phenotype provides a simple

method for selection and also serves to attenuate viral pathogenicity.

In order to make a vaccina vector for VSV, a plasmid was constructed which carried the gene coding for the G glycoprotein." This gene was flanked by sequences of the vaccina virus TK gene. This chimeric gene was introduced into tissue culture cells infected with wild-type vaccina virus so that recombination would take place between the vaccina DNA sequences flanking the G gene in the plasmid and homologous DNA sequences in the intact, replicating vaccina genome, thus creating a population of infectious vaccina virus recombinants carrying the VSV G gene. The inserted G gene inactivates the TK gene so that the recombinants are TK-. It is possible to select the recombinants which express the chimeric G gene by incubating with 5-bromodeoxyuridine. This compound is metabolized by thymidine kinase to a lethal derivative; that is the wild-type vaccina virus particles carrying the active TK gene are inactivated. Particles carrying the chimeric gene, which have an inactivated TK gene, are preferentially selected. The G glycoprotein expressed in cells infected with recombinant vaccina virus is properly glycosylated and cannot be distinguished chemically and immunogenically fron

the authentic glycoprotein of VSV.

The VSV-New Jersey G glycoprotein in the vaccina virus recombinant is capable of protecting cattle against challenge infections." Furthermore, the cattle develop neutralizing antibody to VSV-NJ. These vaccines could produce a very effective way of protecting livestock from this important disease.

The vaccina virus is a double-stranded DNA virus with a genome of approximately 180 kilobases. The host range of the virus is wide, including man, cattle, horses, swine, sheep, goats, mice, and monkeys. It has been demonstrated that as many as 25,000 base pairs of DNA can be removed from nonessential regions of the virus without affecting viral infectivity. The average gene is about 2000 kilobases, as such this implies that a vaccina virus recombinant could be instructed to carry as many as 12 to 15 immunogenic genes taken from a variety of viruses, bacteria or parasites.

The advantage of a recombinant vaccinia virus are that it will express a single gene coding for a single immunogenic protein much like a subunit vaccine. Secondly, there is amplification of the protein through replication of the

vaccina DNA and a number of genes, possibly as many as 12 to 15 may be expressed in one vaccina recombinant. The lyophilized vaccina virus is stable and resistant to drying and heat and as such these vaccines could have a long shelf life at ambient temperature.

Gene Deletion Technology: It is now possible to delete specific genes which will detract from the virulence of organisms. This has been done with the pseudorabies in swine and also with salmonella organisms which affect cattle, sheep and swine. The thymidine kinase gene has been deleted in pseudorabies virus which then renders this virus of low virulence." It is now a commercial vaccine in the United States manufactured by 3 different biologic companies. Aro-A transposon deleted salmonella have been developed and shown to be highly effective for salmonellosis in cattle."

Immunomodulators: Through the use of biotechnology it is now possible to generate large quantities of natural immunomodulators. The immunomodulators, interferons and interleukins have been found to have beneficial effects on immune responsiveness.

Interferons: Interferons have been recognized since the mid-1950's when they were first demonstrated to inhibit or greatly supress the ability of certain viruses to replicate in cell cultures." Interferons are a group of small molecular weight proteins. The three major types of interferons are, IFN-alpha, produced by leukocytes, is a result of stimulation by viruses or double-stranded RNA. IFN-beta is produced by fibroblasts and epithelial cells in response to viruses and double-stranded RNA. The IFN-gamma is a product of T lymphocytes which have been stimulated by specific antigens or nonspecific mitogens. These IFN's have extensive antiviral, antiproliferative, and immunoregulatory activities."

Interferons are capable of inhibiting the replication of a wide variety of microorganisms, including bacteria, fungi, protoza, and both tumor and normal cells. IFN also regulates both humoral and cell mediated immune responses. High doses of IFN- are capable to suppressing the antibody-forming cells in response to antigens, whereas low doses appears to stimulate an increased activity of these same cells. IFN- also regulates the response of B and T lymphocytes to thymus-dependent and- independent antigens. Other immunoregulatory activities of IFN include enhancing

the cytotoxic activity of natural killer cells and the phagocytic function of macrophages." IFN's inhibit viral replication by two mechanisms by blocking the initiation of protein synthesis and by degradation of viral mRNA. IFN also enhances the activity of NK cells and macrophage phagocytosis, inhibits delayed type hypersensitivity reactions, and regulates the production of antibody. IFN is also an effective regulator of the immune response, particularly of B and T lymphocytes, NK cells, and macrophages.

Although interferons can be induced by a number of methods, including the administration of many inducers, the production is quite low and not feasible for commercial purposes. Some inducers of IFN are toxic to human and animals, and IFN is usually metabolized very rapidly within the body.

Recombinant DNA technology, has made it possible to identify the genes that produce the different types of IFN, clone these genes, and mass produce the various subclasses of these particular proteins. These products can now be administered to animals at the time of vaccination, thereby boosting the antibody response. Administration at the time

cattle are faced with stress lessens the chances of stress related shipping fever.

Interleukins: Interleukins (IL) are produced by mononuclear communications which modulate immune responses. There are 6 interleukins IL-1 through IL-6 which have been identified. IL-2 has been cloned from cattle. Interluekin-2 is a glycoprotein produced by T helper cells.[1,2] IL-2 plays a very important role in promoting the clonal expansion of T lymphocytes that have been activated with antigens or mitogens.[3] IL-2, along with IFN-gamma and IL-1, a product of macrophages are all essential for differentiation and proliferation of cytoxic lymphocytes. IL-2 and IL-1 are also important for antibody production by B lymphocytes. In some instances IL-2 has now been used for long-term cultures of lymphocytes in a number of different species.

In vito it is possible to add IL-2 to cultures to generate cytotoxic T lymphocytes, maintain long-term proliferation and cytotoxic cell cultures, stimulate natural killer cell activity, and assist in overcoming some immune dysfunction in lymphocytes taken from individuals with selected immune deficiencies.[4] There is some evidence that IL-2 synthesis may be suppressed by the presence of viruses.

Glucocorticoids that depress immune responses can inhibit IL-2 synthesis. On the other hand, it is possible to override the effects of glucocorticoids by adding exogenous IL-2.

Recombinant DNA technology has now permitted the cloning of IL-2 genes from a number of different species. Human, bovine, mouse, as well as IL-2 from other species have been cloned. It has been demonstrated that both bovine and human IL-2 are capable of augmenting immune responses in cattle.[5] The human IL-2 has been shown to be effective in other species, such as the horse, pig, dog, cat, etc. The responsiveness of these various species to human IL-2 varies to some extent. The addition of these products, along with vaccines sometimes assist in boosting immunological responses to various antigens.

Conclusion: Rapid advancing technological procedures directed at subunit proteins and genetic material are increasing the specificity of diagnostic procedures. The use of novel marker systems which can be amplified through bridging molecules has increased the sensitivity of these systems. Many of these procedures are resulting in animal side tests. This is greatly increasing the use of tests

making it possible for veterinarians to make diagnosis in the field. New improved vaccines produced through rDNA, gene deletion or synthetic peptide technologies are providing new approaches for safe, effective vaccines.

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ABOMASAL DISPLACEMENT, ETIOLOGY, PATHOGENESIS, TREATMENT AND PREVENTION

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INTRODUCTION

Abomasal displacement has been diagnosed since 1898 when the first case of abomasal torsion in a calf was described (10). In 1928 and 1930 (79) the first abomasal torsions were described in adult cows and since then a gradually increasing number of abomasal torsions were recorded. In 1963 Emsbo (20) reported an increasing incidence of abomasal torsions in Denmark and gave a detailed description of the disease. The first case of left displaced abomasum (LDA) was published in 1930 (24), although Moore et al (46) stated in 1954 that they had seen their first case in 1948. The end of the first period of the history of abomasal displacement is marked by the detailed and comprehensive study of Dirksen (16) in 1962. Since then an avalanche of papers on abomasal displacement has been published.
The spate of papers has somewhat subsided. Apparently clinical researchers have lost interest in the subject. One could conclude from this fact that all the questions about etiology, pathogenesis, therapy or prevention have been answered. Nothing is less true, we know only a few answers.
This paper will deal with the history of abomasal displacement and will discuss the results of various studies that have been conducted to elucidate etiology, pathogenesis, treatment and prevention.
Finally it will concentrate on new developments both in therapeutic approach as well as in possible preventive measures.

DEFINITIONS

Most workers investigating the etiology of the displaced abomasum (DA) do not distinguish between left displaced abomasum (LDA) or right displaced abomasum (RDA). Clinically the difference is obvious and RDA is frequently complicated by some form of torsion.
Wensfoort and van der Velden (85) have presented a concept based on a pendulum model and axes centered on the abomasum with which they were able to identify the various forms of abomasal displacement and torsion. With this concept 25 combined displacements could be predicted. Of these 25 possibilities 4 different types of

positions were found during surgery in 217 cows with RDA. The position of the LDA is according to the terms used a flexio of 180-270° to the left and can be compared clinically with the flexio 180-270° to the right without torsion or rotation. In both case passage of food through the abomasum is still possible, a fact that is reflected in the clinical picture of the cows affected.
Although the incidence of LDL still is considerably greater than that of RDL, there are reports that the percentage of RDL is increasing (23, 55).

INCIDENCE, SEASONAL OCCURENCE, BREED AND AGE

Robertson (60) already in 1968 showed in his revieuw that LDA was a world wide problem.
The incidence rate, without specification as to the direction of the displacement has been reported since 1961 were Pinsent (57) found an increased incidence over a period of 5 years (1955-1960). Robertson (60) saw an increase from 3% of the bovine admissions to the Large Animal Hospital of the University of Pennsylvania in 1960 to 30% in 1963. Coppock (12) found an incidence of 1.16% among the affected herds and .35% over the total lactation from all herds (267.844 lactations). Markusfeld (41) noted an increase of the incidence rate from 0.05% over the period 1969-1974 to 1.9% in the period 1975-1976.
Hesselholt and Grymer (31) saw an increase of admissions to the clinic from 16% in 1967 to 24.3% in 1977 and reported an overall incidence of 0.12% - 1.9% in 96400 cows in Denmark.
Sutherland (67) concluded from a 13 years survery in a population of 700 cows that the incidence of abomasal displacement had not increased.
Varden (78) reported an incidence of 0.2 - 0.4% over a 10 year period in a cow practice covering 2400 dairy cows.
Erb and Gröhn (21) found in incidence rate of 1.1% in Holstein cows in the first 21 days after calving. Dohoo and Martin (18) reported a lactational incidence rate of 1.4% from 2875 lactation records of 2008 cows. Robb et al. (59) reported a fairly high incidence of 4.36% in 50 dairy herds.
Pinsent (56) was the first to report a market seasonal incidence after correction for seasonal calving patterns. The majority of the cases occurred between October and April, that is during the stabling season. This was confirmed by others (14, 42, 44, 59, 65). A few reports (18, 78) mentioned no seasonal pattern.

Dirksen (12) reported that most cows had recently calved for the 3rd or 4th time. Most workers agree on the fact that the incidence increases through the 4th to the 6th calving (30, 42, 44, 59, 69, 78).
Occasionally a higher incidence in first calf heifers is reported (8, 84).
Pinsent (56) again was the first to report that Channel Island breeds were at increased risk. This was confirmed by others (34, 42) but Robertson (60) found that the breed distribution of DA cases was similar to that of the whole population.
Most of Pinsents (56) cases occurred during the first week post partum (64.3%) and 11.0% occurred from 1 - 4 weeks post partum.
Others (16, 22, 28, 46, 48, 78, 87) agree that during the period from 2 weeks ante partum to 2 weeks post partum the majority of the cases occur. The median day post partum of diagnosis in a Canadian study was day 8 (18) and in a New York study day 1 to day 15 (22).

MILK PRODUCTION

It has been suggested early that a higher incidence of DA is to be expected in high yielding cows (12, 25, 26, 42, 44, 59). Later studies (59, 18) however found no differences in herd milk yield between high incidence and low incidence herds. Erb and Gröhn (21) concluded from their studies that it is unlikely that a high cow milk yield is a risk factor for LDA.

HERITABILITY

When tracing sire lines higher rates of LDA were found among certain bull lines in two studies (43, 66).

CONCURRENT DISEASES

Dirksen (16) found that in his clinical material a considerable amount of cows had a concurrent disease, 60% had ketosis, 10% had retained placenta and 8% had endometritis.
Hall and Wass (30) suggested that hypocalcaemia predisposed for DA, a suggestion later supported by Varden (78) who reported that 30% of 51 cows with DA had recieved treatment for parturient paresis before displacement was acertained.

These findings, also been reported by others, (14, 60, 78, 84) gained considerable interest in epidemiological studies using bivariate or multivariate analysis. In some of these studies the association between DA and milkfever has been acertained (21) but in others such an association was denied (12, 41). The same opposing results were obtained in studies of other potential risk factors such as retained placenta (41, 18) and metritis (21, 41). Erb and Gröhn (21) concluded that milkfever is a risk factor for LDA and that much of the association is mediated by retained placenta. Mastitis has been ruled out as a risk factor (21). The risk for DA in cows with ketosis was 39.04 times ($P < 0.01$) greater than in cows without ketosis. This relation was confirmed in other studies were clinical ketosis diagnosed prior to LDA was strongly related to LDA (13, 59).

NUTRITION

It was soon after the first publication about DA that the suggestion was put forward that the occurence of DA was caused by the increased use of concentrates in the rations for dairy cows. Later this was specified by pointing out that steaming up (11, 42, 60), rations rich in protein (25) or rich in fat (29) were associated with a high incidence of DA. Nocek et al. (49) devided 289 cows into 3 groups during the dry period and fed all hay, 50% hay/ 50% corn silage or limited corn silage plus liquid protein supplement. They saw 3,4.3 and 6.3% LDA respectively in these groups. The differences however were not significant.
Complete rations, especially those with a high grain percentage were used in herds experiencing a high incidence of DA (28, 44, 50). The avoidance of abrupt ration changes is a frequently given advise (60, 11).
Danish workers point out that in rations low on crude fibre (16% or less) there is excess risk for DA (27).
Robertson (60) however found no correlation between the amount of grain fed and the incidence of LDA within herds. Curtis (13) even found that higher energy intakes in the late dry period were preventive for LDA.
Also Robb et al (59) in their study of 50 dairy herds were not able to find differences in lead feeding, challenge feeding, dry matter, net energy or crude protein between high versus low incidence herds.

HOUSING AND OTHER HUSBANDRY FACTORS

It was suggested that the lack of free movement in tied stalls increased the incidence of DA but, studies of Martin (42) suggested that more case occurred in herds using loose housing. It is shown that on pasture a DA can disappear spontaneously (34).

OTHER FACTORS POSSIBLY ASSOCIATED WITH THE OCCURENCE OF DA

Danish workers, headed by Poulsen (37, 51, 52, 53, 54) have found that in high-yielding cows the acid-base balance changed in alkalotic direction during the year from summer to spring. They also showed that a change in the acid-base balance will induce an atony of the abomasum.
The changes in the acid-base balance in the alkalotic direction taking place during the winter and spring months occurred simultaneously with the increase in beet, beetproducts and concentrate in the ration suggesting that is was dependent on the change in feeding.
In this respect it is remarkable that Markusfeld (41) found that the risk of DA in cows with aciduria was 6.17 times greater ($P < 0.01$) than for those with a normal urine reaction. Metabolic alkalosis in cows namly often is accompanied with paradoxic aciduria (38).

PATHOGENESIS OF DA

Most workers agree on the theory that the factors responsible for displacement of the abomasum can be anatomical and mechanical. There normally may be some displacement of the abomasum before parturition. Lagerlof (40) found that at the end of the gestation period the abomasum is situated left of the midline. Jones (36) found that in 50 cows slaughtered while in dorsal recumbency the abomasum was situated right of midline in 50% of the cows, in 12% of the animals the abomasum was found on the midline and in 30% of the cases it was situated left of the midline.
Weaver (83) using the same procedure saw 61% right of the midline, 28% on the midline and 11% left of the midline. Begg and Whiteford (3) argued that the pregnant uterus forces the abomasum forward and lifts the rumen from the bottom of the abdomen. This brings the abomasum in a semidisplaced position. When after calving the rumen returns to the bottom of the abdomen, the abomasum then is fixed in this semi-displaced position. Gas and ingesta then would accumulate and

the subsequent abomasal dilatation would be followed by displacement.
Sack (62) confirmed that the position of the abomasum could vary in normal cows, but could not relate the position of the abomasum with the roughage/concentatio ratio in the ration.
The suggestion that anatomical factors play a role in the pathogenesis of DA is illustrated by Albert and Ramey (1) describing a patient with hydrops ascites due to chronic liver cirrhosis who developed an LDA. In this respect it is of interest that Markusfeld (41) found that the risk of DA for cows which had twins was 3.25 times greater ($P < 0.01$) than for those with a single calf.
On the other hand it is shown that in cows where a LDA was induced surgically no changes could be found neither clinically nor hematologically (69).
Also the finding that an LDA can exist in cows for a long period without clinical symptoms (2, 35) suggests that other factors than anatomical must be responsible for the disturbances shown by cows suffering from DA. This is supported by the facts that DA is found in bulls, oxes, calves and yearlings and that concurrent diseases, metabolic disturbances and nutritional factors have been identified as risk factor for DA.
Most workers nowadays agree on the fact that abomasal hypomotility or atony are the primary conditions for the development of DA. As a consequence of this hypomotility ingesta and gas is trapped in the abomasum, dilatation and dislocation follows. In the proces of dislocation anatomical factor may play a role. Svendson (68) suggested that the volume of the fore stomachs determines the direction of the dislocation.
It is well known that the motility of the abomasum can be influenced by many factors. Svendson (68) following the suggestion that introduction of large amounts of concentrates had caused the increased incidence of DA examined abomasal contractiona and abomasal gas production after feeding large quantities of concentrates. It was found that feeding large quantities of concentrates reduced the frequency of abomasal contractions and increased the production of gas in the abomasum. Infusion of ruminal contents from an animal fed a high concentrate diet into the abomasum of experimental cows reduced abomasal motility. The same was found for a solution of volatile fatty acids (VFA's) of a similar composition as found in ruminal fluid of a cow on a high concentrate diet. Bolton et al. (5) found that intra abomasal infusion of acetic, propionic or bytyric acid was associated with a marked decrease in abomasal action potential activity and emptying rate. Butyric acid was most effective followed by propionic and acetic acid. Ehrlein and Hill (19) however noticed no effects on abomasal motility when VFA's were infused in the abomasum. Twisselman (70) could

not find an increase in the concentration of VFA's in abomasal fluid after feeding a high concentrate diet although the concentrations of VFA's in the rumen increased considerable. Breukink and De Ruyter (7) compared a high grain ration with an all hay ration and found that after feeding a strong increase in ruminal VFA concentration occurred on the high grain ration and that no changes were seen after feeding hay. There were no changes in the concentration of VFA in the abomasum after feeding the two rations and also between the rations there was no difference in abomasal VFA concentration.
Hatanya et al. (29) found no effect on abomasal motility after introduction of VFA in the abomasum, but they found a decreased motility after the introduction of long chain fatty acids. They suggested that the introduction of considerable amounts of fat in the ration predisposed for abomasal hypomotility.
The role of hypocalcemia in abomasal hypomotility was suggested by Hull and Wase (33) and Poulsen (51). Poulsen (54) stated that the combination of hypocalcemia and alkalosis in high producing cows can lead to serious hypomotility. The findings that cows suffering from DA show alkalosis can also be the consequence of an hypochloremia due to sequestration of chloride in an atonic abomasum (6). Vlaminck et al (82) using broomsulfophtaleine (BSP) as a marker found that in cows with LDA the passage of contents through the gastrointestinal tract was significantly slower. After oral application of calciumcarbonate (20 g/100 ·kg B.W.) in the presence of sodium to increase calcium absorption, the abomasal displacement disappeared in 11 out of 21 animals. However after the treatment was stopped a recurrence was observed frequently.
It is frequently suggested that abomasal motility is influenced directly of indirectly by mediators originating from concomitant disorders such as mastitis, retentio secundinarum and endometritis.
It is shown that prostaglandin (PGE2 en PGF2α) has influence on the spontaneous motility of the antrum pyloricum of the bovine abomasum (72). Vlaminck et al (81) found a small decrease in the rate of emptying of the abomasum when PGE1 and PGE2 was injected intramuscularly. PGF2α showed no effect. Repeated administration of prostaglandin synthesis inhibitors to animals suffering DA had no corrective effect. They concluded that their study did not support a possible role of prostaglandins in the origen of DA.
Studies of antral myo-electric activity, intraluminal pressure and duodenal flow revealed no significant relation between these parameters (80). This may be the consequence of the complexity of the interrelationship of abomasal, pyloric and duodenal activity.

In a study of the myo-electric activity pattern of the abomasal body in adult cows is was found that long lasting periods of inactivity exist, that occurred less then once per day but lasted sometimes several hours independent whether the cow was standing or was lying down (39). If these periods of real inactivity are to be interpreted as periods of real mechanical inactivity, then they could play a rol in the etiology of DA.

In recent years some interesting results have been obtained in studies regarding the relation of fatty infiltration of the liver and DA. Fatty liver is related to impaired fertility and increased incidence of diseases in the early post partum period (58). Holtenius and Niskanen (32) found that cows with LDA had moderate to severe fatty changes of the liver. They suggested that the feeding regime prepartum might influence the concentrations of plasma lipids and the fatty infiltration in the liver. A high energy, low protein feeding especially seemed to predispose for accumulation of fat in the liver cells. The imbalance in the feeding before calving in their opinion may cause the metabolic disturbances resulting in fatty changes of the liver and may cause abomasal displacement.

In a extensive study Muylle et al. (47) showed that cows with DA had severe hepatic lipidosis and ketosis but they saw no glycogen depletion of the liver cells. In cows with DA but without hepatic lipidosis glycogen depletion other liver cells were found. They found evidence of hepatic lipidosis as the main problem in 7 herds suffering from a high incidence of parturient problems such as DA.

Van Dijk et al. (73) also found a higher incidence of fatty liver infiltration in cows suffering from DA compared with normal cows examined during the early postpartum period.

In another study Van Dijk et al. (74) studied blood and liver lipids before and after calving in 22 dairy herds. The incidence of milkfever, retained placenta, endometritis, mastitis, ketosis, abomasal displacement and infertility was recorded in the 49 pluriparous cows involved in the study.

Sixteen cows (33%) were classified as having severe hepatic lipidosis (HL), 26% had moderate HL and 41% mild HL. Of all the cases of periparturient diseases 56% were found in cows with severe HL. The observed lower glucose and high NEFA and 3-hydroxybutyrate concentrations as well as a higher loss of body conditions in severe HL cows suggest a streng relationship between the degree of energy deficiency and that of the fat mobilization as well as between the degree of energy deficiency and the severity of hepatic lipidosis.

Van Meirhaeghe et al. (76) found that cows with DA had higher basal blood glucose levels than control cows independent of their ketotic status. They also found a much lower secretory response of insulin secretion in cows with DA. They

suggested the presence of an isulin insensitivity in these cows (75). The basal insulin levels observed in cows with DA were significantly higher than the expected values in the same post partum period of normal cows. Endogenous as well as exogenous insulin caused a marked decrease of the abomasal emptying rate. They suggested that, since VFA's such as butyrate, iso-valerate and valerate induces a more pronounced insulin response than glucose, insulinogenic substances may be involved in the regulation of abomasal emptying and that disturbance in VFA production and/or metabolism may play a role in the pathogenesis of abomasal displacement.

Insulin insensitivity caused by a decreased number of insulin receptors has been observed in obesitas in man and sheep (77, 45).

These observations suggest that prevention of hepatic lipidosis may also prevent the occurence of DA.

TREATMENT

Displaced abomasum can be treated effectively by several surgical techniques (61).

The close surgical techniques on blind-stich abomasopexy and the bar suture techniques are slightly less succesful (63).

A non surgical treatment by rolling the cow is highly effective but has a high rate of recurrence and a much lower rate of recovery, independent on the additional treatment with calcium infusions, hypertonic saline infusions or oral administration of ammonium chloride (71).

Abomasal torsions or mesentenic torsions have been observed following LDA correction by casting and rolling (71, 64).

The decision which method to use or not to treat and sell the cow is largely dependent on the economic value of the cow.

It was concluded from a Dutch study (9) that surgical treatments had the highest expected monetary value in cows with a lactation value of 10% above herd average An USA study (63) concluded that closed surgical techniques had expected monetary values close to surgical techniques. Rolling is preferred over selling but if an LDA recurs, surgical treatment or selling the cow is preferred over rolling

Studies are under way to evaluate the possibilities of newly developed drugs that may influence abomasal motility and subsequent abomasal emptying. In this respect an abundance of information becomes available regarding the so-called pro-kinetics, substances that increase the motoric activity of the

gastro-intestinal system such as dopamine-antagonists (domperidon, metoclopramide), cholinomimetics (carbachol, physostigmine) and drugs that stimulate the liberation of acetycholine at the neuromuscular junction such as cisapride.
These drugs and there analogues may become important in the treatment of LDA after correction by casting and rolling or even without that.

PREVENTION

From the study of the etiology of DA a certain number of preventive measure can be drawn. Parturition, high milkproduction and aging are inevetable but feeding, management and housing can be controlled. Feeding a high energy diet in connection with low fibre has to be avoided. At least 1/3 of the ration has to exist of fibrous material with fibers of at least 5 mm length. The avoidance of abrupt ration changes is recommended frequently (50). Especially the feeding in late lactation and during the dry period has to gain more interest in order to avoid hepatic lipidosis. An energetic balance is important in this period. Cornsilage for instance may well be excluded from the ration.
It is not possible to prescribe a ration which will prevent DA under all circumstances but it is reasonable to believe that an energetically balanced diet containing sufficient fibrous material in late lactation and during the dry period is of great importance in the prevention of hepatic lipidosis and abomasal displacement. By changing the diet in such a way Wenting (86) observed that in herds with a high incidence of hepatic lipidosis and post-parturient disorders amoung wich DA, the incidence of hepatic lipidosis decrease significantly and no DA occured. One can expect that the coming years more detailed information regarding this method of prevention of DA will become available.

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SUMMARY

The incidence of abomasal displacement continues to show a gradual increase. The need for preventive measures has therefore become a pressing matter. Successful prevention of abomasal displacement is based on adequate knowledge of the aetiology and pathogenesis. The present state of affairs is reviewed on the basis of the literature, personal experience and studies. Obviously, a large number of factors may play a role in the pathogenesis of abomasal displacement. Although the knowledge of the aetiology of abomasal displacement is anything but complete, a number of (probably useful) preventive measures are suggested.

RÉSUMÉ

L'incidence du déplacement de la caillette s'accroit toujours, de sorte que des mesures prèventives deviennent de plus en plus nécessaires. Une prévention effective du déplacement de la caillette dépend de la connaissance de l'étiologie et de la pathogénèse. La situation actuelle à cet égard a été revue à partir de la littérature, des études et des expériences personelles. Bien entendu, beaucoup de facteurs pourraient jouer un rôle dans la pathogénèse du déplacement de la caillette. Bien que notre connaissance de l'étiologie du déplacement de la caillette ne soit pas du tout complète, un nombre de mesures préventives, probablement effectives, ont été suggérées.

ZUSAMMENFASSUNG

Die Häufigkeit der Labmagendislokationfälle nimmt noch immer zu. Dass heisst dass auch das Bedürfnis an wirksame präventive Massnahmen grösser wird.
Es bedarf keines Beweis dass solche Massnahmen gegründet sein müssen auf Kentniss der Etiologie und Pathogenese dieser Erkrankung.
Dieser Beitrag beabsichtigt eine gedrängte Übersicht der Kentniss auf diesem Gebiete zu geben die beruht auf die rezente Literatur und auf persönlichen Erfahrungen und Experimenten. Es ist eine klare Sache dass eine grosze Anzahl manchmal sehr verschiedene Faktoren eine Rolle spielen in der Etiologie der LDL. Mit Bezug auf vielen dieser Faktoren ist noch manches nicht völlig bekannt.
Es wird zum Schlusz eine Anzahl, auf der heutigen Kentniss gegründeten und deshalb am meisten Erfolg versprechenden "Vorsichts massnahmen" suggeriert.

PREDICCION DEL ESTADO DE CAPACIDAD REPRODUCTORA DEL GANADO BOVINO MEDIANTE TECNICAS DE ENZIMO-INMUNO-ANALISIS (EIA)

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Desde el comienzo de la domesticación de los animales salvajes, ha sido una constante preocupación para el hombre seleccionar aquellos que más rendimiento pudieran proporcionarle y, a lo largo de la historia, se han ensayado los métodos y técnicas más variados para conseguir tales objetivos.

No cabe duda que los progresos que se han venido haciendo en Genética, desde hace hace varias décadas, han desembocado en la obtención de unas estirpes con un alto rendimiento, tanto en la producción de carne como en la de leche, refiriendonos concretamente al ganado vacuno, pero que también se pueden hacer extensivos a otras especies, donde las aves y el porcino son ejemplos elocuentes de esos progresos.

Centrándonos en los bóvidos, sabido es que el objetivo, desde el punto de vista estrictamente reproductor, es el conseguir un ternero cada año, lo que no se consigue la mayoría de las veces, por una cantidad de variables que de forma decisiva intervienen en los rendimientos de la explotación.

Se ha calculado, con cierta seguridad, que por cada día que se prolongue el intervalo de paridera -período de tiempo entre dos partos sucesivos- se pierden unas 3 libras esterlinas por animal. Cifra que por si misma puede parecer insignificante, pero si se tiene en cuenta que en paises desarrollados y bien dotados desde el punto de vista ganadero, como puede ser Inglaterra, el intervalo aludido alcanza los 395 días y si esa cantidad se multiplica por el número de animales de una explotación importante, las pérdidas anuales llegan a alcanzar cifras del orden de varios millones de pesetas.

Por otro lado, la introducción de la técnica inmunoenzimática para la determinación de las concentraciones hormonales a lo largo del ciclo reproductor de la vaca ha supuesto una valiosa aportación para dilucidar las causas que llevan a esos aumentos de intervalo de paridera, tan negativos desde el punto de vista económico. Efectivamente, manejada con corrección, esa técnica permite el conocimiento de pequeñas variaciones de concentración de los esteroides sexuales, responsables, en definitiva, del estado reproductor de la hembra.

El disponer, con éxito, de la técnica mencionada no es tarea fácil, pero después de estudios concienzudos, ya que dentro de ella están incluidos muchos factores que pueden alterar sus resultados, se puede utilizar con un índice de fiabilidad de, practicamente, el 100%.

MATERIAL Y METODOS

Uno de los puntos que más contribuye al éxito de la técnica ELISA es el disponer de anticuerpos con títulos adecuados y carentes, lo más posible, de reacciones cruzadas para proporcionarnos una mayor sensibilidad.

Obtención de antisueros

Como animal productor de anticuerpos se han utilizado conejos blancos de Nueva Zelanda, siguiendo el proceder de inoculaciones descrito por MUNRO *et al.* (1984) y mantenidos en condiciones estandar en lo que a dieta, alojamientos, temperatura, humedad, etc. se refiere. Después que las muestras de suero alcanzaran los títulos esperados se sangraron los animales siguiendo la técnica de ILLERA *et al.* (1989) y se diluyeron a las concentraciones precisas, normalmente 1/4.000.

Hemos obtenido anticuerpos frente a estradiol 17β, progesterona y testosterona.

Obtención de muestras

En reses de abasto, de diferente edad y raza, recién sacrificadas, se han recogido, además de muestras de sangre, los dos ovarios con el fin de pesarlos y medirlos así como contar y medir los folículos en ellos contenidos. Se ha recogido líquido folicular para conocer las concentraciones de las tres hormonas mencionadas, al igual que se ha hecho en el plasma procedente de las muestras de sangre recogidas.

Los análisis hormonales, en todos los casos, se han hecho mediante la técnica ELISA de Competición y no se ha precisado extracción, de ningún tipo, para determinar las hormonas en las muestras (plasma y líquido folicular). En algunos casos fue preciso hacer alguna dilución.

Técnica ELISA de Competición

Se han utilizado placas de titulación de poliestireno, provistas de 96 pocillos, (Dynatech). Los pocillos se tapizaron con 100 µl de la dilución del anticuerpo específico, obtenido por nosotros para cada una de las hormonas. Se incubaron las placas a 37ºC, toda una noche. Transcurrido ese tiempo se lava la placa y en cada pocillo se adicionan 50 µl de la mezcla de la muestra a analizar y el conjugado; este conjugado está compuesto por la hormona en cuestión (Steraloids) y como enzima marcadora se ha utilizado la peroxidasa (Sigma). El conjugado se empleó a la dilución de 1/40.000, para el estradiol, 1/40.000 para la progesterona y 1/60.000 para la testosterona. Después de una incubación a temperatura ambiente y una serie de lavados, se adicionaron 100 µl de sustrato específico de la enzima. El sustrato usado fue peróxido de hidrógeno (Merck) y como sustancia cromógena se utilizó la ortofeniendiamina (Dakopatts). La reacción se frenó mediante la adición de 50 µl de ácido sulfúrico 4M (Merck) y el color se cuantificó en un lector de ELISA a 450 nanómetros (Eurogenetics).

Los resultados obtenidos han sido procesados mediante un programa especial del Informatic Department, Universidad de California (Davis, USA) y el programa B.M.D.P. del Centro de Proceso de Datos de la Universidad Complutense de Madrid.

RESULTADOS Y DISCUSION

Los anticuerpos obtenidos por nosotros presentaron las reacciones cruzadas que se indican en la Tabla I.

La sensibilidad alcanzada con la técnica que hemos empleado fue de 0,1 pg/pocillo para progesterona y testosterona, para estradiol 17β fue 0,01 pg/ml; ésto es, eramos capaces de detectar concentraciones tan bajas como dos décimas, o menos, de picogramo por mililitro, tanto en plasma como en líquido folicular y, que sepamos, somos los primeros en haber podido determinar concentraciones de hormonas esteroides, en líquido folicular mediante la técnica ELISA, ya que los casos descritos en la literatura se refieren a análisis mediante Radioinmunoanálisis (BRANTMEIER *et al.*, 1987).

Sabiendo que disponiamos de una técnica idónea para los fines propuestos se han realizado una serie de estudios matemáticos para tratar de encontrar las correlaciones adecuadas que nos pudieran conducir a poder apreciar el estado reproductor de un animal con sólo tener las concentraciones de hormonas en muestras de sangre periferica.

Los folículos encontrados en ambos ovarios se clasificaron en diferentes grupos según edad del animal y alguna característica especial observada en la inspección realizada en la canal, como por ejemplo, estado de nutrición, si estaba o no gestante, posible tratamiento con anabolizantes, etc.. Se midió la cantidad de líquido folicular recogida en practicamente todos los folículos y de allí se tomó la muestra para hacer los análisis hormonales pertinentes; en líneas generales, hemos seguido los consejos que en 1960 dictara RAJAKOCWSKI para este tipo de estudios.

En la tabla II se muestran algunos de los valores del estudio que se hiciera de los ovarios.

Por su parte, la tabla III nos presenta las concentraciones encontradas en el plasma y líquido folicular de las tres hormonas que estamos estudiando ($\bar{x} \pm$ S.E.M.).

En la tabla IV se pueden encontrar los porcentajes de variación de concentración, para las tres hormonas, clasificados según criterios de concentración.

La tabla IV presenta unos valores un tanto artificiosos pero que era necesario mostrar para ver las diferencias tan acusadas que existen en lo que a concentraciones plasmáticas y foliculares se refiere, pudiendo afirmar que hay mayor concentración hormonal en el líquido del folículo que en el plasma. Los valores parecen ofrecer los mismos márgenes de variación que los descritos por otros autores como KRUIP *et al.* (1985), FORTUNE *et al.* (1985) y más recientemente SAVIO *et al.* (1990) en el líquido folicular y los que indican, entre otros, HENRICKS *et al.* (1972) y WISE *et al.* (1982) para la sangre periférica; pero hay que dejar constancia que la metodología es distinta, ellos utilizan RIA y nosotros ELISA, los animales son también de distinta raza y edad y otras cuantas circunstancias también distintas, como pueden ser los estadios del ciclo reproductor, etc.

Sin embargo, nosotros con los resultados expuestos hemos llegado a encontrar unas rectas de regresión que nos permitirán conocer *a priori* cuál es el estado de capacidad funcional ovárica de una hembra determinada conociendo las concentraciones de las hormonas progesterona, estradiol 17β y testosterona en una muestra de plasma.

Las fórmulas son las siguientes:

Para Progesterona:

Niveles foliculares de progesterona = 9,623 X + 85,761

donde X = nivel plasmático de progesterona.

Para Estradiol 17β:

Niveles foliculares de estradiol = 3,064 X + 94,574

donde X = nivel plasmático de estradiol.

Para Testosterona:

Niveles foliculares de testosterona = 1,820 X + 32,964

donde X = nivel plasmático de testosterona.

Se podrían señálar como límites de aceptabilidad de la capacidad reproductora los siguientes:

Progesterona = > 100 ng/ml de líquido folicular.
Estradiol 17β = > 200 ng/ ml de líquido folicular.
Testosterona = Valor impreciso.

Por otro lado, y siguiendo las directrices que marcaran BELLIN y sus colaboradores (1984) podemos clasificar los folículos encontrados en el ovario en atrésicos o funcionales según el cociente:

Concentración de Estradiol / Concentración de progesterona

De tal forma que si el resultado de esa operación es mayor de la unidad podemos asegurar que se trata de folículos **funcionales** mientras que si es menor, los folículos se pueden clasificar como **atrésicos** claro está que para llegar a esto es preciso trabajar con la gónada aislada del organismo.

Podemos concluir diciendo que con las lógicas precauciones que todo tipo de predicción biológica pueda significar presentamos una metódica fácil para tener una idea aproximada del estado de funcionalidad reproductora de hembras bovinas siempre que podamos conocer, mediante ELISA, las concentraciones que de los tres esteroides ováricos más importantes se encuentren en el plasma.

TABLA I- Porcentaje de reacciones cruzadas

Esteroide	Anti-P4	Anti-E2	Anti-T
Progesterona	100,00	0,02	0,01
Estradiol 17B	0,02	100,00	0,01
Testosterona	0,01	0,01	100,00
11α-Hidroxi-P4	20,00	0,02	0,02
17α-Hidroxi-P4	25,00	0,01	0,01
Pregnendiona	0,50	0,01	0,01
Estradiol 17α	0,01	10,00	0,01
Estrona	0,02	15,00	0,01
Estriol	0,01	5,00	0,01
5α-Dihidro-T	0,02	0,01	20,00
Androstenodiona	0,01	0,01	5,00
5B-dihidro-T	0,01	0,02	15,00
Cortisol	0,02	0,02	0,02

TABLA II - Parámetros encontrados en los ovarios (n=50)

	Derecho	Izquierdo
Peso (g)	12,994 ± 0,23	9,681 ± 0,17
Longitud (mm)...	39,904 ± 1,14	36,883 ± 0,98
Anchura (mm) ..	28,275 ± 0,57	24,645 ± 0,34
Número de folículos ..	9,747 ± 1,8	9,132 ± 0,82
Vol. de líquido folicular (ml)	0,531 ± 0,05	0,490 ± 0,01

TABLA III - Valores medios de hormonas en plasma y líquido folicular (n=50)

	Progesterona	Estradiol-17B	Testosterona
Plasma	2,13 ± 0,11ng/ml	20,12 ± 0,76 pg/ml	1,40 ± 0,07 ng/ml
Líquido fol.	115,90 ± 8,00 ng/ml	121,63 ± 9,02 ng/ml	30,81 ± 2,76 ng/ml

TABLA IV- PORCENTAJES DE VARIACION DE CONCENTRACION DE PROGESTERONA, ESTRADIOL 17 ß Y TESTOSTERONA

	En Sangre	En liq. folic.
Progesterona (ng/ml)		
De 0 a 4	74	
De 4 a 8	16	
De 8 a 12	4	
> 12	6	
> 50		50
De 50 a 100		5,5
De 100 a 200		15,5
> 200		30
Estradiol 17 ß (pg/ml)		
< 20	58	
De 20 a 40	22	
De 40 a 60	14	
> 60	6	
(ng/ml)		
< 10		11
De 100 a 200		36
De 200 a 400		25
> 400		28
Testosterona (ng/ml)		
De 0 a 1	40	23
De 1 a 2	28	11
De 2 a 4	18	27
> 4	14	39

AGRADECIMIENTO

Deseamos expresar nuestro agradecimiento a los responsables del Matadero Industrial de Colmenar Viejo y a los del Matadero Municipal de Madrid por las facilidades que nos dieron para la obtención de muestras. Asimismo deseamos agradecer la valiosa ayuda del Sr. Pescador, del Centro de Proceso de Datos de la U.C.M. y las magistrales enseñanzas recibidas de la Dra. C. Munro, de la Universidad de California (Davis, USA), quien nos introdujo en el fascinante mundo del Enzimoinmunoanálisis.

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RESUMEN

Se describe con detalle la técnica ELISA de Competición para determinar hormonas esteroides, tanto en plasma sanguíneo, como en líquido folicular obtenido de reses sacrificadas en los mataderos cercanos a Madrid. Se resalta la importancia de disponer de anticuerpos con títulos altos para que dicha técnica tenga una gran sensibilidad y que, por otro lado, presenten el mínimo de reacciones cruzadas que pudieran enmascarar los resultados. Analizadas un buen número de muestras para que puedan ser tratadas estadísticamente, con buenos índices de significación, se someten a un profundo estudio estadístico con el fin de obtener unas fórmulas matemáticas y así poder predecir, cuanto menos, aproximadamente, en que condición reproductora se encuentra una vaca con sólo conocer las concentraciones plasmáticas de progesterona, estradiol y testosterona. Creemos que sea la primera vez que se describe la aplicación de la técnica ELISA de Competición para determinar hormonas esteroides en líquido folicular.

SUMMARY

In this study, the Competitive ELISA is described for determining steroid hormones on blood plasma or follicular liquids, taken from slaughtered beef cows from the abattoirs in the Madrid area. The importance of making available antibodies with high titres is evident for this technique to have a high sensibility or that on the other hand shows the minimum of cross reactions that could disguise tehe results. A large number of samples were assayed and treated statiscally with good signification coefficients. They were summitted to an exhaustive statistic study to obtain some mathematical formulas so as to be able to predict, approximately, the reproduction condition from cows with only knowing the plasmatic profiles of progesterone, stradiol and testosterone. We think that this is the first time that the application of the Competitive ELISA to determine steroid hormones in follicular liquid has been described.

AUFGABEN, GLIEDERUNG UND ENTWICKLUNG DER WELT-GESELLSCHAFT FÜR BUIATRIK

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EINLEITUNG

Der XVI. Kongress der Welt-Gesellschaft für Buiatrik (1990) ist zugleich ihr 30. 'Geburtstag', was Anlaß dafür bietet, den Werdegang dieser Vereinigung einer rückblickenden Betrachtung zu unterziehen (1, 2, 3). Rinderheilkunde wurde zwar schon im Altertum betrieben: kräftigere praktische und wissenschaftliche Wurzeln schlug diese 'Kunst' jedoch erst geraume Zeit nach Gründung der **tierärztlichen Bildungsstätten** (4). Maßgebend für den Aufbau solcher Schulen waren nicht zuletzt die durch die großen Seuchen des 'Rindviehes' bedingten Verluste und Hungersnöte, obwohl das Pferd zunächst noch die Hauptrolle unter den Haustieren spielte. Zwischen den beiden Weltkriegen rückte das Rind allmählich zum 'Nutztier No. 1' auf und steht seitdem als entscheidender volks- und ernährungswirtschaftlicher Faktor im Mittelpunkt ruraler tierärztlicher Tätigkeit. Dieser Umstand hatte in den letzten Jahrzehnten auch Niederschlag in der tierärztlichen Ausbildung gefunden, sei es durch Schaffung besonderer, mit den verschiedenen Nutztierarten oder nur mit dem Rind befaßter **Hochschulkliniken**, sei es durch die dem Grundstudium folgende Weiterbildung zum **Fachtierarzt für Rinder** (4).

In Zuge dieser Entwicklung wurde 1960 von den Teilnehmern einer auf Initiative **Rosenberger's** in Hannover durchgeführten ersten internationalen Tagung über Rinderkrankheiten der Beschluß gefaßt, eine Weltgesellschaft für Buiatrik (W.G.B.) zu gründen. 1962 konstituierte sich auf dem zweiten derartigen Symposium in Wien ein vorläufiger **Vorstand**. Seine Mitglieder, die Professoren J. Andres/Zürich, K. Diernhofer/Wien, G. Espersen/Kopenhagen und G. Rosenberger/Hannover, legten in Kopenhagen auf dem dritten internationalen Kongress über Rinderkrankheiten, 1964 **Statuten** für die junge Gesellschaft vor, welche von der Mitgliederversammlung einstimmig angenommen wurden.

Danach fallen der W.G.B. folgende **Aufgaben** zu:

- Abhaltung internationaler Kongresse über Rinderkrankheiten
- Vorbereitung und Durchführung des Programmteiles 'Krankheiten der der Wiederkäuer' innerhalb der Kongresse der Welt-Tierärztegesellschaft
- Förderung der Buiatrik in Wissenschaft und Praxis

Zur Verwirklichung ihrer Ziele bedient sich die W.G.B. der nachstehend beschriebenen sowie kommentierten **Gliederung und Arbeitsweise:**

VORSTAND

Die W.G.B. wird von einem aus 5 - 10 **Delegierten** verschiedener Nationalität bestehenden Vorstand geleitet. Diese werden - auf Vorschlag des vorangehenden Vorstandes - von den Teilnehmern der (anläßlich jedes W.G.B. Kongresses) abzuhaltenden Versammlung der

Mitglieder der W.G.B. - und zwar jeweils für eine Dauer von vier Jahren - gewählt (Wiederwahl ist möglich). Anschließend wählen die derart in den Vorstand delegierten Vertreter unter sich den Präsidenten, zwei Vizepräsidenten sowie den Sekretär. Die übrigen Vorstandsmitglieder fungieren als Beisitzer.

An den ebenfalls alle zwei Jahre (jeweils am Vorabend des Kongressbeginns) stattfindenden **Sitzungen des Vorstandes** nehmen des weiteren - als nicht stimmberechtigte Beobachter - die Organisatoren des gerade anstehenden sowie des folgenden W.G.B.-Kongresses, Vertreter des Präsidiums der Welt-Tierärzte-gesellschaft sowie der Redakteur des Mitteilungsblattes der W.G.B. teil. Ehrenmitglieder der W.G.B. sind ebenfalls zur Teilnahme an den Sitzungen ihres Vorstandes befugt. Einzelheiten der personellen Besetzung des W.G.B.-Vorstandes während der ersten 30 Jahre des Bestehens dieser Gesellschaft sind der Übersicht 1 zu entnehmen.

Kommentar: Bislang waren die meisten Vorstandsmitglieder Vertreter der Wissenschaft. Künftig ist - insbesondere im Hinblick auf die Setzung von Schwerpunkten in der Programmgestaltung der W.G.B.- und Welt-Tierärzte-Kongresse - mehr Beteiligung seitens der buiatrischen Praxis anzustreben.

MITGLIEDER

Statutengemäß kann jeder mit der Gesunderhaltung von Rindern befaßte Tierarzt (Praktiker, Wissenschaftler, Labor- und Institutstierarzt, Androloge, Gynäkologe, Obstetriker, Internist, Chirurg, Parasitologe, Pharmakologe, Toxikologe, Hygieniker, Ernährungsfachmann, Pathologe, Immunologe etc.) Mitglied der W.G.B. werden, die auch bereit ist, Nicht-Tierärzte aufzunehmen (maximal 10% der Mitgliederzahl). Früher wurde die **Mitgliedschaft** unmittelbar beim Sekretariat der W.G.B. erworben. Nach Gründung und Angliederung der Nationalen Vereinigungen für Buiatrik (siehe dort), ist diese Regelung fallen gelassen worden: Die Mitglieder nationaler buiatrischer Gesellschaften werden bei Affiliation derselben ohne weiteres Zutun auch Mitglied der W.G.B. Letztere erhebt von ihren Mitgliedern (im Gegensatz zu einigen nationalen Vereinigungen für Buiatrik) keine Beiträge: Die laufenden Kosten des Sekretariates werden von Zuschüssen bestritten, welche diesem statutengemäß von den Organisatoren jedes W.G.B.-Kongresses anzuweisen sind.

Die **Mitgliederversammlung** findet jeweils anläßlich eines W.G.B.-Kongresses und zwar traditionsgemäß am letzten Vormittag desselben statt. Bei dieser Gelegenheit geben Präsident und Sekretär ihre Rechenschaftsberichte, der Sekretär zudem den Kassenbericht ab. Außerdem wird über alle bedeutsamen Entscheidungen abgestimmt (Wahlen zum Vorstand der W.G.B., Assoziation nationaler Vereinigungen für Buiatrik, Nominierung von Kontaktpersonen). Dabei haben nur die an der betreffenden Versammlung teilnehmenden Mitglieder Stimmrecht.

Kommentar: Um Vergleiche - innerhalb der W.G.B. sowie gegenüber den anderen Spezialistenvereinigungen der Welt-Tierärztegesellschaft - zu ermöglichen, sollte die W.G.B. die **Zahl ihrer Mitglieder alle zwei Jahre**, jeweils vor dem anstehenden W.G.B.-Kongress, durch Erfragen der Mitgliederzahl ihrer Tochtervereinigungen feststellen (Umfrage mittels W.G.B.-News letter). Das würde zugleich die Mitgliederwerbung letzterer anregen.

Übersicht 1: Zusammensetzung des Vorstandes der Welt-Gesellschaft für Buiatrik 1960 - 1988 (1, 2, 3)

1	a	b	2 c	d	3				4
1960:					-				-
1962:	Andres	Dierenhofer	Espersen	Rosenberger					-
1964:	Bendixen	Dierenhofer	Amstutz	Rosenberger	Andres	Bratanovic	Charton	Espersen	Grunskell	-
1966:	Bendixen	Dierenhofer	Amstutz	Rosenberger	Andres	Bratanovic	Charton	Espersen	Grunskell	-
1968:	Dierenhofer	Amstutz	Seren	Rosenberger	Andres	Bratanovic	Charton	Espersen	Grunskell	Bendixen[a]
1970:	Dierenhofer	Amstutz	Seren	Rosenberger	Andres	Bratanovic	Charton	Espersen	Grunskell	Stekkov -
1972:	Amstutz	Seren		Bratanovic	Rosenberger	Andres	Espersen	Kapinasse	Grunskell	Mayer Stekkov Diernhofer[a]
1974:	Amstutz	Seren		Videla	Rosenberger	Andres	Bratanovic	Espersen	Grunskell	Mayer Seren Stekkov
1976:	Amstutz	Videla		Kapinasse	Stöber	Espersen	Gentile	Glawischnig	Mayer	Stekkov Weaver André[a],Grunskell[a] Rosenberger[a] Seren[a]
1978:	Amstutz	Videla		Kapinasse	Stöber	Espersen	Gentile	Glawischnig	Mayer	Stekkov Weaver -
1980:	Amstutz	Videla		Kapinasse	Stöber	Breukink	Gentile	Hofmeyr	Mayer	Stekkov Weaver -
1982:	Amstutz	Videla		Kapinasse	Stöber	Breukink	Gentile	Hofmeyr	Mayer	Stekkov Weaver -
1984:	Kapinasse	Mayer		Dubois	Stöber	Berchtold	Cakalfa	Coubrough	Gentile	Talmon Amstutz[f],Videla[f] Hofmeyr[a], Weaver[a]
1986:	Kapinasse	Mayer		Dubois	Stöber	Berchtold	Cakalfa	Coubrough	Gentile	Talmon -
1988:	Kapinasse	Mayer		Dubois	Sekrat	Berchtold	Cakalfa	Coubrough	Gentile	Talmon Stöber[g]

Zeichenerklärung: 1 = Kongreßjahr (Vorstandssitzung); 2 = Vorstand (a = Präsident; b = erster Vizepräsident; c = zweiter Vizepräsident; d = Sekretär); 3 = Beisitzer (erweiterter Vorstand); 4 = Ehrungen (a = Ehrenmitglied; f = Ehrenpräsident; g = Ehrensekretär)

NATIONALE VEREINIGUNGEN FÜR BUIATRIK

Schon bald nach Gründung der W.G.B. entstanden in einigen, Rinderproduktion betreibenden Ländern - ausgehend von Untergruppierungen der nationalen Verbände praktizierender Tierärzte oder von wissenschaftlichen Gesellschaften - eigene nationale Vereinigungen für Buiatrik (oder Rinder-Spezialisten), deren Ziele und Aktivitäten denen der W.G.B. gleichen (Zusammenschluß der mit denselben Aufgaben betrauten Tierärzte, Förderung der Rinderheilkunde durch Symposien und Tagungen auf nationaler Ebene). Die Anregung zu solchen Gründungen ging in der Tat oft von Kontakten ihrer Initiatoren zur W.G.B. sowie von deren Bestreben aus, einen internationalen Kongress über Rinderkrankheiten abzuhalten. Heute sind der W.G.B. 18 nationale Vereinigungen und eine supranationale Gesellschaft für Buiatrik direkt, weitere 5 indirekt angeschlossenen; (ihre Anschriften sind beim Sekretariat der W.G.B. erhältlich). Eine solche Assoziierung erfolgt auf schriftlichen Antrag, dem die Statuten und eine Liste der Mitglieder beizufügen sind; jeder Antrag wird zunächst vom Vorstand der W.G.B. geprüft und dann der nächsten Mitglieder-versammlung zur Zustimmung vorgelegt.

Kommentar: Die auf Übersicht 2 zusammengefaßte Entwicklung solcher Affiliationen ist heute noch in vollem Gange; es steht zu erwarten, daß sich die Liste der angeschlossenen nationalen Vereinigungen in den nächsten Jahren erheblich erweitern wird. Dazu bedarf es - in Erkenntnis des derzeitigen weltweiten sozialen und ökonomischen Umbruches - auch der **tatkräftigen Beratung und Förderung durch die bereits etablierten Tochter-Vereinigungen der W.G.B.**

KORRESPONDENTEN

Über ihren Sekretär hält die W.G.B. nicht nur mit den Büros der angeschlossenen nationalen Vereinigungen, sondern auch mit bestimmten, buiatrisch engagierten Einzelpersonen Kontakt, welche für die Tierärzte ihres Landes die Aufgabe übernommen haben, den einschlägigen **Nachrichtenfluß** in Gang zu halten (Weiterleitung von Meldungen des W.G.B.-News letter an die tierärztliche Fachpresse des betreffenden Landes, Unterrichtung des W.G.B.-Sekretariates über buiatrisch bedeutsame Ereignisse in eben diesem Land). Meist erfolgt die erste Kontaktaufnahme mit solchen Korrespondenten während einer Hospitanz letzterer an tierärztlichen Bildungsstätten in buiatrisch bereits 'aktiven' Ländern. Die bislang aufgrund einer derartigen persönlichen Initiative mit der W.G.B. 'liierten' 45 Länder gehen aus Übersicht 2 hervor (Namen und Anschriften ihrer Korrespondenten oder Kontaktpersonen sind beim Sekretariat des W.G.B. erhältlich).

Kommentar: Die Erfahrungen der W.G.B.-Sekretäre haben gezeigt, daß die Korrespondenz mit den Kontaktpersonen nicht nur eine wichtige **Quelle mutueller Information**, sondern mitunter auch die **'Keimzelle'** einer weiteren nationalen Vereinigung für Buiatrik ist, weshalb sie gebührende Beachtung verdient.

Übersicht 2: Zusammenstellung der an die Welt-Gesellschaft für Buiatrik angeschlossenen nationalen Vereinigungen für Buiatrik sowie der durch Kontaktpersonen assoziierten Länder 1960 - 1988*,**

Gründung	Name der Vereinigung	Angliederung
1968:	Società Italiana di Buiatria:	1968
1965:	Fachgruppe Rinderkrankheiten der Deutschen Vet.-Med. Gesellschaft:	1972
1965:	American Association of Bovine Practitioners:	1972
1972:	Sociedad Latinoamerican de Buiatria:	1974
1972:	Société Française de Buiatrie:	1974
1967:	British Cattle Veterinary Association:	1974
1974:	Australian Association of Cattle Veterinarians:	1974
1973:	Asociación de Veterinarios Españoles Especialistas en Buiatria:	1976
1978:	Indian Society for Buiatrics:	1978
1976:	Nederlandse Groep Geneeskunde van het Rund:	1978
1978:	Israel Association for Buiatrics:	1978
1980:	Rural Practitioners' Group of the South African Veterinary Association	1980
1980:	Irish Cattle Veterinary Association:	1980
1979:	Sociedad Ecuoatoriana de Buiatria:	1984
1981:	Société Belge de Buiatrie:	1984
1972:	Schweizerische Vereinigung für Zuchthygiene und Buiatrik:	1986
1985:	Société Zairienne de Buiatrie:	1988
1956:	Fachgruppe Rinderproduktion der wissenschaftl. Gesellschaft für Veterinärmedizin der Deutschen Demokratischen Republik	1988
1987:	Japanese Association for Buiatrics:	1988

* Folgende nationale Vereinigungen sind der W.G.B. über die **Sociedad Latinoamericana die Buiatria** mittelbar angeschlossen: Sociedad Argentina de Buiatria, Sociedad de Buiatria del Uruguay, Associacao Brasilieira de Buiatria, Asociación Paraguyana de Especialistas en Rumiantes; die Hellenic Society for Physiology of Reproduction and Artificial Insemination ist **über die nationale Kontaktperson** affiliiert.

** In nachstehenden Ländern halten **Kontaktpersonen** (nationale Korrespondenten) mit der W.G.B. Verbindung: Ägypten, Algerien, Argentinien, Australien, Belgien, Brasilien, Bundesrepublik Deutschland, Chile, VR China, Dänemark, Deutsche Demokratische Republik, Equador, Frankreich, Griechenland, Indien, Irland, Island, Israel, Italien, Japan, Jugoslawien, Kanada, Kuba, Marokko, Mexiko, Niederlande, Nigeria, Österreich, Paraguay, Peru, Polen, Rumänien, Schweden, Schweiz, Spanien, Südafrika, Südkorea, Tschechoslowakei, Uganda, U.d.S.S.R., Ungarn, Uruguay, U.S.A., Vereinigtes Königreich, Zaire.

KONGRESSE

Die Mehrzahl der an die W.G.B. angeschlossenen Tochter-Vereinigungen hält in regelmäßigem Rhythmus **nationale Tagungen** über Buiatrik ab, oder beteiligt sich mit **eigenem Programm** an breiter angelegten, praxis- oder wissenschaftsbezogenen Kongressen ihres Heimatlandes. Hierüber berichten sie dem Sekretär der W.G.B., der diese Aktivitäten in seinen alle zwei Jahre zu erstattenden Tätigkeitsbericht aufnimmt.

Die W.G.B. veranstaltet (unter 'Aussparung' der Jahre, in welchen ein Kongress der Welt-Tierärztegesellschaft stattfindet) alle zwei Jahre eine **internationale Tagung über Rinderkrankheiten**, und zwar traditionsgemäß jeweils in einem anderen Land. Vorbereitung und Durchführung dieser Kongresse liegen in den Händen derjenigen nationalen Vereinigung für Buiatrik, die hierzu eingeladen hat. Bewerbungen zur Austragung eines W.G.B.-Kongresses sind mindestens vier Jahre zuvor dem Sekretariat der W.G.B. mitzuteilen, wonach deren Vorstand sowie die Mitgliederversammlung auf ihrer nächsten Sitzung hierüber entscheiden. Die bislang abgehaltenen 15 Kongresse der W.G.B. sind samt Zeitpunkt, Ort, Leiter sowie Vortrags- und Teilnehmerzahl aus Übersicht 3 zu ersehen. Die Aufgliederung der dabei abgehandelten Themen ergibt eine gewisse "Vorliebe" für infektions- und polyfaktoriell bedingte, bestandsweise gehäuft auftretende Leiden, Stoffwechselstörungen, Mangelkrankheiten und Ernährungsfehler, aber auch für chirurgische und medikamentöse Behandlungsverfahren sowie diagnostische Methoden. Organologisch betrachtet, betrafen besonders viele Referate den Verdauungsapparat, die Fortpflanzungsorgane oder den Harnapparat (1,). Der Themenbereich "Verschiedenes" erwies sich bei allen bisherigen internationalen Tagungen über Rinderkrankheiten als "ergiebig" und besonders für den buiatrisch tätigen Praktiker attraktiv.

Als der **Welt-Tierärztegesellschaft** (WTÄG), angeschlossene Spezialistenvereinigung ist die W.G.B. seit 1975 auch verpflichtet, den im Rahmen der alle vier Jahre stattfindenden WTÄG-Kongresse auf die Sektion X, "**Krankheiten der Wiederkäuer**", derselben entfallenden Teil des wissenschaftlichen Programmes vorzubereiten und durchzuführen. Dabei ist das Sekretariat der W.G.B. in besonderem Maße auf die Kooperation der ihr angeschlossenen Vereinigungen sowie der Korrespondenten angewiesen. Auf den Welt-Tierärztekongressen in Thessaloniki/ 1975, Moskau/ 1979, Perth/ 1983 und Montreal/ 1987 entfielen 68, 178, 80 bzw. 109 Vorträge auf diese Sektion. Das Schwergewicht ihrer Themen lag bei den Infektionskrankheiten, den Stoffwechsel- und Ernährungsstörungen (samt Mangelkrankheiten) sowie bei diagnostischen und therapeutischen Maßnahmen (1).

Kommentar: Die **'konkurrierenden' Rhythmen** der von der W.G.B. und der von der WTÄG veranstalteten Kongresse bedingen, daß es drei aufeinanderfolgende Jahre mit je einem 'großen buiatrischen Ereignis' und danach ein Jahr Pause gibt. Das kann zu 'Engpässen' im Vortragsangebot oder zu Belastungen der wiederholt geforderten Vortragenden führen. Eine Änderung der Reihenfolge dieser Tagungen ist daher mehrmals diskutiert, allerdings stets verworfen worden. Für künftige Kongresse ist anzustreben, auch den in der intensiven Rinderproduktion als Berater und Betreuer tätigen **Praktiker** zum 'Reden' zu bringen.

Übersicht 3: Zusammenstellung der Kongresse der Welt-Gesellschaft für Buiatrik 1960 - 1980 (1, 2, 3)

1	2	3	4	5	6	7
I.	1960:	Hannover (Deutschland)	Deutsche Veterinärmedizinische Gesellschaft (Rosenberger)	33	412	10
II.	1962:	Wien (Österreich)	Tierärztliche Hochschule Wien (Diernhofer)	57	500	22
III.	1964:	Kopenhagen (Dänemark)	Kgl. Landw. & Tierärztl. Hochschule Kopenhaben (Bendixen)	68	300	22
IV.	1966:	Zürich (Schweiz)	Vet.-med. Fakultät der Universität Zürich (Andres)	70	400	24
V.	1968:	Opatija (Jugoslawien)	Vereinigter Tierärzteverband Jugoslawiens (Bratanović)	145	575	32
VI.	1970	Philadelphia (USA)	American Association for Bovine Practitioners (Amstutz)	78	608	20
VII.	1972:	London (Ver. Königreich)	British Cattle Veterinary Association (Grunsell)	76	468	29
VIII.	1974:	Mailand (Italien)	Società Italiana di Buiatria (Seren)	84	324	32
IX.	1976:	Paris (Frankreich)	Société Francaise de Buiatria (Espinasse)	175	1130	48
X.	1978:	Mexiko D.F. (Mexiko)	Asociación Mexicana de Medicos Veterinarios Especialistas en Bovinos (Garcia, S. de Aluja)	158	1150	42
XI.	1980:	Tel Aviv (Israel)	Israel Association for Buiatrics (Mayer)	188	700	27
XII.	1982:	Amsterdam (Niederlande)	Nederlandse Groep voor Geneeskunde van het Rund (Breukink)	247	870	45
XIII.	1984:	Durban (Südafrika)	Rural Practitioners' Group of the S.A.V.M.A. (Coubrough)	168	600	23
XIV.	1986:	Dublin (Irland)	Irish Cattle Veterinary Association (Greene,O'Farrell)	270	892	39
XV.	1988:	Palma de Mallorca (Spanien)	Asociación de Veterinarios Españoles Especialistas en Buiatria (Orden, Gonzalo, Partida)	252	900	31

Zeichenerklärung: 1 = laufende Nr.; 2 = Jahr; 3 = Austragungsort (Land); 4 = Veranstalter (Leiter); 5 = Anzahl der Vorträge; 6 = Teilnehmerzahl; 7 = Anzahl beteiligter Länder

BERICHTERSTATTUNG UND INFORMATIONSAUSTAUSCH

Zur Aufrechterhaltung des Kontaktes mit ihren Tochtervereinigungen sowie mit den Korrespondenten bedient sich die W.G.B. - abgesehen von der unmittelbaren Beantwortung von Anfragen - des in unregelmäßigen Zeitabständen (meist einige Monate vor, sowie unmittelbar nach jedem Welt-Kongress über Rinderkrankheiten) zu versendenen **'News-letter'**: Zu seinen Beziehern gehört auch das Präsidium der Welt-Tierärztegesellschaft. Der Inhalt des **'News letters'** ist zur Weiterverbreitung in der tierärztlichen Fachpresse der Mitglieds-Länder bestimmt.
Offizielles Organ der W.G.B. ist die von der American Association for Bovine Practitioners herausgegebene, von Prof. E.I. Williams/ Stillwater-USA redigierte Zeitschrift **"The Bovine Practioner'**: Sie bietet eine hervorragende, breitgestreute und auf neuesten Stand befindliche Fortbildung und übernimmt auch englische Beiträge von Autoren außerhalb der USA. Das jährlich einmal erscheinende umfangreiche Heft ist zu beziehen von Dr.H.E. Amstutz, A.A.B.P., Box 2319, W.Lafayette (Indiana) 47906, USA.

Jeweils anläßlich der Mitgliederversammlung der W.G.B. **berichten** deren **Präsident** und **Sekretär** über die im Verlauf der vergangenen beiden Jahre eingetretenen, die W.G.B. betreffenden Ereignisse (Rechenschafts- und Kassenbericht).

Kommentar: Antwort- und Mitteilungsbereitschaft einzelner affiliierter Vereinigungen für Buiatrik und mancher Korrespondenten ließen in der Vergangenheit zu wünschen übrig; das erschwerte die Vorbereitung eines vollständigen, alle Tochtergesellschaften und Länder umfassenden Berichterstattung oder machte sie unmöglich. Deshalb wird nachdrücklich an die **Pflicht zum gegenseitigen Informationsaustausch** erinnert.

KOOPERATION MIT DER WELT-TIERÄRZTEGESELLSCHAFT

Als assoziiertes Mitglied der WTÄG hat die W.G.B. auf deren Versammlungen Sitz- und Stimmrecht; letzteres 'quantifiziert' sich aus der Zahl ihrer Mitglieder, weshalb es von Bedeutung ist, diese regelmäßig zu erfassen.

Kommentar: Derzeitige und künftige Entwicklung der Aktivitäten der WTÄG werden in zunehmendem Maße von den in ihr vertretenen tierärztlichen Spezialistenvereinigungen bestimmt. Dem ist seitens der WTÄG durch Statutenänderung mit Beteiligung eines Vertreters der verschiedenen Spezialistenvereinigungen am Präsidium Rechnung getragen worden. Auch die W.G.B. sollte sich der hiermit verbundenen Verantwortung bewußt sein.

NACHWUCHSFÖRDERUNG

Üblicherweise erhalten Studierende der Veterinärmedizin bei Teilnahme an W.G.B.-Kongressen (ebenso wie die Vortragenden) einen Nachlaß auf die Tagungsgebühr. Die auf Anregung der niederländischen Groep voor Geneeskunde van het Rund zurückgehende Stiftung **G. Rosenberger-Memorial-Fund** setzt zu jedem W.G.B.-Kongress einen Preis für einen jungen Kollegen aus, der besonders anerkennenswerte Leistungen auf dem Gebiet der Buiatrik erbracht hat. Sekretär dieser Stiftung ist Dr. F. Talmon, Reespoor 5, Lelystad/Niederlande.

SCHRIFTTUM

SCHRIFTTUM

1. Rehkämper, U.: Diss. Tierärztl. Hochschule, Hannover 1985

2. Rosenberger, G.: Dtsch. Tierärztl. Wschr. **69**, 461 (1962); **71**, 593 (1964); **73**, 465 (1966); **75**, 585 (1968); **77**, 550 (1970); **79**, 488 (1972); **81**, 547 (1974); **83**, 509 (1976)

3. Stöber, M.: Dtsch. Tierärztl. Wschr. **67**, 655 u. 684 (1960); **85**, 490 (1978); **88**, 78 (1981); **90**, 158 (1983); **92**, 24 (1985); **94**, 45 (1987); **96**, 82 (1989)

4. Stöber, M.: Berliner Münchener Tierärztl. Wschr. **78**, 461 (1965)

TASKS, STRUCTURES AND DEVELOPMENT OF THE WORLD ASSOCIATION FOR BUIATRICS

SUMMARY

Basing upon the statutes, the reports about its international congresses, and the protocols of the meetings of the W.A.B. board, a review is given on the development of this association.

TACHES, STRUCTURE ET DEVELOPPEMENT DE L'ASSOCIATION MONDIALE DE BUIATRIE

RESUME

En se basant sur les statuts, les rapports concernant les congrès internationaux et les protocoles des réunions du comité de direction de l'A.M.B., une revue est donnée sur le devéloppement de cette association.

TAREAS, ESTRUCTURA Y DESAROLLO DE LA ASOCIACION MUNDIAL DE BUIATRIA

RESUMEN

Basandose sobre las estatutas, las relaciones de sus congresos internacionales y las actas de las reuniones de la junta directiva de la A.M.B., se da una vista general del desarollo de esta asociación.

THE FUTURE OF BOVINE PRACTICE

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Ladies and gentlemen, I would like to thank you and the World Buiatric Congress for the opportunity to be one of the keynote speakers at this years meeting. I have been challenged to look into the future of veterinary medicine from the perspective of a private clinical practitioner. From my vantage point, I see specific challenges that must be dealt with and a limitless number of opportunities that assure a bright future.

We must all acknowledge that the world political climate is a profoundly different one now than it was at the start of 1989. It is difficult to predict what impact the social currents underlying these truly remarkable events will have on veterinary medicine but I will attempt to suggest a few possibilities. The world is progressing to a much more global community than ever before. It is likely that there will be a heavy reordering of resource priorities to the production of consumer foodstuffs in Eastern Europe and the Soviet Union while the "peace dividend" in the west may allow for increased financial resources available for education. Veterinary medical expertise as it applies to food animal productivity will be in demand as never before both by governments and companies seeking to expand opportunities in food production.

With regard to specific challenges facing practice today, we

are increasingly required to give consideration to concerns of food safety, biotechnology and animal welfare. A casual perusal of almost any western newspaper or magazine in the past twelve months reveals headlines and articles pertaining to the safety of the food which we consume. These articles may leave the unsuspecting consumer with the impression that our food supplies have grown ever more contaminated with a wide variety of adulterants ranging from chemicals to every variety of pharmaceutical compound imagineable. The consuming public is led to believe that actresses turned "toxicologists" have a better understanding of risk assesment than any scientific panel embodied to deliberate and report on its findings. For example, in the decision to ban the chemical Alar, used in the United States to control the ripening of apples, it appears that the opinions of actress Meryl Streep were weighted far more heavily that those of legitimate scientific experts. Clearly, we must discard the complacency that our profession has felt with regard to the assumption that science will ultimately prevail over emotion. One only need to look to the trade barriers erected by the EEC against hormone implants used in raising beef in the United States to find another example of emotion and socio-economic criteria triumphing over scientific merit. The same holds true as the debate rages over the use of recombinantly derived products designed to enhance productivity. Global markets cannot afford this trend. On the domestic side, entrepreneurs are already preparing to prey on consumer fears and ignorance by offering for sale so called "natural" or "organic" milk and meat products. If there were ever a word in the english language which

has come to be mis and overused, it is "natural". My challenge to these people is that they should be required to demonstrate according to some measureable scientific criteria that in fact so called natural products are superior. It is irresponsible for us to accept an unsubstantiated claim that these products are somehow better or more wholesome.

Our charge must be to help educate the consuming public with regard to the real threats to food safety, namely microbiological contamination. We must encourage development of measures to control or minimize the likelihood of such contamination. This is not to infer in any way that we should become complacent with regard to responsible pharmaceutical useage and appropriate residue avoidance measures. It is our professional obligation to work to assure the ultimate wholesomeness of foods of animal origin for consumers. Clearly there is much work to be done in developing residue avoidance measures and screening methods for all categories of contaminants. The march of technology manifests itself through our increasing ability to detect minute quantities of chemical and biological residues in food. Unfortunately our ability to detect outpaces the determination of what constitutes an acceptable human health risk.

In the field of biotechnology we have let the genie out of the bottle, now the question becomes can we harness its potential? The debates which are currently raging should serve as a graphic reminder of the promise which nuclear energy first appeared to hold but now have proven unacceptable risks for most societies to tolerate. Never has our agricultural future held so

much promise and yet so much potential for failure due to public acceptance. For example, the controversy surrounding recombinant BST magnifies this point. The word hormone has a very negative connotation in the eyes of most consumers, not because of a poor track record in human food safety. Cancers that developed in the offspring of DES treated mothers resulted from use of the compound as therapy for human infertility, NOT from the consumption of DES in the food chain. Few know, much less care that BST is a polypeptide protein hormone biologically inert in humans and that is degraded through digestion the same as any other ingested protein.

For years, our emphasis has been to search for theraputic agents that could "cure " an animals' malady. Perhaps no better example exists than that of masititis and yet despite great advances in antimicrobial therapy, the disease is as prevalent as ever. Despite the fact that no strain of S. Agalactia has ever demonstrated resistance to penicllin and numerous control strategies have been devised which effectively eliminate the organism from milking herds, the organism continues to flourish in most dairying areas of the world. Our ability to manipulate and enhance the bovine immune system while still in its infancy, will eventually enable us to reduce or eliminate such universal problems as bovine mammary disease, enteric disease and respiratory disease. Our greatest challenge will be to educate the consuming public about the intrinsic safety of this technology. Often the most convincing mechanism turns out to be the economic benefits that utilization of these methods brings both to the producer and consumer.

Throughout my talk, I have alluded to improved productivity and consumer confidence, animal welfare is an integral component of these concerns. Our affluent societies demand that the days of painful procedures performed without anesthesia or analgesia draw to a close. Veterinarians may be surprised to find that employment of these agents yield benefits beyond those accruing directly to the animal. Producers and veterinarians have a fundamental and overriding concern for the welfare of the animals under their charge. As veterinarians, we must constantly devise new methods which address the concerns of the unapprenticed public. At the same time, we must continually revisit what have been the standard paradigms of todays agricultural practices. Yet changes in husbandry practices must be made with an understanding of the physiological and behavioral requirements of the species involved. We must also be aware that like Newton's Laws of physics, we often create equal and opposite reactions when we devise new methodologies. For example, when we move cattle from confined contact to pastures, we may be trading protozoan parasite problems for those caused by helminths. Are we equipped to diagnose and deal with those new problems? Likewise, I have personnally observed schemes which were successful in reducing neonatal mortality only to become victims of their own success. The increasing number of surviving calves simply overwhelmed the facilities and resources available to raise them. It would be easy to label our initial success at decreasing neonatal mortality as inhumane due to the problems created as the calves got older. As this example shows, animal welfare challenges will

continue to confront us in all phases of animal production.

As the profession moves into the twenty first century, we must recognize the changing demographics at work from within. Gone are the days when applicants to veterinary schools came primarily from agrarian backgrounds. We must realize that if we are to produce sufficient numbers of graduates to enter the food animal arena, we must actively recruit and foster the interests of the best and the brightest. We can no longer assume that our new graduates have an appreciation of the subtleties and economics of today's animal husbandry principles and methods. Yet this understanding lies at the heart of any successful production medicine program. Despite the predictions that computer aided learning and XPERT systems will play a major role in veterinary education, I remain skeptical that these can become viable substitiutes for clinical experience. One only need think about the theory and knowledge of pregnancy diagnosis versus the manual skills of palpation and its vagaries to know that we must still provide our students with hands on experience.

One of the universal roadblocks to effecting change remains in our communications skills. In food animal veterinary medicine, we have been reluctant to recognize that perhaps our greatest challenge is to effect change on the part of managers and workers on any given livestock operation. We all have been faced with situations where we can easily correct a disease or other technical problem with currently available knowledge only to be thwarted and frustrated by an owner, manager or worker who refuses to make needed changes. When we propose a new program for clients they are faced with essentially four choices. They can

work additional time and implement the changes. They can put off some of the normal daily routine and replace it with the new program. They can hire additional help to accomplish the added duties, or they can do nothing. This last choice most often prevails since inertia to change is a fundamental aspect of human nature. There are a whole host of skills which the profession must learn in this arena before we are going to realize tangible results in our programs. All too often our clients judge us by the competence we demonstrate in the more mundane aspects of individual cow treatement and find it difficult to embrace more complicated concepts of production medicine programs.

Finally, with a certainty, the practice of veterinary medicine in the ensuing decades will be vastly different due to developments in food safety, biotechnology, animal welfare and changing demographics. It is certain that our future is bright and filled with opportunity for those willing to meet the challenges.

EL TORO EN EL MEDIO RURAL LATINOAMERICANO: SU CIRUGIA

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INTRODUCCION

Como consecuencia de la evolución en la aplicación de sistemas de explotación extensiva en el Uruguay, y en otros paises de América, la patología del toro ha merecido mayor atención por parte del hacendado y del Médico Veterinario. La necesidad de obtener mejores resultados económicos y aumentar los índices de procreo, ha estimulado a una más detallada observación y un reclamo más asiduo de la opinión clínica sobre machos y hembras.

Si bien no existen estudios concluyentes, se sabe que la vida útil promedio de un toro, en un sistema de explotación extensiva en el Uruguay, no supera los tres o cuatro años; variados son los factores que inciden para que sea así, destacándose la alimentación, el manejo y las afecciones quirúrgicas, como los más importantes.

En nuestro medio, el toro de razas de carne a partir de los siete u ocho meses de edad realiza un período de vida en grupo, a campo mejorado o racionado a campo, previo a su comercialización o utilización que se concreta entre los 24 y 36 meses de edad.

Este período de vida colectiva tiene una patología de grupo muy especial, destacándose las consecuencias de la monta entre ellos, anomalía frecuente que causa lesiones en el pene y en el aparato locomotor, además de perforaciones intestinales. También la papilomatosis viral es común en ésta época de la vida de los reproductores con un alto porcentaje de presencia en la mucosa prepucio-peneana.

Una vez ingresado al rodeo otros son los elementos que juegan para impedir o dificultar que el toro trabaje en forma normal.

De la observación práctica se puede concluír que existen hechos que inciden en forma negativa sobre un número elevado de machos, aunque no se han publicado trabajos que determinen su real importancia. Citamos los siguientes:

a. toros que entran al rodeo por primera vez, y que con anterioridad fueron sobrealimentados para presentarlos a la venta
b. toros jóvenes que hacen sus primeras experiencias reproductivas en rodeos de vaquillonas
c. toros que son descuidados en el período de invierno, o de descanso sexual; toros que son utilizados fuera de los porcentajes adecuados o que deben tabajar en campos muy sucios o irregulares
d. toros que no se controlan periódicamente en sus posibilidades de realizar el servicio, como tampoco en las manifestaciones de afecciones
e. la herencia, muy mencionada en la literatura, no sabemos hasta qué grado influye en nuestra explotación comercial
f. debido a la situación sanitaria del Uruguay, las enfermedades infecciosas comunes han dejado de tener importancia especial para el toro. Lamentamos decirlo, pero este concepto no se puede aplicar a todos los paises de América Latina.

En el estudio realizado por el autor, sobre 1621 fichas clínicas de toros atendidos en explotación extensiva, se comprobó que el 62% correspondían a patologías de las áreas prepucio-peneanas y del aparato locomotor, repartiéndose en partes iguales cada uno de los grupos (31%), y desde luego muy superiores en frecuencia a cualquier otro grupo patológico por el cual se haya reclamado atención veterinaria.

En Tacuarembó, Uruguay, un grupo de 25 veterinarios integrantes de la Sociedad de Medicina Veterinaria del Uruguay, realizó un trabajo muy interesante, en combinación con el Ministerio de Ganadería, Agricultura y Pesca, relacionado con este tema. su finalidad era determinar las condiciones de 554 toros previo el entore, en 26 establecimientos de explotación extensiva. Sus resultados confirmaron el alto número de toros afectados que existen en la ganadería extensiva. Se determinó que el 13.5% de los 554 reproductores examinados se debían considerar como no aptos para el trabajo a que iban a ser destinados y sólo el 33% de los establecimientos tenían la totalidad de sus toros aptos. Hecho corroborado en Cerro Largo (Uruguay) dónde otro grupo de 21 veterinarios comprobó, en un trabajo similar al anterior, que el 20.60% de los toros a usar por los hacendados no eran aptos para la reproducción. En esta oportunidad se evaluaron 267 reproductores en 10 establecimientos.

Por último, merece una referencia el hecho de que los propietarios, muchas veces eliminan reproductores por afecciones espectaculares pero curables, y además, no consultan por otras tratables, pero menos llamativas.

Se deduce de lo que antecede que si se toman en cuenta los seis aspectos mencionados, podremos elevar las condiciones del toro en explotación extensiva y mejorar su manejo, logrando un aumento del número de procreos y la prolongación de su vida útil.

PROCESOS PATOLOGICOS DEL PENE Y DEL PREPUCIO

Los procesos de los que nos ocuparemos en esta publicación pueden producir, de inmediato o a mediano plazo, la incapacidad del toro para completar el acto sexual, o sea terminar en una impotencia copulatoria. Podemos hablar de tres tipos de situaciones que se presentan con relación a toros con incapacidad de servir: a. impotencia coeundi, b. inhibición psíquica y c. falta de líbido.

En relación a lo que nos referimos en este trabajo se debe destacar la primera condición; la segunda situación puede estar relacionada con los procesos que estudiaremos, como una consecuencia de fallidos y repetidos intentos. La falta de líbido no será materia de este trabajo pero el veterinario la debe tener en cuenta en el diagnóstico diferencial.

Refiriéndonos a la impotencia coeundi debemos recordar que un importante grupo de afecciones que se encuentran fuera del área prepucio-peneana la pueden originar. Citaremos como ejemplo las siguientes:

a. afecciones locales graves: hernia inguinal, vesiculitis, cáncer de ojo etc.
b. enfermedades infecciosas que afectan el aparato locomotor: aftosa, dermatitis interdigital
c. alteraciones de la columna vertebral: desviaciones, osteítis, trauma.
d. afecciones del aparato locomotor con dolor o dificultades mecánicas: callo interdigital

La impotencia coeundi de un toro se traduce, cuando nos referimos al individuo, como la falta de fecundación de las vacas para las que estaba dispuesto; pero cuando hablamos del toro como parte de un grupo trabajando en forma conjunta, lo debemos relacionar a un menor índice de preñez del rodeo, salvo que otro u otros reproductores del conjunto sean capaces de sustituirlo. Esto implica el uso de un porcentaje superior al necesario y por consiguiente un factor económico negativo en la explotación.

Como ejemplo en el mencionado trabajo de Tacuarembó existieron rodeos que se enteraron hasta al 7%, lo que sólo se explica por no tener información cierta de cuáles son las verdaderas posibilidades de los toros a utilizar.

EXAMEN CLINICO

El exámen de un reproductor exige, en principio, una revisación general para observar en conjunto cuales son las condiciones en que el animal se encuentra. Volvemos a recalcar que muchos procesos, pueden alterar gravemente la actividad del toro.

En cuanto a la semiología concreta, tenemos las siguientes etapas a realizar: a. inspección en pié, sin anestesia ; b. inspección en actividad ; c. inspección manual o mecánica, en pie o en decúbito, con anestesia o tranquilizantes.

a. Se comienza por una inspección en pie sin ninguna medicación. Para ello el toro se pone en el cepo. Allí los animales se pueden revisar comodamente: su cabeza, sus ojos, sus patas, los ganglios y aún hacer un tacto rectal. Para el pene se les ata el miembro posterior a un poste trasero o lateral, del lado opuesto al observador. No muy tirante que moleste al animal, pero lo suficiente para que proteja al técnico.

b. A lo largo del desarrollo de cada uno de los temas se podrá relacionarlos con la falla apreciada en la observación de alguna de estas etapas. Es común no encontrar vacas en celo; en oportunidades el toro se inhibe por nuestra presencia, o posee dolores o inhibiciones originadas en fallidos y repetidos intentos anteriores. Para algunos casos clínicos puede resultar peligroso este intento de monta, agravándose la pa-

tología presente. Esto debe ser sobrepesado por el veterinario. De lo que antecede se deduce que frecuentemente debemos orientarnos, y pasar a las etapas semiológicas siguientes, tan sólo con la información proporcionada por el propietario sin poder comprobar la conducta del macho. Las"pruebas de capacidad de servicio" son importante fuente de información en relación a defectos en esta actividad, muy especialmente cuando nos referimos a situaciones clínicas que no producen incapacidad total y sólo son observables luego de repetidos intentos.

c. Por último, si no hemos logrado aún realizar un diagnóstico definitivo, podemos recurrir a un tranquilizante o a la anestesia para continuar la inspección. Los toros, si caen dentro del cepo por efecto de la anestesia, pueden llegar a correr peligro de muerte, a lo que se debe prestar atención. En el caso de inyectar un anestésico o preanestésico, cuando se aplica ya se tiene todo dispuesto para realizar el acto operatorio si ello resultara lo indicado.

Deseo agregar que difícilmente en nuestra región ganadera, el colega tiene a su alcance aparatos especiales para estudios más o menos concretos.

CIRUGIA - PRE Y POST OPERATORIO - ANESTESIA

La totalidad de los procesos que mencionaremos, encuentran su solución en un acto operatorio de mayor o menor enverga dura, considerándose la cirugía como necesaria.

El toro criado a campo no es tan fácilmente manejable. Por su seguridad y la del personal, se hace necesario tomar las máximas precauciones. En primer lugar el animal siempre debe ser manipulado en un cepo o potro. El tubo o manga es un recurso cuando no existen otros medios, pero las precauciones deben ser máximas.

Al sujetar un bovino en uno de los medios mencionados con la finalidad de administrarle un producto de efecto general, se debe tomar la precaución de impedirle que retroceda una vez liberado. Esto se logra con el cierre de la puerta trasera del cepo o con una tranca detrás del toro. Esto es con la finalidad de evitar que luego no quiera volver al lugar de salida y pueda sufrir los efectos de la anestesia dentro del aparato de contención.

Consideramos que la xilacina es un producto sumamente útil para trabajar en el medio, al cual nos referimos;no hemos tenido problemas con ella manejándonos a dosis no altas.

Existen condiciones bajo las cuales es posible realizar la anestesia de la mejor forma posible. En nuestra opinión debe existir un buen conocimiento de los siguientes elementos: 1.el sujeto, 2.el anestésico, 3.la técnica operatoria y 4.el medio ambiente.

1. El sujeto: es muy importante una correcta información de la salud del sujeto, no referido en especial a la noxa operable; lesiones a nivel pulmonar, circulatorio o hepático, entre otras, deben ser tenidas en consideración. Su edad, el estado, la raza, la idiosincrasia, también se deben observar. Pero en nuestra opinión, el ayuno es el elemento que más debemos atender. Pero en general, los toros alimentados a campo sin ayuno, no presentan problemas de timpanización, ni vómitos. Opinamos que cualquier ayuno es útil, pero que deben recomendarse 36 a 48 horas para tener seguridad. El hacendado debe conocer este hecho y estar prevenido de ello cuando solicita la presencia del técnico.

2. El anestésico o preanestésico: es preferible que el veterinario se mueva con un reducido grupo de productos y de vías de administración. Esto lleva a un dominio más correcto de sus efectos, su variabilidad y de las combinaciones anestésicas posibles.

Si pretendemos mantener el animal en pie, por ejemplo para una operación de cancer de ojo, administramos propionil promacina al 1%, 1cc 1/4 cada 100 Kg de peso vivo, vía i/m. Si necesitamos que el toro caiga , le inyectamos por la misma vía xilacina, de 1 cc a 1 cc 1/4 cada 100 Kg de peso. Después nos movemos con la lidocaína,en inyección local o regional, al 2%.

Es trascendente conocer con qué elementos contamos para combatir los efectos del producto utilizado, cuando las circunstancias lo requieren. Estas en general son dos: a. presencia de efectos no deseables y b. suspensión o disminución de los efectos una vez terminado el acto operatorio.

Los efectos negativos más destacables por la aplicación de xilacina están en la sialorrea, el timpanismo, la hipoventilación, la regurgitación y posibles injurias

a causa del decúbito. Con la finalidad de que, ni la saliva ni el contenido ruminal regurgitado, puedan ser aspirados, se pone la cabeza en posición declive.

3. La técnica operatoria: para utilizar en la mejor forma posible un anestésico, el cirujano debe conocer correctamente la técnica operatoria y sus posibles variantes. El tiempo que ello le ha de insumir y la posición en que debe sujetar al animal.

4. El medio ambiente: La tranquilidad pre y post-operatoria es lo más recomendable. Lo primero, para lograr un buen efecto anestésico, y lo segundo, para evitar fracasos en la técnica aplicada. En el post-operatorio, el hecho de que alguien observe al animal para que no tome posiciones anormales, hasta que logre suficientes fuerzas, es recomendable. Los días siguientes al acto operatorio son trascendentes; como norma no hemos podido trabajar con asepsia, sino solamente con antisepsia y en un medio ambiente favorable. Siempre recomendamos dejar al toro libre y sin otros bovinos; el ejercicio lo consideramos muy importante. El edema, parece ser el elemento a combatir, y no presentándose problemas en el toro por el uso de diuréticos, los aplicamos regularmente. Los antibióticos de amplio espectro son la norma, pero apreciamos mucho el uso de sulfas combinadas con trimetropin. Algún desinflamatorio completa el arsenal terapéutico más frecuente.

LA EXPLOTACION EXTENSIVA EN LATINOAMERICA: patología del toro

En el Uruguay, próximo a 100.000 toros se encuentran trabajando en establecimientos de explotación extensiva. No conocemos información sobre la cantidad que lo hacen en los demás paises latinoamericanos, pero fuera de duda ellos sumarán varios millones.

La información de los casos clínicos atendidos reltará una ayuda para quien se inicia en esta actividad , más teniendo en cuenta que el 84% de los animales atendidos curaron con el tratamiento, lo que se puede considerar excelente en una terapia a campo.

A continuación presentamos una descripción de algunas de las patologías que hemos visto. Estas apreciaciones son más o menos importantes en las diversas zonas que integran el conjunto de paises que forman Latinoamérica.

1. ABSCESOS GANGLIONARES: Pueden encontrarse en cualquier ganglio, pero la presentación más frecuente es en ganglio submandibular o subglótico, En realidad suele ser más de un ganglio. Se encuentran próximos al ángulo de la mandíbula algo profundos, lateralmente a la glandula submaxilar y al musculo esternocefálico o entre ambos. En el acto operatorio se deben cuidar los ramales de la yugular y de las venas maxilares interna y externa. Para su extirpación se practica un corte paralelo al borde mandibular. Cuando el ganglio está saliente se practican dos cortes en tajada de sección. Se extrae la totalidad de la masa tumoral que rodea el absceso teniendo precauciones en la parte más profunda. Ante cualquier vaso que sangre abundantemente se liga. Sutura a puntos continuos de la piel. Previo a la realización del acto operatorio se trata de desinflamar y delimitar la zona, para ello se pueden aplicar antibióticos, diuréticos y yoduro de sodio.

2. AMPUTACION DEL DEDO EN MITAD DE lera. FALANGE: Se debe realizar buena limpieza previo a operar. Debemos aplicar un torniquete arriba del nudo. Incisión de piel en la linea media del dedo desde la mitad de la primer falange; al llegar a la pezuña el corte se divide en dos sobre el rodete; en la parte posterior se vuelve a hacer un corte solamente. A continuación se libera la parte osea; luego con un fetótomo o con una sierra se corta, por su parte media, la primer falange. El corte es preferible hacerlo levemente oblicuo hacia abajo y adentro. Se debe tener seguridad que se ha extraido todo lo alterado; ligar algún vaso si sangra. Dejar drenaje y vendar. En el postoperatorio se administran antibióticos, diuréticos, fenilbutazona.

3. CANCER DE OJO: Enucleación total del globo ocular y parpados. Se realizá la incisión acompañando a un centímetro el borde palpebral o la masa tumoral, cuando esta está ubicada sobre uno de los parpados . Sangra profusamente, pero la hemorragia se suele detener con prontitud. Una vez realizada la enucleación se aplica una torunda de algodón embebida en agua oxigenada, de 10 a 15 volúmenos. Luego se procede a suturar en guarda griega la piel con hilo o nylon. Dentro de la herida quirúrgica se aplica un poco de los usados para mastitis. El resultado final es una cicatriz que no afecta la estética del animal.

Extirpación del tumor solamente:
Extirpación de pequeños tumores sobre córnea y borde conjuntivo corneal: en este caso es conveniente prolapsar el ojo para trabajar con seguridad. Esto no siempre se consigue con facilidad, para lograrlo nos ayudamos con la parte posterior de una pinza o tijera. Se extirpa el tumor a filo de bisturí, en posición paralela a la superficie corneal y luego cauterizamos la base del tumor.
Extirpación de un tumor sobre tercer párpado: con dos hemostáticas se pinza el tercer párpado a unos 3 o 4 mm por detrás del tumor. Se extirpa con un corte neto a filo de bisturí. Cauterizamos sobre las pinzas. En este caso y en el anterior conviene una vez operado aplicar un colirio líquido.

4. CALLO INTERDIGITAL : Se practica una incisión fuera del borde del callo interdigital, de manera que al iniciar la extirpación de la tumoración no quede ningún resto de ella en el interdigito. Se extrae la masa tumoral totalmente. Se colocan directamente sobre los tejidos sangrantes compresas de gasa embebidas en solución alcohólica de cloranfenicol al 10%. Se venda ajustado y por fuera con alpillera se conforma un zapato. Se deja la venda por cinco días.

5. ANILLOS GRUESOS, PLIEGUES Y ESTENOSIS GRAVES DEL CONDUCTO PREPUCIAL: Si el anillo es muy estrecho, de forma que no permite el paso del pene, se debe ampliar la abertura, incidiéndolo. Una vez protruído el pene, realizar dos incisiones completas circulares de la mucosa, una superior y otra inferior al anillo. Disecar mucosa y todo el tejido fibroso entre ambas incisiones, sin tocar albugínea ni ligamento dorsal. Una vez extraído este tejido, realizar 2 a 4 puntos en las túnicas elásticas para no dejar espacios muertos. Los realizaremos entrando por mucosa proximal, se pasa el hilo de ácido poliglicólico tomando varias veces el conjuntivo, se llega a la mucosa distal y se reintroduce la aguja haciendo el camino inverso para volver a mucosa proximal, donde se anuda. De estos se pueden hacer dos puntos opuestos o cuatro en cruz. Se debe tener la máxima precaución de no girar el pene, las mucosas se deben enfrentar en su situación normal. Se sutura con el mismo material a puntos separados la mucosa.

6. LLAGA PREPUCIAL: Pasos operatorios: a) incisión de piel del prepucio en semicírculo anterior y superior a la masa tumoral. b) incisión de piel hacia posterior en V invertida (de 3 a 5 centímetros) para completar el semicírculo del punto anterior. c) se profundiza la totalidad de ambas incisiones hasta llegar a la mucosa. Se diseca todo el conducto sin incidir la mucosa. d) corte transversal completo de la mucosa, tratando de conservar la mayor parte posible de mucosa sana. e) pequeña incisión longitudinal de la mucosa en la línea media posterior, de un largo de 2 a 3 cms. f) se hacen dos puntos en vainas elásticas para evitar espacios muertos, con hilo de ácido poliglicólico N°2. Tratamos de ligar vasos lo menos posible para no dejar elementos extraños en la zona operatoria. Actuamos especialmente con pinzas por forcipresión, dejándoles unos minutos en cada vaso sangrante. En la casi totalidad de los casos esto es suficiente para detener la hemorragia. g) unión a puntos separados mucosa-piel. Utilizando hilo de lino 00. Se administran abundantes antibióticos y diuréticos y se mantiene al paciente bajo observación, ya que suelen formarse coágulos que se retiran a los 3 a 7 días.

7. HEMATOMA PENEANO Y PERIPENEANO: La técnica quirúrgica, consiste en la extracción del coágulo. Esto se logra bajo los efectos de xilacina intramuscular. El acto operatorio se debe realizar entre el séptimo y el décimo día posteriores al accidente, con el fin de asegurarse que no existirá una hemorragia. Realizamos un corte de piel vertical al eje peneano, lo profundizamos a través del subcutáneo y de las túnicas elásticas. Cuidamos no lesionar los músculos retractores. Extraemos al máximo los restos de sangre: aplicamos enzimas proteolíticas (estreptoquinasas y estreptodornasa de 25.000 a 50.000 U.I.) y antibióticos. Dejamos una mecha por 48 a 72 horas; aplicamos 2 o 3 puntadas en piel con material no reabsorvible, fijando la mecha a una de ellas. No nos preocupamos por suturar la lesión primitiva de la albugínea, ni las que realizamos con el corte operatorio en las vainas conjuntivas. Somos exigentes en la limpieza del coágulo. En trabajos a campo no consideramos recomendable dejar puntos interiores y sólo los aplicamos bajo la más estricta necesidad. Todo toro atendido, sea operado o no, se deja sin trabajar por un período mínimo de 45 días. Los reproductores operados se tratan con antibióticos y diuréticos y se

dejan a campo , de forma que puedan realizar ejercicio regularmente.

RESUMEN
La patología del toro ha merecido mayor atención, pero aún no se ha logrado mejorar el tiempo de su vida útil y su producción. Entre otros efectos, lo que tratamos puede producir impotencia coeundi, lo que se traduce por faltas de servicio o sustitución por otros toros en el rodeo. El exámen clínico de los reproductores se efectúa en tres etapas: 1. inspección en pie, 2.inspección en actividad y 3.inspección con anestecia o tranquilizantes. Para este tercer punto consideramos como más conveniente el uso de la propionil promacina al 1%, la xilacina al 2% y la lidocaína al 2%. Para aplicárlos se necesita conocimiento del sujeto, del anestésico, de la técnica operatoria y del medio ambiente. Se relatan varias técnicas operatorias más frecuentemente usadas en el toro a campo. Visto los éxitos obtenidos se considera muy útil de conocer por los cirujanos.

RESUME
La patologie du taureau à reçu une plus grande attention mais on n'a pas encore pu améliorer le temps de sa vie utile et sa production. Entre d'autres effects ce que nous essayons peut produire une impuissance coeundi, ce qui se traduit par manque des services au remplacement par d'autres taureaux dans le troupeau.
L'examen clinique des reproducteurs s'effectue en trois stages: 1.inspection sur pied, 2.examen fonctionnel et 3. inspection avec anesthesie ou tranquillizants. Pour ce troisième point, nous considerons plus convenient l'usage de la propionil promacine au 1%, la xilacina au 2% el la lidocaine au 2%. Pour les utilizer l'on a besoin de connaître le sujet, l'anesthesie, la technique d'operation el du milieu. Nous informons diverses techniques d'operation qui sont plus souvent utilisés avec le taureau en liberté. En vue des succès obtenus on considere qu'ils sont très a etre connus par les chirurgiens.

SUMMARY
The patology of the bull has received major attention but was impossible to improve the time of utilization and his production. Between other effects what we treated can produce impotentia coeundi, with the consequences of problems in service or need of substitution of bulls in the herd.
The clinical examination of breeding bulls is developed in three steps: 1.Inspection on fod; 2.inspection in activity and,3.inspection with anestetics or sedatives. In the third step is considered more appropiate to use propionil promacine 1%, xilacine 2% and lidocaina 2%, but first in necessary to know the individual, the anestesic, the surgical technique and the environment. We relatet several surgical thechniques more frecuently used in field bulls. As the results were successfull can be interesting to be knoworky veterinary surgeons.

TRABALHOS

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INHALTSVERZEICHNIS

INFLUENCE OF BOVINE SOMATOTROPIN ON SUBCLINICAL AND CLINICAL MASTITIS

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INTRODUCTION

Recombinant bovine somatotropin (rBST) has been shown to enhance milk production in the lactating cow (1-7). Producer and consumer acceptance and U.S. Food and Drug Administration approval for the field application of rBST is based upon the demonstration that such treatment does not produce deleterious effects upon the health of treated animals (1-8). Mastitis is one of the most costly diseases affecting dairy cattle and the influence of rBST treatment on mastitis could be significant. The purpose of this investigation was to determine the influence of rBST treatment on subclinical and clinical mastitis.

MATERIALS AND METHODS

Animals--Lactating Holstein cows of mixed parity were blocked according to parity and calving date and were randomly allocated to 4 levels of rBST treatment. Random allocation was performed separately for cows of parity 1 and for parities 2-5. The period studied included October 1986 until December, 1988. All treated cows were housed on one of 2 North Carolina Department of Agriculture research dairies located in the coastal plains of North Carolina. Each dairy consisted of >150 lactating cows. On dairy A, 60 cows were studied for a first lactation and 43 of the same cows were studied for a second lactation. On dairy B, 32 cows were studied for a single lactation, but all cows had been treated with the same dose of rBST during the previous lactation. Treated cows were housed with the entire lactating cow herd and were identified as to level of rBST treatment by unique animal identification and by color-coded neck chains. Cows were fed a corn silage-based total mixed ration with grain concentrate and whole cotton seeds and a minimum of 1.8 kg of hay/cow daily. Cows were milked twice daily in automated parlors and standard mastitis control procedures were practiced. The herds were on comprehensive dairy herd health programs, with bi-weekly visits by a veterinarian.

rBST treatment--Cows were allocated to one of 4 levels of rBST (0, 5.2, 10.3, and 16.5 mg of active available drug per day). The rBST (Agriculture Research Division, American Cyanamid, Princeton, NJ) was supplied in color-coded vials and refrigerated at 4 C until administered. Administration of rBST was via subcutaneous injection in the area of the tail fold, alternating sides daily. Administration was from early lactation (28-35 days in milk) until the earlier of either the end of lactation or 400 days postpartum.

Milk sampling--Quarter milk samples for microbiological analysis were collected aseptically in duplicate from each cow at trial entry and at dry-off (9). Single quarter milk samples were aseptically collected at 60-day intervals during the trial. For milk samples from cows giving a change in infection status, quarter milk samples were collected in duplicate until duplicate samples were in agreement.

Clinical mastitis--Cows were observed by milkers at each milking for evidence of clinical mastitis, defined as the presence of grossly abnormal milk or mammary gland. Duplicate milk samples were aseptically collected from affected quarters of cows with clinical mastitis prior to treatment with a commercial lactating mastitis infusion product. All signs of clinical mastitis and treatments administered were recorded by milkers.

Microbiological analysis--Standard microbiological techniques were used in microbiological analysis of milk samples (10-11). For milk samples of cows with clinical mastitis, 0.01 and 0.05 or 0.10 ml (after January, 1988) of milk were plated onto the surfaces of Columbia agar plates. Major pathogens were considered as Staphylococcus aureus (positive by tube coagulase), Streptococcus agalactiae, Str. dysgalactiae, Str. uberis, Escherichia coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., Actinomyces pyogenes, and Nocardia spp. Minor pathogens included coagulase negative Staphylococci, Streptococcus spp. excluding those above, Corynebacterium spp., Serratia marcescens, Prototheca spp., and yeasts.

Indices of mastitis--Indices of mastitis monitored (9) included:

1. Point prevalence of intramammary infection (IMI): Prevalence of IMI by cow for major and minor pathogens by rBST treatment level was determined from milk samples collected at trial entry, 60-day intervals during the study and at dry-off.
2. Occurrence of new IMI: New IMI detected at samplings during the trial or as a result of clinical mastitis were evaluated by level of rBST treatment.
3. Duration of IMI: Duration of IMI (new IMI and IMI existing at trial entry) was evaluated on the basis of the maximum duration of any infection within a cow during each lactation.
4. Incidence of clinical mastitis: Incidence of clinical mastitis by level of rBST treatment was evaluated for the entire period of study.

Statistical analysis--Infection prevalence data was analyzed by a repeated measures categorical analysis procedure which considered frequency of responses among rBST treatment levels over time (CATMOD Procedure, SAS, Inc., Cary, NC). Remaining data were analyzed using a modified Mantel-Haenzel test (FREQ Procedure, SAS, Inc., Cary, NC).

RESULTS

Milk production response to rBST treatment was dose-dependent, similar to previous reports and is reported elsewhere (12). There were no significant differences in somatic cell concentrations in milk from cows among treatment groups (12).

Point prevalence of IMI--The prevalence of IMI by treatment level and collection period are given in Table 1. Prevalence of IMI by major and minor pathogens did not differ significantly among rBST dose levels (P=0.06 for major pathogens and P=0.67 for minor pathogens). Neither major (P=0.11) nor minor pathogen (P=0.09) cow prevalence infection rates varied significantly over time during the period of treatment.

Table 1--Point prevalence of IMI by major pathogens by treatment level and period of collection for Dairy A for first lactation*

	Treatments--% cows infected (no. sampled in parentheses)			
Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	20%(15)	47%(15)	27%(15)	27%(15)
2nd	13%(15)	53%(15)	33%(15)	20%(15)
3rd	13%(15)	53%(15)	33%(15)	33%(15)
4th	8%(12)	62%(13)	31%(13)	36%(11)
7th--dry off	21%(14)	47%(15)	33%(12)	36%(14)

IMI by major pathogens for Dairy A for second lactation*

Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	25%(8)	27%(11)	27%(11)	15%(13)
2nd	25%(8)	27%(11)	27%(11)	31%(13)
3rd	25%(8)	27%(11)	27%(11)	31%(13)
4th	25%(8)	40%(10)	27%(11)	31%(13)
7th--dry-off	25%(8)	60%(10)	22%(9)	23%(13)

IMI by major pathogens for Dairy B for first lactation

Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	0%(7)	43%(7)	23%(13)	0%(5)
2nd	0%(7)	43%(7)	15%(13)	0%(5)
3rd	0%(7)	43%(7)	8%(13)	0%(5)
4th	0%(7)	43%(7)	8%(12)	0%(5)
5th	14%(7)	33%(6)	0%(10)	0%(3)
6th--dry-off	14%(7)	29%(7)	15%(13)	0%(5)

IMI by minor pathogens for Dairy A for first lactation*

Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	20%(15)	40%(15)	20%(15)	13%(15)
2nd	13%(15)	47%(15)	20%(15)	27%(15)
3rd	13%(15)	40%(15)	20%(15)	27%(15)
4th	17%(15)	38%(13)	23%(13)	18%(11)
7th--dry-off	21%(14)	27%(15)	17%(12)	7%(14)

IMI by major pathogens for Dairy A for second lactation*

Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	38%(8)	36%(11)	9%(11)	15%(13)
2nd	25%(8)	45%(11)	18%(11)	15%(13)
3rd	25%(8)	64%(11)	9%(11)	23%(13)
4th	25%(8)	60%(10)	9%(11)	15%(13)
7th--dry-off	25%(8)	70%(10)	33%(9)	23%(13)

IMI by minor pathogens for Dairy B for first lactation

Collection	16.5 mg	10.3 mg	5.2 mg	0 mg
1st--trial entry	14%(7)	29%(7)	15%(13)	20%(5)
2nd	29%(7)	29%(7)	23%(13)	20%(5)
3rd	14%(7)	43%(7)	23%(13)	40%(5)
4th	14%(7)	43%(7)	17%(12)	40%(5)
5th	14%(7)	33%(6)	20%(10)	67%(3)
6th--dry-off	29%(7)	29%(7)	23%(13)	60%(5)

*Due to small sample size, data from 5th and 6th collections on dairy A are not presented.

Total new infections (TNI)--Total new IMI acquired during the trial are given in Table 2 and did not differ significantly among treatment groups (P=0.48). Total new IMI by major pathogens did not differ significantly (P=0.20) among treatment groups. Similarly, total new IMI by minor pathogens did not differ significantly (P=0.21) among treatment groups.

Clinical mastitis--Reported clinical cases of mastitis by treatment groups are given in Table 2. Because of the small number of cases, these data were not analyzed statistically. However, there was no indication of differences among treatment groups.

Table 2--Frequencies of all new IMI (major and minor pathogens) and clinical mastitis by treatment groups

	Frequency of new IMI					
Treatment	0	1	2	3	Total*	Clinical#
16.6	21	9	0	0	30	4
10.3	21	8	2	2	33	3
5.2	30	6	3	0	39	4
0	21	8	3	1	33	4
TOTAL	93	31	8	3	135	15

*=Cow lactations studied. #=No. cows affected with clinical mastitis.

Duration of infections--Maximum duration of IMI within cows by treatment levels is given in Table 3. Duration of new or existing IMI gave no clearly significant differences among dose levels (P=0.08 for duration of new IMI and P=0.06 for existing IMI). A tendency was observed for a dose-related increase in maximal duration of existing IMI during the second lactation in Dairy A.

Table 3--Mean±S.E.M. maximal duration of IMI within cows for new and existing IMI

Maximal duration (days) of new IMI				
Dairy/lactation	16.5 mg	10.3 mg	5.2 mg	0 mg
A, first	28.6±15.3	82.6±25.9	25.1±20.5	35.0±14.0
A, second	19.8±19.8	57.1±25.9	10.6± 5.6	37.1±20.4
B, first	42.1±37.7	43.7±31.0	33.8±23.4	70.8±40.7

Maximal duration (days) of existing IMI				
Dairy/lactation	16.5 mg	10.3 mg	5.2 mg	0 mg
A, first	58.3±27.2	126.2±37.9	102.4±35.7	52.8±28.5
A, second	137.8±51.8	131.1±40.0	92.4±39.7	55.2±29.2
B, first	7.1± 7.1	125.6±53.2	44.8±25.1	54.2±54.2

DISCUSSION

Prevalence of IMI did not vary among treatments over time during the period of study. This portion of the experiment was designed to determine if there was any association between rBST dose level and prevalence of IMI. If rBST had detrimental effects on treated cows with respect to susceptibility to IMI or elimination of existing IMI, one would expect to see differences in IMI prevalence rates. The present study did not indicate any consistent differences in this regard. No significant differences were noted in the maximal duration of new or existing IMI.

No significant differences were observed in the occurrence of new IMI or cases of clinical mastitis during our study. Because of the small number of clinical cases observed in the present trial studies employing larger numbers of animals or analysis of data combined from several experiments may be indicated in order to address the issue of whether rBST treatment influences the incidence of clinical mastitis. However, the present study does not provide evidence for deleterious effects of rBST treatment on the incidence of new infections or of clinical mastitis.

There was no direct evidence provided in this study for any detrimental influence from rBST treatment on subclinical and clinical mastitis. The dairies studied were similar to commercial Southeastern dairies, indicating that these results should apply to actual field use of rBST.

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SUMMARY

The purpose was to investigate the influence of treatment of lactating cows with recombinant bovine somatotropin (rBST) on subclinical and clinical mastitis. Lactating Holstein cows of mixed parity were randomly allocated to 4 levels of rBST (0, 5.2, 10.3, and 16.5 mg/day) administered into the tail fold area from early lactation (28-35 days in milk) to the end of lactation. On dairy A, 60 cows were studied for the first lactation and 43 of the same cows were studied for 2 complete lactations. On dairy B, 32 cows were studied for a single lactation, but all cows had been treated with the same dose of rBST during the previous lactation. Duplicate quarter milk samples were aseptically collected from all cows at trial entry, at 60-day intervals during the trial and at trail end or dry-off. All cases of clinical mastitis were recorded and milk samples were collected from affected quarters for microbiological analysis. Milk samples were analyzed by standard microbiological procedures. Preliminary analysis of data indicated that treatment with rBST did not adversely affect incidence, prevalence and duration of subclinical intramammary infections or incidence of clinical mastitis.

RESUMEN

El objetivo fué investigar el tratamineto con somatotropina bovina de tipo recombinante en vacas lactantes con mastitis clínica o subclínica. Vacas holstein lactantes de diferente parto fueron assignadas al azar a uno de cuatro tratamientos: 0, 5.2, 10.3 o 16.5 mg/día de somatotropina administrada en la comisura de la cola. El tratamiento se inició a los 28-35 días de lactación y continuó hasta el final del período. En el rancho A 60 vacas se estudiaron en una primera lactacion y 43 de las mismas en 2 lactaciones completas. En rancho B, 32 vacas se estudiaron por una lactación. Muestras de leche de cada cuarto se colectaron por duplicado al inicio del tratamiento, cada 60 días durante el tratamiento y al momento de secar a la vaca. Todos los casos de mastitis clínica se anotaron y se colectaron muestras de leche de los cuartos afectados para análisis bacteriológico. Muestras de leche se analisaron utilizando procedimientos microbiológicos de rutina. Resultados preliminares indican que el tratamiento con somatotropina bovina de tipo recombinante no afecta de manera adversa la incidencia prevalencia o duración de infecciones intramamarias subclínicas ó la incidencia de mastitis clínica.

SOMMAIRE

L'objet a été examiné les effets de la Bovine Somatotropine recombinée (rBST) sur mastite clinique et sous-clinique des vaches laitieres. Des vaches laitieres Holstein on été assignée au hasard a 4 groupes de traitement avec rBST (0, 5.2, 10.3 et 16.5 mg/jour), injecté dans la pli de queue. La durée de traitement a été de 28-35 jours en lactation a la fin de lactation. Dans Laiterie A, 60 vaches ont été etudié pour la premiere lactation, et 43 de les mêmes vaches ont été etudié pour 2 lactations complet. Dans Laiterie B, 32 vaches ont été etudié pour une lactation. Les doubles prélèvements de lait ont été collecté par une methode aseptique dans tous les vaches au debut d'essai, en des intervalles de 60 jours durant l'essai, et a la fin d'essai. Les cas des mastites cliniques ont été enregistré et des prélèvements de lait ont été collecté en les quartiers affectes pour analyses microbiologiques. Des prélèvements de lait ont, été analysé par les techniques normal. L'analyses preliminaires des données ont été indiqué que le traitement avec rBST n'affecte pas l'incidence, prevalence ou la duree des infections intramammaires sous-clinique, ou l'incidence de mastite clinique.

ZUSAMMENFASSUNG

Den Einfluss von "recombinant bovine somatotropin" (rBST) auf Mastitis wurde in laktierenden Kühen untersucht. Laktierende Holsteiner Kühe wurden zufallsweise in 4 ahnliche Gruppen verteilt die entweder 0, 5.2, 10.3 oder 16.5 mg/Tag rBST bekamen. Die Injektiones wurden in der Schwangfalte von 28-35 Tag post partus bis zum Ende des Laktierens verabreicht. An des Molkerei A wurden 60 Kühe für einen Zyklus studiert, und 43 davon für einen Zweiten. Bei Molkerei B wurden 32 Kühe für einen Zyklus studiert. Doppelte Milchproblem von jedem Viertal wurden am Anfang des Studie, alle 60 Tage, und am Ende aseptisch gesammelt. Jeder Fall klinischen Mastitis wurde registriert und die entsprechende Milchprobe mikrobiologisch untersucht. In der vorläufigen analyse konnten keine nachteiligen Einflüsse von rBST auf Mastitis oder anderer Enterentzundungen fertgestellt werden.

INFLUÊNCIA DO TEMPO DECORRIDO PÓS-PARTO SOBRE O APARECIMENTO DE CIO EM VACAS NELORE, COM BEZERRO AO PÉ, SINCRONIZADAS COM CLOPROSTENOL SÓDICO.

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INTRODUÇÃO

O manejo reprodutivo de rebanhos de corte criados extensivamente na confluência das regiões Centro Oeste e Amazônica, tem sido prejudicado pela dificuldade em se estabelecer um período mais homogêneo para a realização das coberturas. A grande irregularidade no intervalo entre parto e a manifestação do primeiro cio exige o emprego de maior número de reprodutores em atividade sexual e provoca sensível alargamento na estação de monta.

A presença do bezerro junto à mãe até o seu desmame natural, prática comum no sistema de criação extensiva, parece exercer grande influência no surgimento do cio pós-parto e ser responsável pelo retardo da concepção.

Com o surgimento da Prostaglandina e seus análogos, passou-se a utilizar a sincronização de cio como técnica para adequar a estação de monta e permitir o emprego do cruzamento industrial nos grandes rebanhos de corte, para fins de confinamento, através da Inseminação Artificial.

Como a região apresenta períodos distintos de seca (abril/ setembro) e chuvas (outubro/março), os nascimentos ocorrem do meio para o final da estação seca, época mais propícia para a sobrevivência dos bezerros. O retorno da atividade sexual das vacas paridas se distribui no início da estação chuvosa, quando as pastagens se recuperam e passam a servir como fonte de alimento.

Devido as parições ocorrerem na seca, a aplicação de um agente sincronizante no início da estação chuvosa, recairá sobre vacas com diferentes intervalos de tempo entre o parto e o tratamento. A menor ou maior proximidade do parto no momento da aplicação do agente sincronizante pode levar a diferentes resultados no número de vacas que entrão em cio. A ação do medicamento torna-se mais ou menos eficiente conforme se distancia ou encurta o período parto-tratamento (8). Este trabalho foi elaborado objetivando verificar o período mais próximo do parto em que maior número de vacas responde à aplicação do Cloprostenol sódico, um análogo da Prostaglandina.

MATERIAIS E MÉTODOS

O experimento foi realizado em fazenda localizada ao Norte do Estado do Mato Grosso, na região amazônica. A precipitação pluviométrica no mês do experimento (outubro) foi de 436 mm e a temperatura variou de 22,2ºC a 32,4ºC com média de 27,3ºC.

Procurou-se desenvolver o trabalho dentro das condições normais de manejo da propriedade, escolhendo-se a época mais propícia, início da estação das chuvas, quando as condições de pastagens passaram a permitir o retorno da atividade reprodutiva.

O rebanho de fêmeas estudadas fazia parte de uma população de 30.000 bovinos e foi escolhido ao acaso, sem qualquer critério de seleção, a não

ser a presença do bezerro junto à mãe.

Foram utilizadas 1816 vacas da raça Nelore, com bezerros de idade variando entre 30 a 239 dias, subdivididas em lotes ao acaso e distribuídas em sete pastos formados de capim colonião (Panicum maximum). O sistema de criação era extensivo, com permanência constante do bezerro junto à mãe. Os animais tinham livre acesso às aguadas e aos cochos contendo sal mineralizado. As pastagens encontravam-se recuperadas, após a chegada das chuvas, fornecendo um bom suporte alimentar.

As vacas numèricamente identificadas foram mantidas separadas dos reprodutores após o parto. Os bezerros, ao nascerem, foram marcados à ferro com um número que correspondia ao mês de nascimento.

Todas as vacas receberam uma primeira dose de 0,125 mg de Cloprostenol sódico aplicada via intravulvo-submucosa (ivsm) e após 11 dias o mesmo tratamento foi repetido nas fêmeas que não manifestaram cio à primeira aplicação. Cada tratamento foi realizado simultaneamente em todos os lotes, dentro de um período não superior a 48 horas, independentemente da fase do ciclo estral em que as fêmeas se encontravam.

As vacas que entraram em cio foram inseminadas artificialmente com sêmen de reprodutores das raças Aberdeen angus, Fleckvieh e Pardo suiço. Ao término do experimento todas as fêmeas, inseminadas ou não, foram colocadas com touros da raça Nelore.

A verificação do cio era realizada duas vezes ao dia. Não foram utilizados rufiões para a identificação das fêmeas. Os vaqueiros reuniam o lote de vacas pela manhã e o mantinha sob observação. As vacas montadas por suas companheiras eram assinaladas com tinta de cor azul e deixadas junto às demais. À tarde os vaqueiros juntavam novamente as vacas e faziam a segunda observação. As fêmeas que manifestavam cio, não assinaladas anteriormente, recebiam uma marca com tinta de cor vermelha. No final do período de observação, todas as vacas marcadas de azul e vermelho eram levadas, sem os bezerros, para o curral. As vacas identificadas pela cor azul eram anotadas e inseminadas na tarde do mesmo dia e as de vermelho, na manhã do dia seguinte.

RESULTADOS

Para melhor avaliação estatística dos resultados obtidos, as vacas foram estratificadas em 6 classes, de acordo com as idades dos bezerros, com intervalo de 30 dias, sendo que na última classe foram reunidas todas as vacas que havian parido há mais de 180 dias.

Os resultados numéricos da pesquisa são apresentados no quadro 1 e gráfico 1, anexos. A proporção de vacas em cio dependeu estatisticamente ($P<0,01$) do período pós-parto no qual foi realizado o tratamento.

As manifestações de cio decorrentes da aplicação do agente sincronizante Cloprostenol sódico foram baixas (47,10%) nas vacas com intervalo parto-tratamento inferior a 60 dias. Com o aumento do intervalo para 60-89 dias e 90-119 dias, o número de vacas em cio aumentou alcançando os coeficientes de 56,18% e 59,32%, respectivamente. A resposta de maior significação estatística foi obtida do período compreendido entre 120-149 dias do parto, quando 74,65% das vacas tratadas manifestaram cio.

Estes resultados revelaram que existe uma ou mais influência inibidora da proximidade do parto sobre o retorno da atividade reprodutiva da vaca de corte.

As vacas tendem a demonstrar pouca evidência de cio quando o intervalo parto-primeiro cio é curto (5). Estimativas da ocorrência de manifestações de cios pós-parto, delineadas com intervalos de 15 dias revelaram que o percentual de vacas em cio aumentava conforme alongava o intervalo parto-primeiro cio (30 dias - 6%; 45 dias - 44%; 60 dias - 70%; 75 dias - 87%; 90 dias - 96%; 105 dias - 99%; e 120 dias - 100% (3). A maior ocorrência de cios no 120º dia pós-parto, coincidiu com a resposta máxima encontrada neste trabalho (120-149 dias). Igual resultado foi obtido em gado mestiço holando-zebu, quando maior ciclicidade das vacas tratadas com Cloprostenol (85,7%) foi obtida no intervalo médio de 135,1 dias pós-parto (1).

A falta de atividade cíclica do ovário no início do pós-parto elimina a principal condição para o sucesso da sincronização de cio com Prostaglandinas, que é a presença de um corpo lúteo ativo (2). A redução do efeito do agente sincronizante neste período também foi constatada em vacas que receberam implantes auriculares de SMB (Syncro-Mate-B). A melhor resposta ao tratamento ocorreu quando os implantes foram removidos aos 50-60 dias que aos 20-30 dias pós-parto (8).

Trabalhos realizados em vacas de corte mastectomizadas demonstraram que a principal condição atuante como inibidora da manifestação do cio pós-parto era a presença do bezerro junto à mãe (6). No sistema de criação extensiva, predominante na região onde este trabalho foi desenvolvido, as vacas permanecem em constante contato com suas crias até que a desmama ocorra naturalmente. Este tipo de manejo pode ser responsável pela menor ocorrência de manifestações de cio no período imediato ao parto e pelo maior distanciamento do período de melhor resposta à sincronização.

Pelos resultados obtidos, pode-se concluir que em um programa de cruzamento industrial em fazendas situadas ao Norte do Estado do Mato Grosso e regiões vizinhas, a melhor época para sincronização de cio com emprego de Cloprostenol sódico, na dosagem de 0,125 mg, via intravulvo submucosa, será no mês de novembro, utilizando-se de vacas paridas no mês de julho.

O trabalho foi desenvolvido na estação das chuvas por ser um período de maior ciclicidade das vacas e de melhor resposta à ação da Prostaglandina (7).

A dosagem do medicamento e a via de aplicação utilizada foram baseadas nas conclusões de CHAUHAN et alii (4). O tratamento apresentou satisfatória resposta de vacas em cio, principalmente à partir dos 120 dias pós-parto e representou uma redução de custo significativa, por corresponder a 25% da dosagem IM recomendada pelo laboratório.

A constatação, em pesquisa anterior (7), do comportamento predominantemente homossexual das vacas em cio no período chuvoso, permitiu a contenção de elevadas despesas na compra de machos destinados a rufião.

RESUMO

Objetivando verificar no tempo decorrido após o parto, o período de melhor resposta à ação de agentes sincronizantes, utilizou-se 1816 vacas Nelore, com bezerros de idade variando entre 30-239 dias, criados extensivamente em pastagens de capim colonião (Panicum maximum).

Todas as vacas receberam uma primeira dose de 0,125 mg de Cloprostenol sódico, aplicado via intravulvo-submucosa e após 11 dias o mesmo tratamento foi repetido nas fêmeas que não manifestaram cio à primeira aplicação.

Para identificar o período de melhor resposta ao tratamento, as vacas foram distribuídas em 6 classes, de acordo com a idade dos bezerros, com intervalo de 30 dias.

60,96% do total de vacas tratadas manifestaram cio, sendo 23,56% ao primeiro tratamento e 37,40% no segundo.

A resposta com maior significação estatística ($P<0,01$) ao emprego do

Cloprostenol sódico foi obtida no período de 120-149 dias pós-parto quando 74,65% das vacas tratadas manifestaram cio.

SUMMARY

This research was carried out with the objective to determine the best answer to the action of synchronizations during the post-partum period. It was used 1816 Nelore cows with calves of 30-239 days old, raised extensively in pastures of Panicum maximum in the Amazonic region.

Every cow received one dose of 0,125 mg of Sodium Cloprostenol intravulvo-submucosal and a second dose was given 11 days after the first treatment to those cows which did not show estrus signs.

To identify the period that showed the best results for the treatment the cows were divided into 6 groups according to the calves age, with 30 days interval.

60,96% of the cows showed estrus, 23,56% after the first treatment and 37,40% after the second one.

The best answer (P<0,01) to the treatment of Sodium Cloprostenol was obtained in the 120-149 days post-partum period when 74,65% of the treated cows showed estrus.

RESUMÉ

Ayant pour objectif la vérification, durant le temps decouru après la mise à bas, de la période la plus favorable à l'action d'agents synchronisants, nous avons utilisé 1816 vaches Nelore, ayant des veaux d'âges differents, variant de 30 à 239 jours, élevés extensivement dans des champs d'herbe Panicum maximum.

Toutes ces vaches ont reçu une première dose de 0,125 mg de Cloprostenol de sodio, appliqué par voie intravulvo-submuqueuse. Après 11 jours, le même traitement a été répété chez les femelles qui n'avaient pas manifesté la chaleure à la première application.

Pour reconnaitre la période la plus favorable au traitement, les vaches ont été réparties en 6 groupes, selon l'âge de leurs veaux, obéissant à un intervalle de 30 jours.

60,96% du total des vaches traitées ont manifesté la chaleure, respectivement 23,56% au premier traitement et 34,40% au second.

La réponse, ayant la plus grande signification statisque (P<0,01) à l'usage du Cloprostenol de sodio, a été obtenue pendant la periode de 120 à 149 jours après la mise à bas, lorsque 74,65% des vaches traitées ont manifesté la chaleure.

RESUMEN

Objetivando verificar en el tiempo recorrido después del parto el periodo de mejor respuesta a la acción de agentes sincronisantes, se utilisó 1816 vacas Nelore, en becerros con edad variando entre 30-239 dias, criados extensivamente en pastos de hierva Panicum maximum.

Todas las vacas recibieron una primera dosis de 0,125 mg de Cloprostenol sodico, aplicada via intravulvo-submucosa y después de 11 dias el mismo tratamiento fué repetido en las hembras que no manifestaron celo a la primera aplicación.

Para identificar el periodo de mejor respuesta al tratamiento, las vacas fueron distribuidas en 6 clases, de acuerdo con la edad de los becerros, con intervalo de 30 dias.

60,96% del total de vacas tratadas manifestaron celo, siendo 23,56% con el primero tratamiento y 37,40% con el segundo.

La respuesta con mayor significado estatistico (P<0,01) al empleo de Cloprostenol sodico fué obtenida en el periodo de 120-149 dias después del parto, cuando 74,65% de las vacas manifestaron celo.

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Período Pós-Parto	Fêmeas tratadas		Fêmeas que responderam ao tratamento						Fêmeas que não responderam ao tratamento	
			1ºtratam.		2ºtratam.		TOTAL			
DIAS	nº	%	nº	%	nº	%	nº	%	nº	%
30 - 59	138	7,60	26	18,84	39	28,26	65	47,10	73	52,90
60 - 89	728	40,09	140	19,23	269	36,95	409	56,18	319	43,82
90 -119	472	26,00	117	24,79	163	34,53	280	59,32	192	40,68
120 -149	284	15,64	80	28,17	132	46,48	212	74,65	72	25,35
150 -179	100	5,50	35	35,00	41	41,00	76	76,00	24	24,00
>180	94	5,17	30	31,92	35	37,23	65	69,15	29	30,85
TOTAL GERAL	1816	100,00	428	23,56	679	37,40	1107	60,96	709	39,04

QUADRO 1: Sincronização de cio em Vacas Nelore, com bezerro ao pé, no período de 30 a 239 dias pós-parto, com emprego de Cloprostenol Sódico (número e percentual).

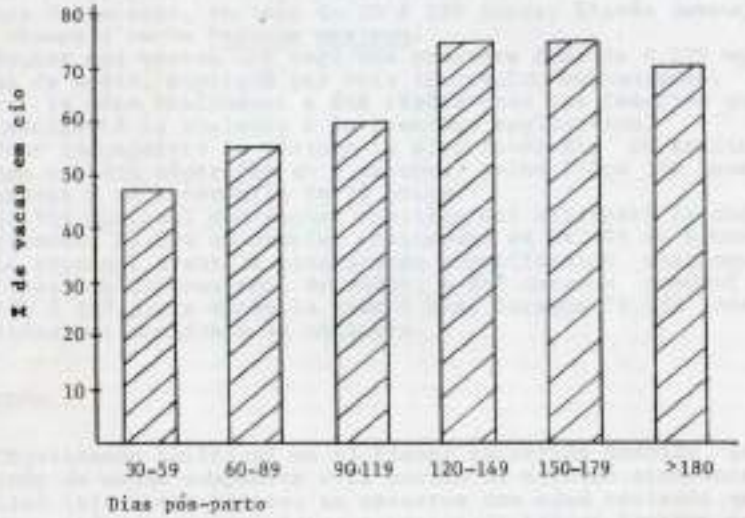

GRÁFICO 1: Percentual de Vacas Nelore, com bezerro ao pé, que manisfestaram cio após tratamento com Cloprostenol Sódico, no período de 30 a 239 dias pós-parto.

INMUNOGLOBULINAS Y CELULAS CONTENEDORAS DE INMUNOGLOBULINAS EN EL TRACTO GENITAL DE TOROS VACUNADOS Y DESAFIADOS CON TRITRICHOMONAS FOETUS

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INTRODUCCION

La tricomoniasis bovina es una enfermedad venérea causada por Tritrichomona foetus. La enfermedad está presente en grandes rodeos de carne provocando pérdidas por abortos, piometra e infertilidad. El tratamiento de los toros infectados no es práctico bajo condiciones de manejo extensivo y es costoso. La presencia de cepas químio-resistentes enfatiza el riesgo de los tratamientos (1). El control de la tricomoniasis bovina mediante la vacunación de los toros con vacuna formolada con antígeno de célula completa o proteína de membrana de T.foetus en adjuvante oleoso (2,3) crea nuevas expectativas.

Aunque existe información referente a la concentración de inmunoglobulinas en fluidos genitales de toros normales y toros con campylobacteriosis (4,5) no existe similar información referente a toros infectados con T.foetus. Muchos aspectos de la inmunidad local y el posible rol de la misma en la enfermedad permanecen desconocidos. En el presente trabajo se cuantificarán los niveles de inmunoglobulinas en fluidos genitales y las células contenedoras de inmunoglobulinas (CCI) en tejidos prepuciales y glándulas sexuales accesorias (GSA) en toros vacunados y desafiados con T.foetus.

MATERIALES Y METODOS

Animales experimentales

Se utilizaron 11 toros cruzas Bos indicus (1/2 a 3/3 Brahman con Shorthorn) de 5,5 años provenientes de un rodeo libre de brucelosis, tricomoniasis y campylobacteriosis del noreste de Queensland, Australia.

Vacuna y vacunación

Se utilizó una vacuna a base de proteína de membrana de T.foetus var. brisbane la cual contenía 500 ug de proteína por dosis en 5 ml. de aceite mineral como adjuvante oleoso (3). La vacuna fue gentilmente preparada y suplida por CSIRO, Parkville, Victoria, AUSTRALIA. Siete de los toros fueron vacunados tres veces por vía subcutánea a intervalos mensuales. Los remanentes 4 toros sirvieron como controles sin vacunar.

Muestras y muestreos

Los lavajes prepuciales fueron obtenidos mediante infusión de 10 ml de solución salina fosfatada bufferada pH 7,2 conteniendo azida de sodio al 0,01% como bacteriostático. Los lavajes fueron centrifugados a 2.000 G a 4°C por 10 minutos y luego congelados a - 70°C hasta su uso.

Las muestras de semen fueron recolectadas mediante masaje rectal o bien por electroeyaculación. El semen fue centrifugado y se le

agregó azida de sodio como ya se mencionó conservandose a -70°C hasta su uso. Las muestras prepuciales y seminales se extrajeron antes de la vacunación, un mes después de la tercera dosis y una semana después del desafío. Después del desafío experimental se extrajeron muestras prepuciales para cultivo (6).

Desafío experimental

Un mes después de la tercera dosis de vacuna, todos los toros fueron desafiados intraprepucialmente con 5 ml de cultivo de 36 h de T.foetus cepa vacunal en caldo-fusión hígado con una concentración de protozoos viables de 7 x 10^6/ml.

Cuantificación de inmunoglobulinas

Se utilizó antisuero bovino monoespecífico IgA, IgM, IgG_1 y IgG_2 mediante la técnica de inmunodifusión simple radiada (6).

Muestras post-mortem

Los toros fueron faenados una semana posterior al desafío. El tracto genital completo fue recolectado y examinado (7). Secciones de tejidos fueron fijadas en formol al 10% y en Bouin's y procesadas rutinariamente.

Inmunohistoquímica

Se utilizó la técnica de peroxidasa - antiperoxidasa (PAP) (8) en muestras de tejidos de pene, prepucio y BSA. La cuantificación de CCI se realizó como se describió (8).

Estadística

Se obtuvieron los valores medio, relación, error standart o desvio standart. Un repetido análisis de varianza fue realizado sobre los datos después de la transformación logarítmica. Para todas las comparaciones post-logarítmicas se utilizó el método de SCHEFFE'S (9).

RESULTADOS

Cultivos

El examen de los cultivos de T.foetus obtenido a los 7 días post-desafío mostró que sólo dos toros controles fueron infectados. Todos los toros vacunados fueron negativos.

Histopatología

No se encontraron lesiones en los tejidos prepuciales ni en las BSA atribuidas a T.foetus. No se observó tricomonas en los cortes de pene o prepucio teñidos con hematoxilina y eosina. Se observaron acúmulos focales de células plasmáticas, macrófagos y linfocitos en pene y prepucio. Dichos acúmulos fueron más obvios en la dermis cercana al epitelio. Sin embargo, algunas células plasmáticas y neutrófilos fueron a veces localizadas dentro del epitelio escamoso estratificado. En las áreas caudales del pene y fornix se observaron criptas bien desarrollados rodeadas por acúmulos prominentes de células plasmáticas. Fueron notorios folículos linfoides en la dermis en todos los toros, el algunas áreas formaban linfo-epitelio. No se observó tendencia con respecto a la presencia o prevalencia de los folículos linfoides y acúmulo celular y el estado vacunal de los toros.

Niveles de inmunoglobulinas en fluidos

Las concentraciones de inmunoglobulinas en el plasma seminal son mostrados en la Tabla 1. No se observaron diferencias estadísticas ($P > 0.05$) para la concentración de ningún tipo de inmunoglobulina o relación entre o dentro de los toros vacunados y controles. IgA fue la inmunoglobulina de mayor concentración en ambos grupos (rango de 0.39 mg/ml a 0.58 mg/ml) para los toros vacunados y de 0.30 mg/ml a 0.35 mg/ml para los toros controles. Solo trazas de inmunoglobulinas fueron encontrados en los lavajes prepuciales.

Células contenedoras de inmunoglobulinas

El porcentaje promedio de CCI en tejidos prepuciales se muestra en la Table 2. Tampoco se observó diferencias estadísticas entre grupos de animales para cada clase específica de CCI. Las CCIgG fueron las más prevalentes (78%) en ambos grupos de toros. CCIgA fueron las segundas en prevalencia (20-21%). Las CCI fueron localizadas en acúmulo en la parte apical de las papilas dermales, cercano al epitelio. La distribución de CCI en las BSA es mostrada en la Tabla 3. Las vesículas seminales fueron excluidas del análisis cuantitativo por su baja prevalencia de CCI. En próstata y glándulas bulbouretrales prevaleció las CCIgA, mientras que en las ampollas lo fueron las CCIgG. Tampoco hubo diferencias significativas ($P > 0.05$) entre cada clase de CCI y grupo de toros.

DISCUSION

En este trabajo no fue posible establecer ningún tipo de relación entre vacunación y concentración de inmunoglobulinas o prevalencia de CCI en fluidos genitales o tejidos, respectivamente.

El desafío con antígeno vivo no indujo ningún cambio en la prevalencia de CCI en los tejidos de los toros previamente vacunados. Estos hallazgos no apoyan la hipótesis de que la inmunidad de T.foetus en toros vacunados podría ser manifestada por una elevada prevalencia de células plasmáticas e incremento en la concentración de Ig en respuesta a T.foetus.

Histológicamente, la infiltración de células plasmáticas en la lámina propia del tejido prepucial se incrementa con la edad probablemente debido al efecto de una repetida exposición antigénica a nivel prepucial (6). La presencia de folículos linfoides o linfoepitelio en el fornix y pene han sido mencionados por otros autores (10).

El hallazgo de que IgA fue la Ig predominante en el plasma seminal podría deberse a algún grado de producción local y transporte selectivo. La incapacidad de demostrar cualquier nivel de Ig en el fluido prepucial podría deberse al volumen del fluido instilado en la cavidad prepucial el cual tendría un efecto diluyente sumado a la en concentración del líquido recolectado posteriormente del lavado. Sin embargo, mediante la técnica de ELISA (6) se observaron títulos positivos contra T.foetus en muestras post-desafío de toros vacunados, lo que explicaría una baja producción de anticuerpos locales. La baja carga de T. foetus y la naturaleza asintomática de la infección podría determinar una baja respuesta inmune local

contrariamente a lo que ocurriría con la vaca infectada con T.foetus.

En este estudio la prevalencia de CCI en el prepucio es coincidente con los hallazgos previos de otros autores (10).

Si bien parece lógico asumir el rol local de las CCI en la dermis prepucial y peniana, la cual podría contribuir al nivel de inmunoglobulinas en la cavidad prepucial, el mecanismo de transferencia involucrado es aún desconocido. La naturaleza escamosa del epitelio estratificado y la falta de glándulas secretorias dificultaría la difusión de Ig a través de dichas áreas. La presencia de Ig provenientes de las BSA, al menos parcialmente, parece más probable de ocurrir.

La introducción de antígenos de T.foetus en la cavidad prepucial probablemente evoca ambas respuestas inmunes: local y sistémica. Otros aspectos involucrados en la respuesta inmune en el huesped tal como la relación entre la población de células T y la interacción del linfoepitelio y tejido linfoideo en la cavidad prepucial y el estado de infección restan por ser aclarados.

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RESUMEN

Siete toros adultos fueron vacunados en tres oportunidades con una vacuna oleosa de T.foetus quedando 4 toros con controles. Una semana después del desafío intraprepucial con T.foetus, todos los toros fueron faenados. Se determinó la concentración de IgA, IgG_1, IgG_2 e IgM en muestras de fluido prepucial y plasma seminal. No se observaron diferencias estadísticas entre las concentraciones de IgA, IgG_1 e IgG_2 en el plasma seminal de toros controles y vacunados.

Mediante la técnica de peroxidasa-antiperoxidasa se identificaron células contenedoras de inmunoglobulinas de los isotipos IgA, IgM, IgG, IgG_1 e IgG_2 en las glándulas sexuales accesorias, tejido prepucial y pene. No se observaron diferencias estadísticas entre los grupos vacunados y control.

SUMMARY

Seven adult bulls were vaccinated on three occasions with a T.foetus vaccine in an oil adjuvant, 4 other bulls remained as controls. One week after intraprepucial challenge with T.foetus the bulls were slaughtered. Preputial fluid and seminal plasma samples were screened for the presence of IgA, IgG_1, IgG_2 and IgM. The concentration of IgA; IgG_1 and IgG_2 in the seminal plasma showed no statistical difference between vaccinated and control bulls at any time. The peroxidase-antiperoxidase technique was used to identify immunoglobulin containig cells of the isotypes IgA, IgM, IgG, IgG_1 and IgG_2 in the accessory sex glands, preputial and penile tissues. No statistical differences for immunoglobulin containing cells between vaccinated and control animals were found.

RÉSUMÉ

Sept taureaux on été vaccinés en trois opportunités avec un vaccin avec adjuvant huileu de T.foetus et quatre taureaux ont été gardés comme temoins. Une semaine après avoir été mis en contact avec T.foetus (voie preputiale) les animaux ont été abattus. La concentration d' IgA, IgG_1, IgG_2 et IgM a été determine dans des échantillons des secretrions preputiales et du plasma seminal. Des différences statistiques n'ont pas été observés dans les concentrations de IgA, IgG_1 e IgG_2 dans le plasma seminal des taureaux vaccinés et temoins. A travers la technique de la peroxidase-antiperoxidase on a pu identifier des cellules ayant immunoglobulines des isotypes IgA, IgM, IgG, IgG_1 et IgG_2 dans les glandes annexes, le tiesu preputiale et la penis. Des differences statistiques n'ont pas été observés entre le group vaccinè et le group temoin.

Tabla 1: Niveles de Inmunoglobulinas (media ± DS, mg/ml) y relación IgG_1/IgG_2 en el plasma seminal de toros vacunados y desafiados con T.foetus.

Tiempo de muestreo	IgA	IgG_1	IgG_2	IgG^1/IgG_2
Pre Vacunación	0.58±0.30	0.16±0.04	0.10±0	1.61±0.44
Post Vacunación	0.39±0.22	0.13±0.06	0.10±0	1.31±0.22
Post desafío	0.52±0.44	0.22±0.06	0.10±0	2.01±0.70

Tabla 2: Media porcentual de células contenedoras de inmunoglobulinas en el tejido prepucial de toros vacunados y/o desafiados con T.foetus.

Grupo	IgA	IgM	IgG	IgG_1	IgG_2	IgG_1/IgG_2
Vacunado (n: 7)	20.1	3.6	78.0	39.6	39.1	1.0
Control (n: 4)	21.0	3.5	78.0	42.0	40.5	1.0

Tabla 3: Media porcentual de células contenedoras de inmunoglobulinas en las glándulas sexuales accesorias de toros vacunados y/o desafiados con T.foetus.

Organo	Grupo*	IgA	IgM	IgG	IgG_1	IgG_2	IgG_1/IgG_2
Ampolla	V	11.7	6.9	80.7	42.1	39.6	1.1
	C	11.0	6.8	79.5	40.8	39.5	1.0
Prostata	V	88.7	6.0	7.0	4.1	3.0	1.4
	C	89.3	4.3	6.0	3.5	2.8	1.3
Glándulas Bulboure trales	V	88.7	4.6	4.9	3.7	2.9	1.4
	C	9.3	4.0	5.0	4.0	3.3	1.4

* V: vacunado C: control

SELECTION STRATEGIES TO IMPROVE FEMALE FERTILITY IN DAIRY CATTLE

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INTRODUCTION

The structure of dairy cow populations provides good possibilities for epidemiological approaches in breeding against production diseases, if a recording system for veterinary data is working on a regional basis. In Israel health data are collected by veterinarians of the Hahaklait, the mutual society for clinical veterinary services and livestock insurance (Mayer, 1986). The veterinary service is based on a weekly, twice weekly or even more frequent basis depending on herd size. All events related to the health of a cow are computerized and integrated in the data bank of Israel Cattle Breeders' Association (ICBA). The objective of this analysis is to prove if the disease data recorded in Israeli Holsteins can be used in breeding for disease resistance and which parameters would be the most efficient ones.

MATERIAL AND METHODS

The dataset analysed comprised information from about 35,000 dairy cows freshening in the years 1983 to 1985. The cows involved in this investigation were housed in 102 Kibbutzim herds and completed the first, second or third lactation (Table 1). Cows with the birth-types fetotomy, ceasarean section, abortion and twins were discarded.

The analysis was restricted to the most frequent disorders: retained placenta, abnormal lochia, endometritis, anestrus and cystic ovaries. The disease frequencies were analysed by a mixed

model procedure for ordered categorical data. The approach uses a threshold model, in which the observed category is determined by the value of an underlying unobservable continuous response that follows a mixed linear model. The threshold model enables to analyse data, which can be recorded only on a categorical scale, but show a polygenic inheritance. Variance components are estimated by a Bayes-like approach. The estimator maximizes the marginal a posteriori probability density function. An iterative scoring algorithm is applied to solve the nonlinear equations.

In the second approach the reaction of cows to fertility disorders is estimated (Distl, 1988). The reaction to fertility disorders was measured by culling rate and A.I.-parameters like first insemination success, number of inseminations, overall conception rate and days to first breeding after calving. The estimation procedure is developed in following way: Reaction to fertility disorders is defined by the difference between the production parameter without the influence of fertility disorders and the production parameter affected by fertility disorders. The production without the influence of fertility disorders can be estimated using the subpopulation of healthy cows, which are exposed to the same environmental conditions as the diseased cows.

The estimation procedure takes into account for environmental and genetic effects and in this way the genetic part of reaction to fertility disorders can be estimated (Table 2).

The effects regarded in the applied mixed models were: herd-year of calving or insemination, month of calving or insemination, interaction of calving difficulty and sex of calf, preceding pregnancy duration, age of the cow at calving, sire of cow.

RESULTS AND DISCUSSION

The additive-genetic component of traits showing polygenic inheritance is characterized by the heritability. Genetic improvement of traits with low heritability estimates is difficult and expensive. The heritability estimates from the paternal half sib analysis for the frequencies of fertility disorders under the threshold model are rather low and almost in the range from 0.5% to 3.5% (Table 3). The highest heritability estimates were found for the frequency of anestrus and the lowest for the frequency of cystic ovaries. If all fertility disorders were combined in

one categorical trait, the heritability estimates amounted to 2.5% (first lactation), 2.2% (second lactation) and 5.4% (third lactation).

Using the reaction to fertility disorders measured by the culling rate and A.I.-parameters, the analysis revealed that the heritability estimates were in the range of 10% in the first and second lactation and in the range of 15% to 20% in the third lactation (Table 4).

Model calculations for the genetic progress showed that the use of the reaction parameters would give the opportunity to breed efficiently against the production disorders analysed (Table 5). Index I is based on milk yield and first insemination success of progeny groups in the first lactation. Index II includes besides milk yield the first insemination success in the subpopulation without fertility disorders and the reaction of cows to fertility disorders. Also in an open MOET-breeding scheme (Multiple Ovulation and Embryo Transfer) selection for fertility seems to be justified (Table 6). Even if the accuracy of the index is much lower in comparison to a progeny test, considerable genetic progress can be expected. In the case that selection is based only on half and full sib information, the selection response in a MOET-scheme might be reduced by 40%-50% in comparison to a progeny test with 50-90 daughters. The genetic progress in comparison to usual selection strategies for fertility based on A.I.-data could be increased in a progeny test by 40%.

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TABLE 1 Basic statistics of the data

	Lactation number		
	1	2	3
Number of cows	23,081	14,792	8,286
Number of sires	155	146	103
Disease frequency (%)			
Retained placenta	4.1	6.5	6.9
Abnormal lochia	8.8	6.8	6.4
Endometritis	8.7	7.2	7.9
Anestrus	34.6	35.5	31.8
Cystic ovaries			
- with anestrus	3.5	4.8	3.9
- with nymphomania	2.6	3.4	3.1

TABLE 2 Statistical model for analysis of reaction of cows to fertility disorders

I. YO = E(YO) + e = Xb + e;
II. Y1 = E(Y1) + e = E(YO) + E(D) + e;
Xb = expectations for herd, stage of lactation, genetic factors, ... (= systematic components);
E(D) : expected effects of the disease on systematic influences like herd, genetic factors, ...;
YO : production record of a healthy cow;
Y1 : production record of a cow being diseased;
E(.) : expectation for (.); e: error component;
III. Reaction to disease
= E(YO)-Y1 = E(YO)-E(YO)-E(D)-e = -E(D)-e;

TABLE 3 Estimates of heritability (%) using the threshold model for frequencies of reproductive disorders in the first three lactations

	Lactation number		
	1	2	3
Retained placenta	2.08	1.30	6.11
Abnormal lochia	3.14	1.52	1.70
Endometritis	3.41	1.62	0.22
Anestrus	4.22	3.61	7.02
Cystic ovaries			
- with anestrus	0.58	0.49	0.39
- with nymphomania	1.09	0.50	0.19
All fertility disorders	2.53	2.19	5.41

TABLE 4 Heritability estimates (%) for the reaction to fertility disorders in Israeli-Holsteins

	Lactation number		
Parameter	1	2	3
Interval to first breeding	2.83	6.78	8.92
First insemination success	10.36	11.73	19.16
Overall conception rate	7.33	8.89	13.25
Reciprocal value for number of inseminations*	9.19	9.70	14.79
Culling rate	6.33	13.64	11.12

* 1/number of insemination in the service period; if the cow was culled it takes the value zero.

TABLE 5 Comparison of the selection response per generation between index-I and index-II

	Selection response			
	natural		monetary in DM	
	size of progeny groups			
	50	90	50	90
Index-I				
milk-yield	228.5	243.8	57.15	60.95
first insemination success (%)	-0.55	-0.59	-1.40	-1.47
			55.75	59.48
Index-II				
milk-yield (kg)	228.6	243.9	57.15	60.98
first insemination success (%)	-0.60	-0.62	-1.50	-1.55
(subpopulation without fertility disorders) reaction to fertility disorders (%) (measured by first insemination success)	2.34	2.42	22.42	23.18
			78.07	82.61

TABLE 6 **Expected selection response per generation for reaction to fertility disorders applying an open nucleus breeding programme with MOET**

sources of information FS	HS	PG	r_{AI}	MY	FI-N	REC	monetary (DM) total
4	-	-	0.393	105.5	-0.30	1.16	36.74
6	-	-	0.448	120.2	-0.34	1.31	41.75
2	4	-	0.348	93.3	-0.27	1.03	32.52
4	4	-	0.420	112.8	-0.37	1.23	39.06
4	10	-	0.445	119.4	-0.33	1.29	41.38
2	2	50	0.857	229.9	-0.61	2.35	78.46
4	4	50	0.861	230.9	-0.61	2.36	78.81
4	10	50	0.862	231.3	-0.61	2.36	78.91
2	2	90	0.911	244.4	-0.62	2.42	82.73
4	4	90	0.912	244.8	-0.62	2.42	82.83
4	10	90	0.913	244.9	-0.62	2.42	82.86

FS: Full sibs; HS: Half sibs; PG: progeny; r_{AI} : accuracy of selection index; MY: milk yield
FI-N: first insemination success in subpopulation without fertility disorders
REC: reaction to fertility disorders measured by first insemination success

SUMMARY

Two approaches based on epidemiological data are compared in their efficiency to breed for disease resistance in Israeli dairy cattle. In the first approach a threshold model was applied to estimate the recurrence risk for disease in half sib groups. Heritability estimates in the threshold model were rather low (h^2 < 4.0%). In the second approach the reaction of cows to diseases was estimated. The analysis releaved that heritability estimates of reaction to diseases are rather high and range almost from 9% to 15%. A progeny breeding programme including the reaction to disease could increase the efficieny by 40%. The newly developed approach enables also a selection for fertility in nucleus breeding programmes with MOET.

ZUSAMMENFASSUNG

Zwei Ansätze für die Zucht auf Krankheitsresistenz wurden an epidemiologischen Daten von Israeli-Holstein Kühen auf ihre Effizienz getestet. Im ersten Ansatz wird ein Schwellenmodell angewandt, um das Krankheitsrisiko für väterliche Halbgeschwistergruppen zu schätzen. Die Heritabilitätsschätzwerte im Schwellenmodell sind ziemlich niedrig (h^2 <4,0%). Im zweiten Ansatz wird die Reaktion der Kühe auf Krankheiten geschätzt. Die Analyse zeigte, daß die Heritabilitätsschätzwerte der Reaktion auf Krankheiten im Bereich zwischen 9% und 15% liegen und damit relativ hoch sind. Ein Nachkommentest, der auf dem Ansatz der Reaktion auf Krankheiten basiert, würde die Effizienz eines herkömmlichen Zuchtprogrammes auf Fruchtbarkeit um 40% erhöhen. Der neu entwickelte Ansatz ermöglicht auch eine Selektion auf Fruchtbarkeit in Nukleuszuchtprogrammen mit MOET.

FACTORS INFLUENCING THE FERTILITY OF NULLIPARA HEIFERS

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INTRODUCTION

The fertility of nullipara heifers (NH) as measured by indices like: a) Conception Rate (CR), b) length of the oestrus cycle and c) percentage of late returns to oestrus after insemination (12) differs markedly from the fertility of the cows in the same herds. In Israeli dairy herds the difference in CR between NH groups and cows in milk are, on average, about 24% (13), these differences remaining constant for periods lasting many years (8). On the other hand considerable variations in the CR of NH do exist between different herds. It was suggested that fertility in females is most accurately measured in heifers (6). The objective of this study was to determine the factors influencing the Conception Rate of NH in Israeli dairy herds.

MATERIALS AND METHODS

An extensive epidemiological study was performed during the years 1987/88 in orders to establish factors connected with herd fertility levels. 60 Kibutz industrial sized dairy herds milking an average of 300 cows and containing about 100 NH each, were analysed. Within the framework of this study, factors influencing the fertility of NH were investigated in the herds surveyed. The CR of NH was calculated from the computerised records of each herd and compared with the CR of milking cows in the same herd. All feeding data were analysed according to the herd records. The number of scheduled heat observations for each herd was recorded.

The Nullipara Heifers were assigned to two comparison groups according to the following criteria: a) Conception Rate of the cows of above 45% or below 40%; and b) Conception rate of the NH of above or below 59%.

The statistical evaluation of the feeding factors of the NH herd groups was performed by applying the simple linear model: $Y_{ij}=m+HKL_i+e_{ij}$. Y_{ij} = dependent variable of ij herd; m = model constant; KHL_i = group of herds (i=1,2); e_{ij} = a residual component.

RESULTS

In 30 herds having a fair conception rate of the cows of above 45% (average 48%), that of the NH was 63.8%, while in 30 other herds with a low CR in cows of below 40% (average 35%) that of the NH was of but 55.4% (p<0.001). The correlation between the CR of NH and of cows was low, in the below 40% herds 0.239, and -0.273 in the 45% and above ones.

The dry matter percentage of the roughage fed to NH groups located in the below 40% CR herds was 44% compared to 57.9% fed to NH groups located in the above 45% CR herds (p<0.006) (Table 1).

The amounts of hay fed were significantly lower and the amounts of concentrates fed were significantly higher in NH groups located in the below 40% CR herds compared to NH groups located in the above 45% CR herds (Table 1).

TABLE 1: Least square means for Feeding Factors to Nullipara Heifers in herds with Conception Rates of below 40% and above 45% in Cows.

Feeding Factor	Conception Rates of Multiparas		Significance
	below 40%	above 45%	
Dry Matter (D.M.) kg	8.19±0.17	8.31±0.17	0.622
Metabolised Energy Mcal	17.50±0.63	17.48±0.61	0.979
Protein %	9.10±0.36	9.09±0.35	0.894
Energy Mcal : Protein kg	1.97±0.08	1.95±0.08	0.863
Roughage % from total DM	44.00±3.50	57.90±3.40	0.006***
Hay kg	0.03±0.21	0.80±0.20	0.012**
Concentrates kg	1.69±0.24	0.91±0.24	0.027*

When the nullipara groups were divided according to their CR with 59% the dividing point, amounts of roughage, hay and concentrates fed in the below 59% and the above 59% CR groups were extremely similar to those fed when the division was performed to the NH location in the below 40% and above 45% CR Herds (Table 1 and Table 2).

TABLE 2: Least square means for Feeding factors to Nullipara groups having Conception Rates of below and above 59%.

Feeding Factor	Conception Rates of Nulliparas		Significance
	below 59%	above 59%	
Dry Matter (D.M.) kg	8.19±0.17	8.33±0.17	0.554
Metabolised Energy Mcal	17.70±0.61	17.20±0.63	0.586
Protein %	8.96±0.35	9.30±0.36	0.506
Energy Mcal : Protein kg	2.04±0.08	1.88±0.08	0.128
Roughage % from total DM	44.53±3.41	58.36±2.54	0.007***
Hay kg	0.03±0.20	0.86±0.21	0.006***
Concentrates kg	1.72±0.23	0.82±0.24	0.009**

There appears to be a tendency to a wider energy/protein ratio in the below 59% CR nullipara heifer groups (Table 2).

A comparison of NH groups having a CR of above 55% with those having a CR of below 50% showed a lower protein content of the ration in the below 50% CR group ($p<0.042$).

DISCUSSION

The correlation between the Conception Rate of NH and those of the cows was low, thus the fertility level of those two groups in the herds seems to be quite independent from each other which coincides with other findings (13,7). On the other hand NH in herds with low CR of cows had highly significant lower CRs compared to NH in herds with a fair CR of the cows. This apparent contradiction can but be explained by the existence of factors in the herds surveyed having a low CR which depress NH fertility. One of these factors was the low content of roughage in the ration fed NH, particularly a low content of hay. The detrimental effect of low roughage rations on the fertility of dairy cows was found in many previous investigations on dairy herd fertility in Israel, where lack of pasture and the high alternative value of water for irrigation sharply limit it's supply (5,2,10,1). The feeding of a diet containing but 40% of roughage from total dry matter to adult Holstein bulls, was connected with significantly lower spermatozoa concentrations compared to that of bulls fed a 60% roughage containing ration (3). Our investigation showed that the ration fed to NH with a low CR contained 44% of roughage on a dry matter basis, compared to 57.9% fed the fair CR nullipara heifer groups. It thus appears that a low roughage content of the ration is a risk factor for the fertility not only of lactating cows but to non-lactating dairy cattle as well.

In the NH groups having a CR above 59%, the relationship energy/protein tended to be narrower than that of the below 59% ones (Table 2). Similar data were found by (4). Relative amounts of protein and energy yielding nutrients are likely to determine the net efficiency of absorbed nutrients (11). The lowered protein levels found in the NH groups having a CR of below 50% supplies additional evidence on the influence of the protein factor on the NH conception rate.

In conclusion it appears that the level and the quality of the roughage fraction, the protein density and the energy/protein ratio should be considered as risk factors for the fertility of Nullipara Heifers.

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SUMMARY

An extensive epidemiological study was performed in order to ascertain the factors connected with herd fertility levels. 60 Kibutz industrial sized dairy herds milking an average of 300 cows and containing about 100 nullipara heifers each, were analyzed. Within the framework of this study, factors influencing the fertility of Nullipara Heifers (N.H.) were investigated.

The conception rate of N.H. was connected with the conception rate (CR) of the dairy cows in each herd. In 30 herds having a fair CR of 48% in cows that of the N.H. was 63.8%, while in 30 other herds having a low CR of 35% that of the N.H. was of but 55.4% ($p<0.001$).

The total Dry Matter content of the roughage fed N.H. groups having a CR of above 59% was 58.4%, compared to 44.5% in N.H. groups having a CR of below 59% ($p<0.007$).

The amount of hay fed were significantly higher ($p<0.006$) in the above 59% CR groups when compared to the below 59% CR groups of N.H.

The Energy/Protein ratio tended to be wider in the below 59% CR groups of N.H.

A comparison of N.H. groups having a CR of above 55%, with N.H. groups having a CR below 50% showed that the protein content of the ration was significantly lower in the below 50% CR groups ($p<0.042$).

RESUME

Une étude épidémiologique de grande envérgure a été faite dans le but d'établir les facteurs liés a la fécondité dans les troupeaux laitiers.

60 troupeaux laitiers type Kibutz, ayant une moyenne de 300 vaches allaitantes et contenant chaqu'un environ 100 genisses nullipares (G.N.) ont été analysés. Dans le cadre de cette étude les facteurs ayant une influence sur la fécondité des G.N. ont été récherchés.

Le taux de conceptions (TC) des G.N. a été lie à celui des vaches en lait dans chaque troupeau. Dans 30 troupeaux ayant un TC des vaches de 48% celui des G.N. a été de 63.8%, tandis que dans 30 autres troupeaux ayant un mauvais TC des vaches, celui des G.N. n'a été que de 55.4% ($p<0.001$).

La quantité de matière sèche d'origine fourragère consommée par les groupes de G.N. ayant un TC au dessus de 59% etait 58.4% comparée a 44.5% dans groupes de G.N. ayant un TC en dessous de 59% ($p<0.007$).

Les quantitées de foins consommées etaient plus hautes dans les groupes avec TC depassant 59% que dans ceux ayant un TC en dessous de 59% ($p<0.006$).

La relation énergie/protéines avait tendance a être plus large dans les groupes de G.N. ayant un TC en dessous de 59%.

Une comparaison entre groupes de G.N. ayant des TC depassant 55% avec groupes de G.N. ayant des TC en dessous de 50% montrait que le contenu de la ration en protéines etait plus bas dans les groupes avec un TC en dessous de 50% ($p<0.042$).

ZUSAMMENFASSUNG

Eine extensive epidemiologische Forschung wurde mit dem Ziel durchgefuehrt die Faktoren die mit der Fruchtbarkeit in den Milchkuhherden verbunden sind zu determinieren.

60 Kibutz Milchherden die einen Durchschnitt von 300 Kuehen melken und die je weitere 100 Nullipara-Faersen (N.F.) mitzuechten, wurden analysiert. In diesen Studien wurden die Faktoren die die Fruchtbarkeit der N.F. beeinflussen speziell erforscht.

Die Konzeptionsrate (KR) der N.F. war mit der allgemeinen KR der Kueho in Milch in denselben Herden verbunden. In 30 Herden die eine akzeptable 48% KR in Kuehen erreichten war die der N.F. 63.8%, waehrend in 30 anderen Herden mit schwacher, unter 35% KR in Kuehen die N.F. nur 55.4% erreichten ($p<0.001$).

Die aus Rauhfutter stammende Trockensubstanz war 58.4% in der Futterration der N.F. Gruppen mit einer KR von ueber 59%, und nur 44.5% in den N.F. Gruppen mit einer unter 59% liegenden KR ($p<0.007$).

Die Quantitaeten von Heu waren signifikant hoeher ($p<0.006$) in den ueber 59% KR als in den unter 59% liegenden KR Gruppen.

Die Energie/Protein Bilanz war breiter in den unter 59% KR Gruppen.

Ein Vergleich von N.F. Gruppen die eine KR von ueber 55% aufwiesen, mit Gruppen die eine KR von unter 50% hatten, zeigten das der Proteingehalt der Rationen in den unter 50% KR Gruppen signifikant tiefer war ($p<0.042$).

EPIDEMIOLOGIA DE LA ULCERA PREPUCIAL BOVINA EN EL URUGUAY.-

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INTRODUCCION

La Ulcera Prepucial Bovina (UPB), postitis ulcerativa, acrobustitis, o como se la denomina comunmente "llaga de prepucio",es una afección de importancia en nuestro país. (8)(9)

Esta enfermedad se traduce básicamente por lesiones ulcerativas de la piel del prepucio y mucosa del orificio prepucial, afectando toros de diferentes razas, edades y estado nutricional, con grados variables de morbilidad que pueden llegar hasta el 100%, pudiendo en los casos graves llevar al descarte del reproductor. (6)(8)(9)

El agente etiológico se mantiene en discusión.

Brook et al. (1965), estudiaron la relación entre la urea de la orina y la facilidad de infección. Según los autores, debe haber una estrecha relación entre el pH de la orina y los niveles proteicos que determinan la afección prepucial. (2)

Riet et al. (1982), no pudieron demostrar la participación del Corynebacterium renale, mas confirmaron que la prevalencia de la enfermedad aumenta con el mejoramiento de las condiciones nutricionales. (10)

Southcott (1965), desarrolló la UPB en toros Jersey mediante la escarificación de la mucosa prepucial y posterior inoculación con C.renale.(11

Anon (1967), en 50 toros afectados con un 100% de morbilidad encontró un gran crecimiento de C. renale a partir de las muestras obtenidas de las lesiones, mejorando la condición de los mismos mediante antibioticoterapia. (1)

Riet et al. (1978,1979) elaboraron una hipótesis por cuanto la UPB sería una dermatitis debida primariamente al amoníaco producido al ser hidrolizada la urea por C. renale, explicando la mayor incidencia en toros con una ingesta de elevado contenido proteico ya que como consecuencia eliminan mayor cantidad de nitrógeno en forma de urea. (8)(9)

Huck et al. (1971) reportaron casos de balanopostitis asociados al virus IBR. (4)

Riet et al. (1978,1979), realizaron sus estudios en la raza Hereford encontrando una morbilidad del 59.7%. (8)(9)

Parsonsons (1972), estudiando la incidencia en varias razas, encontró tasas de morbilidad del 18% en Hereford, del 7.1% en Aberdeen Angus, 7.1% para Shorthorn y 9% para la raza Brahman y sus cruzas. (7)

Riet Correa et al. (1978,1979) encontró que las categorías más afectadas eran las de toros de 18 a 36 meses, con un 44,4% de grados 3 de severidad de infección. (8)(9)

El objetivo del presente trabajo es el estudio de los factores epizootiológicos de la enfermedad en condiciones de campo.

MATERIALES Y METODOS

1. Se estudiaron 1349 toros pertenecientes a 28 establecimientos rurales, de las razas Hereford (418), Polled Hereford (558), Simmenthal-Fleckvieh (238) y Aberdeen Angus (135).

2. Se clasificaron las lesiones de acuerdo con la clasificación propuesta por F. Riet et al. (8)(9)

3. Se remitieron muestras para aislamiento bacteriológico y despiste serológico de IBR al CIVet MC Rubino.

4. Se analizaron los datos de acuerdo a: incidencia por raza, grado de afección por raza, incidencia por categoría dentro de cada raza.

5. Se realizó análisis estadístico de los resultados mediante la prueba de X^2 (Chí cuadrada).

RESULTADOS

De las lesiones se aisló C. renale y C. hoffmanii.

Las muestras remitidas para realizar análisis serológico a IBR dieron positivas con un título de 1:8.

Las variaciones debidas al mejoramiento de las condiciones nutricionales (ver tabla I) estudiadas para la raza Hereford fueron altamente significativas ($P<0.005$) mostrando que los toros con mejores condiciones nutricionales se verían mas afectados.

El 36.69% de los toros se encontraron afectados. Sin embargo, la distribución de la enfermedad por raza no fue uniforme ($P<0.005$) variando ampliamente de una raza a otra. (ver tabla I)

La raza más afectada fue la Hereford (61.44%), seguida de la Fleckvieh (36.97%), la Polled Hereford (26.52%), encontrando que la raza Aberdeen Angus casi no es afectada por la enfermedad siendo su morbilidad casi nula (2.2%).

Estudiando los grados de afección dentro de cada raza, se encontró que su distribución es también desigual ($P<0.005$), mostrando una clara tendencia a la presentación de casos leves, grados 1 y 2 de la clasificación de Riet. (ver tabla II)

Con respecto a la edad, se realizaron estudios sólo en las razas Hereford y Fleckvieh (ver tabla III). La raza Hereford, si bien los resultados porcentuales muestran a los toros de 18 a 36 meses como los más afectados, estadísticamente la tendencia es a la uniformidad ($P=0.10$). En Fleckvieh, los resultados son altamente significativos ($P< 0.005$), mostrando desuniformidad en su distribución, con una clara tendencia a mostrarse afectados los toros de 3 y 4 años.

DISCUSION

Konte (1988) afirma que el C. renale es un habitante natural de la flora prepucial, siendo un potencial patógeno del mismo. (5)

Doherty (1985), aisló C. hoffmanii de lesiones de postitis en carneros.(3) Los autores creen que es importante incluirlo entre los probables causantes de la UPB.

Si bien se encontró que los factores nutricionales aumentan la inci-

ESTABLECIMIENTO	RAZA	CALIDAD DE NUTRIENTES	TOTAL TOROS	ENFERMOS	% MORBILIDAD
1	H	CN	12	9	0.75
2	H	CN	12	9	0.75
3	H	CN+P	80	78	0.97
4	H	CN+P	87	75	0.86
5	H	CN	23	13	0.43
6	H	CN	21	2	0.09
7	H	CN	1	1	1.00
8	H	CN	12	7	0.58
9	H	CN	53	23	0.43
10	H	CN	50	12	0.24
11	H	CN	3	3	1.00
12	H	CN	52	19	0.36
13	H	CN	12	5	0.41
			418	256	0.6144
14	PH	CNM	137	41	0.29
15	PH	CNM	209	51	0.24
16	PH	CNM	212	56	0.26
			558	148	0.2652
17	F	CN+P	51	20	0.39
18	F	CN+P	15	10	0.66
19	F	CN	2	0	0.00
20	F	CNM	29	11	0.37
21	F	CNM	57	9	0.15
22	F	CNM	51	14	0.27
23	F	CNM	33	24	0.72
			238	88	0.3697
24	AA	CN	21	2	0.09
25	AA	CN	20	1	0.05
26	AA	CN	1	0	0.00
27	AA	CN	3	0	0.00
28	AA	CN	90	0	0.00
			135	3	0.02222
28			1349	495	0.3669

TABLA I.- H: Hereford; PH: Polled Hereford; F: Fleckvieh; AA: Aberdeen Angus; CN: Campo Natural; M: Mejorado; P: Pradera.
Detalle de establecimientos y casos estudiados.

RAZA	%º1	%º2	%º3	%º4
HEREFORD	0.3710	0.2656	0.1953	0.1679
POLLED HEREFORD	0.4130	0.4347	0.1086	0.0434
FLECKVIEH	0.4923	0.2615	0.1384	0.1076
ABERDEEN ANGUS	1	0	0	0

TABLA II.- Tasas de morbilidad según grado de afección de acuerdo a cada raza.

	HEREFORD			FLECKVIEH		
EDAD	S	E	%M	S	E	%M
BLL	47	46	0.494	4	2	0.333
6D				2	7	0.777
4D	33	37	0.528	8	16	0.666
2D	60	147	0.710	9	11	0.550
0L	17	31	0.645	17	8	0.320

TABLA III.- Morbilidad por categoría. S: Sanos; E: Enfermos; %M: tasa de morbilidad.

dencia de la enfermedad, se piensa que esto no es concluyente por si solo para el desarrollo de la enfermedad.

Con respecto al agente etiológico, frente al panorama descrito en la introducción de este trabajo, los autores expresan que la hipótesis planteada por Riet et al. (1978) de que la UPB sería una dermatitis debida primariamente al amoníaco producido al ser hidrolizada la urea por el C. renale, explicando la mayor incidencia en toros con una ingesta de elevado contenido proteico ya que como consecuencia eliminan mayor contenido de nitrógeno en forma de urea, es la que explica mejor el desarrollo de la enfermedad. (8)(9) No obstante, habría que introducir ciertas modificaciones a la misma, como incluir al C. hoffmanii y no descartar la probable participación del virus IBR como factor predisponente. (3)(4)

Las variaciones raciales encontradas indican que la raza Hereford es la más afectada al igual que otros autores. (1)(2)(6)(7)(8)(9)

No se encontraron referencias bibliográficas respecto a la menor incidencia de Polled Hereford frente a Hereford.

Nielsen opina que los Aberdeen Angus se afectan en menor grado dada la pigmentación del prepucio. (6)

Parsonsons sin embargo refuta a Nielsen diciendo que se afectan en igual forma que otras razas. (7) Los autores creen que las postitis descritas por Parsonsons podrían ser eversiones de mucosa prepucial tan frecuentes en la raza, infectadas a posteriori.

En los trabajos de F. Riet encontramos un mayor porcentaje de casos

graves (44,4% de grados 3) que los hallados por los autores de este trabajo (19.53% º3) (8)(9). Si consideramos que la medida de los grados de afección por el clínico es totalmente subjetiva, igualmente se cree que esta diferencia pueda expresar una atenuación de la cepa de campo o una respuesta inmunitaria por parte de los toros.

La edad de toros Fleckvieh afectados (3 y 4 años) coincide con el rango de edad descrito por Parsonsons. (7)

En la raza Hereford, si bienla distribución tiende a la uniformidad, los toros de 18 a 36 meses de edad reúnen la mayor cantidad de casos porcentuales concordando apenas con lo observado por Riet. (8)(9)

Por el tipo de asistencia que se presta a los establecimientos atendidos no se ceryó conveniente realizar estudios acerca de clima y epoca del año.

CONCLUSIONES

1. El agente etiológico estaría dado por la interacción de C. renale y C. hoffmanii con el aumento de las condiciones nutricionales de los toros, no pudiendose descartar al virus IBR como factor predisponente.
2. La UPB afectó en forma desigual las razas en estudio.
3. La raza Aberdeen Angus presentó marcada resistencia a la UPB.
4. En lo que respecta a otras razas, la más susceptible es la Hereford, seguida de la Fleckvieh y la Polled Hereford.
5. Hay que resaltar el diferente comportamiento frente a la enfermedad de Hereford y Polled Hereford.
6. Los grados de afección se distribuyeron desigualmente siendo relativamente pocos los casos de gravedad.
7. En la raza Hereford las categorías se afectaron en forma similar con ligera tendencia hacia las categorías más jóvenes (18 a 36 meses).
8. En la raza Fleckvieh los toros más afectados fueron los de 3 a 4 años de edad.

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RESUMEN

Se estudiaron algunos factores epizootiológicos de la Ulcera Prepucial Bovina en 1349 toros pertenecientes a las razas Hereford (418), Polled Hereford (558), Simmenthal-Fleckvieh (238) y Aberdeen Angus (135).

Se discute la etiología de la Ulcera Prepucial, entendiendo que es el resultado de la interacción de C. renale y C. hoffmanii con el aumento de las condiciones nutricionales, no pudiendo descartar al virus IBR como factor predisponente.

La raza Hereford fuela mas afectada (61.44%), seguida de la Fleckvieh (36.97%) yla Polled Hereford (26.52%). La raza Aberdeen Angus no se mostró casi afectada (2.2%)

En la raza Fleckvieh, los toros de 3 y 4 años fueron los más afectados. En las otras razas la edad no fue significativa.

La mayoría de los toros se afectaron en forma leve, no habiendo casi casos de gravedad.

SUMMARY

Some epizoothiology factors of preputial ulcer in bulls has been studied in 1349 bulls belong race's Hereford (418), Polled Hereford (558), Simmenthal-Fleckvieh (238) and Aberdeen Angus (135).

Aetiology agent of preputial ulcer was discussed, understanding that preputial ulcer was the result of nutritional condition and the bacters: C.renale and C. hoffmanii. IBR virus participation was no descarted.

Hereford proved to be most affected (61.44%), in second place Fleckvieh (36.97%), and Polled Hereford (26.53%). Aberdeen Angus was no significative (2.2%).

In Fleckvieh, three and four years old bulls were the most affected. In the other races the age was no significative.

Very little amount of bulls were seriously affected.

RÉSUMÉ

On a étudié quelques facteurs epizootiologiques de l'ulcère prépucial bovine en 1349 taureaux qui appartient aux races: Hereford (418), Polled Hereford (558), Simmenthal-Fleckvieh (238) et Aberdeen Angus (135).

On a discuté l'étiologié de l'ulcère prépucial bovine en entendu qu'il est le résultat de l'interaction de C. renale et C. hoffmanii avec l'augment de conditions alimentaires, sans décartér le virus IBR comme facteur prédisposant.

La race Hereford a été la plus abattu (61.44%), suivi la race Fleckvieh (36.97%) et la race Polled Hereford (26.52%). La race Aberdeen Angus ne se trouve presque abattu (2.2%)¿

Dans la race Fleckvieh, les taureaux de 3 et 4 ans out été les plus abattus. En autres races l'âge n'a pas été significatif.

La plupart des taureaux ont été abattus lègérment sans trouver de cas désespérés.

KRANKHEITSVERLAUF SOWIE FSH- UND LH-BLUTSPIEGEL VOR UND NACH BUSERELIN-APPLIKATION BEI KÜHEN MIT FOLLIKEL-THEKA-ZYSTEN

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EINLEITUNG

Zur Behandlung der Follikel-Theka-Zysten des Rindes werden seit Beginn der 70er Jahre GnRH- Analoge eingesetzt, und zwar zunächst das Dekapeptid Gonadorelin in einer Dosierung von 0,1 mg (8) bzw. 0,5 mg (5), später das Nonapeptid Buserelin in einer Dosierung von 20 µg (7).

Aufgrund von Untersuchungen an bovinen Hypophysenvorderlappen-Zellkulturen wird angeregt, die Frage der GnRH-Dosierung bei Kühen mit Follikel-Theka-Zysten zu überprüfen (1). Deshalb versuchten wir zu klären, ob nach Applikation des GnRH-Agonisten "Buserelin" (Receptal(R)-Hoechst) in hoher Dosierung (50 µg) bessere Behandlungserfolge zu erzielen sind als mit der empfohlenen Dosierung (20 µg).

MATERIAL UND METHODIK

Versuchstiere

Untersucht wurden 21 Kühe und 4 Färsen (vorwiegend "Deutsche Schwarzbunte"). Die Zysten wurden im Ø 16,4 Wochen post partum diagnostiziert (4,5 bis 83,5 Wochen p.p.). Elf Tiere zeigten längere Zeit keine äußeren Brunstsymptome, drei weitere Kühe hatten p.p. einmal gebullt, danach blieb die Brunst aus. Unregelmäßig mit teilweise längeren Brunstperioden rinderten 10 Kühe. Von einem Tier fehlten Angaben. Zweiundzwanzig Tiere zeigten ein Einfallen der Beckenbänder sowie ein Vulvaödem.

Die 25 Tiere wurden zweimal im Abstand von 8 bis 12 Tagen gynäkologisch untersucht, um sicher zu sein, daß es sich um persistierende Follikel-Theka-Zysten als Ursache einer Subfertilität handelte. Sowohl bei der 1. als auch bei der 2. Untersuchung wurde eine Blutprobe zur Progesteronbestimmung entnommen. Nach dem Zentrifugieren des Blutes wurde das Plasma bei -20°C bis zur Hormonbestimmung gelagert.

Die Behandlung der Versuchstiere erfolgte einige Tage nach der Zweituntersuchung, d.h. nach Erhalt des Ergebnisses der Progesteronuntersuchung, mit Buserelin in zwei unterschiedlichen Dosierungen, und zwar alternierend (Gruppe A und B). In der Gruppe A (Ø Progesteronwert 0,52 ± 0,17 ng/ml Plasma) wurden 12 Kühen 20 µg Buserelin, in der Gruppe B (Ø Progesteronwert 0,46 ± 0,26 ng/ml Plasma) 13 Tieren 50 µg Buserelin intramuskulär appliziert. Kurz vor und 2 Std. nach der Buserelingabe wurden Blutproben für die FSH- und LH-Bestimmungen entnommen. Acht bis 12 Tage nach der Behandlung wurden die Kühe wieder gynäkologisch untersucht sowie Blutproben zur Progesteronbestimmung entnommen. Mit Ausnahme von 6 zur Schlachtung vorgesehenen Kühen erfolgte bei den anderen Tieren die Besamung bei Auftreten der ersten Brunst post medicationem. Nicht umrindernde Tiere wurden 6 bis 8 Wochen post inseminationem auf Trächtigkeit untersucht, umrindernde Tiere besamt.

Prof. Dr. D. Schams und Dr.Dr. habil. E. Schallenberger, Freising-Weihenstephan, sei für die FSH- und LH-Bestimmungen herzlich gedankt.

HORMONBESTIMMUNGEN

Die Bestimmung von Progesteron (6) sowie von FSH und LH (11) im Blutplasma erfolgte nach einem radioimmunologischen Verfahren.

ERGEBNISSE

Klinische Ovarbefunde sowie Progesteronwerte

12 Tage post medicationem war bei 17 der 25 behandelten Kühe mit Follikel-Theka-Zysten ein Gelbkörper fühlbar. Die Blutproben wiesen einen Progesterongehalt von Ø 8,3 ± 7,0 ng/ml (Gruppe A) und Ø 5,2 ± 1,9 ng/ml (Gruppe B) auf. Zwei Tiere hatten 12 Tage nach der Behandlung hohe Progesteronwerte, ohne daß ein Gelbkörper palpiert werden konnte. Sie kamen 20 bzw. 23 Tage nach der Behandlung in Brunst. Die erste Brunst post medicationem trat bei den 8 Kühen der Gruppe A Ø 24,6 ± 8,1 Tage, bei den 11 Kühen der Gruppe B Ø 22,6 ± 11,1 Tage auf. Bei einer Kuh der Gruppe A sowie 2 Kühen der Gruppe B war die Gelbkörperbildung post medicationem verzögert. Die Tiere hatten zum Zeitpunkt der Nachuntersuchung (10. Tag post med.) einen Progesteronwert von 0,2, 0,6 bzw. 0,3 ng/ml; ein Gelbkörper war nicht zu palpieren. Die erste Brunst trat 10, 11 bzw. 29 Tage nach der Buserelingabe auf. Bei 3 weiteren Kühen war ebenfalls 12 Tage post medicationem weder ein Gelbkörper fühlbar noch Progesteronwerte über 1 ng/ml festzustellen. Auch konnte später kein Gelbkörper palpiert werden.

Eine Färse und 3 Kühe der Gruppe A und 2 Färsen sowie eine Kuh der Gruppe B blieben azyklisch oder wurden bald nach der Behandlung geschlachtet. Das Erstbesamungsergebnis der 8 Tiere in Gruppe A betrug 25%, das der 11 Tiere der Gruppe B 45,5%. Das Gesamtträchtigkeitsergebnis lag in Gruppe A bei 55,6%, in Gruppe B bei 72,7%. Zwei Tiere der Gruppe A wurden später mit der PRID-Spirale bzw. mit 20 µg Buserelin nachbehandelt und wurden nach einer Besamung tragend. Damit erhöhte sich die Gesamtträchtigkeitsrate der Tiere in Gruppe A auf 77,8%. Der Besamungsindex betrug in Gruppe A 2,0, in Gruppe B 1,5. Das Intervall zwischen Behandlung und Trächtigkeit betrug bei den Tieren der Gruppe A Ø 47,6 ± 26,8, bei den Tieren der Gruppe B 42,6 ± 26,1 Tage.

FSH- und LH-Werte vor und nach Buserelinverabreichung

Die Ø Basiswerte von FSH (Blutentnahme unmittelbar vor der Buserelingabe) unterschieden sich nur wenig zwischen den Gruppen (Gruppe A (n=12): Ø 47,3 ± 9,9 ng/ml; Gruppe B (n=13): Ø 50,1 ± 13,2 ng/ml). Der Anstieg von FSH 2 Std. nach Buserelingabe war jedoch in den beiden Gruppen unterschiedlich (Anstieg: Gruppe A: Ø um 7,6 ng/ml; Gruppe B: Ø um 26,3 ng/ml). Während bei 5 Tieren der Gruppe A die FSH-Basiswerte höher als die Endwerte waren, konnte dies bei nur 3 Tieren der Gruppe B beobachtet werden. Die LH-Basiswerte unterschieden sich zwischen den beiden Gruppen nicht. Zwei Stunden nach Buserelinverabreichung betrug der Anstieg der LH-Durchschnittswerte bei den 12 Tieren der Gruppe A 31,8 ng/ml, bei den 13 Tieren der Gruppe B sogar 45,3 ng/ml.

In der Gruppe A entsprach der Anstieg der LH-Konzentration einer 14,8-fachen Steigerung bezogen auf den Ausgangswert, während es für FSH nur zu einer 1,2-fachen Steigerung führte. In der Gruppe B war nach der Behandlung mit 50 µg Buserelin eine 19,8-fache Steigerung von LH festzustellen. Bei den FSH-Werten betrug die Steigerung nur das 1,5-fache des Anfangswertes. In dieser Gruppe lag bei einem Tier ein extremer FSH-Endwert von 200 ng/ml vor. Für den FSH- und LH-Endwert ergaben sich signifikante Abweichungen zwischen den verschieden hohen Dosierungen

Übersicht 1: FSH- und LH-Werte vor und 2 Std. post medicationem von 25 Kühen mit Follikel-Theka-Zysta

Tier Nr.	FSH Basis-Wert	FSH End-Wert	LH Basis-Wert	LH End-Wert	C.l. oder Pg 1 *	1. Brunst p. medic. (in Tagen)	danach Zyklus	Anzahl Besamungen	Intervall zw. Beh. u. Trächt.
Gruppe A									
15	52,8	84,8	2,6	20,0	-	--	-	-	Schlachtt.
19	62,6	52,5	1,3	38,0	+	15	+	6	neg.
22	84,8	66,9	2,6	40,0	+	19	+	2	40 Tage
23	58,8	82,8	2,5	28,5	+	7	+	-	Schlecht.
26	37,3	77,9	2,4	48,5	+	10	+	7	neg.
33	53,1	84,2	3,2	25,5	-	37	-	-	neg.
34	39,0	67,8	2,5	36,5	+	25	+	2	52 Tage
37	36,8	60,6	2,4	38,5	+	20	+	1	20 Tage
43(Färse)	35,3	46,4	2,2	47,0	+	7	+	-	Schlecht.
52	35,0	33,5	2,0	31,8	-	29	+ (verzög.)	4	neg.
62	57,1	54,8	2,4	18,5	+	24	+	4	91 Tage
65	51,9	69,6	1,9	33,5	+	36	+	1	35 Tage
	Ø 47,3	Ø 54,9	Ø 2,3	Ø 34,1	* 6-12 d p.med.	Ø 24,2			Ø 47,6
	± 9,9	± 15,7	± 0,5	± 9,6		± 8,1			± 26,8
Gruppe B									
7(Färse)	51,5	113,2	1,6	80,5	+	38	+	1	38 Tage
14	48,8	82,4	2,2	53,8	+	47	+	1	47 Tage
20(Färse)	37,9	83,8	2,3	44,5	-	--	-	-	Schlachtt.
21	74,1	96,7	2,9	88,5	+	23	+	2	66 Tage
24(Färse)	63,4	200,0	2,4	69,5	+	?	+	-	Schlecht.
25	46,6	82,3	3,0	58,5	+	20	+	1	20 Tage
40	30,2	47,6	2,7	51,5	+	28	+	1	20 Tage
48	51,2	63,6	1,9	41,0	+	24	+	3	neg.
58	52,8	75,2	2,8	41,5	+	14	-	1	14 Tage
59	61,4	56,5	2,1	33,0	+	20	+	4	neg.
60	38,6	48,8	2,5	53,5	-	11	+ (verzög.)	3	91 Tage
63	61,9	51,6	1,8	26,5	+	22	+	2	45 Tage
66	32,1	31,7	2,8	30,0	-	10	+ (verzög.)	6	neg.
	Ø 50,1	Ø 76,4	Ø 2,4	Ø 47,7	* 6-12 d p.med.	Ø 22,6			Ø 42,6
	± 13,2	± 43,1	± 0,5	± 15,7		± 11,1			± 26,1

(α = 0,1; t-Test) (Übersicht 1). Die Endwerte von FSH und LH der beiden Tiere, bei denen 10 Tage post medicationem kein Corpus luteum palpiert werden konnte, entsprachen denen der anderen Tiere. Die bereits 10 bis 14 Tage nach der Behandlung in Brunst gekommenen 3 Tiere zeigten hinsichtlich ihrer FSH- und LH-Werte ebenfalls keine Besonderheiten.

DISKUSSION

Wegen der vorwiegend in den ersten 6 Wochen post partum gehäuft auftretenden Selbstheilung der Ovarzysten sowie der Tatsache, daß Follikel-Theka-Zysten nicht immer eine Subfertilität bedingen müssen, wurden die Zystentiere sorgfältig ausgewählt. Eine baldige Selbstheilung bei unseren Versuchstieren war insofern wenig wahrscheinlich, als die Erstuntersuchung im Mittel 124,5 Tage post partum erfolgte und die Tiere ein-, z.T. zweimal im Abstand von 8 bis 12 Tagen nachuntersucht wurden. Zusätzliche Veränderungen an Beckenbändern (Einfallen) und Vulva (Ödem) sowie Progesteronwerte unter 1 ng/ml Plasma bei den Eingangsuntersuchungen sicherten die Diagnose. Die ermittelten ø FSH-Werte vor der Buserelinapplikation (Gruppe A: ø 47,3 ± 9,9; Gruppe B: ø 50,1 ± 13,2 ng/ml Plasma) entsprachen den von anderen Autoren (2, 4) bei Kühen mit Follikel-Theka-Zysten registrierten Werten (53,1 ± 22 bzw. 52 ± 5 ng/ml). Sie lagen jedoch unter den von weiteren Autoren (2, 11) angegebenen Werten für regelmäßig rindernde Kühe. Die von uns beobachtete hohe LH- und z.T. auch hohe FSH-Ausschüttung bei Kühen mit Follikel-Theka-Zysten steht im Gegensatz zu In-vitro-Versuchen an entsprechenden bovinen Hypophysenvorderlappen-Zellkulturen (1).

Während alle Versuchstiere 2 Std. nach der Buserelinapplikation mit einem deutlichen LH-Peak reagierten, wurden bezüglich der FSH-Werte bei 17 Kühen ein Anstieg und bei 8 Versuchskühen (5 der Gruppe A und 3 der Gruppe B) ein Abfall der FSH-Werte nach der Medikation festgestellt. Dieser Abfall hatte keinen Einfluß auf die Gelbkörperinduktion sowie auf den Zeitpunkt des Auftretens der 1. Brunst post medicationem. Bei den beiden Tieren, die azyklisch blieben, waren sowohl die FSH-Basis- als auch die FSH-Endwerte hoch (52,8 bzw. 37,9 ng/ml zu 64,0 bzw. 83,9 ng/ml). Die von uns ermittelten LH-Werte 2 Std. nach Buserelinverabreichung liegen mit 34,1 ± 9,6 ng/ml (20 ug) und 47,7 ± 15,7 ng/ml (50 ug) deutlich unter den von anderen Autoren (2) nach Applikation von 20 ug Buserelin angegebenen LH-Werten (75 ng/ml). Das von uns ermittelte LH/FSH-Verhältnis war mit (0,62 [20 µg] bzw. 0,63 [50 µg]) niedriger als das anderer Autoren (0,78) (2). Die bei höherer Buserelindosierung größere Ausschüttung sowohl von FSH als auch von LH ist bezüglich der Zyklusinduktion (in 88% der Fälle möglich) nicht entscheidend. Der ø Progesterongehalt im peripheren Blut am 10. bis 12. Tag post medicationem betrug bei den 9 Kühen der Gruppe A ø 8,3 ± 7,0 ng/ml und bei den 10 Tieren der Gruppe B ø 5,19 ± 1,9 ng/ml, d.h. es wurde ein funktionstüchtiger Gelbkörper gebildet. Das Intervall zwischen Behandlung und Auftreten der 1. Brunst (24,4 ± 8,1 vs. 22,6 ± 11,1 Tage) entspricht den Angaben anderer Autoren (3) mit ø 20,1 ± 1,5 Tagen. Sie konnten ebenfalls große Unterschiede im Auftreten der ersten Brunst post medicationem beobachten (Schwankungen von 9 bis 27 Tagen). Unterschiede zwischen den Gruppen A und B bestanden dagegen sowohl beim Erstbesamungsergebnis (25,0% vs. 45,5%), beim Gesamtträchtigkeitsergebnis (55,6% vs. 72,7%) sowie beim Intervall zwischen Behandlung und Trächtigkeit (47,6 ± 26,8 Tage vs. 42,6 ± 26,1 Tage). Auch lag der Besamungsindex mit 2,0 bei Tieren der Gruppe A (20 µg Buserelin) höher als bei Tieren der Gruppe B (50 µg Buserelin) mit 1,5. Dies dürfte jedoch nicht mehr der Behandlung zugeschrieben werden können. Durch die

Buserelingabe kann lediglich - durch die provozierte LH-Ausschüttung - eine Luteinisierung der Zystenwand und/oder eine Ovulation eines bereitstehenden Tertiärfollikels mit anschließender Bildung eines funktionstüchtigen Gelbkörpers bewirkt werden. Dies ist - von wenigen Ausnahmen abgesehen - in beiden Gruppen erreicht worden. Nicht zu erklären ist das verzögerte Einsetzen des Zyklus bei 3 Tieren. Ebenfalls unklar bleibt die ausgebliebene Zyklusinduktion bei 3 Tieren. Ob bei diesen Tieren die Zystenwand so atrophisch war, daß keine Luteinisierung mehr möglich war, keine Tertiärfollikel vorhanden waren oder LH-Rezeptoren an den Ovarien fehlten, konnte nicht ermittelt werden. Der Anteil der Kühe, bei denen nach Buserelinverabreichung kein Progesteronanstieg erfolgte, entspricht mit 24% den Ergebnissen anderer Autoren (9, 10), die je 20% ermittelten, überein. Das um 29 Tage verzögerte Einsetzen des 1. Zyklus nach der Behandlung bei einer Färse der Gruppe A (Progesteronwert 10 Tage p.med. 0,3 ng/ml) muß nicht der Behandlung, sondern einer Selbstheilung zugeschrieben werden. Inwieweit die verzögerte Zyklusinduktion bei Kühen (10 bzw. 11 Tage nach Buserelingabe) durch die Medikation bedingt ist, bleibt unklar. Der ausbleibende Behandlungserfolg bei 2 Versuchstieren, d.h. die fehlende Ausbildung eines Gelbkörpers mit nachfolgender Brunst, ist mit den vorliegenden Hormonbefunden nicht zu erklären. Bei einer Färse fehlten anamnestische Daten, bei dem anderen Tier handelte es sich um eine bereits 9jährige Kuh mit 6 Abkalbungen, die in früheren Jahren bereits mehrfach umgerindert hatte, Ovarialzysten aufwies und einmal Zwillinge abortiert hatte.

ZUSAMMENFASSUNG

Bei Färsen und Kühen mit Follikel-Theka-Zysten sind nicht die provozierte FSH-, sondern die LH-Ausschüttung für den Behandlungserfolg (d.h. die Bildung eines Gelbkörpers) von Bedeutung. Die nach Buserelingabe (20 bzw. 50 µg) bei 3 Kühen beobachtete 10, 11 bzw. 29 Tage verzögerte Zyklusinduktion dürfte kein Buserelin-, sondern ein Selbstheilungseffekt gewesen sein. Als Ursache des Therapieversagens bei 3 weiteren Kühen sind entweder eine weitgehende Atrophie der Zystenwand (dadurch keine Luteinisierung möglich), ein Fehlen eines zur Ovulation zu induzierenden Tertiärfollikels oder ein Fehlen von LH-Rezeptoren an den Ovarien in Betracht zu ziehen. Das unterschiedliche Erstbesamungs- sowie Gesamtträchtigkeitsergebnis nach Applikation von 20 bzw. 50 ug Buserelin zugunsten der höheren Dosierung kann nicht mehr der Medikation zugeschrieben werden.

SUMMARY

In heifers and cows with follicular cysts not the induced FSH-, but the LH-secretion is essential for the effect of treatment (i.e. the formation of a corpus luteum). The by 10, 11 or 29 days, respectively, delayed induction of the estrous cycle observed in 3 cows after application of buserelin (20 or 50 µg) might not be due to an effect of buserelin, but to a self-adjusting mechanism. As a cause for the failure of treatment in 3 other cows are either an almost atrophic wall of the cyst (therefore no luteinization possible), the absence of a Graafian follicle to be induced to ovulate, or the absence of LH-receptors at the ovaries to be taken into consideration. The different non-return-rate after the first service and also the different pregnancy rate after application of 20 or 50 µg buserelin in favor of the higher dosage cannot be attributed to the medication.

RESUMO

No tratamento de quistos foliculínicos em novilhas e vacas (isto é, para que se induza a formação de um corpo luteo) é de importância a libertação de LH, e não de FSH. Apos a aplicação de buserelina (20 ou 50 µg) em 3 vacas portadoras de quistos foliculínicos foi observada a indução do ciclo estrico, respectivamente em 10, 11 e 29 dias. Estes resultados não deven ser imputados ao efeito da buserelina, mas sim a uma cura expontânea. O insucesso terapeutico registrado em 3 outras vacas tratadas podera ter tido como causa duma acentuada atrofia da parede quistica (estando assim impossibilitada a luteinização desta), a ausência de um folículo terciário que pudesse ser induzido em ovulação na altura do tratamento ou a falta de receptores para LH. A variabilidade dos resultados da primeira inseminação, assim como o número total de gestações observadas nos 25 animais em tratamento, apos a aplicação de respectivamente 20 ou 50 µg de buserelina, apontam para um maior sucesso apos a aplicação da dose mais elevada. No entanto estes resultados não permitem concluir da responsabilidade da buserelina na sua obtenção.

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Die Behandlung von mittleren Zitzenstenosen mittels Autotransplantation von Vulvaschleimhaut

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EINLEITUNG

Mittlere Stenosen sind Veränderungen der Schleimhaut und der Mittelschicht im Bereich der Zitzenzisterne. Sie werden durch eine gedeckte Verletzung oder eine Euterentzündung während der Galtzeit verursacht. Zitzenstenosen führen in der Regel zu einer Milchabflussstörung (1). Bei Stenosen im Bereich der Zitzenzisterne findet man häufig bindegewebige Proliferationen, deren Prognose betreffend Maschinenmelkbarkeit auch nach der operativen Resektion des veränderten Materials sehr schlecht ist (3,6) Die herkömmliche opertative Resektion des wuchernden Gewebes (5,6) führte nur in wenigen Fällen zu einer restitutio ad usum.
Im folgenden soll eine neue therapeutische Massnahme beschrieben werden: Um eine erneute Proliferation von Bindegewebe in der Zitzenzisterne zu verhindern, wurde nach der Thelotomie und dem Entfernen des Proliferationsgewebes, Vulvaschleimhaut auf das freiliegende Bindegewebe der Zitze autotransplantiert.

MATERIAL UND METHODEN

Material
Zur Versuchsgruppe gehörten 12 Kühe, welche wegen einer Milchabflussstörung, verursacht durch eine mittlere und zum Teil gleichzeitig hohe Stenose, an die Klinik für Geburtshilfe des Tierspitals Zürich eingeliefert wurden. Die Kühe gehörten der Schweizer Braunviehrasse, der Simmentaler Fleckviehrasse, der Schwarzfleckviehrasse und deren Kreuzungen an. Sieben Kühe wiesen eine mittlere, fünf Kühe eine hohe und mittlere Stenose auf.

Methodik
-Diagnose
Die Diagnose einer mittleren Stenose in der Zitzenzisterne konnte durch Palpation, Sondierung, Röntgenaufnahme im Doppelkontrastverfahren (5) oder mittels Ultraschall (4) zuverlässig gestellt werden.
-Operationstechnik
Die gefastete Kuh wurde unter leichter Sedation (Xylazin 3-5mg/100kg KG i/v) auf dem Operationstisch seitlich gelagert und die Zitze mittels Ringblock, die Vulva durch subcutane Infiltration mit Lidocain 2% anästhesiert. Nach der Vorbereitung der Operationsfelder wurde die Zitze routinemässig eröffnet (1) und das veränderte Gewebe reseziert. Mit dem Dermatom nach Goulian (Hausmann AG, Zürich, Schweiz) wurde die zu transplantierende Schleimhaut (Schichtdicke 12 - 1,2mm) von der Vulva abgetragen. Das Transplantat wurde mit Vicryl 4-0 mittels Kürschnernaht in die Zitzenzisterne eingenäht. Der Verschluss der Thelotomiewunde geschah im üblichen Rahmen (1). Bei 6 Kühen wurde zusätzlich versucht, durch die Verwendung von Vasokonstriktiva (POR-8, Sandoz, Basel, Schweiz), Fibrinkleber (Tissucol, Immuno AG, St. Gallen, Schweiz) und das temporäre Einsetzen eines Silicontubus eine bessere Haftung des Transplantates zu erreichen. Die Tiere wurden für 10 Tage am betroffenen Viertel unter antibiotischen Schutz vorübergehend trockengestellt und die

Milch im Abstand von drei Tagen mittels einer Zitzenkanüle passiv abgelassen. Die Vulvaverletzung wurde mit Formazibazol (Ciba Geigy, Basel, Schweiz) abgedeckt.

-Auswertung

Der Erfolg der Operation wurde vier Wochen post operationem anhand der Maschinenmelkbarkeit festgestellt.

RESULTATE

Tabelle 1 zeigt die Melkbarkeit der zwölf operierten Kühe unterteilt nach Lokalisation der Stenose und unterschiedlicher Operationstechnik vier Wochen nach der Operation.

Tabelle 1:Melkbarkeit von zwölf Kühen mit Zitzenstenosen nach Thelotomie und Autotransplantation von Vaginalschleimhaut vier Wochen nach der Operation:

Lokalisation	Zusätze[a]	n	Melkbarkeit gut	schlecht
mittlere	nein	4	4	-
	ja	3	-	3
mittlere & hohe	nein	2	-	2
	ja	3	-	3

a)= Vasokonstriktiva, Fibrinkleber, Silikontubus

Die Zusammenstellung zeigt, dass die Operation nur bei Tieren mit mittleren Stenosen gelang. Lagen mittlere und gleichzeitig hohe Stenosen vor, war die Operation bezüglich späterer Melkbarkeit erfolglos. Von allen Kühen, bei denen zusätzlich zur Naht Vasokonstriktiva, Fibrinkleber oder Silikontubi verwendet wurden, konnte in keinem Fall eine befriedigende Melkbarkeit wiederhergestellt werden.

DISKUSSION

Autotransplantationen stellen in der humanmedizinischen Chirurgie keine grossen Schwierigkeiten dar. Bei unserer Methode ist die zu transplantierende Schleimhaut in genügender Menge vorhanden und auch leicht mobilisierbar. Trotz der guten Verträglichkeit des Fibrinklebers und den Vorteilen eines Vasokonstriktors in der Humanmedizin, um ein Ablösen des Transplantates durch einschiessendes Blut nach Entfernen der Klemmen an der Zitzenbasis zu verhindern, scheinen sämtliche zusätzliche Manipulationen (Vasokonstriktor, Fibrinkleber, Silikontubus) an der Zitze die Heilung negativ zu beeinflussen. Das beste Resultat wurde erzielt, indem das Transplantat nur am Wundrand eingenäht wurde. Das Ablösen des Transplantates wird sicher auch durch die einschiessende Milch verhindert, die das Transplantat an das Bindegewebe drückt. Bristol (1989) transplantierte Maulschleimhaut in die Zitzenzisterne. Zu diesem Zweck musste eine Vollnarkose durchgeführt werden. Zudem implantierte er einen Silikontubus in die Zitzenzisterne, was zur Entfernung des Tubus eine Reoperation der Zitze erforderlich machte (2). Die hier beschriebene Operationstechnik fordert zwar vom Operateur einiges chirurgisches Geschick, ist aber abgesehen davon leicht durchzuführen und stellt für den Patienten keine unverhältnismässige Belastung dar. In Regionen, in denen der Landwirt bereit ist, eine angemessene Summe für die

Wiederherstellung der Melkbarkeit der Kuh auszugeben, stellt dieses Verfahren eine erfolgversprechende Alternative zu den herkömmlichen und zudem meist erfolglosen Methoden dar.

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ZUSAMMENFASSUNG

Mittlere und hohe Stenosen in der Zitze führen zu Milchabflussstörungen, welche mit den herkömmlichen Operationstechniken nur in den seltensten Fällen behoben werden können. Die hier beschriebene Methode, bei der an der sedierten Kuh, Schleimhaut von der Vulva in die Zitzenzisterne autotransplantiert wird, stellt eine Alternative dar, weil dadurch ein Wiederauftreten der Proliferation von Bindegewebe in der Zitze verhindert werden kann. Verschiedene Operationstechniken wurden ausprobiert. Das beste Resultat wurde dabei erzielt, wenn das Transplantat nur in die Zitze eingenäht wurde. Hohe Stenosen konnten auch mit dieser Methode nicht behoben werden.

SUMMARY

Surgery of the teat after middle and upper stenosis were often unsuccessfull. In this paper we describe a technique, which can be achieved under normal sedation, by autotransplanting a graft from the vulva to the connective tissue of the teat. Several techniques are described. The best results were achieved, when the graft was only fixed with fine hydrolytic suture material. Stenosis of the upper part of the teat cistern could not be successfully treated with this method.

RESUME

Les techniques chirurgicales habituelles s'avèrent très souvent inefficaces dans les cas de sténoses médianes et hautes du trayon. La présente étude décrit une méthode d'autotransplantation de muqueuse vaginale dans la citerne du trayon. Cette opération est praticable sous sédation et présente l'avantage d'empêcher la prolifération post opératoire de tissu conjonctif. Plusieurs techniques sont décrites. Les meilleurs résultats sont observés lorsque la greffe n'est fixée qu'avec un matériel de suture résorbable aussi fin que possible. Cette méthode ne permet toutefois pas un traitement efficace des sténoses hautes.

ESTUDO IMUNOLÓGICO CLÍNICO DO USO EM DOSE REDUZIDA DA VACINA CONTRA A BRUCELOSE (AMOSTRA 19) EM BÚFALAS ADULTAS DE REBANHO PROBLEMA

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INTRODUÇÃO

A brucelose causada pela B.abortus é uma zoonose amplamente disseminada, de importância mundial para a saúde pública e a economia, sendo primariamente descrita como doença de bovinos. Entretanto, há evidências de que seja de igual importância entre os bubalinos, como apontam os levantamentos epidemiológicos internacionais (1,2) e nacionais(3,4,5).

Diante das dificuldades encontradas no controle e erradicação da brucelose no rebanho bovino, particularmente no que diz respeito aos indivíduos adultos infectados, onde o isolamento ou a eliminação dos mesmos pode ser uma medida insatisfatória, desenvolveu-se o método alternativo da vacinação destes animais com dose reduzida da vacina tradicional (amostra 19) (6). Diversos estudos neste sentido, provam ser este um método eficiente para o controle da disseminação da enfermidade no rebanho, restringindo os casos clínicos e diminuindo o número de animais acometidos, com a vantagem de provocar pouca interferência nas provas sorológicas normalmente utilizadas para o diagnóstico (7,8,9,10).

O objetivo do presente trabalho é avaliar os efeitos do uso da vacina contra a brucelose (amostra 19) em dose reduzida num rebanho de bubalinos naturalmente infectado.

MATERIAL E MÉTODOS

Animais experimentais e manejo

Utilizaram-se 67 búfalos (Bubalus bubalis) adultos, sendo 63 fêmeas e 4 machos, de raças variadas, provenientes de 16 rebanhos de diferentes regiões do País, agrupados em Araçatuba-SP, com prevalência de 3,17% a 14,28% em diferentes testes sorológicos, sem antecedentes de vacinação contra a brucelose. Os animais receberam acompanhamento clínico diário e foram mantidos en regime de alimentação de pasto mais suplementação com volumosos e mistura mineral à disposição e em regime de monta natural.

Período experimental

O período experimental de 18 meses foi dividido em pré-vacinação: mês 0; pós-vacinação: meses 1 a 6 e pós-revacinação: meses 7 a 18.

Vacinação e revacinação

Procedeu-se a vacinação das 63 fêmeas por instilação na conjuntiva de uma dose reduzida, com $2x10^9$ bactérias/dose, obtida pela diluição adequada, com solução fisiológica estéril, de vacina contra a brucelose (amostra 19)*, oficialmente controlada, contendo, no mínimo, $60x10^9$ bactérias viáveis/dose, no mês 0.

No mês 6, as fêmeas foram revacinadas, por via subcutânea, com igual dose reduzida da mesma vacina.

Colheita das amostras

Amostras mensais de sangue foram colhidas de cada animal, submetendo-

* Vacina Liofilizada Contra Brucelose-Pfizer S.A.

se o soro às provas sorológicas, do mês 0 ao 12 e ainda nos meses 15 e 18.

Provas sorológicas

Utilizaram-se as provas de soroaglutinação rápida (SAR) e do cartão (CARTÃO) para o acompanhamento sorológico mensal, incluindo-se as provas do Rivanol (RIVANOL) e de fixação do complemento (FC) na última colheita do 18º mês.

Todos os testes foram realizados utilizando-se de material controlado e dos métodos recomendados oficialmente.

Na interpretação dos resultados das provas RIVANOL e FC utilizaram-se os critérios atualmente preconizados pelo "The Uniform Methods and Rules" (UMR) do "Cooperative Brucellosis Eradication Program"(CBEP) dos EUA,revisados em 1979 e postos em prática a partir de 1980 (11), os quais consideram, para animais vacinados, na prova RIVANOL, positivo a 1/50 ou mais = positivo; e na FC, 50% de fixação em 1/10 até menos de 25% de fixação a 1/40 = suspeito; e 25% de fixação a 1/40 ou mais = positivo.

RESULTADOS E DISCUSSÃO

Os resultados das provas sorológicas do período pré-vacinação, pós-vacinação nos meses 1, 3 e 5, e, pós-revacinação nos meses 7, 9, 15 e 18, são expressos em porcentagem e estão apresentados nas Figuras 1 e 2. Os resultados obtidos nas demais colheitas não estão apresentados em virtude de serem pouco representativos na tendência observada na avaliação dos dados.

A porcentagem de animais positivos observada no período pré-vacinação indica que a prevalência de brucelose no rebanho estudado situa-se entre os valores descritos na literatura estrangeira (1,2), e, bem próxima aos citados por autores brasileiros (3,4,5), sugerindo que o rebanho represente uma amostra significativa do que está ocorrendo nas populações de búfalos de várias regiões do País.

Os resultados no período pós-vacinação indicam aumento do número de animais positivos logo no primeiro mês, não chegando a se manifestar em todas as búfalas, com redução gradual nos meses subsequentes, chegando a valores inferiores à prevalência inicial (Fig.1). Isto indica que o comportamento sorológico dos búfalos à vacinação com dose reduzida na conjuntiva foi bastante semelhante ao dos bovinos, como anteriormente observado (7,8,12).

O aumento na porcentagem de positivos à SAR, no mês 5 (Fig.1), em comparação aos resultados iniciais e aos do CARTÃO, sugere um problema da própria prova, que é de eficiência inferior especialmente quando empregada no diagnóstico de animais que estão em período pós-vacinal (8).

Após a revacinação observou-se fenômeno semelhante ao encontrado no período pós-vacinação com aumento expressivo da porcentagem de positivos logo aos 30 dias, sendo 100% no CARTÃO, havendo decréscimo gradual no número de reagentes positivos e sendo a queda mais lenta em relação ao tempo do que aquela observada no período anterior (Fig.2), caracterizando a resposta anamnéstica proporcionada pela segunda exposição ao antígeno.

Comparando-se os resultados pré-vacinal e final verificou-se redução do número de animais reagentes positivos (Fig.1 e 2), o que se assemelha ao observado em bovinos de rebanhos problema (7,8), bem como, diminuição na porcentagem de suspeitos à SAR (Fig.1 e 2).

As provas sorológicas utilizadas neste trabalho apresentam níveis diferentes de sensibilidade e especificidade tanto para bovinos (7,8,13) quanto para bubalinos (14), sendo o teste do CARTÃO de grande sensibilidade e as provas do RIVANOL e de FC de maior especificidade, o que torna as duas últimas mais eficientes e,portanto, indicadas na fase de 6 meses ou mais pós-vacinação (7,13). Diante deste fato os resultados pode-

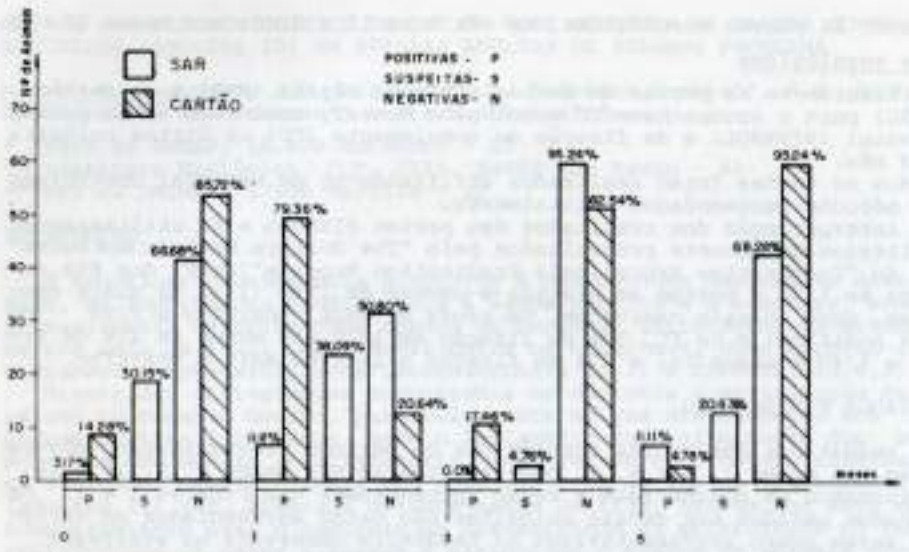

FIGURA 1- QUANTIFICAÇÃO PELOS RESULTADOS NOS TESTES SAR E DO CARTÃO PARA BRUCELOSE DE 63 BÚFALAS ADULTAS ANTES E EM DIFERENTES MESES APÓS A VACINAÇÃO COM DOSE REDUZIDA DE VACINA B 19

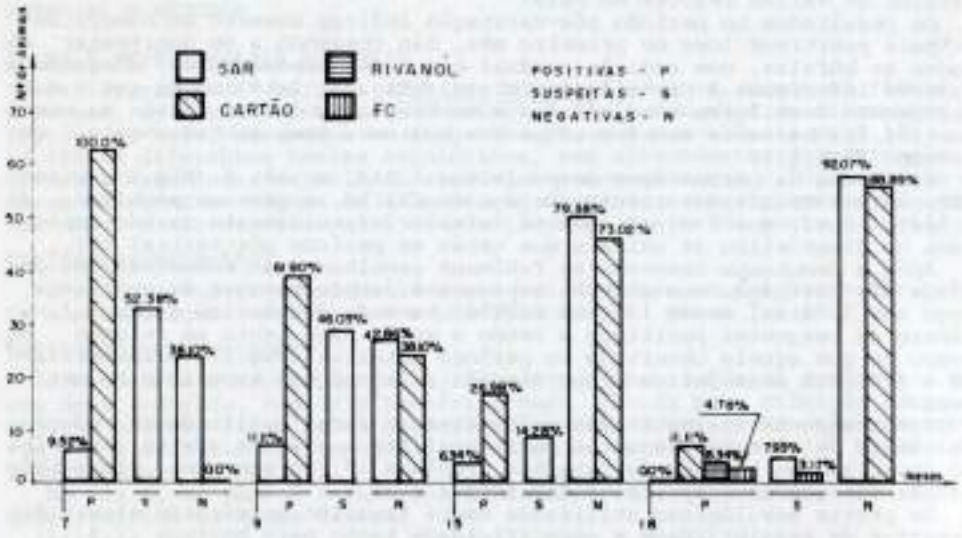

FIGURA 2- QUANTIFICAÇÃO PELOS RESULTADOS NOS TESTES SAR E DO CARTÃO, E, RIVANOL E FC NO 18º MÊS, DE BÚFALAS ADULTAS VACINADAS COM DOSE REDUZIDA DE VACINA B19 NA CONJUNTIVA E 6 MESES DEPOIS REVACINADAS EM IGUAL DOSE DA MESMA VACINA VIA SUB-CUTÂNEA EM VÁRIOS MESES PÓS-REVACINAÇÃO.

riam ser melhor avaliados comparando-se os dados do diagnóstico inicial do CARTÃO (Fig.1) com os resultados finais do RIVANOL e FC (Fig.2) percebendo-se efetivamente a diminuição do número de animais positivos aos 12 meses pós-revacinação.

Ao contrário do que seria esperado, e já fora observado com a introdução da infecção em rebanho de animais não vacinados (15), o procedimento de vacinação das fêmeas adultas com dose reduzida da vacina tradicional controlou efetivamente a disseminação da infecção limitando o aborto a um único caso, mantendo o índice de fertilidade no rebanho(16), reduzindo o número de fêmeas reagentes positivas e não afetando os machos, os quais se mantiveram negativos durante todo o período experimental. Estas observações permitem concluir que a vacinação de búfalas adultas de um rebanho infectado, com dose reduzida de vacina B19 é um método eficiente de intervenção no controle da brucelose nesta espécie animal.

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RESUMO

Em um grupo de 67 búfalos (63F e 4M) adultos de diversas raças, sem antecedentes de vacinação e com prevalência de suspeitas e de positivas para a doença, foi aplicada nas fêmeas dose reduzida com $2x10^9$ bactérias vivas/dose, da vacina contra a brucelose (amostra B-19). Pós-vacinação, de todos os animais no primeiro mês e nos subsequentes foi colhido sangue sendo o sôro submetido às provas sorológicas de soroaglutinação rápida (SAR) e do teste do cartão (CARTÃO). Aos seis meses revacinou-se as búfalas com dose igual, da mesma vacina por via sub-cutânea, continuou-se o exame do sôro por mais 6 meses e a partir do sexto mês por mais duas vezes com intervalos de três meses entre as colheitas. Na última vez além das provas citadas os soros dos animais inicialmente positivos e/ou positivos em alguma prova anterior foram submetidos também ao teste do Rivanol e da fixação de complemento (FC). Os resultados obtidos permitem concluir que a vacinação com dose reduzida da vacina B-19 de búfalas adultas de rebanho com prevalência entre aquelas encontradas no País, frente a diferentes métodos habituais de diagnóstico, evita a disseminação da doença, limita a doença clínica e diminue o núemro de animais rea

gentes positivos no decorrer de 3-5 meses pós-vacinação ou de 12 meses pós-revacinação.

RESUMO

En um grupo de 67 búfalos (63 hembras e 4 machos) adultos de diversas razas, sin antecedentes de vacunación y con prevalencia de animales sospechosos y positivos para la enfermedad, fue aplicado en las hembras dosis reducidas con $2x10^9$ bactérias vivas por dosis de vacuna contra la brucelosis (Muestra B-19). Posteriormente a la vacunación de todos los animales se obtubieron muestras de sangue durante los primeiros meses, siendo el suero sometido a pruebas serológicas de soroaglutinación rápida (SAR) y el "CARD TEST". A los seis meses se revacunaron las hembras por via subcutánea, con dosis iguales de la misma vacuna, y se continuó el exámen del suero por seis meses más, a partir del sexto mes se realizaron otras dos pruebas, siendo las muestras recolectadas con intervalo de tres meses. Después del último muestreo, además de las pruebas citadas, el suero de los animales que eran positivos inicialmente e/ó de los que resultaron positivos en alguna prueba posterior, fué sometido a los testes Rivanol y Fijación de complemento (FC). Los resultados obtenidos usando diferentes métodos de diagnóstico permiten concluir, que la vacunación de las hembras adultas de rebaño entre los prevalescientes en el País, evita la diseminación de la enfermedad, limita las manifestaciones clínicas y disminuye el numero de animales reaccionantes en el período de 3 a 5 meses posterior a la vacunación é de 12 mese después de la revacunación.

SUMMARY

In a group composed of 67 buffaloes (63 females and 4 males), all adult animals belonging to various breeds, with no prior vaccination records, some positive and some suspected of having brucellosis, the females were inoculated with small doses containing $2x10^9$ live bacteria / dose of brucellosis vaccine (B-19 strain). In the first and subsequent months post-vaccination, blood samples of all animals were collected and the sera used for serologial tests, i.e., the rapid serum agglutination test (RSAT) and the Card Test (CT). Six months later, the buffalo cows were revaccinated with an equal dose of the same vaccine, by the subcutaneous route, and their sera were then examined for six more months and after this, 2 more times at a 3-months interval. In the last time, besides the two tests mentioned, sera of those animals, positive at the beginning and/or positive for some of the tests previously used were also submitted to the Rivanol test and to the complement fixation test (FC). The results obtained allow us to conclude that vaccination with a reduced dose of the B-19 vaccine, of adult buffalo cows of a herd in which the prevalence is similar to that regularly recorded for the Country when usual diagnostic methods are employed, avoids dissemination of the disease, restrains the overt disease and decreases the number of positive cases in a period of time ranging from 3 to 5 months post-vaccination or 12 months after revaccination.

NIVELES PLASMATICOS DE PROGESTERONA EN RECEPTORAS DE EMBRIONES CONGELADOS DETERMINADOS POR ELISA TEST.

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INTRODUCCION

La transferencia de embriones bovinos (sean frescos o congelados), como biotecnología aplicada, reconoce varias etapas que se inician con la hembra dadora y finaliza con las receptoras.

Durante los últimos años se investiga con mayor énfasis la receptora y su influencia sobre el porcentaje de preñez, relacionándola con la raza, edad, estado sanitario, nivel nutricional y productivo (4,9,10,11); por el momento los resultados son contradictorios. También debe establecerse un perfil endocrinológico que asegure la potencial fertilidad de la receptora y el establecimiento satisfactorio de una preñez (12,13), por cuanto, luego de la transferencia, es decisivo un cuerpo lúteo (C.L) funcional, que a su vez dependerá de las señales lutеotróficas y antiluteolíticas del embrión.

Aún no está definida la concentración de progesterona (P_4) necesaria para que un embrión sobreviva durante la tercera semana post-implante; pero sí se han demostrado fallas en la concepción cuando la concentración de P_4 es inferior a 1 ng/ml (12,13,14,15,16).

Por otro lado numerosas publicaciones señalan que los mayores índices de preñez, se logran con concentraciones de P_4 que oscilan entre 2 y 5 ng/ml (13,14,16,18,19, 20,21).

Este trabajo se propone establecer las siguientes relaciones:

1) Niveles plasmáticos de P_4 y porcentaje de preñez.
2) Concentración de P_4 y calidad del cuerpo lúteo.
3) Calidad del embrión, niveles plasmáticos de P_4 y porcentaje de preñez.

MATERIALES Y METODOS

Durante el año 1986, en el Centro de Transferencia Embrionaria "La Josefina", ubicado en San Antonio de Areco (Buenos Aires), fueron destinadas 163 vaquillonas Holando Argentino,(cuyas edades y pesos oscilaron entre 18 y 24 meses, y 350 y 470 kg., respectivamente) a la transferencia de embriones congelados.

El programa de sincronización utilizado fue la doble aplicación de Cloprostenol 500 ug. (análogo de la prostaglandina $F_{2\alpha}$), con un intervalo de 11 días. Entre las 48-96 hrs. después de la última inyección, presentaron celo manifiesto un total de 146 vaquillonas (74,07%).

El día de la transferencia las vaquillonas fueron sometidas a tacto rectal a los fines de evaluar: a) presencia y tamaño del/los folículo/s; b) localización y calidad de cuerpo lúteo. Para determinar la calidad del cuerpo lúteo se estableció la siguiente escala subjetiva:

- 1 ó muy bueno: 15 a 25 mm de diámetro, con corona manifiesta
- 2 ó bueno: 15 a 20 mm de diámetro, sin corona manifiesta
- 3 ó regular: menos de 15 mm de diámetro, sin corona, con un folículo en cualquiera de los ovarios.

- 4 ó malo: cuerpo lúteo muy pequeño, acompañado de un folículo con un diámetro de 5 a 15 mm.

En base a ésta escala se seleccionaron 111 animales (68,0%) con cuerpo lúteo de tipo 1 ó 2 para ser transferidos quirúrgicamente (5). Antes de la misma se extrajeron dos muestras de sangre en tubos con heparina, los cuales fueron centrifugados y el plasma resultante almacenado a -22 °C hasta su procesado. La concentración de P_4 fue determinada por enzimoinmunoensayo (Elisa) semicuantitativo (Ovucer) en los rangos de 0,5, 1.0 y 5.0 ng/ml.

Los embriones utilizados fueron clasificados, después de la descongelación, en calidad 1 ó excelente y calidad 2 ó buena, según técnica ya descripta (5,6).

Todas las vaquillonas transferidas se observaron dos veces al día para determinar el intervalo de tiempo transcurrido en retornar al celo, de aquellas que no quedaron preñadas. Según el mismo, el celo se clasificó como:

fisiológico	18 a 23 días
prolongado	24 a 36 días
extenso	más de 37 días

La preñez fue diagnosticada por tacto rectal entre los días 45 y 60 posteriores a la transferencia. En el análisis estadístico se utilizó el Test X^2 (Ji cuadrado).

RESULTADOS

Sobre un total de 111 receptoras seleccionadas y transferidas, 56 de ellas (50.45%) presentaron preñez positiva al tacto rectal.

Las receptoras fueron divididas en dos grupos, A y B, de acuerdo al análisis de P_4 plasmática. El grupo A lo constituyeron todos los animales cuyo plasma fue positivo a los patrones de 1 y 5 ng/ml. El grupo B engloba a las receptoras negativas a todos los patrones ó positivas a 0.5 ng/ml.

Según Cuadro N°1, dentro del grupo A, de las 78 vaquillonas (70.2%;78/111) se obtuvo preñez en 53 de ellas (67.9%), mientras que para el grupo B, de 33 receptoras (29.7%;33/111) sólo resultan preñadas 3 animales (9.09%). El porcentaje de preñez entre ambos grupos, muestra diferencias altamente significativas ($P<0.01$).

El Cuadro N°2 muestra el ordenamiento de las receptoras transferidas y los resultados de preñez en función de los niveles de P_4 plasmáticos. Los porcentajes de preñez para cada uno de los grupos fue: 0.0%, 12.28%, 66.6% y 69.0%. Las diferencias son altamente significativas ($P<0.01$) para cada uno de los niveles de P_4.

El Cuadro N°3 establece la relación entre la calidad del cuerpo lúteo, nivel plasmático de P_4, receptoras transplantadas y preñadas. Como puede observarse no hay diferencias significativas con el parámetro calidad del cuerpo lúteo, pero sí la hay con los niveles de P_4 plasmáticos.

Además, se observaron las 25 receptoras del grupo P_4 mayor a 1 ng/ml que no quedaron preñadas; 17 de ellas (68.0%) retornaron al celo en un período fisiológico (18 a 23 días); 5 (20%) tuvieron celo en un intervalo prolongado (23 a 36 días); mientras 3 receptoras (12%) manifestaron un intervalo inter-estrual post-transferencia mayor de 37 días, posiblemente debido a mortalidad embrionaria.

Como puede observarse en el Cuadro N°4, no existe significación estadística entre la calidad del embrión (excelente ó bueno) y la preñez lograda; pero vuelve a ser significativa la preñez versus el nivel plasmático de la progesterona.

Se analizó el grado de repetibilidad del Test Elisa, evaluando las muestras por radioinmunoensayo. El coeficiente de correlación (r) fue 0.87 (22).

Los resultados de Hiraco y col. (23) concluyen que el Elisa test para P_4 plas-

CUADRO N°1. Relación Porcentaje de preñez - Nivel plasmático de progesterona.

Concentración P_4 plasmática	N° recept. transf. (%)	N° recept. preñad(%)
Grupo A (1 ng/ml)	78 (70.2%)	53 (67.9) ^{a}
Grupo B (1 ng/ml)	33 (29.8%)	3 (9.09) ^{b}
TOTAL	111 (100)	56

a,b: letras diferentes indican diferencias significativas ($P<0.01$)

CUADRO N°2. Porcentajes de preñez para cada concentración de P_4 plasmática el día de la transferencia.

Niveles de P_4 plasmática	N° recept. transf. (%)	N° recept. preñ. (%)
Negativo	12 (10.8)	0 (0.0) ^{a}
Positivo a 0.5 ng/ml	21 (18.9)	3 (14.3) ^{b}
Positivo a 1.0 ng/ml	36 (32.4)	24 (66.6) ^{c}
Positivo a 5.0 ng/ml	42 (37.8)	29 (69.0) ^{d}
TOTAL	111 (100)	56 (50.2)

a,b,c,d: letras diferentes indican diferencias significativas ($P<0.01$)

CUADRO N°3. Porcentajes de preñez según niveles plasmáticos de P_4 y su relación con la calidad del cuerpo lúteo.

Calidad del C.L.	Conc. de P_4	N° recept. transf. (%)	N° preñadas (%)
1	mayor a 1ng/ml	63 (80.7)	43 (68.2) ^{a}
2	mayor a 1ng/ml	15 (19.3)	10 (66.6) ^{a}
TOTAL		78 (100)	53 (67.9) ^{A}
1	menor a 1 ng/ml	26 (78.7)	3 (11.5) ^{c}
2	menor a 1 ng/ml	7 (21.3)	0 (0.0) ^{c}
TOTAL		33 (100)	3 (9.0) ^{C}

a,a y A,C: letras diferentes indican diferencias significativas ($P<0.01$)

CUADRO N°4. Porcentaje de preñez según calidad del embrión y nivel de P_4 en recept.

Calidad embrión	N° embr. transf.	P_4 recept.	N° recept. preñ. (%)
1 (excel.)	56	mayor a 1ng/ml	39 (69.6) ^{a}
2 (buena)	22	mayor a 1ng/ml	14 (63.6) ^{a}
TOTAL	78		53 (67.9) ^{A}
1 (excel.)	17	menor a 1 ng/ml	2 (11.7) ^{b}
2 (buena)	11	menor a 1 ng/ml	1 (9.09) ^{b}
TOTAL	28		3 (10.7) ^{B}

a,b y A,B: letras diferentes indican diferencias significativas ($P<0.01$)

mática, posee la suficiente sensibilidad y precisión para ser usado "a campo"; con un coeficiente de correlación con el radioinmunoanálisis (R.I.A) de 0.97 ($P<0.001$).

COMENTARIO

El objetivo de ésta experiencia fue evaluar el Elisa test (P_4) como un criterio adicional en la selección de receptoras al momento de la transferencia; con la idea de incrementar los índices de preñez.

Los resultados obtenidos coinciden con otras publicaciones, donde los mejores índices de preñez se logran en aquellas receptoras que presentan niveles de P_4 plasmática entre 1 y 5 ng/ml [13,16,18,19,22). En nuestro caso llega al 67.9% (53/78).

La selección de una hembra receptora se rige,en primera instancia, por la observación de un celo manifiesto entre las 48 y 96 hs. posteriores a la sincronización; luego el diagnóstico de un cuerpo lúteo, clasificándolo en forma subjetiva, de acuerdo al tamaño, presencia de corona, elasticidad del tejido luteal, en sincronismo con el día del celo de referencia [20). Cualquier alteración en éstos parámetros es suficiente para eliminar la receptora del programa. Lamentablemente, ninguno de éstos criterios permite abrir juicio sobre la secreción hormonal del cuerpo lúteo (Cuadro N°3). No existe relación entre la calidad subjetiva del cuerpo lúteo y los niveles de P_4, por cuanto el 29.2% de receptoras con cuerpo lúteo-calidad 1, registraron concentración de P_4 inferior a 1 ng/ml, y el 31.8% de receptoras con calidad de cuerpo lúteo 2 también registraron concentración d P_4 inferior a 1 ng/ml (Cuadro N°3).

El ritmo de secreción de P_4 por parte del cuerpo lúteo es importante por cuanto permite que exista una sincronización entre el medio intrauterino y el desarrollo del embrión [12,23). Un aumento prematuro de dicho esteroide durante el metaestro iniciaría un asincronismo entre ambos, similar a la situación que ocurre después de la transferencia de un embrión a una receptora que presentara celo 2 a 3 días antes que la donante (4,12,24). Es sabido que la sobrevivencia del embrión transferido es mayor cuando las concentraciones plasmáticas de P_4 en el 7° a 8° día del ciclo son moderadas. Si bien no se conocen con exactitud los niveles mínimos que aseguran la viabilidad embrionaria, puedan considerarse adecuadas concentraciones de P_4 entre los 2 y 5 ng/ml [12,13,14,16,18,19,20).

En éste estudio los porcentajes de preñez fueron 67.9% y 9.09% para aquellas receptoras con niveles de P_4 entre 1 y 5 ng/ml, y menos de 1 ng/ml respectivamente (Cuadros N°1 y 2). La bibliografía cita que con menos de una concentración de P_4 de 2 ng/ml, los porcentajes de preñez oscilan entre el 8.3 al 35.3%, situación que se presenta en un 21 a 24% de las receptoras [14,18,19,20]; no estableciéndose preñez alguna con niveles inferiores a 1 ng/ml [16,22]. Esto corresponde con nuestros resultados (Cuadros N°1 y 2).

Finalmente los niveles decisivamente altos (más de 6.0 ng/ml) no presentan una correlación positiva con el establecimiento de la preñez [12). Esto puede deberse a una errónea observación del celo, especialmente cuando se maneja un gran número de hembras sincronizadas.

La calidad de los embriones también influye sobre los índices de preñez [2,3). No se establecieron diferencias significativas entre aquellos embriones de calidad excelente y buena, siempre que fueran transferidos a receptoras con P_4 superiores a 1 ng/ml; pero sí resultaron significativas para receptoras con P_4 menores a 1 ng/ml (Cuadro N°4).

CONCLUSIONES

Reconocemos la utilidad de un test rápido para la determinación de la concentración de P_4 en plasma el día de la transferencia, por cuanto: a) permitirá la selección de receptoras que brindarán mejores y mayores probabilidades de quedar preñadas; b) permitirá la reutilización de receptoras en breve plazo, con niveles hormonales inadecuados (hasta 35%) sin esperar el retorno al celo o el diagnóstico de vacuidad después de la transferencia.

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RESUMEN

El objetivo de ésta experiencia fue evaluar el Elisa test para determinación de progesterona, como un criterio adicional en la selección de receptoras en el momento de la transferencia embrionaria, con la finalidad de incrementar los índices de preñez.

SUMMARY

This experience aim was to evaluate the Progesterone Elisa test as an aditional screening method for recipients at the embryo transfer time.
This method should result in increase of the pregnancy rates.

SUMARIO

O trabalho objetivou avaliar o emprego do Elisa Test para a determinação de progesterona, como mais um critério na seleção de receptoras no momento da transferência embrionária, com a finalidade de obter maiores índices de gravidez.

PROLONGATION OF CORPUS LUTEUM LIFESPAN IN HEIFERS BY INTRAMUSCULAR ADMINISTRATION OF RECOMBINANT BOVINE INTERFERON ALPHA$_1$1.

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INTRODUCTION

In cattle maternal recognition of pregnancy, characterized by a sustained secretion of progesterone by the corpus luteum (CL), occurs on day 14-17 post estrus. At this time the embryo must signal its presence to prevent luteolysis. Indeed, regulation of the lifespan of the corpus luteum by the conceptus is critical for a successful pregnancy because, in cattle, the corpus luteum is the major source of progesterone during pregnancy. Between day 15 and 25 the conceptus secrete specific protein called bovine trophoblast protein 1 (bTP-1) which directly or indirectly prolong the lifespan of the corpus luteum and prevent return to ovarian cyclicity (3). Recent studies have demonstrated that bTP-1 has a high degree of sequence homology with interferon of the alpha class (2). In addition it has been shown that recombinant bovine interferon alpha11 (rBolFN$\alpha_1$1) administered in utero into cyclic cows from day 15.5 to 21 after estrus delayed luteolysis (4). Similarly twice daily intramuscular injections of rBolFN$\alpha_1$1 from day 15 to 19 after estrus extend estrus intervals (5).

It is well recognized that early embryo losses, occuring around the time of maternal recognition of pregnancy constitute a major economic problem to the animal industry (1). If some embryonic mortality occurs naturally because the conceptus is deficient in producing the signal (bTP-1) to prevent luteolysis, embryonic death could be reduced by pharmacological manipulation of lifespan of the corpus luteum. The purpose of our study was to demonstrate if daily systemic treatment of cycling heifers with various doses of rBolFN$\alpha_1$1 can prolong the lifespan of CL.

MATERIALS AND METHODS

Animals

Simmental heifers, 18-20 months old, weighing 350-420 kg were housed in an indoor pen and fed corn silage and hay. Palpation per rectum of the reproductive organs was performed before the beginning of the trial to monitor ovarian activity and uterus size. Only animals with no detectable reproductive disorders were enrolled in the study.

Estrus detection

Estrus behaviour was observed for at least 30 min three times a day (07:00, 13:00 and 18:00h) starting on day 14 post estrus. The heifers standing to be mounted by other heifers without attempts to escape were considered to be on heat. The results of the observation was confirmed by determination of progesterone concentrations in plasma: levels declined to a value below 1 ng/ml before and during estrus.

Sample collection

Blood samples were collected from the coccygeal vein once daily (10:00h) beginning on day 14 post estrus and continuing until either estrus was detected or day 30 post estrus, for determination of progesterone concentration. Plasma was harvested by centrifugation at 1000 g for 20 min at 4° and frozen at -20°C until assayed.

Progesterone assay

The concentrations of progesterone in plasma were measured using an enzyme linked immunosorbent assay (ELISA, OVUCHECK® Cambridge Veterinary Science, Cambridge, UK) and an automated microtiter spectrophotometer set at 405 nm. Limit detection of the assay was 0.5 ng/ml. The intra assay precision at 1 ng/ml progesterone in plasma was 10%.

Recombinant Bovine Interferon Alpha$_1$1

rBolFN$\alpha_1$1 was produced in E. coli by recombinant DNA technology, purified to homogeneity as determined by SDS polyacrylamide gel electrophoresis and formulated as a lyophilized dry substance. Stock preparation had a minimum specific activity of 10^7 working units/mg protein as determined by plaque inhibition of vesicular stomatitis virus grown in Madin. Darby bovine kidney cells.

Each animal received daily either 10 or 20 mg of rBolFN$\alpha_1$1 intramuscularly or an equivalent volume of placebo (8ml). Studies were performed blind: the personnel involved in the trials were unaware of the nature of treatment administered.

Study design

32 Simmental heifers were synchronized using a standard double injection (11 days apart) of prostaglandin F2α (Dynolytic® The UpJohn Company). Animals were allocated to treatment groups that were balanced for weight of heifers. Treatment with 20 mg of rBolFN$\alpha_1$1 were administered daily from day 14 to 20 post estrus. Only animals with a functional CL on day 14 as demonstrated by plasma progesterone levels > 1ng/ml were admitted into the study. Animals were observed for signs of heat up to day 33 after estrus and blood samples collected daily.

In another similar experiment 12 dairy heifers were treated once a day intramuscularly with 10mg of rBolFN$\alpha_1$1 or a placebo from day 14 to 19 post estrus.

Statistical analysis

Treatment effect on length of estrus cycle were determined by one tailed Student t. test.

RESULTS AND DISCUSSION

In the first experiment administration of rBolFN$\alpha_1$1 extended significantly ($p<0.01$) the length of the estrus cycle (24.08 ± 3.38 days vs 20.74 ± 3.22 days for the controls). Similarly the interval from estrus until progesterone concentrations declined to a value below 1 ng/ml was 23.15 ± 3.21 days for the rBolFN$\alpha_1$1 treated animals and 19.5 ± 2.83 for the control heifers. (table 1).

Similarly, in experiment 2, interferon treated heifers had prolonged cycles ($p<0.05$) when compared with the placebo treated group (table 2). Mean interestrus intervals were respectively 23.4 ± 3.78 and 19.5 ± 1.61 days. Means for the period of functional lifespan of the CL (measured by progesterone levels >1 ng/ml) were 21.8 ± 3.03 and 18.33 ± 1.254. Luteal lifespan was longer in the interferon treated group as compared with that in the control group.

Therefore, daily systemic administration of various doses of rBolFN$\alpha_1$1 can prolong functional lifespan of the CL and length of estrus cycle. It is known, that high rates of embryonic loss occur during the time of maternal recognition of pregnancy day 14-24 (1). This period is critical for the conceptus because it must rescue the corpus luteum from luteolysis by secreting bTP-1. It is therefore possible that some embryos do not produce an optimal level of bTP-1 at the right time, if for instance their development is retarded or in asynchrony with the endometrium of the dam. It has been shown for example that heat stress causes a large decrease in bTP-1 secretion by the conceptus in utero (6). Anasynchrony between the embryo and the uterus of the cow is also possible after embryo transfer. Likewise, in heifers or cows with a short estrus cycle, luteolysis occurs earlier than normal before the conceptus can produce bTP-1 to ensure its survival. Avoiding embryonic loss by injecting bTP-1 or its homologous proteins interferons to maintain corpus luteum function is therefore a possible mean of increasing fertility in cattle. Other approaches, using luteotrophic factors like human chorionic gonadotrophin have been described with some success (8).

		Interestrus Intervals	Functional Lifespan of CL
Placebo	(n=19)	20.74 ± 3.22	19.5 ± 2.84
rBolFN$\alpha_1$1	(n=13)	24.08 ± 3.38	23.15 ± 3.21

Table 1: Experiment 1 - Effect of daily administration of rBolFN$\alpha_1$1 (20 mg from day 14 to 20) on cycle length and lifespan of CL (mean ± standard deviation)

		Interestrus Interval	Functional lifespan of CL
Placebo	(n=6)	19.5 ± 1.61	18.33 ± 1.25
rBolFN$\alpha_1$1	(n=6)	23.4 ± 3.78	21.8 ± 3.03

Table 2 Experiment 2 - Effect of daily administration of rBolFN$\alpha_1$1 (10 mg from day 14 to 19 postestrus)

The mechanisms that are engaged to extend the corpus luteum function are only partially understood. Interferons might extend the lifespan of the corpus luteum by either inhibiting prostaglandin F2α release from the uterus or inducing resistance to PGF2α in the corpus luteum. Indeed it has been shown (7) that PGF2α and PGE2 secretion by cultured ovine endometrial cells can be inhibited by both oTP-1 or human interferon alpha.

The results presented in this study support further investigation of the role of interferons as polypeptide hormones in establishment of pregnancy.

ABSTRACT

In cattle, maternal recognition of pregnancy, which is signified by a sustained secretion of progesterone by the corpus luteum (CL), occurs on day 13-17 post-estrus. At this time the embryo must signal its presence to prevent release of prostaglandin F2α, luteolysis and resumption of ovarian cyclicity. Extension of CL lifespan is accomplished by secretion of bovine trophoblast protein (bTP-1). Recent studies have demonstrated that bTP-1 has a high degree of sequence homology with interferon of the alpha class.

Early embryo losses, occurring around the time of maternal recognition of pregnancy constitute a major economic problem to the cattle industry. If some embryonic mortality occurs naturally because the conceptus is deficient in producing the signal (bTP-1) to prevent luteolysis, embryonic death could be reduced by pharmacological manipulation of lifespan of the CL.

Intramuscular administration of recombinant bovine interferon-alpha11 extends luteal lifespan in cattle. For example daily injection of 20mg of interferon to Simmental heifers from day 14 to 20 post-estrus significantly extended interestrus intervals (24.08 ± 3.38 days versus 20.74 ± 3.22 days for the controls $p<0.01$). Similarly the interval from estrus until progesterone concentrations declined to a value below 1 ng/ml was 23.15 ± 3.21 days for the rBolFN$\alpha_1$1 treated animals and 19.5 ± 2.83 for the control heifers. The effects depend upon the dose and length of treatment.

Therefore, an interferon alpha molecule may regulate luteal function in cattle: this property could be used to reduce embryonic mortality in cattle and thus to increase fertility.

RESUME

Chez les bovins, la reconnaissance maternelle de gestation, qui se traduit par un maintien de la sécrétion de progesterone par le corps jaune survient au jour 13-17 après oestrus. A cette époque l'embryon doit signaler sa présence pour prévenir la sécrétion de prostaglandine F2α, la lutéolyse et la reprise de la cyclicité ovarienne. La prolongation de la survie du corps jaune est obtenue par sécrétion d'une proteine, la trophoblastine ou bTP-1. Des études récentes ont montré que bTP-1 présente une homologie importante avec les interférons de classe alpha.

La mortalité embryonnaire précoce, survenant aux alentours de la reconnaissance maternelle de gestation est un problème economique important pour les éleveurs de bovins. Si certaines mortalités embryonnaires sont provoquées par une déficience en production de bTP-1 une manipulation pharmacologique de la survie du corps jaune pourrait avoir un impact positif sur la fertilité chez les bovins.

Une injection intra-musculaire d'interféron recombinant alpha bovin (rBolFN$\alpha_1$1) prolonge la survie du corps jaune. Par exemple, une injection journalière de 20 mg d'interferon à des génisses de race Simmental du jour 14 au jour 20 après oestrus a prolongé l'intervalle entre 2 oestrus de façon significative (24,08 ± 3,38 jours contre 20,74 ± 3,22 jours pour le groupe placebo $p<0.01$). De même l'intervalle entre l'oestrus et le jour ou le taux de progestérone chute en dessous de 1 ng/ml fut 23,15 ± 3,21 jours pour les animaux traités avec rBolFN$\alpha_1$1 et 19,5 ± 2,83 jours pour les témoins. L'effet dépend de la dose et de la durée du traitement.

En consequence, l'interféron peut assurer le maintien d'un corps jaune fonctionnel chez les bovins: cette propriété peut être utilisée pour réduire la mortalité embryonnaire et améliorer la fertilité.

RESUMO

Nos bovinos, o reconheicimento materno da gravidez que se segue nos dias 13-17 depois do estro, é definido pela continuação da secreção de progesterona pelo corpo lúteo. Neste período o embrião deve se mostrar presente para evitar a secreção de prostaglandinas F2α, a luteólise e a retomada do ciclo ovariano. A prolongação de vida do corpo lúteo se faz pela secreção de uma proteína, a trofoblastina ou bTP-1. Estudos, realizados recentemente, mostraram que a bTP-1 tem um homologia importante com os interferões da classe alfa.

A mortalidade do embrião prematuro, que aparece por volta do reconhecimento materno da gravidez é um problema económico importante para os criadores de gado. Se algumas mortalidades embrionárias são provocadas por uma deficiência da produção de bTP-1, uma manipulação farmacológica sobre a vida do corpo lúteo poderia ter um impacto positivo na fertilidade dos bovinos.

Uma injeção intra muscular de interferão recombinante alfa bovino (rBolFN$\alpha_1$1) prolonga a vida do corpo lúteo. Por exemplo, uma injeção diária de 20 mg de interferão em novilhas da raça Simmental do dia 14 ao dia 20 depois do estro, prolongou o intervalo entre 2 estros de maneira significativa (24,8 ± 3,38 dias contra 20,74 ± 3,2 dias para o grupo placebo $p<0.01$). No mesmo intervalo entre o estro e o dia no qual a quantidade de progesterona cai a níveis inferior a 1 ng/ml. foi 23,15 ± 3,21 dias para os animais que foram tratados com rBolFN$\alpha_1$1 e 19,5 ± 2,83 dias para os controle. O efeito depende da dose e da duração do tratamento.

Por consequência, o interferão pode manter a vida do corpo lúteo e sua função nos bovinos: esta particularidade pode ser utilizada para diminuir a mortalidade embrionária e melhorar a fertilidade.

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Milch im Abstand von drei Tagen mittels einer Zitzenkanüle passiv abgelassen. Die Vulvaverletzung wurde mit Formazibazol (Ciba Geigy, Basel, Schweiz) abgedeckt.
-Auswertung
Der Erfolg der Operation wurde vier Wochen post operationem anhand der Maschinenmelkbarkeit festgestellt.

RESULTATE

Tabelle 1 zeigt die Melkbarkeit der zwölf operierten Kühe unterteilt nach Lokalisation der Stenose und unterschiedlicher Operationstechnik vier Wochen nach der Operation.

Tabelle 1:Melkbarkeit von zwölf Kühen mit Zitzenstenosen nach Thelotomie und Autotransplantation von Vaginalschleimhaut vier Wochen nach der Operation:

Lokalisation	Zusätze[a)]	n	Melkbarkeit gut	schlecht
mittlere	nein	4	4	-
	ja	3	-	3
mittlere & hohe	nein	2	-	2
	ja	3	-	3

a)= Vasokonstriktiva, Fibrinkleber, Silikontubus

Die Zusammenstellung zeigt, dass die Operation nur bei Tieren mit mittleren Stenosen gelang. Lagen mittlere und gleichzeitig hohe Stenosen vor, war die Operation bezüglich späterer Melkbarkeit erfolglos. Von allen Kühen, bei denen zusätzlich zur Naht Vasokonstriktiva, Fibrinkleber oder Silikontubi verwendet wurden, konnte in keinem Fall eine befriedigende Melkbarkeit wiederhergestellt werden.

DISKUSSION

Autotransplantationen stellen in der humanmedizinischen Chirurgie keine grossen Schwierigkeiten dar. Bei unserer Methode ist die zu transplantierende Schleimhaut in genügender Menge vorhanden und auch leicht mobilisierbar. Trotz der guten Verträglichkeit des Fibrinklebers und den Vorteilen eines Vasokonstriktors in der Humanmedizin, um ein Ablösen des Transplantates durch einschiessendes Blut nach Entfernen der Klemmen an der Zitzenbasis zu verhindern, scheinen sämtliche zusätzliche Manipulationen (Vasokonstriktor, Fibrinkleber, Silikontubus) an der Zitze die Heilung negativ zu beeinflussen. Das beste Resultat wurde erzielt, indem das Transplantat nur am Wundrand eingenäht wurde. Das Ablösen des Transplantates wird sicher auch durch die einschiessende Milch verhindert, die das Transplantat an das Bindegewebe drückt. Bristol (1989) transplantierte Maulschleimhaut in die Zitzenzisterne. Zu diesem Zweck musste eine Vollnarkose durchgeführt werden. Zudem implantierte er einen Silikontubus in die Zitzenzisterne, was zur Entfernung des Tubus eine Reoperation der Zitze erforderlich machte (2). Die hier beschriebene Operationstechnik fordert zwar vom Operateur einiges chirurgisches Geschick, ist aber abgesehen davon leicht durchzuführen und stellt für den Patienten keine unverhältnismässige Belastung dar. In Regionen, in denen der Landwirt bereit ist, eine angemessene Summe für die

Wiederherstellung der Melkbarkeit der Kuh auszugeben, stellt dieses Verfahren eine erfolgversprechende Alternative zu den herkömmlichen und zudem meist erfolglosen Methoden dar.

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ZUSAMMENFASSUNG

Mittlere und hohe Stenosen in der Zitze führen zu Milchabflussstörungen, welche mit den herkömmlichen Operationstechniken nur in den seltensten Fällen behoben werden können. Die hier beschriebene Methode, bei der an der sedierten Kuh, Schleimhaut von der Vulva in die Zitzenzisterne autotransplantiert wird, stellt eine Alternative dar, weil dadurch ein Wiederauftreten der Proliferation von Bindegewebe in der Zitze verhindert werden kann. Verschiedene Operationstechniken wurden ausprobiert. Das beste Resultat wurde dabei erzielt, wenn das Transplantat nur in die Zitze eingenäht wurde. Hohe Stenosen konnten auch mit dieser Methode nicht behoben werden.

SUMMARY

Surgery of the teat after middle and upper stenosis were often unsuccessfull. In this paper we describe a technique, which can be achieved under normal sedation, by autotransplanting a graft from the vulva to the connective tissue of the teat. Several techniques are described. The best results were achieved, when the graft was only fixed with fine hydrolytic suture material. Stenosis of the upper part of the teat cistern could not be successfully treated with this method.

RESUME

Les techniques chirurgicales habituelles s'avèrent très souvent inefficaces dans les cas de sténoses médianes et hautes du trayon. La présente étude décrit une méthode d'autotransplantation de muqueuse vaginale dans la citerne du trayon. Cette opération est praticable sous sédation et présente l'avantage d'empêcher la prolifération post opératoire de tissu conjonctif. Plusieurs techniques sont décrites. Les meilleurs résultats sont observés lorsque la greffe n'est fixée qu'avec un matériel de suture résorbable aussi fin que possible. Cette méthode ne permet toutefois pas un traitement efficace des sténoses hautes.

ESTUDO IMUNOLÓGICO CLÍNICO DO USO EM DOSE REDUZIDA DA VACINA CONTRA A BRUCELOSE (AMOSTRA 19) EM BÚFALAS ADULTAS DE REBANHO PROBLEMA

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INTRODUÇÃO

A brucelose causada pela B.abortus é uma zoonose amplamente disseminada, de importância mundial para a saúde pública e a economia, sendo primariamente descrita como doença de bovinos. Entretanto, há evidências de que seja de igual importância entre os bubalinos, como apontam os levantamentos epidemiológicos internacionais (1,2) e nacionais(3,4,5).

Diante das dificuldades encontradas no controle e erradicação da brucelose no rebanho bovino, particularmente no que diz respeito aos indivíduos adultos infectados, onde o isolamento ou a eliminação dos mesmos pode ser uma medida insatisfatória, desenvolveu-se o método alternativo da vacinação destes animais com dose reduzida da vacina tradicional (amostra 19) (6). Diversos estudos neste sentido, provam ser este um método eficiente para o controle da disseminação da enfermidade no rebanho, restringindo os casos clínicos e diminuindo o número de animais acometidos, com a vantagem de provocar pouca interferência nas provas sorológicas normalmente utilizadas para o diagnóstico (7,8,9,10).

O objetivo do presente trabalho é avaliar os efeitos do uso da vacina contra a brucelose (amostra 19) em dose reduzida num rebanho de bubalinos naturalmente infectado.

MATERIAL E MÉTODOS

Animais experimentais e manejo

Utilizaram-se 67 búfalos (Bubalus bubalis) adultos, sendo 63 fêmeas e 4 machos, de raças variadas, provenientes de 16 rebanhos de diferentes regiões do País, agrupados em Araçatuba-SP, com prevalência de 3,17% a 14,28% em diferentes testes sorológicos, sem antecedentes de vacinação contra a brucelose. Os animais receberam acompanhamento clínico diário e foram mantidos em regime de alimentação de pasto mais suplementação com volumosos e mistura mineral à disposição e em regime de monta natural.

Período experimental

O período experimental de 18 meses foi dividido em pré-vacinação: mês 0; pós-vacinação: meses 1 a 6 e pós-revacinação: meses 7 a 18.

Vacinação e revacinação

Procedeu-se a vacinação das 63 fêmeas por instilação na conjuntiva de uma dose reduzida, com $2x10^9$ bactérias/dose, obtida pela diluição adequada, com solução fisiológica estéril, de vacina contra a brucelose (amostra 19)*, oficialmente controlada, contendo, no mínino, $60x10^9$ bactérias viáveis/dose, no mês 0.

No mês 6, as fêmeas foran revacinadas, por via subcutânea, com igual dose reduzida da mesma vacina.

Colheita das amostras

Amostras mensais de sangue foram colhidas de cada animal, submetendo-

* Vacina Liofilizada Contra Brucelose-Pfizer S.A.

se o soro às provas sorológicas, do mês 0 ao 12 e ainda nos meses 15 e 18.

Provas sorológicas

Utilizaram-se as provas de soroaglutinação rápida (SAR) e do cartão (CARTÃO) para o acompanhamento sorológico mensal, incluindo-se as provas do Rivanol (RIVANOL) e de fixação do complemento (FC) na última colheita do 18º mês.

Todos os testes foram realizados utilizando-se de material controlado e dos métodos recomendados oficialmente.

Na interpretação dos resultados das provas RIVANOL e FC utilizaram-se os critérios atualmente preconizados pelo "The Uniform Methods and Rules" (UMR) do "Cooperative Brucellosis Eradication Program"(CBEP) dos EUA,revisados em 1979 e postos em prática a partir de 1980 (11), os quais consideram, para animais vacinados, na prova RIVANOL, positivo a 1/50 ou mais = positivo; e na FC, 50% de fixação em 1/10 até menos de 25% de fixação a 1/40 = suspeito; e 25% de fixação a 1/40 ou mais = positivo.

RESULTADOS E DISCUSSÃO

Os resultados das provas sorológicas do período pré-vacinação, pós-vacinação nos meses 1, 3 e 5, e, pós-revacinação nos meses 7, 9, 15 e 18, são expressos em porcentagem e estão apresentados nas figuras 1 e 2. Os resultados obtidos nas demais colheitas não estão apresentados em virtude de serem pouco representativos na tendência observada na avaliação dos dados.

A porcentagem de animais positivos observada no período pré-vacinação indica que a prevalência de brucelose no rebanho estudado situa-se entre os valores descritos na literatura estrangeira (1,2), e, bem próxima aos citados por autores brasileiros (3,4,5), sugerindo que o rebanho represente uma amostra significativa do que está ocorrendo nas populações de búfalos de várias regiões do País.

Os resultados no período pós-vacinação indicam aumento do número de animais positivos logo no primeiro mês, não chegando a se manifestar em todas as búfalas, com redução gradual nos meses subsequentes, chegando a valores inferiores à prevalência inicial (Fig.1). Isto indica que o comportamento sorológico dos búfalos à vacinação com dose reduzida na conjuntiva foi bastante semelhante ao dos bovinos, como anteriormente observado (7,8,12).

O aumento na porcentagem de positivos à SAR, no mês 5 (Fig.1), em comparação aos resultados iniciais e aos do CARTÃO, sugere um problema da própria prova, que é de eficiência inferior especialmente quando empregada no diagnóstico de animais que estão em período pós-vacinal (8).

Após a revacinação observou-se fenômeno semelhante ao encontrado no período pós-vacinação com aumento expressivo da porcentagem de positivos logo aos 30 dias, sendo 100% no CARTÃO, havendo decréscimo gradual no número de reagentes positivos e sendo a queda mais lenta em relação ao tempo do que aquela observada no período anterior (Fig.2), caracterizando a resposta anamnéstica proporcionada pela segunda exposição ao antígeno.

Comparando-se os resultados pré-vacinal e final verificou-se redução do número de animais reagentes positivos (Fig.1 e 2), o que se assemelha ao observado em bovinos de rebanhos problema (7,8), bem como, diminuição na porcentagem de suspeitos à SAR (Fig.1 e 2).

As provas sorológicas utilizadas neste trabalho apresentam níveis diferentes de sensibilidade e especificidade tanto para bovinos (7,8,13) quanto para bubalinos (14), sendo o teste do CARTÃO de grande sensibilidade e as provas do RIVANOL e de FC de maior especificidade, o que torna as duas últimas mais eficientes e,portanto, indicadas na fase de 6 meses ou mais pós-vacinação (7,13). Diante deste fato os resultados pode-

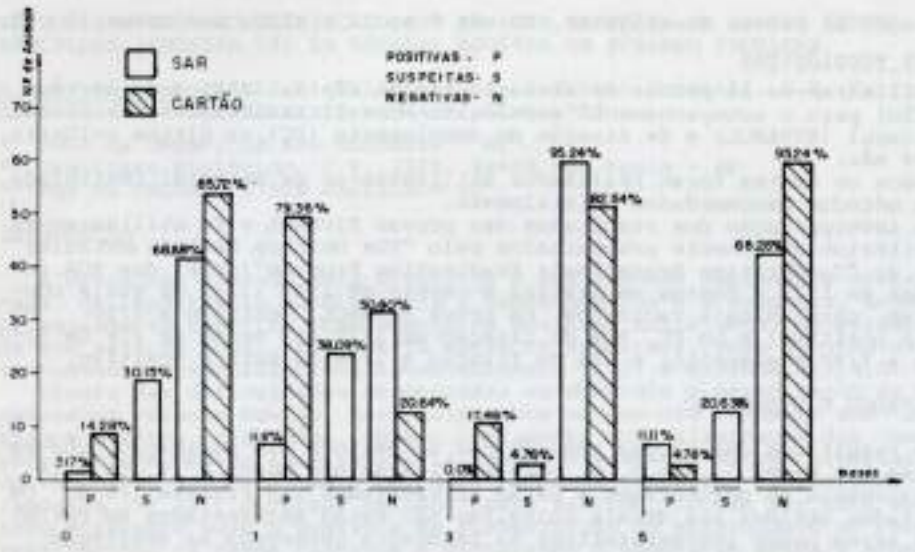

FIGURA 1- QUANTIFICAÇÃO PELOS RESULTADOS NOS TESTES SAR E DO CARTÃO PARA BRUCELOSE DE 63 BÚFALAS ADULTAS ANTES E EM DIFERENTES MESES APÓS A VACINAÇÃO COM DOSE REDUZIDA DE VACINA B 19

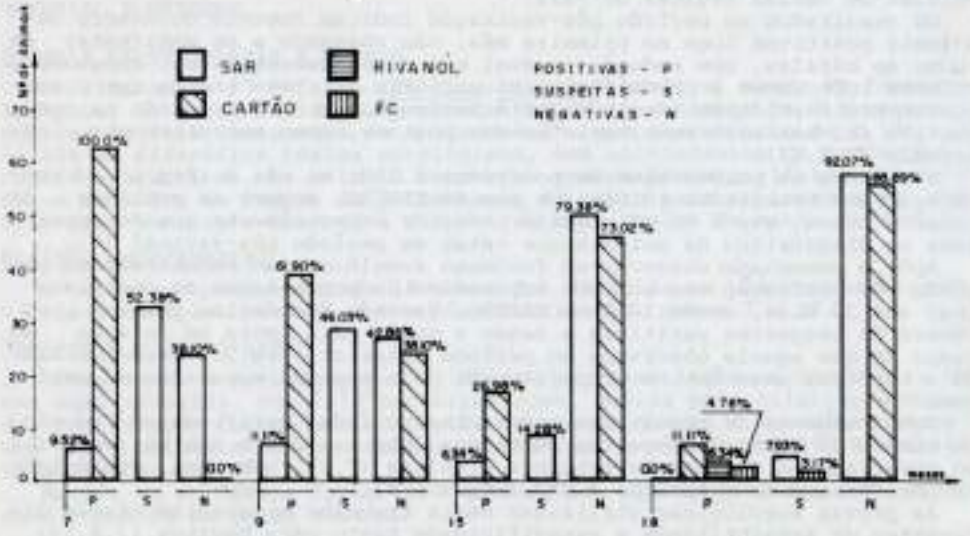

FIGURA 2- QUANTIFICAÇÃO PELOS RESULTADOS NOS TESTES SAR E DO CARTÃO, E, RIVANOL E FC NO 18º MÊS, DE BÚFALAS ADULTAS VACINADAS COM DOSE REDUZIDA DE VACINA B19 NA CONJUNTIVA E 6 MESES DEPOIS REVACINADAS EM IGUAL DOSE DA MESMA VACINA VIA SUB-CUTÂNEA EM VÁRIOS MESES PÓS-REVACINAÇÃO.

riam ser melhor avaliados comparando-se os dados do diagnóstico inicial do CARTÃO (Fig.1) com os resultados finais do RIVANOL e FC (Fig.2) percebendo-se efetivamente a diminuição do número de animais positivos aos 12 meses pós-revacinação.

Ao contrário do que seria esperado, e já fora observado com a introdução da infecção em rebanho de animais não vacinados (15), o procedimento de vacinação das fêmeas adultas com dose reduzida da vacina tradicional controlou efetivamente a disseminação da infecção limitando o aborto a um único caso, mantendo o índice de fertilidade no rebanho(16), reduzindo o número de fêmeas reagentes positivas e não afetando os machos, os quais se mantiveram negativos durante todo o período experimental. Estas observações permitem concluir que a vacinação de búfalas adultas de um rebanho infectado, com dose reduzida de vacina B19 é um método eficiente de intervenção no controle da brucelose nesta espécie animal.

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RESUMO

Em um grupo de 67 búfalos (63F e 4M) adultos de diversas raças, sem antecedentes de vacinação e com prevalência de suspeitas e de positivas para a doença, foi aplicada nas fêmeas dose reduzida com $2x10^9$ bactérias vivas/dose, da vacina contra a brucelose (amostra B-19). Pós-vacinação, de todos os animais no primeiro mês e nos subsequentes foi colhido sangue sendo o sôro submetido às provas sorológicas de soroaglutinação rápida (SAR) e do teste do cartão (CARTÃO). Aos seis meses revacinou-se as búfalas com dose igual, da mesma vacina por via sub-cutânea, continuou-se o exame do sôro por mais 6 meses e a partir do sexto mês por mais duas vezes com intervalos de três meses entre as colheitas. Na última vez além das provas citadas os soros dos animais inicialmente positivos e/ou positivos em alguma prova anterior foram submetidos também ao teste do Rivanol e da fixação de complemento (FC). Os resultados obtidos permiten concluir que a vacinação com dose reduzida da vacina B-19 de búfalas adultas de rebanho com prevalência entre aquelas encontradas no País, frente a diferentes métodos habituais de diagnóstico, evita a disseminação da doença, limita a doença clínica e diminue o número de animais re

gentes positivos no decorrer de 3-5 meses pós-vacinação ou de 12 meses pós-revacinação.

RESUMO

En un grupo de 67 búfalos (63 hembras e 4 machos) adultos de diversas razas, sin antecedentes de vacunación y con prevalencia de animales sospechosos y positivos para la enfermedad, fue aplicado en las hembras dosis reducidas con $2x10^9$ bactérias vivas por dosis de vacuna contra la brucelosis (Muestra B-19). Posteriormente a la vacunación de todos los animales se obtubieron muestras de sangue durante los primeiros meses, siendo el suero sometido a pruebas serológicas de soroaglutinación rápida (SAR) y el "CARD TEST". A los seis meses se revacunaron las hembras por via subcutánea, con dosis iguales de la misma vacuna, y se continuó el exámen del suero por seis meses más, a partir del sexto mes se realizaron otras dos pruebas, siendo las muestras recolectadas con intervalo de tres meses. Después del último muestreo, además de las pruebas citadas, el suero de los animales que eram positivos inicialmente e/ó de los que resultaron positivos en alguna prueba posterior, fué sometido a los testes Rivanol y Fijación de complemento (FC). Los resultados obtenidos usando diferentes métodos de diagnóstico permitem concluir, que la vacunación de las hembras adultas de rebáno entre los prevalescientes en el País, evita la diseminación de la enfermedad, limita las manifestaciones clínicas y disminuye el numero de animales reaccionantes en el período de 3 a 5 meses posterior a la vacunación é de 12 mese después de la revacunación.

SUMMARY

In a group composed of 67 buffaloes (63 females and 4 males), all adult animals belonging to various breeds, with no prior vaccination records, some positive and some suspected of having brucellosis, the females were inoculated with small doses containing $2x10^9$ live bacteria / dose of brucellosis vaccine (B-19 strain). In the first and subsequent months post-vaccination, blood samples of all animals were collected and the sera used for serologial tests, i.e., the rapid serum agglutination test (RSAT) and the Card Test (CT). Six months later, the buffalo cows were revaccinated with an equal dose of the same vaccine, by the subcutaneous route, and their sera were then examined for six more months and after this, 2 more times at a 3-months interval. In the last time, besides the two tests mentioned, sera of those animals, positive at the beginning and/or positive for some of the tests previously used were also submitted to the Rivanol test and to the complement fixation test (FC). The results obtained allow us to conclude that vaccination with a reduced dose of the B-19 vaccine, of adult buffalo cows of a herd in which the prevalence is similar to that regularly recorded for the Country when usual diagnostic methods are employed, avoids dissemination of the disease, restrains the overt disease and decreases the number of positive cases in a period of time ranging from 3 to 5 months post-vaccination or 12 months after revaccination.

NIVELES PLASMATICOS DE PROGESTERONA EN RECEPTORAS DE EMBRIONES CONGELADOS DETERMINADOS POR ELISA TEST.

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INTRODUCCION

La transferencia de embriones bovinos (sean frescos o congelados), como biotecnología aplicada, reconoce varias etapas que se inician con la hembra dadora y finaliza con las receptoras.

Durante los últimos años se investiga con mayor énfasis la receptora y su influencia sobre el porcentaje de preñez, relacionándola con la raza, edad, estado sanitario, nivel nutricional y productivo (4,9,10,11); por el momento los resultados son contradictorios. También debe establecerse un perfil endocrinológico que asegure la potencial fertilidad de la receptora y el establecimiento satisfactorio de una preñez (12,13), por cuanto, luego de la transferencia, es decisivo un cuerpo lúteo (C.L) funcional, que a su vez dependerá de las señales luteotróficas y antiluteolíticas del embrión.

Aún no está definida la concentración de progesterona (P_4) necesaria para que un embrión sobreviva durante la tercera semana post-implante; pero sí se han demostrado fallas en la concepción cuando la concentración de P_4 es inferior a 1 ng/ml (12,13,14,15,16).

Por otro lado numerosas publicaciones señalan que los mayores índices de preñez, se logran con concentraciones de P_4 que oscilan entre 2 y 5 ng/ml (13,14,16,18,19, 20,21).

Este trabajo se propone establecer las siguientes relaciones:

1) Niveles plasmáticos de P_4 y porcentaje de preñez.
2) Concentración de P_4 y calidad del cuerpo lúteo.
3) Calidad del embrión, niveles plasmáticos de P_4 y porcentaje de preñez.

MATERIALES Y METODOS

Durante el año 1986, en el Centro de Transferencia Embrionaria "La Josefina", ubicado en San Antonio de Areco (Buenos Aires), fueron destinadas 163 vaquillonas Holando Argentino,(cuyas edades y pesos oscilaron entre 18 y 24 meses, y 350 y 420 kg., respectivamente) a la transferencia de embriones congelados.

El programa de sincronización utilizado fue la doble aplicación de Cloprostenol 500 ug. (análogo de la prostaglandina $F_{2\alpha}$), con un intervalo de 11 días. Entre las 48-96 hrs. después de la última inyección, presentaron celo manifiesto un total de 146 vaquillonas (74,0%).

El día de la transferencia las vaquillonas fueron sometidas a tacto rectal a los fines de evaluar: a) presencia y tamaño del/los folículo/s; b) localización y calidad de cuerpo lúteo. Para determinar la calidad del cuerpo lúteo se estableció la siguiente escala subjetiva:

- 1 ó muy bueno: 15 a 25 mm de diámetro, con corona manifiesta
- 2 ó bueno: 15 a 20 mm de diámetro, sin corona manifiesta
- 3 ó regular: menos de 15 mm de diámetro, sin corona, con un folículo en cualquiera de los ovarios.

- 4 ó malo: cuerpo lúteo muy pequeño, acompañado de un folículo con un diámetro de 5 a 15 mm.

En base a ésta escala se seleccionaron 111 animales (68,0%) con cuerpo lúteo de tipo 1 ó 2 para ser transferidos quirúrgicamente (5). Antes de la misma se extrajeron dos muestras de sangre en tubos con heparina, los cuales fueron centrifugados y el plasma resultante almacenado a -22 °C hasta su procesado. La concentración de P_4 fue determinada por enzimoinmunoensayo (Elisa) semicuantitativo (Ovucar) en los rangos de 0,5, 1.0 y 5.0 ng/ml.

Los embriones utilizados fueron clasificados, después de la descongelación, en calidad 1 ó excelente y calidad 2 ó buena, según técnica ya descripta (5,6).

Todas las vaquillonas transferidas se observaron dos veces al día para determinar el intervalo de tiempo transcurrido en retornar al celo, de aquellas que no quedaron preñadas. Según el mismo, el celo se clasificó como:

fisiológico	18 a 23 días
prolongado	24 a 36 días
extenso	más de 37 días

La preñez fue diagnosticada por tacto rectal entre los días 45 y 60 posteriores a la transferencia. En el análisis estadístico se utilizó el Test χ^2 (Ji cuadrado).

RESULTADOS

Sobre un total de 111 receptoras seleccionadas y transferidas, 56 de ellas (50.45%) presentaron preñez positiva al tacto rectal.

Las receptoras fueron divididas en dos grupos, A y B, de acuerdo al análisis de P_4 plasmática. El grupo A lo constituyeron todos los animales cuyo plasma fue positivo a los patrones de 1 y 5 ng/ml. El grupo B engloba a las receptoras negativas a todos los patrones ó positivas a 0.5 ng/ml.

Según Cuadro N°1, dentro del grupo A, de las 78 vaquillonas (70.2%;78/111) se obtuvo preñez en 53 de ellas (67.9%), mientras que para el grupo B, de 33 receptoras (29.7%;33/111) sólo resultan preñados 3 animales (9.09%). El porcentaje de preñez entre ambos grupos, muestra diferencias altamente significativas (P<0.01).

El Cuadro N°2 muestra el ordenamiento de las receptoras transferidas y los resultados de preñez en función de los niveles de P_4 plasmáticos. Los porcentajes de preñez para cada uno de los grupos fue: 0.0%, 12.28%, 66.6% y 69.0%. Las diferencias son altamente significativas (P<0.01) para cada uno de los niveles de P_4.

El Cuadro N°3 establece la relación entre la calidad del cuerpo lúteo, nivel plasmático de P_4, receptoras transplantadas y preñadas. Como puede observarse no hay diferencias significativas con el parámetro calidad del cuerpo lúteo, pero sí la hay con los niveles de P_4 plasmáticos.

Además, se observaron las 25 receptoras del grupo P_4 mayor a 1 ng/ml que no quedaron preñadas: 17 de ellas (68.0%) retornaron al celo en un período fisiológico (18 a 23 días); 5 (20%) tuvieron celo en un intervalo prolongado (23 a 36 días); mientras 3 receptoras (12%) manifestaron un intervalo inter-estrual post-transferencia mayor de 37 días, posiblemente debido a mortalidad embrionaria.

Como puede observarse en el Cuadro N°4, no existe significación estadística entre la calidad del embrión (excelente ó bueno) y la preñez lograda; pero vuelve a ser significativa la preñez versus el nivel plasmático de la progesterona.

Se analizó el grado de repetibilidad del Test Elisa, evaluando las muestras por radioinmunoensayo. El coeficiente de correlación (r) fue 0.87 (22).

Los resultados de Hireco y col. (23) concluyen que el Elisa test para P_4 plas-

CUADRO N°1. Relación Porcentaje de preñez - Nivel plasmático de progesterona.

Concentración P_4 plasmática	N° recept. transf. (%)	N° recept. preñad (%)
Grupo A (1 ng/ml)	78 (70.2%)	53 (67.9) [a]
Grupo B (1 ng/ml)	33 (29.8%)	3 (9.09) [b]
TOTAL	111 (100)	56

a,b: letras diferentes indican diferencias significativas (P<0.01)

CUADRO N°2. Porcentajes de preñez para cada concentración de P_4 plasmática el día de la transferencia.

Niveles de P_4 plasmática	N° recept. transf. (%)	N° recept. preñ. (%)
Negativo	12 (10.8)	0 (0.0) [a]
Positivo a 0.5 ng/ml	21 (18.9)	3 (14.3) [b]
Positivo a 1.0 ng/ml	36 (32.4)	24 (66.6) [c]
Positivo a 5.0 ng/ml	42 (37.8)	29 (69.0) [d]
TOTAL	111 (100)	56 (50.2)

a,b,c,d: letras diferentes indican diferencias significativas (P<0.01)

CUADRO N°3. Porcentajes de preñez según niveles plasmáticos de P_4 y su relación con la calidad del cuerpo lúteo.

Calidad del C.L.	Conc. de P_4	N° recept. transf. (%)	N° preñadas (%)
1	mayor a 1ng/ml	63 (80.7)	43 (68.2) [a]
2	mayor a 1ng/ml	15 (19.3)	10 (66.6) [a]
TOTAL		78 (100)	53 (67.9) [A]
1	menor a 1 ng/ml	26 (78.7)	3 (11.5) [c]
2	menor a 1 ng/ml	7 (21.3)	0 (0.0) [c]
TOTAL		33 (100)	3 (9.0) [C]

a,c y A,C: letras diferentes indican diferencias significativas (P<0.01)

CUADRO N°4. Porcentaje de preñez según calidad del embrión y nivel de P_4 en recept.

Calidad embrión	N° embr. transf.	P_4 recept.	N° recept. preñ. (%)
1 (excel.)	56	mayor a 1ng/ml	39 (69.6) [a]
2 (buena)	22	mayor a 1ng/ml	14 (63.6) [a]
TOTAL	78		53 (67.9) [A]
1 (excel.)	17	menor a 1 ng/ml	2 (11.7) [b]
2 (buena)	11	menor a 1 ng/ml	1 (9.09) [b]
TOTAL	28		3 (10.7) [B]

a,b y A,B: letras diferentes indican diferencias significativas (P<0.01)

mática, posee la suficiente sensibilidad y precisión para ser usado "a campo"; con un coeficiente de correlación con el radioinmunoanálisis (R.I.A) de 0.97 ($P<0.001$).

COMENTARIO

El objetivo de ésta experiencia fue evaluar el Elisa test (P_4) como un criterio adicional en la selección de receptoras al momento de la transferencia; con la idea de incrementar los índices de preñez.

Los resultados obtenidos coinciden con otras publicaciones, donde los mejores índices de preñez se logran en aquellas receptoras que presentan niveles de P_4 plasmática entre 1 y 5 ng/ml [13,16,18,19,22). En nuestro caso llega al 67.9% (53/78).

La selección de una hembra receptora se rige,en primera instancia, por la observación de un celo manifiesto entre las 48 y 96 hs. posteriores a la sincronización; luego el diagnóstico de un cuerpo lúteo, clasificándolo en forma subjetiva, de acuerdo al tamaño, presencia de corona, elasticidad del tejido luteal, en sincronismo con el día del celo de referencia (20). Cualquier alteración en éstos parámetros es suficiente para eliminar la receptora del programa. Lamentablemente, ninguno de éstos criterios permite abrir juicio sobre la secreción hormonal del cuerpo lúteo (Cuadro N°3). No existe relación entre la calidad subjetiva del cuerpo lúteo y los niveles de P_4, por cuanto el 29.2% de receptoras con cuerpo lúteo-calidad 1, registraron concentración de P_4 inferior a 1 ng/ml, y el 31.8% de receptoras con calidad de cuerpo lúteo 2 también registraron concentración de P_4 inferior a 1 ng/ml (Cuadro N°3).

El ritmo de secreción de P_4 por parte del cuerpo lúteo es importante por cuanto permite que exista una sincronización entre el medio intrauterino y el desarrollo del embrión (12,23). Un aumento prematuro de dicho esteroide durante el metaestro iniciaría un asincronismo entre ambos, similar a la situación que ocurre después de la transferencia de un embrión a una receptora que presentara celo 2 a 3 días antes que la donante (4,12,24). Es sabido que la sobrevivencia del embrión transferido es mayor cuando las concentraciones plasmáticas de P_4 en el 7° a 8° día del ciclo son moderadas. Si bien no se conocen con exactitud los niveles mínimos que aseguran la viabilidad embrionaria, pueden considerarse adecuadas concentraciones de P_4 entre los 2 y 5 ng/ml (12,13,14,16,18,19,20).

En éste estudio los porcentajes de preñez fueron 67.9% y 9.09% para aquellas receptoras con niveles de P_4 entre 1 y 5 ng/ml, y menos de 1 ng/ml respectivamente (Cuadros N°1 y 2). La bibliografía cita que con menos de una concentración de P_4 de 2 ng/ml, los porcentajes de preñez oscilan entre el 8.3 al 35.3%, situación que se presenta en un 21 a 24% de las receptoras [14,18,19,20]; no estableciéndose preñez alguna con niveles inferiores a 1 ng/ml [16,22]. Esto corresponde con nuestros resultados (Cuadros N°1 y 2).

Finalmente los niveles decisivamente altos (más de 6.0 ng/ml) no presentan una correlación positiva con el establecimiento de la preñez (12). Esto puede deberse a una errónea observación del celo, especialmente cuando se maneja un gran número de hembras sincronizadas.

La calidad de los embriones también influye sobre los índices de preñez (2,3). No se establecieron diferencias significativas entre aquellos embriones de calidad excelente y buena, siempre que fueran transferidos a receptoras con P_4 superiores a 1 ng/ml; pero sí resultaron significativas para receptoras con P_4 menores a 1 ng/ml (Cuadro N°4).

CONCLUSIONES

Reconocemos la utilidad de un test rápido para la determinación de la concentración de P_4 en plasma el día de la transferencia, por cuanto: a) permitirá la selección de receptoras que brindarán mejores y mayores probabilidades de quedar preñadas; b) permitirá la reutilización de receptoras en breve plazo, con niveles hormonales inadecuados (hasta 35%) sin esperar el retorno al celo o el diagnóstico de vacuidad después de la transferencia.

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RESUMEN

El objetivo de ésta experiencia fue evaluar el Elisa test para determinación de progesterona, como un criterio adicional en la selección de receptoras en el momento de la transferencia embrionaria, con la finalidad de incrementar los índices de preñez.

SUMMARY

This experience aim was to evaluate the Progesterone Elisa test as an aditional screening method for recipients at the embryo transfer time.
This method should result in increase of the pregnancy rates.

SUMARIO

O trabalho objetivou avaliar o emprego do Elisa Test para a determinação de progesterona, como mais um critério na seleção de receptoras no momento da transferência embrionária, com a finalidade de obter maiores índices de gravidez.

PROLONGATION OF CORPUS LUTEUM LIFESPAN IN HEIFERS BY INTRAMUSCULAR ADMINISTRATION OF RECOMBINANT BOVINE INTERFERON ALPHA$_I$1.

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INTRODUCTION

In cattle maternal recognition of pregnancy, characterized by a sustained secretion of progesterone by the corpus luteum (CL), occurs on day 14-17 post estrus. At this time the embryo must signal its presence to prevent luteolysis. Indeed, regulation of the lifespan of the corpus luteum by the conceptus is critical for a successful pregnancy because, in cattle, the corpus luteum is the major source of progesterone during pregnancy. Between day 15 and 25 the conceptus secrete specific protein called bovine trophoblast protein 1 (bTP-1) which directly or indirectly prolong the lifespan of the corpus luteum and prevent return to ovarian cyclicity (3). Recent studies have demonstrated that bTP-1 has a high degree of sequence homology with interferon of the alpha class (2). In addition it has been shown that recombinant bovine interferon alpha1 (rBoIFNα_I1) administered in utero into cyclic cows from day 15.5 to 21 after estrus delayed luteolysis (4). Similarly twice daily intramuscular injections of rBoIFNα_I1 from day 15 to 19 after estrus extend estrus intervals (5).

It is well recognized that early embryo losses, occurring around the time of maternal recognition of pregnancy constitute a major economic problem to the animal industry (1). If some embryonic mortality occurs naturally because the conceptus is deficient in producing the signal (bTP-1) to prevent luteolysis, embryonic death could be reduced by pharmacological manipulation of lifespan of the corpus luteum. The purpose of our study was to demonstrate if daily systemic treatment of cycling heifers with various doses of rBoIFNα_I1 can prolong the lifespan of CL.

MATERIALS AND METHODS

Animals

Simmental heifers, 18-20 months old, weighing 350-420 kg were housed in an indoor pen and fed corn silage and hay. Palpation per rectum of the reproductive organs was performed before the beginning of the trial to monitor ovarian activity and uterus size. Only animals with no detectable reproductive disorders were enrolled in the study.

Estrus detection

Estrus behaviour was observed for at least 30 min three times a day (07:00, 13:00 and 18:00h) starting on day 14 post estrus. The heifers standing to be mounted by other heifers without attempts to escape were considered to be on heat. The results of the observation was confirmed by determination of progesterone concentrations in plasma: levels declined to a value below 1 ng/ml before and during estrus.

Sample collection

Blood samples were collected from the coccygeal vein once daily (10:00h) beginning on day 14 post estrus and continuing until either estrus was detected or day 30 post estrus, for determination of progesterone concentration. Plasma was harvested by centrifugation at 1000 g for 20 min at 4° and frozen at -20°C until assayed.

Progesterone assay

The concentrations of progesterone in plasma were measured using an enzyme linked immunoabsorbent assay (ELISA, OVUCHECK® Cambridge Veterinary Science, Cambridge, UK) and an automated microtiter spectrophotometer set at 405 nm. Limit detection of the assay was 0.5 ng/ml. The intra assay precision at 1 ng/ml progesterone in plasma was 10%.

Recombinant Bovine Interferon Alpha$_1$1

rBoIFN$\alpha_1$1 was produced in E. coli by recombinant DNA technology, purified to homogeneity as determined by SDS polyacrylamide gel electrophoresis and formulated as a lyophilized dry substance. Stock preparation had a minimum specific activity of 10^7 working units/mg protein as determined by plaque inhibition of vesicular stomatitis virus grown in Madin Darby bovine kidney cells.

Each animal received daily either 10 or 20 mg of rBoIFN$\alpha_1$1 intramuscularly or an equivalent volume of placebo (8ml). Studies were performed blind: the personnel involved in the trials were unaware of the nature of treatment administered.

Study design

32 Simmental heifers were synchronized using a standard double injection (11 days apart) of prostaglandin F2α (Dynolytic® The UpJohn Company). Animals were allocated to treatment groups that were balanced for weight of heifers. Treatment with 20 mg of rBoIFN$\alpha_1$1 were administered daily from day 14 to 20 post estrus. Only animals with a functional CL on day 14 as demonstrated by plasma progesterone levels > 1ng/ml were admitted into the study. Animals were observed for signs of heat up to day 33 after estrus and blood samples collected daily.

In another similar experiment 12 dairy heifers were treated once a day intramuscularly with 10mg of rBoIFN$\alpha_1$1 or a placebo from day 14 to 19 post estrus.

Statistical analysis

Treatment effect on length of estrus cycle were determined by one tailed Student t. test.

RESULTS AND DISCUSSION

In the first experiment administration of rBoIFN$\alpha_1$1 extended significantly ($p<0.01$) the length of the estrus cycle (24.08 ± 3.38 days vs 20.74 ± 3.22 days for the controls). Similarly the interval from estrus until progesterone concentrations declined to a value below 1 ng/ml was 23.15 ± 3.21 days for the rBoIFN$\alpha_1$1 treated animals and 19.5 ± 2.83 for the control heifers. (table 1).

Similarly, in experiment 2, interferon treated heifers had prolonged cycles ($p<0.05$) when compared with the placebo treated group (table 2). Mean interestrus intervals were respectively 23.4 ± 3.78 and 19.5 ± 1.61 days. Means for the period of functional lifespan of the CL (measured by progesterone levels >1 ng/ml) were 21.8 ± 3.03 and 18.33 ± 1.254. Luteal lifespan was longer in the interferon treated group as compared with that in the control group.

Therefore, daily systemic administration of various doses of rBoIFN$\alpha_1$1 can prolong functional lifespan of the CL and length of estrus cycle. It is known, that high rates of embryonic loss occur during the time of maternal recognition of pregnancy day 14-24 (1). This period is critical for the conceptus because it must rescue the corpus luteum from luteolysis by secreting bTP-1. It is therefore possible that some embryos do not produce an optimal level of bTP-1 at the right time, if for instance their development is retarded or in asynchrony with the endometrium of the dam. It has been shown for example that heat stress causes a large decrease in bTP-1 secretion by the conceptus in utero (5). Anasynchrony between the embryo and the uterus of the cow is also possible after embryo transfer. Likewise, in heifers or cows with a short estrus cycle, luteolysis occurs earlier than normal before the conceptus can produce bTP-1 to ensure its survival. Avoiding embryonic loss by injecting bTP-1 or its homologous proteins interferons to maintain corpus luteum function is therefore a possible mean of increasing fertility in cattle. Other approaches, using luteotrophic factors like human chorionic gonadotrophin have been described with some success (8).

		Interestrus Intervals	Functional Lifespan of CL
Placebo	(n=19)	20.74 ± 3.22	19.5 ± 2.84
rBoIFN$\alpha_1$1	(n=13)	24.08 ± 3.38	23.15 ± 3.21

Table 1 Experiment 1 - Effect of daily administration of rBoIFN$\alpha_1$1 (20 mg from day 14 to 20) on cycle length and lifespan of CL (mean ± standard deviation)

		Interestrus Interval	Functional lifespan of CL
Placebo	(n=6)	19.5 ± 1.61	18.33 ± 1.25
rBoIFN$\alpha_1$1	(n=6)	23.4 ± 3.78	21.8 ± 3.03

Table 2 Experiment 2 - Effect of daily administration of rBoIFN$\alpha_1$1 (10 mg from day 14 to 19 postestrus)

The mechanisms that are engaged to extend the corpus luteum function are only partially understood. Interferons might extend the lifespan of the corpus luteum by either inhibiting prostaglandin F2α release from the uterus or inducing resistance to PGF2α in the corpus luteum. Indeed it has been shown (7) that PGF2α and PGE2 secretion by cultured ovine endometrial cells can be inhibited by both oTP-1 or human interferon alpha.

The results presented in this study support further investigation of the role of interferons as polypeptide hormones in establishment of pregnancy.

ABSTRACT

In cattle, maternal recognition of pregnancy, which is signified by a sustained secretion of progesterone by the corpus luteum (CL), occurs on day 13-17 post-estrus. At this time the embryo must signal its presence to prevent release of prostaglandin F2α, luteolysis and resumption of ovarian cyclicity. Extension of CL lifespan is accomplished by secretion of bovine trophoblast protein (bTP-1). Recent studies have demonstrated that bTP-1 has a high degree of sequence homology with interferon of the alpha class.

Early embryo losses, occurring around the time of maternal recognition of pregnancy constitute a major economic problem to the cattle industry. If some embryonic mortality occurs naturally because the conceptus is deficient in producing the signal (bTP-1) to prevent luteolysis, embryonic death could be reduced by pharmacological manipulation of lifespan of the CL.

Intramuscular administration of recombinant bovine interferon-alpha11 extends luteal lifespan in cattle. For example daily injection of 20mg of interferon to Simmental heifers from day 14 to 20 post-estrus significantly extended interestrus intervals (24.08 ± 3.38 days versus 20.74 ± 3.22 days for the controls $p<0.01$). Similarly the interval from estrus until progesterone concentrations declined to a value below 1 ng/ml was 23.15 ± 3.21 days for the rBolFNα$_1$1 treated animals and 19.5 ± 2.83 for the control heifers. The effects depend upon the dose and length of treatment.

Therefore, an interferon alpha molecule may regulate luteal function in cattle: this property could be used to reduce embryonic mortality in cattle and thus to increase fertility.

RESUME

Chez les bovins, la reconnaissance maternelle de gestation, qui se traduit par un maintien de la sécrétion de progestérone par le corps jaune survient au jour 13-17 après oestrus. A cette époque l'embryon doit signaler sa présence pour prévenir la sécrétion de prostaglandine F2α, la lutéolyse et la reprise de la cyclicité ovarienne. La prolongation de la survie du corps jaune est obtenue par sécrétion d'une protéine, la trophoblastine ou bTP-1. Des études récentes ont montré que bTP-1 présente une homologie importante avec les interférons de classe alpha.

La mortalité embryonnaire précoce, survenant aux alentours de la reconnaissance maternelle de gestation est un problème économique important pour les éleveurs de bovins. Si certaines mortalités embryonnaires sont provoquées par une déficience en production de bTP-1 une manipulation pharmacologique de la survie du corps jaune pourrait avoir un impact positif sur la fertilité chez les bovins.

Une injection intra-musculaire d'interféron recombinant alpha bovin (rBolFNα$_1$1) prolonge la survie du corps jaune. Par exemple, une injection journalière de 20 mg d'interféron à des génisses de race Simmental du jour 14 au jour 20 après oestrus a prolongé l'intervalle entre 2 oestrus de façon significative (24,08 ± 3,38 jours contre 20,74 ± 3,22 jours pour le groupe placebo $p<0.01$). De même l'intervalle entre l'oestrus et le jour où le taux de progestérone chute en dessous de 1 ng/ml fut 23,15 ± 3,21 jours pour les animaux traités avec rBolFNα$_1$1 et 19,5 ± 2,83 jours pour les témoins. L'effet dépend de la dose et de la durée du traitement.

En conséquence, l'interféron peut assurer le maintien d'un corps jaune fonctionnel chez les bovins: cette propriété peut être utilisée pour réduire la mortalité embryonnaire et améliorer la fertilité.

RESUMO

Nos bovinos, o reconhecimento materno da gravidez que se segue nos dias 13-17 depois do estro, é definido pela continuação da secreção de progesterona pelo corpo lúteo. Neste período o embrião deve se mostrar presente para evitar a secreção de prostaglandina F2α, a luteólise e a retomada do ciclo ovariano. A prolongação de vida do corpo lúteo se faz pela secreção de uma proteína, a trofoblastina ou bTP-1. Estudos, realizados recentemente, mostraram que a bTP-1 tem um homologia importante com os interferões da classe alfa.

A mortalidade do embrião prematuro, que aparece por volta do reconhecimento materno da gravidez é um problema econômico importante para os criadores de gado. Se algumas mortalidades embrionárias são provocadas por uma deficiência da produção de bTP-1, uma manipulação farmacológica sobre a vida do corpo lúteo poderia ter um impacto positivo na fertilidade dos bovinos.

Uma injeção intra muscular de interferão recombinante alfa bovino (rBolFNα$_1$1) prolonga a vida do corpo lúteo. Por exemplo, uma injeção diária de 20 mg de interferão em novilhas da raça Simmental do dia 14 ao dia 20 depois do estro, prolongou o intervalo entre 2 estros de maneira significativa (24,8 ± 3,38 dias contra 20,74 ± 3,2 dias para o grupo placebo $p<0.01$). No mesmo intervalo entre o estro e o dia no qual a quantidade de progesterona cai a níveis inferior a 1 ng/ml, foi 23,15 ± 3,21 dias para os animais que foram tratados com rBolFNα$_1$1 e 19,5 ± 2,83 dias para os controle. O efeito depende da dose e da duração do tratamento.

Por consequência, o interferão pode manter a vida do corpo lúteo e sua função nos bovinos: esta particularidade pode ser utilizada para diminuir a mortalidade embrionária e melhorar a fertilidade.

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NUTRITION AND REPRODUCTIVE PERFORMANCE IN FINNISH DAIRY COWS

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INTRODUCTION

Conception difficulties in cows are a rather common problem on Finnish dairy farms. Such difficulties prolong the calving interval, resulting in economic consequences especially in high-yielding cows. The mean calving interval in Finnish dairy herds is about 2 weeks longer than the theoretical optimum of 365 days. Many factors , including inappopriate management and clinical abnormalities, may contribute to this reduced fertility. It has commonly been suggested that nutrition greatly affects the fertility of dairy cows.

In preventive medicine certain examinations, such as food analysis and metabolic profiles may be carried out on a herd basis, however, among individual cows in a herd the reproductive efficiency varies considerably. The metabolic profile test has been used to monitor the metabolic state of a dairy herd and particularly to assess the adequacy of dietary intake (11). These tests have sometimes been disappointing, however, often because reference animals are difficult to select and blood reference values and their variations remain poorly known. Moreover, the results are often contradictory (13). Some reports have suggested that low energy levels impair fertility (3). Others have shown no significant effects on fertility (2). Low level of blood glucose influences fertility negatively (8). Glucose level is low 8-9 weeks postpartum and then higher when fertility is better (7). Conversely, Blowey *et al.* (1), Kappel *et al.* (6) and Rowlands *et al.* (14) found no relationship between blood glucose and fertility.

Often cows are fed rather intensively before parturition, which leads to an accumulation of body fat. Body fat is mobilised in early lactation when the energy requirement exceeds energy intake, resulting in shifts of the energy metabolism. During energy deficiency, glucose values tend to decrease, whereas concentrations of acetoacetate and ß-hydroxybutyrate increase. The correlation between blood glucose, acetoacetate and ß-hydroxybutyrate and energy balance is weak (4).

Previously was found that the type of feed can significantly affect reproductive efficiency in dairy cows (9).The silage group took a significantly longer time for uterine involution, had a lower fertility rate at first insemination, and the interval from calving to conception was longer than in the hay-urea group. In the silage group low energy level in early puerperium caused a delay in onset of ovary activity and of uterine involution (10). The blood glucose had also a significant effect on the reproductive performance.

Materials and Methods

Animals

In this study a total of 50 cows calving the third time were included. The calving season was from April to September. Cows with a gestation period of less than 260 days were regarded as abnormal and were excluded from the study. All animals included were pregnant before 160 days postpartum and had a normal and easy calving (unassisted or assisted by one person) and they had no clinically detectable disturbances of the genital tract during the postpartum period.

Management and feeding

The experimental cows were housed indoors on a reseach farm and fed individually with feed produced on the same farm. Daily feed intake and feed refusals were recorded. The cows were divided according to the type of feed into a hay-urea group (n = 26) and a silage group (n = 24). Both groups were fed a concentrate containing 2/3 barley and 1/3 oats according to the daily milk yield in accordance with Finnish feeding standards. The energy value was calculated in Finnish Feed unit (f.u). The amount of concentrate depended on daily milk yield as follows: up to 15 kg FCM (4% fat corrected

milk) 0.32 f.u (feed unit)/kg FCM was given in the hay-urea group and 0.24 f.u/kg FCM in the silage group; for higher milk yield than 15 kg FCM 0.02 f.u/5kg FCM was added. All animals in the hay-urea group were allowed free access to dry hay, and two per cent urea was added to the concentrate as a source of nitrogen. The silage group was allowed free access to grass silage and the amount of hay was limited to 1 kg per day. The rations were designed to contain the same amounts of nutritiens in terms of energy and digestible crude protein. The cows were allowed to eat twice a day: in the morning from 6 to 10 am and in the afternoon from 2 to 6 pm. The average milk yield of the herd was about 5500 kg. External signs of heat were checked 3 times daily by the herdsmen.

Clinical examinations

The cows were examined clinically 3 times a week between calving and the first insemination, which normally took place at the first heat, up to 8 weeks after the previous delivery. The examination included an inspection of the vulva and perineum as well as a rectal palpation of the cervix, uterine horns and ovaries. The diameters of cervix and both uterine horns at the external bifurcation were estimated by palpation and reported in centimeters. One examiner carried out all studies, but previous findings were not available during the subsequent examinations.

The uterine involution was considered complete when both horns were nearly symmetrical and no further change took place between two consecutive examinations. For each animal the time was recorded when the uterus was within or at the edge of the pelvic cavity, i.e., in the pelvic cavity position (P.C.P.). The end of the cervical involution was the time required for the mid-cervix diameter to reach the nadir. The interval from parturition to the appearance of the first palpable follicle was recorded. The pregnancy rate at the first insemination and the interval from calving to conception were determined for each cow. The pregnancies were confirmed by rectal palpation 6-7 weeks after insemination and in any doubtful case the examination was repeated 1-2 weeks later. The pregnancy of all cows was followed for a period of more than 3 months.

Blood samples

Blood samples were taken 10, 15, 30 and 50 days postpartum in the morning between 0900 1000h. The blood was drawn from the jugular vein, then one blood sample was allowed to clot and was centrifuged. Serum was received for analysis of glucose on the same day. The other sample was taken into heparinized tube and a blood samples of 0.5 ml was immediately after sampling precipitated with 2.0 ml of 0.6 M perchlorid acid. The precipitated samples were kept frozen at -20°C until analyzed.

Before analysis of acetoacetat (AA) and 3-ß-hydroxybutyrate (HB), the acid samples were centrifuged and the supernatant was treated with 3 N KOH and kept on ice for 30 minutes. The $KClO_4$-precipitate was then removed by centrifugation. The clear supernatant was used for the analysis of AA and 3-HB by kinetic enzymatic methods using 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) as a catalytic enzyme. AA was determined by the method of Price *et al.* (12) and HB according to Hansen and Freier (5). The analyses were performed with a Gilford System 3500 analyzer (Gilford Instrument Laboratories Inc., Ohio, USA). Glucose was determined by the GOD-glucose test (Boehringer Mannheim Gmbh Diagnostica, FRG).

The means (±S.D.) of the measurements were calculated. Student's *t*-test between groups was used in the statistical comparison of the results. Correlations were evaluated by regression analysis.

RESULTS

The correlation between blood glucose and ketone bodies were negative during the follow-up period. Only by day 30 were both correlations significant ($p<0.05$). The correlations between glucose and AA and between glucose and HB were similar.

Relationships between energy level and fertility parameters varied at different times postpartum period (Table 1). An increased level of ketone bodies only at day 10 did not significantly influence either the interval from calving to conception or the interval from calving to the first insemination, but a later disturbance at days 30 and 50 significantly influenced the interval from calving to the first

Table 1. Relationships between metabolic balance (acetoacetat and ß-hydroxybutyrate) at different times postpartum and fertility parameters. F1 = first palpable follicle; CC = interval from calving to conception; AI = interval from calving to first insemination; AIn = number of inseminations per conception; r = regression coefficient; Adj. r^2 = adjusted r-squared; p = probability.

Stage (days)	Parameter	r	adj. r^2	p
10	CC	0.350	0.077	0.078
15	CC	0.531	0.247	0.001
	AIn	0.427	0.143	0.016
	F1	0.332	0.071	0.072
30	CC	0.408	0.126	0.024
	F1	0.473	0.189	0.003
	AI	0.387	0.108	0.036
50	CC	0.275	0.030	0.206
	F1	0.382	0.105	0.036
	AI	0.398	0.116	0.032
10+15	CC	0.654	0.364	0.0004
	AIn	0.523	0.193	0.018
10+15+30	CC	0.684	0.372	0.001
	AIn	0.549	0.174	0.052
	F1	0.512	0.143	0.065
10+15+ 30+50	CC	0.737	0.421	0.001
	AIn	0.696	0.346	0.006
15+30+50	CC	0.705	0.409	0.0004
	AIn	0.672	0.354	0.002
	F1	0.526	0.156	0.056
30+50	CC	0.461	0.128	0.058
	F1	0.524	0.200	0.012
	AI	0.451	0.117	0.071

Table 2. Effect of blood ketone bodies on fertility. The animals were divided on the basis of blood acetoacetate (AA) levels (mmol/l) into normal and ketotic animals and on the basis of blood ß-hydroxybutyrate (HB) levels (mmol/L) into normal and high-level groups. AI = interval (days) between calving and the first insemination; CC = interval (days) from calving to conception; AIn = inseminations (n) per conception; PR% = pregnancy rate at the first insemination; * $p < 0.05$, ** $p < 0.01$

	N	AI (days)	CC (days)	PR (%)	AIn (n)
Normal AA	28	71.8±8.9	76.5±18.8	86	1.14
Subclin AA	16	75.0±11.9	93.1±23.9 *	56	1.69
Norm HB	34	70.2±6.8	77.0±19.6	85	1.18
High HB	10	78.9±12.4*	101.2±20.3**	40	1.90

insemination. Similarly, high level of ketone bodies in blood at days days 15 and significantly affected the interval from calving to conception. The adverse influence of blood ketone bodies on fertility depended on the duration and timing of the elevated levels; the longer and later in puerperium the cows have elevated ketone bodies, the lower the fertility will be. The energy level in early puerperium had a greater influence on the interval from calving to conception, whereas in late puerperium it was more important as regards to the interval from calving to the first insemination. Low energy balance in puerperium influenced the number of inseminations per conception.

The earlier the ovary activity began, the shorter was the interval from calving to first insemination ($p<0.001$) and to conception ($p<0.01$). The onset of ovary activity was influenced by energy level at days 15, 30 and 50.

The energy level in early puerperium had more influence on the interval from calving to conception, whereas the energy level in late puerperium had more influence on the interval from calving to first insemination.

The cows were divided into non-ketotic and ketotic groups according to the AA levels by day 50 postpartum (Table 2); cows were regarded as non-ketotic (n=28) if they had an AA concentration between 0.01-0.07 mmol/l and the rest were considered to be ketotic (n=16). The non-ketotic cows were inseminated earlier than the ketotic ones, at 71.8±8.9 days and 75.0±11.9 days,respectively. The interval from calving to conception was shorter in the non-ketotic group than in the ketotic group 76.5±18.8 days and 93.1±23.9 days, respectively ($p<0.05$). The non-ketotic cows needed an average of 1.14 inseminations per conception, whereas ketotic ones required 1.69.The pregnancy rate at first insemination was better in the non-ketotic group than in the ketotic group,75% and 40%, respectively. When the cows were divided according to the HB concentration into the normal cows and ketotic ones, the fertility traits for the 2 groups were similar (Table 2).

In conclusion, the results of this study indicate that the energy balance significantly affect reproductive efficiency in dairy cows. Low energy level during puerperium influenced the lengh of the interval from calving to conception, and low energy level in late puerperium affected the of first insemination. Therefore, if shorter calving intervals are desired, adequate nutrition is essential before and after calving.

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SUMMARY

The effect of nutrition on involution of the genital tract and on fertility was evaluated Finnish dairy cows fed with home-produced feed. The time required for complete uterine involution, onset of ovarian activity, pregnancy rate at the first insemination, and the interval from calving to conception were obtained for each cow. To study the relationships between energy levels and reproductive traits serum glucose and blood acetoacetat (AA) and ß-hydroxybutyrate (HB) were measured 10, 15, 30 and 50 days postpartum. The low energy balance in puerperium prolonged the interval from calving to the first insemination and the interval from calving to conception. The energy level in early puerperium had a greater influence on the interval from calving to conception, whereas in late puerperium it was more important as regards the interval from calving to the first insemination. The adverse influence of ketone bodies on fertility depended on duration and timing of their increased levels; the longer and later in puerperium the cow has ketone bodies, the lower is the fertility.

RESUME

L'effet de la nutrition sur l'involution de l'appareil génital et sur la fécondité a été évalué sur les vaches laitières finlandaises nourries avec du fourrage produit à la ferme. Le temps nécessaire pour l'involution utérine complète, le début de l'activité ovarienne, le taux des gestations à la première insémination et l'intervalle entre le vêlage et la conception ont été obtenus pour chaque vache. Pour étudier les rapports entre les niveaux d'énergie et les traits reproductifs, le glucose du sang et l'acétoacétate sanguin (AA) et l'hydroxybutyrate ß (HB) ont été mesurés 10, 15, 30 et 50 jours après parturition. L'équilibre bas d'énergie dans la période puerpérale a prolongé l'intervalle entre le vêlage et la première insémination et d'intervalle entre le vêlage et la conception. Le niveau d'énergia dans la période puerpérale primaire avait une influence plus grande sur l'intervalle entre le vêlage et la conception, tandis que dans la période puerpérale avancée, il était plus important par rapport à l'intervalle entre le vêlage et la première insémination. L'action contraire des corps cétoniques sur la fécondité dépendait de la durée et du moment de leurs niveaux élevés; plus la durée de la présence des corps cétoniques chez la vache dans la période puerpérale est longue et plus elle est tardive, plus faible est la fécondité.

ZUSAMMENFASSUNG

Das Fortpflanzungsgeschehen von Kühen in der postpartum Phase wurde in einer Fütterungsversuch in einer Versuchsherde mit genauer Fütterungs- und Leistungskontrolle untersucht. 10, 15, 30 und 50 Tage postpartum wurden Blutproben genommen und Azetoazetat und ß-Hydroxybutyrat bestimmt und als Indikator der Energiebilanz benutzt. Die niedrige Energiebilanz während des Puerperiums verlängerte sowohl die Rastzeit als auch die Güstzeit. Im Frühpuerperium die Energiebilanz beeinflußte mehr die Güstzeit als die Rastzeit, während im Spätpuerperium die niedrige Energiebilanz mehr die Zeitpunkt der ersten Besamung beeinflußte. Die nachteilige Einfluß der Ketonkörpern auf Fruchtbarkeit war abhängig von Dauer und Höhe des Energiemangels im Puerperium.

USE OF SYNCRO-MATE B TO INDUCE OESTRUS IN DAIRY CATTLE

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INTRODUCTION

The failure to detect oestrus is a major cause of suboptimal fertility in dairy cattle (1). In cycling cattle oestrus may be induced by administration of prostaglandin F2alpha or its analogues during the luteal phase. The variable interval between prostaglandin injection and oestrus (2) however requires careful oestrus detection for optimal results.

Syncro-Mate B is a progestagen method that can be used in all cattle, cycling or non-cycling, at any point in the oestrus cycle to induce oestrus and ovulation. This field trial was carried out to study the fertility in dairy heifers and lactating dairy cows treated with Syncro-Mate B and inseminated at fixed times following treatment, irrespective of oestrus observation.

MATERIALS AND METHODS

Animals

189 heifers and 162 lactating cows of the Friesian, Holstein-Friesian or MRY breed on 30 commercial dairy farms in the surroundings of Boxmeer, The Netherlands.

Before admission to the trial all animals were examined clinically. only healthy animals without abnormalities of the genital tract were selected.

During the trial period the cattle remained housed and fed according to the normal procedures on the farm.

Treatment

The animals were treated with Syncro-mate B and, depending on age and ovarian status, 500 I.U. PMSG.

Syncro-mate B consists of a silastic implant containing 3 mg of Norgestomet and an oily solution for injection containing 3 mg Norgestomet plus 5 mg oestradiol-valerate. On the day of treatment the injectable solution was administered i.m. in the neck and the implant was placed subcutaneously in the middle part of the ear.

The implants were removed 10 days later at which moment all cows and the heifers with inactive ovaria were injected with 500 I.U. PMSG (Folligon®, Intervet International), i.m. in the neck.

Insemination

Regardless of oestrus behaviour, the cows were inseminated at 48 and 72 hours after removal of the implant. The heifers were inseminated once at 48 hours. Some heifers however were also inseminated twice depending on oestrus observation by the farmer.

Pregnancy

All animals, except 8, were tested for pregnancy by rectal palpation between 5 and 9 weeks after insemination. Cattle that conceived after re-insemination, i.e. not on the induced oestrus, were regarded as not pregnant.

Observations

Oestrus detection was carried out by the farmer during the treatment period and until 72 - 96 hours after implant removal.

The inseminator scored the oestrus at the time of insemination as 1) good, 2) moderate or 3) not in oestrus, based on the appearance and palpation of the genital tract.

RESULTS

Oestrus during treatment

23 animals showed signs of oestrus during the treatment period. Of these cattle, inseminated at the fixed times (48 and 72 hours) after implant removal, 15 became pregnant i.e. a conception rate of 65,2%.

Oestrus after implant removal

The oestrus response as observed by the farmer and assessed by the inseminator is summarised in Table 1 and 2.

Table 1 : The oestrus response after implant removal observed by the farmer

Oestrus observation	Number of animals	Percentage pregnant*
Standing oestrus	193	62
Oestrus signs	43	14
No oestrus observed	73	24
No detection performed	42	

* of those animals that were checked for oestrus

Table 2 : The oestrus response after implant removal scored by the inseminator

Oestrus score	Number of animals	Percentage pregnant*
Good	250	76
Moderate	65	28
Not in oestrus	14	4
Not assessed	22	

* Of those animals for which the oestrus was scored

Pregnancy

Table 3 shows the results of the pregnancy test for the heifers and cows. Heifers conceived significantly better than cows ($p < 0,01$).

Table 3

	Number of animals treated	Not checked for pregnancy	Percentage of animals pregnant*
Heifers	189	6	66,6
Cows	162	2	51,2

* Of those tested for pregnancy
$p < 0,01$ Chi-square test, animals not tested for pregnancy excluded.

Animals with inactive ovaries, i.e. that were not cycling at the start of treatment had a pregnancy rate of 63%, similar to that of the cycling cattle of which 59% became pregnant on the induced oestrus (Table 4).

Table 4 : Pregnancy rate of cycling and non-cycling animals

Ovarian status before treatment	Number of animals treated	Percentage of animals pregnant*
Cycling	305	59
Non-cycling	46	63

* Of those tested for pregnancy
N.S. 0,05, Chi-square test

The pregnancy rate was significantly related to the oestrus response as observed by the farmer and assessed by the inseminator, see Table 5.

Table 5 : Pregnancy rate grouped by oestrus response

Oestrus response (observations)	Number of animals	Percentage of animals pregnant*	Oestrus response (score)	Number of animals	percentage of animals pregnant*
Standing oestrus	193	62[a)]	Good	250	64[b)]
Signs of oestrus	43	53	Moderate	65	54
Not observed	73	44	Not in oestrus	14	7

* of those tested for pregnancy
a) $p < 0,25$ Chi-square test b) $p < 0,01$ Chi-square test

Effect of Insemination

The cows were all inseminated at 48 and 72 hours after implant removal. 62% of the heifers was inseminated once at 48 hours and the rest twice, at the same time as the cows.

The conception rate of the groups of heifers was 64% and 65% respectively.

DISCUSSION

In cycling females the Syncro-mate B treatment effectively blocks follicle development and maturation, as is demonstrated by the low (7,5%) percentage of animals that showed signs of oestrus during the treatment and the normal fertility of these animals when inseminated at the fixed times after implant removal.

The oestrus response was high : 76% was observed in oestrus by the farmer and 96% was considered by the inseminator to be in oestrus after the treatment.

Following fixed time insemination, irrespective of the type of oestrus response, 51,2% of the cows and 66,6% of the heifers conceived. The conception rate of the cows may be improved by administration of prostaglandin $F2\alpha$ two days before implant removal, as is recommended for lactating dairy cattle (3).

In heifers a double insemination did not improve the pregnancy rate.

In females not cycling before treatment, the Syncro-Mate B treatment was able to induce a fertile oestrus; 63% of these cattle conceived on the induced oestrus.

CONCLUSION

The Syncro-Mate B system provides an effective and simple means for controlled breeding in cycling and non-cycling dairy cattle.

The predictable and precise oestrus response after implant removal allows fixed time insemination, thus eliminating the need for oestrus detection.

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SUMMARY

189 heifers and 162 lactating cows on commercial dairy farms were treated with Syncro-mate. The cows and the non-cycling heifers received 500 I.U. of PMSG at implant removal. Regardless of oestrus behaviour the cows were inseminated at 48 and 72 hours after removal of the implant. The heifers were inseminated once at 48 hours or twice as the cows.

In 7,5% of the animals some signs of oestrus were observed during treatment. On inseminated at the fixed times after implant removal 65,2% of them conceived.

After treatment 76% of the cattle was observed in oestrus by the farmer and 96% was considered by the inseminator to be in oestrus.

Following fixed time insemination 66,6% of the heifers conceived and 51,2% of the cows. A double insemination did not improve the conception rate of the heifer.

In females not cycling before treatment Syncro-mate B was able to induce a fertile oestrus on which 63% conceived.

It can be concluded that Syncro-mate B provides a predictable and precise oestrus response following treatment thus eliminating the need for oestrus detection.

RESUMÉ

189 génisses et 162 vaches en production dans des exploitations laitieres ont été traitées avec Syncro-mate B. Les vaches et les génisses en repos sexuel ont reçu une injection de 500 U.I. de PMSG lors du retrait de l'implant. Les vaches ont été inséminées sans détection des chaleurs deux fois 48 heures et 72 heures après le retrait de l'implant. Les génisses ont été inséminées une fois à 48 heures ou deux fois comme les vaches.

7,5% des femelles ont montré quelques signes de chaleurs durant le traitement. 65,2 des celles femelles sont devenues gravide sur l'oestrus induit lors du retrait de implant.

Après traitement 75% des animaux ont été observé en chaleurs par l'éleveur et 96% considerés "en chaleurs" par l'inséminateur.

La fertilité sur l'oestrus induit a été de 66,6% chez les génisses et 51,2% chez les vaches. Une double insémination n'améliorat pas la fertilité chez les génisses.

Syncro-mate B a induit des chaleurs fertiles chez les femelles en repos sexuel; 63% de ces femelles a été fédondées à l'oestrus induit.

On peut donc conclure que Syncro-mate B induit des chaleurs fertiles bien synchronisés aussi bien chez les femelles cyclés que les femelles non-cyclées, permettent ainsi leur insémination sans avoir à détecter les chaleurs.

RESÚMEN

Se sometieron a tratamiento con Syncro-Mate B 182 novillas y 162 vacas en lactación de fincas lecheras comerciales. Las vacas, así como las novillas fuera de ciclo recibieron 500 UI de PMSG. Las vacas fueron inseminadas 48 y 72 horas después de retirado el implante, independiente del aparecimiento de señales de estro. Las novillas se inseminaron una vez a las 48 horas o dos veces como realizado con las vacas.

En 7,5% de los animales se notaron algunas señales de estro durante el tratamiento. El 65,2% de los animales concibieron al ser inseminados en el periodo previamente fijado, posterior a la remoción del implante.

Luego del tratamiento el 76% de los animales mostraron (según el granjero) señales de estro y el inseminador consideró que el 96% estaba en el celo.

Siguiendo el plan de inseminación establecido se obtuvo un porcentaje de 66,6% de concepción en las novillas y de 51,2% en las vacas. La repetición de la inseminación en las novillas no mejoró la tasa de concepción.

El tratamiento con Syncro-Mate B en hembras fuera de ciclo indujo el aparecimiento del estro y se obtuvo un porcentaje de concepción del 69%.

Se puede concluir que después del tratamiento con Syncro-Mate B se produce el estro como respuesta precisa y predecible, eliminando así la necesidad de la detección del celo.

EFFECT OF FSH ON EXPERIMENTAL INDUCTION OF BOVINE LUTEAL HYPOPLASIA

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INTRODUCTION

Luteal hypoplasia has sometimes been encountered in infertile cows. However, little is known about its' etiology at present. In our previous experiment it seems possible that luteal hypoplasia is prone to occur when ovulation takes place in the early stage of follicular maturation (4). The present study intends to clarify that stimulation to follicular maturation by the administration of follicle stimulating hormone (FSH) can give some efffect on the luteal formation after ovulation, so that the FSH treatment can reduce the occurrance of luteal hypoplasia.

MATERIALS AND METHODS

Experimental cows : A total of 10 adult cows were used. Some of them were used more than once, so that a total of 12 experiments could be performed. Their body weights averaged 528 kg, ranging from 360 to 753 kg.

Experimental production of luteal hypoplasia : Luteal hypoplasia was produced by the following method of artificially induced ovulation in the early stage of follicular development. At first, each cow received an intramuscular injection of 0.8 mg prostaglandin $F_{2\alpha}$ analogue ($PGF_{2\alpha}$-A, ONO-1052, Ono Pharmaceutical Co., Ltd., Osaka, Japan) in the mid-luteal stage, 10 - 11 days after spontaneous ovulation, followed by two intramusular injections of 100 µg of gonadotrophin releasing hormone analogue (GnRH-A, Fertirelin acetate, Takeda Chemical Industries, Ltd., Osaka, Japan), each 16 hr (groups A and B) or 32 hr (groups C and D) after injection of $PGF_{2\alpha}$-A. The interval between GnRH-A injections was 60 min according to Kittok et al.(1). In our previous experiment (4), luteal function in cows treated with the above-mentioned procedure of induced ovulaiton was very poor.

Treatment with FSH : The FSH used was Antrin (Denka Seiyaku Co., Ltd.). Two dosages of 10 Armour units (AU) each, or a total of 20 AU, were injected by the intramuscular route. When the time of injection with $PGF_{2\alpha}$-A was regarded as 0 hr, FSH was administered to each group at -16 and 0 hr (group A), 0 and 16 hr (groups B and C), or 16 and 32 hr (group D). Groups A, B, C and D consisted of 2, 2, 4 and 4 cows, respectively. Two cows, one from group A and one from group B, were sacrificed 10 days after induced ovulation to examine the condition of luteinization macroscopically.

Estimation of plasma progesterone level : Plasma progesterone levels were estimated by radioimmunoassay (2,3), the fractuation and purification by chromatography being skipped.

RESULTS

Group A : Ovulation with silent estrus took place in two cows, H-4 and N-J, of group A, 16.5 and 33.5 hr respectively after GnRH-A injection, or 25.0 hr on the average. In cow H-4, a small corpus

luteum was formed after induced ovulation, showing 1.5 cm in longer diameter 7 days after induced ovulation. It remained this size up to 13 days after induced ovulation, then regressed. As a short estrus appeared in this cow 20 days after induced ovulation, the subsequent estrous cycle was almost normal. Cow N-J was sacrificed 10 days after induced ovulation to examine the condition of luteinization macroscopically. The left ovary weighed 5.9 g and the right ovary 5.0 g. At the site of induced ovulation in the left ovary there was a small almost spherical corpus luteum 1.5 cm in longer diameter with a central cavity of 0.4 cm in diameter. The cross section of the corpus luteum was dark orange in color. Morever, the ovary contained four follicles, 0.8, 0.6, 0.5 and 0.5 cm in diameter, respectively. In a cross section of the right ovary, a small corpus luteum, 0.5 cm in longer diameter, was seen. This was previously formed and regressed after injection with $PGF_{2\alpha}$-A. No follicle greater than 0.5 cm in diameter were seen in the ovary.

Group A in Fig. 1 exhibits plasma progesterone levels in cows H-4 and N-J after induced ovulation. In cow H-4 the level showed a slight increase for 3 days after induced ovulation. Then, it increased rapidly to 6.2 ng/ml 5 days after induced ovulation. After that, it began to decrease, reaching 1.0 ng/ml 8 days after induced ovulation. It then increased to 7.6 ng/ml and remained at a high level until the 13 th day, when it began to decrease rapidly. The plasma progesterone level averaged 5.6 ng/ml in cow H-4 over the period from 5 to 13 days after induced ovulation. Cow N-J showed a slow increase for 7 days

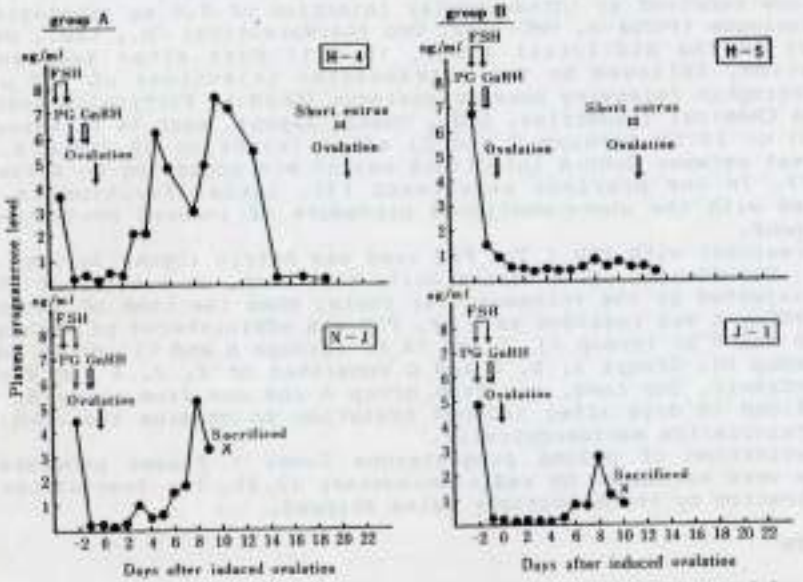

Fig. 1. Plasma progesterone patterns in cows injected with FSH before induced ovulation (groups A and B).

after induced ovulation with the level rising rapidly to 5.2 ng/ml on the eighth day. In brief, small corpora lutea were formed in these two cows. Judging from the plasma progesterone level, however, luteal function was improved to some extent in these cows by treatment with FSH.

Group B : In two cows belonging to group B, H-5 and J-1, ovulation took place 30.5 and 25.5 hr, respectively, or 28.0 hr on the average, after injection with GnRH-A. No estrous behavior was observed in either of the two cows from injection with GnRH-A to ovulation. Cow H-5 had a small corpus luteum 1.5 cm in longer diameter 10 days after induced ovulation. It manifested short estrus 2 days later, it's estrous cycle being reduced remarkably. Cow J-1 was sacrificed 10 days after induced ovulation. The left ovary weighed 12.0 g and the right ovary 13.3 g. In the right ovary a small, very slender corpus luteum of yellow ocher color was noticed at the exact site where induced ovulation had taken place. Its' cut surface was 2.2 cm in longer diameter. The ovary also contained four follicles, 1.1, 1.0, 0.6 and 0.6 cm in diameter, respectively. The left ovary contained a corpus luteum which had regressed after injection with $PGF_{2\alpha}$-A and two follicles 0.6 cm in diameter.

Group B in Fig. 1 presents the plasma progesterone levels in cows H-5 and J-1 after induced ovulation. In cow H-5, the level showed no increase after induced ovulation, but remained lower than 1.0 ng/ml (0.5 ng/ml on the average) for 12 days after induced ovulation until a short estrus appeared. In cow J-1, the level continued to be low after induced ovulation. It increased to 2.7 ng/ml 8 days after induced ovulation, but began to decrease on the following day, being 0.8 ng/ml on the day it was sacrificed. In short, the FSH injection gave little effect on plasma progesterone levels in group B.

Group C : Four cows in this group, H-4, H-6, S-78 and S-0110, were artificially ovulated 18 to 42 hr, or 26.8 hr on the average, after GnRH-A injection. No estrous behavior was observed in any cow from this group during the period from GnRH-A injection to ovulation. In cow H-6, one follicle from each ovary was ruptured almost simultaneously with GnRH-A injection. Two corpora lutea, 1.5 and 2.0 cm in longer diameter, were seen in either ovary, respectively, 12 days after induced ovulation. In cow H-6, estrus appeared 21 days after induced ovulation and a follicle ruptured the following day. In cow S-78, a corpus luteum 1.6 cm in longer diameter was noticed at the site of ovulation 5 days after induced ovulation. It later developed rather remarkably, being 2.5 cm in longer diameter 10 days after induced ovulation. Silent estrus was found in this cow 22 days after induced ovulation. In cow S-0110, a small corpus luteum 2.2 cm in longer diameter was seen 10 days after induced ovulation and normal estrus 9 days later, when the corpus luteum was reduced to 0.5 cm in longer diameter. In the remaining cow, H-4, a small corpus luteum was formed, being 1.5 cm in longer diameter 12 days after induced ovulation. Normal-like estrus appeared in this cow 18 days after induced ovulation. The corpora lutea formed in cows H-4, H-6 and S-0110 were all small as mentioned above, but did not regress so soon after developed.

Blood samples were collected from two cows, S-78 and S-0110, from group C. Fig.2 shows the group C plasma progesterone levels after induced ovulation. In cow S-78, the level rose from 2.6 to 4.4 ng/ml within 3 - 4 days after induced ovulation and continued the high level for 10 days. The level was essentially the same in cow S-0110. It increased to 3.9 ng/ml in 5 days and maintained a high degree (5.6

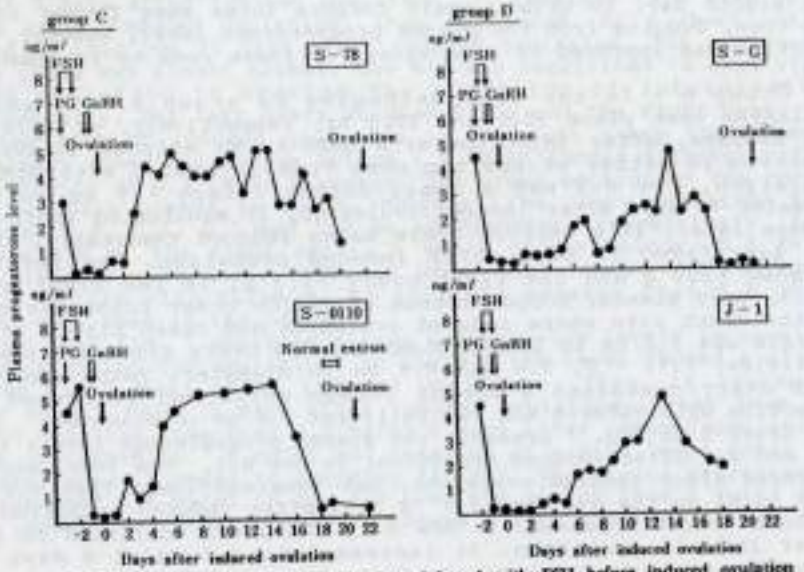

Fig. 2. Plasma progesterone patterns in cows injected with FSH before induced ovulation (groups C and D).

ng/ml on the average) for 14 days after induced ovulation before beginning to decrease. Thus, the plasma progesterone level in these cows resembled those in the normal estrous cycle. An improved function of corpus luteum by the FSH treatment was observed in group C.

Group D : Four cows, belonging to this group S-G, J-1, H-1 and S-44, ovulated 16 - 26 hr, or 21.1 hr on the average, after GnRH-A injection. The ovulation was accompanied with silent estrus in all cases. In cow S-G, the corpus luteum, 2.5 cm in longer diameter 7 days after induced ovulation, was very slender like that of the sacrificed cow from group B. This cow showed silent estrus again 21 days after induced ovulation. Also, in cow J-1 the corpus luteum was slender and similar that of cow S-G, reaching a maximum size of 2.5 cm in longer diameter 18 days after induced ovulation. This cow exhibited persistent estrus 27 and 28 days after induced ovulation. The corpus luteum formed in cow H-1 was very slender, 3.2 cm in longer diameter 5 days after induced ovulation. This was reduced to 2.0 cm in longer diameter 13 days after induced ovulation. The subsequent estrus with short duration appeared 10 days later. In the remaining cow from group D, S-44, a slender corpus luteum, 2.0 cm in longer diameter, was formed 7 days after induced ovulation. As it regressed early, the subsequent estrus occurred 4 days later. Thus, the estrous cycle was reduced distinctly, but the estrus was almost normal in nature.

Blood samples were collected from two cows, S-G and J-1, of group

D. Fig.2 presents plasma progesterone levels from group D cows after induced ovulation. This level rose slowly in both cows, reaching 4.7 ng/ml in cow S-G in 14 days and 4.8 ng/ml in cow J-1 13 days after induced ovulation, before falling gradually. In short, all corpora lutea were slender in the four cows of group D. In cow S-44 , the corpus luteum regressed early. Judging from the plasma progesterone levels in cows S-G and J-1, functional activity of the corpora lutea was insufficient. Treatment with FSH, therefore, failed to display the same improving effect on luteal formation in group D as observed in group C.

DISCUSSION

According to Onuma et al. (6), the follicle stimulating effect of FSH in cows depends on the number of injections rather than on the total dose. In the present experiment, the goal of FSH treatment was not to induce superovulation, but rather to stimulate follicular development after luteal regression induced by $PGF_{2\alpha}$-A . The treatment is successful if only one follicle is developed. In a preliminary experiment, cows were treated with 5 AU of FSH four times at 8 hr intervals, the total dosage of the hormone being 20 AU. They received $PGF_{2\alpha}$-A simultaneously with the first FSH injection. Finally, they were injected with GnRH-A 32 hr after the first FSH injection. As a result, many follicles developed in the ovaries. This treatment was too strong to stimulate single follicule development. Therefore, in the present experiment the number of injections of FSH was reduced. Cows were twice injected with 10 AU of the hormone at a 16 hr interval, the total dosage being 20 AU. This procedure developed only one or two follicles exceeding 1.0 cm in diameter in the ovaries of each cow immediately before ovulation. The number of follicles ruptured was only one in all the cows, except one case in group C in which two follicles ruptured. It was ascertained that this procedure of FSH treatment was sufficient to meet the purpose of the present experiment.

The interval between GnRH-A injection and ovulation was examined in four experimental groups, the average intervals being 25.0, 28.0, 26.8 and 21.1 hr in groups A, B, C and D, respectively. In the previous experiment (4), the intervals were 37.5 hr in groups A and B, and 20.0 hr in groups C and D without FSH, respectively. Ovulation was induced in the early stage of follicular development after injection with $PGF_{2\alpha}$-A, regardless of FSH treatment.

In the present experiment, the cows of group A had small corpora lutea, but luteal function was improved in them. In the cows of group B, however, luteal function was poor. Of the four cows of group C, three had small corpora lutea, but luteal function was improved in them. In the cows of group D, FSH treatment displayed some effect, although it was minimal. Thus, FSH treatment improved luteal function in groups A, C and D. Among these groups, however, a large difference was observed in the degree of improvement in the luteal function. The degree was the highest in group C and the lowest in group B, being moderate in groups A and D. It is very interesting to note the interval between the first FSH injection and GnRH-A injection and also the interval between $PGF_{2\alpha}$-A injection and GnRH-A injection. Both were relatively long in group C, while relatively short in group B. In groups A and D, however, these intervals were not uniform. These results clearly indicate there is a close relationship with the interval between injections of FSH or $PGF_{2\alpha}$-A and GnRH-A;

that is, between the degree of follicular maturation at the time of GnRH-A injection and the function of corpus luteum.

In the other experiment, the present authors induced ovulation in cows having a developing follicle by GnRH-A injection. But, administration of hCG to such cows could not exert any luteotrophic effect on the lutinization process (5). From these results, it is suggested that whether luteal function would take place satisfactorily or not, might be determined at the time of ovulation. Therefore, it is presumed that luteal hypoplasia is caused when ovulation occurs before granulosa cells and theca interna cells of the follicle are sufficiently sensitized to gonadotrophin and acquired an ability to change themselves to lutein cells.

In conclusion, it was elucidated that luteal hypoplasia was caused that a follicle is immature when ovulation took place.

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SUMMARY.

It is well known that if ovulation is artificially induced in an immature follicle in the mid-cycle of cows, luteal formation after ovulation is arrested and a hypoplastic corpus luteum is formed. This study was undertaken to investigate if the function of corpus luteum after ovulation is related with the development of ovulating follicle by using FSH. Using 12 cows, 10 or 11 days after spontaneous ovulation, induced ovulation was achieved by administration of 0.8 mg of prostaglandin $F_{2\alpha}$ analogue (PGF$_{2\alpha}$-A) followed by the injection of 200 µg of gonadotrophin releasing hormone analogue 16 hr (groups A and B) or 32 hr (groups C and D) after. A dosage of 10 Armour units (AU) of FSH was administered by intramuscular injections, twice at 16 hr interval, starting either -16 hr in group A, 0 hr in groups B and C or 16 hr in group D after PGF$_{2\alpha}$-A treatment. Luteinization after these treatments was checked by rectal palpation, plasma progesterone levels and macroscopic findings at slaughter. Luteal function in groups A and C were improved, compared with groups B and D. Group C showed almost the same plasma progesterone pattern as that of the normal estrous cycle. However, in groups B and D, the improving effect of FSH on luteal formation was slight or poor. In conclusion, it was confirmed that the cause of luteal hypoplasia is closely related to the degree of follicular maturation at the time of ovulation.

PROTEINOGRAMA DO COLOSTRO DE VACAS BUBALINAS (Bubalus bubalis L.) DA RAÇA MURRAH NO MOMENTO DO PARTO

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INTRODUÇÃO

A bûfala possui placenta semelhante a da vaca bovina, isto é, do tipo epitélio-corial, múltipla ou cotiledonária (6) o que permite supor o impedimento da transferência de anticorpos via placenta, advindo daí, variação da susceptibilidade dos neonatos às condições ambientais decorrentes da ingestão do colostro. Estudos realizados em países como a Índia e a Itália, revelam que a taxa de mortalidade em bezerros bubalinos é variável, sem contudo ultrapassar os 30% (5,7). Entre os vários fatores predisponentes à mortalidade de neonatos tem-se destacado a diarréia, debilidade e a pneumonia (9,8,10,1,5). No Paquistão Chaudhry (2) cita as deficiências de manejo dos recém-nascidos, tais como acesso colostro, tratamento inadequado do cordão umbilical e ausência de tratamento anti-helmíntico, como principais fatores predisponentes às enfermidades. Mediante tais estudos, podemos inferir que o mesmo ocorre com os bezerros bubalinos na região Amazônica, onde as condições sanitárias e profiláticas deficientes favorecem a ocorrência de taxas elevadas de mortalidade.

O presente trabalho teve como objetivo o estudo do perfil eletroforético do colostro de bûfalas visando facilitar o diagóstico de estados de imunodeficiência em recém-nascidos decorrentes da variação da concentração de gamaglobulina presente no colostro.

MATERIAIS E MÉTODOS

Foram estudados o colostro de 17 vacas bubalinas da raça Murrah no momento do parto, pertencentes ao Centro de Pesquisa Agropecuária do Trópico Úmido (CPATU) da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), criados em condições semelhantes de manejo na fazenda Experimental "Dr. Felisberto Camargo" em regime semi-intensivo, em pastoreio constante de pastagens nativas e suplementação proteíca e mistura mineral especial preconizada pelo CPATU da EMBRAPA.

Colheita das amostras e obtenção do soro

Logo após o nascimento dos bezerros e antes da ingestão do colostro, foram colhidas amostras de 50 mL as quais foi adicionado 1 mL de renina comercial, levou-se à banho-maria, de 40 a 45 graus Célsius de temperatura até a coagulação da caseína e separação do soro (3) sendo conservados a -18 graus Célsius em "freezer" em tubos de teflon arrolhados.

Determinação da proteína total

Após o descongelamento das amostras à temperatura de 25 à 27 graus Célsius, determinou-se a concentração de proteína total através do método do Biureto com leitura em espectrofotômetro.

Determinação eletroforética das frações proteícas do colostro

As frações eletroforéticas foram obtidas utilizando-se fitas gelatinizadas de acetato de celulose em solução tampão de

dietilbarbiturato de sódio (veronal) a 8,4% e pH 8,6. As corridas foram realizadas em sistema de semi-microeletroforese com duração de 30 minutos, 230 volts e volume de 1 microlitro. A coloração empregada foi a do negro de amido e transparentização recomendada por Moura (4). Os resultados foram obtidos em percentuais (%) através de leitura em densitômetro integrador automático de eletroforese e à seguir, calculados os valores em grama por cento (g%) a partir da proteína total, constituindo a proporção de cada fração proteíca do colostro.

Análise estatística

As análises estatísticas incluíram a determinação dos valores médios, desvio padrão, coeficiente de variação, intervalo de confiança e amplitude variação (11) das variáveis proteína total, albumina, alfaglobulina, betaglobulina e gamaglobulina presentes nas amostras do colostro.

RESULTADOS

Na tabela 1 são mostrados os valores médios, desvio padrão, coeficiente de variação, intervalo de coenfiança e a amplitude de variação da proteína total, albumina, alfaglobulina, betaglobulina e gamaglobulina obtidos pelo método do Biureto e eletroforese respectivamente no colostro de búfalas no momento do parto.

Observa-se as elevadas concentrações de proteína total (13,33 g/100mL) e gamaglobulina (10,95 g/100 mL) o que comprova a importância do colostro para a espécie durante as primeiras fases de vida, já que não há transferência de anticorpos através da placenta conforme foi referido anteriormente. A concentração de gamaglobulina representou 82% de toda a proteína colostral.

TABELA 1. Proteinograma eletroforético do colostro de vacas bubalinas no momento do parto (g/100 mL).

	X	DP	CV	IC LI	IC LS	AV
Proteína total[1]	13,33	3,43	25,72	11,50	15,16	8,00-19,00
Albumina[2]	0,49	0,21	44,60	0,35	0,63	0,20- 1,00
Alfaglobulina[2]	1,05	0,47	45,12	0,74	1,35	0,40- 1,80
Betaglobulina[2]	0,87	0,55	63,37	0,45	1,30	0,90- 1,40
Gamaglobulina[2]	10,95	3,08	28,18	8,99	12,92	6,40-16,00

[1] Biureto
[2] eletroforese
X média aritmética
DP desvio padrão
CV coeficiente de variação
IC intervalo de coenfiança
LI (limite inferior)
LS (limite superior)
AV amplitude de variação

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SUMMARY

The colostrum serum of 17 buffalo cows of the Murrah breed belonging to CPATU/EMBRAPA, were studied to determinate the electrophoretic profile. We obtain the colostral serum using 1 mL of comercial renin in the colostrum sample and than maintaining it in a double boiler until the casein coagulation and the serum separation according to DAM (1968). To determinate the total protein concentration was used the Biutet method with the spectrophotometer reading. The separation of colostral serum proteins, was made in a semimicroelectrophoresis system using the gelatinized band of cellulose acetat in a buffer solution of veronal at 8,4% and pH of 8,6 with run of 30 minutes of duration at 230 of volts and a volume of 1 microlitre according MOURA (1987). The results were in g/100 mL: total protein 13,33 ± 3,43 ; albumin 0,49 ± 0,21 ; alphaglobulin 1,05 ± 0,47 ; betaglobulin 0,87 ± 0,55 ; gammaglobulin 10,95 ± 3,08. The high concentration of gammaglobulin confirm the colostrum importance to this spcecies during the first period of life now that don't heve any antiserum transfer from placenta.

RÉSUMÉ

Les sérums colostraux de 17 vaches bubalines de race Murrah appartenant au CPATU/EMBRAPA ont été étudiés dans le but de déterminer le profil d'électrophorèse. Pour obtenir le sérum colostral, on a ajoute aux échantillons 1 mL de rénine commerciale et maintenu l'ensemble au bain-marie jusq'à coagulation de la caséine et séparation du serum, comme préconisé par DAM (1968). Pour la détermination de la concentration de protéine totale, on a utilisé la métode de Biureto avec lecture au spectrophotometre. La séparation des protéines sériques colostrales a été réalisée de cellulose gélatinisées en solution tampon de vérona à 8,4% et pH de 8,6 en séquences de 30 minutes à 230 volts et volume de 1 microlitre, selon ce que recommande MOURA (1987). Les résultats obtenus, en g/100 mL, ont été les suivants: proteíne totale 13,33 ± 3,43; albumine 0,49 ± 0,21 ; alphaglobuline 1,05 ± 0,47 ; betaglobuline 0,87 ± 0,55 ; gammaglobuline 10,95 ± 3,08. La concentration élevée de gammaglobuline démontre l'importance du colostrum pour l'espèce durant les premières phases de la vie, vu qu'il n'y a pas de transfert d'anticorps autravers du placenta.

RESUMEN

Los sueros calostrales de 17 vacas bubalinas de la raza Murrah pertencientes a el CPATU/EMBRAPA fueron estudiados con el objetivo de determinar el perfil eletroforético. Para obtención del suero calostral fué adicionado a las mustras 1 mL de renina comercial y mantenidas en baño maria hasta la coagulación de caseína y separación del suero conforme preconiza DAM (1968). Para determinación de la proteína total fué utilizado el método de el Biureto com lectura en espectrofotômetro. La separación de las proteínas séricas calostrales fuê hecha en sistema de semimicroelectroforese utilizânose cientas gelatinizadas de acetato de celulosa en solución buffer de veronal a 8,4% y pH 8,6 en transcurso de tiempo de 30 minutos de duratión y volumen de 1 microlitro segun MOURA (1987). Los resultados fueron los siguientes en g/100 mL: proteína total 13,33 ± 3,43 ; albumina 0,49 ± 0,21 ; alfaglobulina 1,05 ± 0,47 ; betaglobulina 0,87 ± 0,55 ; gamaglobulina 10,95 ± 3,08. La elevada concentración de gamaglobulina comprueva la importância del calostro para la espécie durante las primeras fases de vida ya que no hay transferência de anticuerpos através de la placenta.

RESUMO

Os soros colostrais de 17 vacas bubalinas da raça Murrah pertencentes ao CPATU/EMBRAPA foram estudados com o objetivo de se determinar o perfil eletroforérico. Para obtenção do soro colostral foi adicionado às amostras 1 mL de renina comercial e mantidas em banho-maria até a coagulação da caséina e separação do soro conforme preconiza DAM (1968). Para determinação da concentração de proteína total foi utilizado o método do Biureto com leitura em espectrofotômetro. A separação das proteínas séricas colostrais foi feita em sistema de semimicroeletroforese utilizando-se fitas gelatinizadas de acetato de celulose em solução tampão de veronal a 8,4% e pH 8,6 em corridas de 30 minutos de duração a 230 volts e volume de 1 microlitro segundo MOURA (1987). Os resultados foram os seguintes em g/100 mL: proteína total 13,33 ± 3,43 ; albumina 0,49 ± 0,21 ; alfaglobulina 1,05 ± 0,47; betaglobulina 0,87 ± 0,55 ; gamaglobulina 10,95 ± 3,08. A elevada concentração de gamaglobulina comprova a importância do colostro para a espécie durante as primeiras fases da vida já que não há transferência de anticorpos através da placenta.

EARLY BOVINE PREGNANCY DIAGNOSIS BY A BATTERY OPERATED PORTABLE ULTRASONIC SCANNER THE "ULTRA-SCAN"

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INTRODUCTION

The accurate and early detection of pregnant and non-pregnant cows has become a key of good breeding management, for it is an essential factor for monitoring and controlling herd fertility. Rectal palpation of the uterus and its contents has been the standard method of pregnancy diagnosis for many years. The accuracy of this method and the earliest time at which pregnancy can be recognized is dependent upon the experience of the veterinary surgeon and on the pregnancy diagnosis criteria used. It has the advantage , compared with laboratory methods , in that an opinion can be given immediately after examination on the farm, and if the cow is found not to be pregnant, explanation may be given for its failure and some treatment implemented. A significant embrionic death after early pregnancy diagnosis (40 days of gestation) may be disadvantage of this method (1).

Although rapid progesteron assays of plasma, serum or milk also can be used to detect pregnancy on the farm, the accuracy of this method is only 75% for cows that are pregnant and nearly 100% for cows that are not pregnant, if the testing is done on the 21st or 24th day after breeding (1). In another study, a qualitative cow-side milk progesteron EIA test was compared with a quantitative milk progesteron RIA test (2). The positive predictive values for pregnancy were 59.3% and 61%, while the negative predictive values for non-pregnancy were 85.7% and 78.4%, respectively.

The use of transrectal ultrasonography of the uterus for early diagnosis of bovine pregnancy has been reported by several authors during recent years. Besides these studies only a limited number of reports are available regarding reliability and accuracy of this technique for early pregnancy diagnosis (2-6). The present study was designed to determine the predictive accuracy of transrectal ultrasound scanning of the uterus, under field conditions, by a battery operated portable ultrasonic scanner the "ULTRA-SCAN".

MATERIALS AND METHODS

Two to eleven year old cows, Hungarian Red Pied and crossbreds with red-and-white Dutch Friesian, were used. Between 17 and 47 days after A.I., cows (n=400) were tested by a portable ULTRA-SCAN V8 from Alliance Medical Inc., Montreal, Canada, with a 5 MHz rectal sector transducer, reaching a focal length of 5-6 cm from the surface of the probe. After removal of the faeces the transducer was inserted into the rectum and was moved from the cervix toward both the left and the right uterine horn. Transversal excursions were made to image the entire uterus. After each examination the transducer was cleaned. No palpation of the genital track took place before or after the ultrasound examinations, which were all performed and interpreted by the same investigator. A cow was consider to be pregnant when, on the monitor an irregularly shaped, non-echogenic black spot (or spots)

appeared within the uterine lumen, representing the fluid field conceptus. Pregnancy was confirmed by rectal palpation of the uterus between 6 and 8 weeks after breeding.

The days of gestation on which the ultrasound investigations took place were arranged as follows:

- between 17 and 24 days after A.I.
- between 25 and 29 days after A.I.
- between 30 and 39 days after A.I.
- between 40 and 47 days after A.I.

According to the final rectal pregnancy diagnosis, the results were grouped as follows: correct positive diagnosis (a), incorrect positive diagnosis (b), correct negative diagnosis (c) and incorrect negative diagnosis (d). From these, the sensitivity (100 x a/a+d), the specificity (100 x c/c+b), the positive predictive value (100 x a/a+b) and the negative predictive value (100 x c/c+d) of the test were calculated.

RESULTS

Quantitative results of the 400 ultrasound examinations are given in Table 1. With transrectal sector scanning of the uterus between day 17 and 24 after A.I. 77% (17 from the 22 tested) of the pregnant cows were correctly diagnosed as pregnant, while 47% (30 from the 64 tested) of the non-pregnant cows were correctly diagnosed as non-pregnant. Between day 25 and 29 after A.I., among the 58 pregnant animals 54, i.e. 93% were diagnosed correctly. Among the 43 non-pregnant cows, 42 (97.6%) were diagnosed correctly. From 30 days onwards after breeding, 100% reliable results could be obtained both for positive and negative pregnancy diagnosis.

DISCUSSION

The use of real-time B-mode ultrasound scanning for pregnancy detection has arisen historically from human use where, until recently, the examination was made transabdominally. This required a greater depth of penetration for the sound waves and for this purpose a 3.0 or 3.5 MHz probe was used. These probes were transfered to the first veterinary applications. The accuracy of a 3.0 or 3.5 MHz ultrasound probe for the detection of the conceptus after day 20 has been investigated by several authors (3-6). Accuracy was defined as the proportion of pregnant and non-pregnant cattle (as determined by a manual transrectal examination) that were ultrasonically diagnosed as pregnant and non-pregnant, respectively.

In the first study (3), due to a high number of false positive diagnoses, the accuracy of these scanning results were only accurate after day 50. In the second study (4), 201 cattle were scanned with a 3.0 MHz linear array probe 21 to 70 days after insemination. There was complete agreement on 80 cattle scanned on days 56 to 70 of pregnancy. The other scanning results, especially the ones made between day 42 and 49, were more accurate than those in the first study. In the third study (5), 309 ultrasonic examinations were performed during the first trimester of pregnancy using a 3.5 MHz real-time sector probe. Near 100% reliable results could be obtained for positive diagnosis from 45 days onwards. The fourth study (6) showed similar accuracy of positive and negative diagnoses, using a 3 MHz linnear-array probe.

More recently, probes of higher frequency (5.0 or 7.5 MHz) have been tried in transrectal examination for pregnancy in cattle. Pierson

TABLE 1. Pregnancy diagnosis in cows with a 5 MHz sector probe

Grouping and evaluation	Test performed during days after A.I. 17 to 24	25 to 29	30 to 39	40 to 47
Diagnosis pregnant correct (a)	17	54	87	36
Diagnosis pregnant incorrect (b)	5	4	-	-
Diagnosis not pregnant correct (c)	30	42	56	24
Diagnosis not pregnant incorrect (d)	34	1	-	-
Sensitivity (%) 100 x a/a+d	33	98	100	100
Specificity (%) 100 x c/c+b	86	91.3	100	100
Positive predictive value (%) 100 x a/a+b	77	93	100	100
Negative predictive value (%) 100 x c/c+d	47	97.6	100	100

and Ginther (7) used a 5 MHz probe and were able to report the presence of discrete non-echogenic areas within the uterus at days 12 and 14. Curran and others (8), using a 5 MHz probe, detected embrionic vesicle at a mean of 11.7 days in a group of heifers in which the embrio proper was identified between 19 and 24 days, when fetal heart beats were also seen. The earliest detection of pregnancy with a 7.5 MHz real-time linear array probe was at 9 days when a vesicle was imaged within the lumen of the uterine horn (9). The early conceptus was seen at day 13 within the vesicle. Despite these studies, there is only a critical work regarding the accuracy of conceptus detection with a high frequency (5 MHz) probe (2). It is expected, however, because of increased resolution, that pregnancy can be diagnosed with a high degree of accuracy at a much earlier stage than that reported for 3.0 and 3.5 MHz probes. Pieterse and others (2) proved this supposition because the real-time ultrasound examination with a 5 MHz linear array probe between day 26 and 33 day after A.I. was more accurate than that reported for a lower frequency probe.

The results of our study show transrectal ultrasound scanning of cows with a 5 MHz real-time sector probe between days 25 and 29 after breeding was more reliable than that reported by Pieterse and others (2). At the same time, 100% reliable results could be obtained both for positive and negative diagnosis from 30 days onwards.

It may be concluded from this study that ultrasound imaging of the uterine horn in transverse planes is more useful for the initial detection of the conceptus than those in longitudinal or in oblique planes. This fact may be the reason why our scanning results are more accurate than those described in the literature using the same ultrasound frequency. Since the ULTRA-SCAN operates with rechargeable batteries and could be recharged from a car battery, it makes ultrasonographyc examinations in field circumtances considerably easier.

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SUMMARY

A battery operated portable scanner with a 5 MHz sector probe was used to make early pregnancy diagnoses in 400 milk cows (Hungarian Red Pied and crossbreds with red-and-white Dutch Friesian) between 17 and 47 days after the last insemination. The irregular shaped non-echogenic areas within the uterus were used as criterions for pregnancy. The ultrasound diagnostic findings were systematically confirmed by rectal palpation between 6 to 8 weeks after A.I.

One hundred per cent reliable results could be obtained both for positive and negative diagnoses from 30 days after breeding onwards. Between days 25 and 29 after A.I. our results were also very reliable, which may be accounted for by the ultrasound imaging in transverse planes.

RÉSUMÉ

Un appareil portable (scanner), fonctionné avec des batteries, ayant une probe sectorielle de 5 MHz a été utilisé pour la détéction précoce de la gestation chez 400 vaches laitieres (race tacheté rouge hongroise et croisée avec de la frisienne néderlandaise) entre les 17 et 47 jours après la dernière insemination. Le critère de la gestation a été une ombre non-échogénique irrégulière dans l'utéros. La diagnose ultrasonique a été systématiquement confirmée par une palpation réctale pendant les 6-8 semaines après l'I.A.

Les résultats sûrs a 100 p.c. peuvent être obtenir et pour les cas positive et négatives des le 30ème jour après l'insémination. Entre les 25 et 29 jours l'I.A. nos résultats ont été aussi de toute confiance si l'on fait l'observation ultrasonique dans un plan transversal.

ZUSAMMENFASSUNG

Ein batteriebetriebener Sonograph, dessen Sonde eine Kapazität von 5 MHz aufwies, wurde für die Diagnostizierung der Frühträchtigkeit bei 400 Milchküchen (Ungarisches Fleckvieh und deren Kreuzungen mit der Rotweissen Holländischen Friesischen Rasse) zwischen den 17. und 47. Tagen nach der letzten Inseminierung verwendet. Die unregelmässigen echoarmen Zonen im Uterus wurden als Kriterium der Trächtigkeit betrachtet. Die ultraschalldiagnostische Befunde wurden in 6-8 Wochen nach der Besamung regelmässig auch mit rektaler Palpation bestätigt.

Hundertprozentig zuverlässige Ergebnisse konnten sowohl hinsichtlich der positiven als auch der negativen Diagnose vom 30. Tag an nach der Brunst gewonnen werden. Auch die Ergebnisse, die zwischen dem 25. und 29. Tag nach der Inseminierung gewonnen wurden, waren sehr zuverlässig, was auf die Ultraschallabbildung in transversaler Ebene zurückzuführen ist.

INFLUENCE OF BODY CONDITION ON OVARIAN FUNCTION, HEAT DETECTION PREGNANCY AND HORMONAL TREATMENTS

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INTRODUCTION

The energy and protein supply, which meets the requirements of the high lactating dairy cow, is a prerequisite of its reproductive functions. The postpartum negativ energy balance developes according to the metabolic demands of the high milk yield, fodder shortages as well as a sequel of diseases, endo- and ectoparasitic load for instance. Inadequate feeding lenghthened anestrus in beef cows (7). The degree of the negativ energy balance within 3 weeks p.p. was related to the time of the first ovulation p.p., which occurred about 10 d after the maximum energy deficit (2,3). Energy deficiency was related to increased fat mobilisation, elevated serum progesterone values around estrus as well as a higher incidence of silent heat (6). Ovarian dystrophy was a sequel of underfeeding (1,4).
Body condition scoring is a method of assessing the nutritional status of the dairy cow independent of its frame size. The body condition is scored by visual inspection and palpation. The score range was proposed from 0 to 5 (8) or 1 - 8 (5). Cows with an increase in body condition during lactation had a longer calving to conception interval (8).

MATERIAL AND METHODS

At Moc Chau State Farm 1869 Holstein Frisian cows were examined gynaecologically in 1988 and 1989. The examination includes rectal palpation. Vaginal inspection was done in cows with suspected heat or without palpable functional bodies of the ovaries. In the cows with uncertain diagnosis the examination was reapeated after 14 d. Body condition of each cow was scored. Ovarian dystrophy was treated by 100 - 500 IU PMSG (Intergonan, Vemie). Luteolysis was done by i.v.-injection of 250 g cloprostenol (Estrumate, Coopers).

RESULTS

The body condition of the dairy changed significantly within one year. In 1988 resp. 1989 13% resp. 74% of the examined cows showed a light moderate or better body condition (tab.1). The enhanced body condition was the sequel of the higher fodder supply, adequate fodder regime and closer endo- and ectoparasite control.

tab. 1 Body Condition of examined Cows

Dairy /heifer unit		Cows with body condition emaci-ated 1	very poor 2	poor moderate 3	light moderate 4	moderate and more 5,>5	total
1989							
cows	n	26	61	132	237	375	831
	%	3	7	16	29	45	831
1988							
cows	n	147	449	309	91	42	1038
	%	14	43	30	9	4	100

The reproductive function of the cows was related to the individual body condition. The incidence of ovarian dystrophy decreased from 74% resp. 45% in emaciated cows to 9% resp. 4% in cows of the moderate or better body condition score within 42 - 120 d p.p. resp. >120 d p.p. (tab. 2,3). The ratio of cows with detected heats (i.e. inseminated but not confirmed pregnant) increased from 4% to 26% within 42 - 120 d p.p. (tab. 2). In the cows > 120 d there was no relation between body condition and detected heats (tab. 3). The ratio of pregnant cows increased from 0% resp. 19% in the emaciated cows to 46% resp. 79% in cows of the moderate or better body condition score within 42 - 120 d p.p. resp. 120 d p.p. (tab. 2,3).

tab. 2 Relation of the Body Condition and the Ovarian Function, the Detected Heats and the Pregnancies in Cows within 42 - 120 d after the last calving

Findings		Cows with Body Condition 1	2	3	4	5,>5
cows with ovarian dystrophy	n	70	102	50	28	9
	%	74	49	28	29	9
cows with ovarian function	n	21	69	82	21	18
	%	22	33	47	21	19
cows with detected heat (inseminated)	n	4	25	31	23	25
	%	4	12	18	23	26
cows pregnant	n	0	11	9	26	45
	%	-	6	7	27	46
cows examined	n	95	209	176	98	97
	%	100	100	100	100	100

tab. 3 Relation of the Body Condition and the Ovarian Function, the Detected Heats and the Pregnancies in Cows more than 120 days after the last Calving

Findings		Cows with Body Condition 1	2	3	4	5,>5
cows with ovarian dystrophy	n	32	60	43	18	13
	%	45	22	14	8	4
cows with ovarian function	n	15	44	62	33	20
	%	20	16	20	15	6
cows with detected heat (inseminated)	n	12	66	76	53	36
	%	16	24	25	24	11
cows pregnant	n	14	101	124	116	246
	%	19	38	41	53	79
cows examined	n	74	271	305	220	315
	%	100	100	100	100	100

After cloprostenol injection and terminated inseminations 31 of 56 cows were pregnant. The pregnancy rate was highest in cows with the body condition score 3 and 4 (tab. 4). After 46 PMSG-treatments 18 cows developed follicles, 13 corpora lutea and 15 showed no ovarian function (tab. 5)

DISCUSSION

Body condition scoring is a practical method of assessing the nutritional status of the dairies. From the reproductive viewpoint the dairy should be fed at least in body score 4 during the post partum and the service period to achieve expressed heat signs and normal pregnancy rates. The body condition of the individual cow seems to be an important aspect of treatment prognosis. Cows should be at least in body condition 3 to exspect normal pregnancy rates after luteolysis. Body condition 2-3 seems to be necessary to induce follicular growths, ovulation and corpus luteum development by PMSG treatment.

tab. 4 Body Condition and Pregnancy Rate after Cloprostenol Injection

		Cows examined	Number of Cows pregnant	not pregnant
cows with body condition	1	4	0	4
"	2	22	11	11
"	3	19	13	6
"	4	8	7	1
"	5,>5	3	0	3
total		56	31	25

tab. 5 Body Condition and Ovarian function 14 d after PMSG-treatment

		Cows examined	cows with the ovarian finding C.luteum	follicle	no function
cows with body condition	1	11	4	1	6
"	2	23	10	9	4
"	3	12	8	2	2
total		46	22	12	12

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ZUSAMMENFASSUNG

1869 Kühe und 429 Färsen wurden gynäkologisch untersucht. 675 Kühe befanden sich innerhalb von 42 - 120 Tagen und 1185 über 120 Tagen nach dem Abkalben. Die Zuchtkondition wurde mit Hilfe eines Punktesystems bewertet. Im Jahr 1988 bzw. 1989 wurde die Zuchtkondition von 147 (14%) bzw. 26 (3%) Kühen als kachektisch, von 61 (7%) bzw. 449 (43%) als sehr mager, von 132 (16%) bzw. 309 (30%) als mager, 237 (29%) bzw. 91 (9%) als etwas zu mäßig und 42 (4%) bzw. 375 (45%) als gut oder zu mastig beurteilt. Körperkondition, Ovarfunktion, Brunstanzeichen und Trächtigkeitsrate standen in Beziehung zueinander. Innerhalb von 42 - 120 Tagen nach dem Abkalben zeigten 70 (74%) der 95 kachektischen Kühe Ovardystrophie, 21 (22%) stille Brunst, 4 (4%) normale Brunstzeichen; keine Kuh war tragend. 102 (49%) von 207 Kühen in sehr schlechter Körperkondition zeigten Ovardystrophie, 69 (33%) stille Brunst, 25 (12%) normale Brunstzeichen und 11 (6%) waren tragend. 50 (28%) von 176 Kühen in schlechter Körperkondition zeigten Ovardystrophie, 82 (47%) stille Brunst, 31 (18%) normale Brunstzeichen und 9 (7%) waren tragend. 28 (29%) von 98 Kühen mit etwas zu mäßiger Körperkondition zeigten Ovardystrophie, 21 (21%) stille Brunst, 23 (23%) normale Brunstanzeichen und 26 (27%) waren tragend. 9 (9%) der 97 Kühe mit guter oder zu mastiger Zuchtkondition zeigten Ovardystrophie, 18 (19%) stille Brunst, 25 (26%) normale Brunstanzeichen und 45 (46%) waren tragend. In der Milchviehherde konnte eine zystische Degeneration der Ovarien nicht festgestellt werden. Die Ovardystrophie wurde mit 100 - 300 IE PMSG behandelt. Nach Stillbrunst wurde bei Kühen zwischen dem 10. und 17. Zyklustag eine Luteolyse eingeleitet sowie 56 h und 80 h p.injectionem zweimalig besamt. Der Behandlungserfolg (Stimulierung der Ovarfunktion und normale Brunstanzeichen) war von der Körperkondition der Kühe abhängig.

SUMMARY

1869 cows were examined gynaecologically. 675 cows were within 42 - 120 d and 1185 after 120 d after calving. The body condition was evaluated by a scoring system. 26 cows (3%) vs. 147 (14%) were scored emaciated in 1877 resp. 1989, 61 (7%) vs. 449 (43%) very poor, 132 (16%) vs. 309 (30%) poor, 237 (29%) vs. 91 (9%) light moderate and 375 (45%) vs. 42 (4%) moderate or more. There was a close relationship of body condition, ovarian function, heat signs and pregnancies. Within 42 - 120 days after calving 70 (75%) of 95 cows with an emaciated body condition showed ovarian dystrophy, 21 (22%) silent heat, 4 (4%) normal heat and none was pregnant. 102 (49%) of 207 cows with a very poor body condition showed ovarian dystrophy, 69 (33%) silent heat, 25 (12%) normal heat an 11 (6%) were pregnant. 50 (28%) of 176 cows with a poor body condition showed ovarian dystrophy, 82 (47%) silent heat, 31 (18%) normal heat and 9 (7%) were pregnant. 28 (29%) of 98 cows with a light moderate body condition showed ovarian dystrophy, 21 (21%) silent heat, 23 (23%) normal heat and 26 (27%) were pregnant. 9 (9%) of 97 cows with a moderate or a higher body condition showed ovarian dystrophy, 18 (19%) silent heat, 25 (26%) normal heat and 45 (46%) were pregnant. In cows 120 d after calving the relationship was evident as well. There was no case of cystic degeneration within the dairy. Ovarian dystrophy was treated by 100 - 500 IU PMSG. In cows with silent heat and a corpus luteum within 10 - 17 d of the cycle luteolysis was induced followed by two terminated artificial inseminations 56 and 80 h after the injection of PGF2a analogue. Treatment success i.e. induced ovarian function and normal heats depended on the individual body condition.

RESUME

1869 vaches ont fait l'objet d'un examen gynécologiyue. 675 vaches avaient vêlé entre 42 et 120 jours auparavant et 1185 plus que 120 jours auparavant. Les conditions d'élevage ont été analysées par le biais d'un système de points. En 1988 et 1989, 147 vaches (14%) étaient dans un état extrêmement maigres, 26 (3%) ou 61 (7%) ou 449

(43%) étaient très maigres, 132 (16%) ou 309 (30%) maigres, 237 (29%) ou 91 (9%) d'un poids moyen et 375 (45%) ou 42 (4%) en bon état ou trop grosses. Il existe une relation entre la condition physique, la fonction ovarienne, les symptômes du rut et les taux de gestation. Entre 42 et 120 jours après le vêlement, 70 (74%) des 95 vaches très maigres révélaient une dystrophie ovarienne, 21 (22%) un rut sans signe apparent, 4 (4%) un rut normal; Aucune vache n'était en gestation. 102 (49%) des 207 vaches en très mauvaise condition physique ont révélé une dystrophie ovarienne, 69 (33%) un rut sans signe apparent, 25 (12%) un rut normal et 11 (6%) étaient en gestation. 50 (28%) des 176 vaches en mauvaise condition physique ont rélélé une dystrophie ovarienne, 82 (47%) un rut sans signe apparent, 31 (18%) un rut normal et 9 (7%) étaient en gestation. 28 (29%) des 98 vaches d'une condition physique moyenne ont rélélé une dystrophie ovarienne, 21 (21%) un rut sans signe apparent, 23 (23%) un rut normal et 26 (27%) étaient en gestation. 9 (9%) des 97 vaches en bonne condition physique ou trop grosses ont rélévé une dystrophie ovarienne, 18 (19%) un rut sans signe apparent, 25 (26%) un rut normal et 45 (46%) étaient en gestation. Il n'a été constaté aucune dégénération cystique des ovaires dans le troupeau. La dystrophie ovarienne a été traitée par 100 á 500 UI de PMSC. Le rut sans signe apparent a été traité par une lutéolyse entre le 10ème et le 17ème jour du cycle et en les fécondant par deux fois, á savoir 56 h et 80 h aprés les injections. Le succés du traitement (stimulation des fonctions ovariennes et signes normaux de rut) était tributaire de la condition physique des vaches.

RESUMO

1869 vacas foram examinadas ginecologicamente. Com as vacas os estudos foram realizados 42 até 120 dias (675 vacas) respectivamente mais de 120 dias (1185 vacas) depois do parto. Nos anos 1988 e 1989 a condição geral dos animais foi analisado como "emaciada" em 147 animais (14%) e 26 animais (3%), como "muito magro" em 449 animais (43%) e 61 animais (7%), como "magro" em 309 animais (30%) e 132 animais (16%), como "moderado" em 91 animais (9%) e 237 animais (29%) e como "bom" ou melhor em 45 animais (4%) e 375 animais (45%) respectivamente. Foi observado uma boa relação entre condição, funcionamento dos ovários, sintomas do cio e número de gestações. No período de 42-120 dias depois do parto 70 vacas (74%) emaciadas mostraram distrofia dos ovários, com 21 (22%) faltaram sintomas visiveis do cio e 4 (4%) ficaram com cio normal; nenhuma das vacas era prenha. 102 vacas (49%) muito magras mostraram distrofia dos ovários, com 69 (33%) faltaram sintomas do cio, 25 (12%) ficaram com cio normal e 11 (6%) eram prenhas. Das 176 vacas magras 82 (47%) ficaram sem sintomas do cio, 31 (18%) com cio normal e 9 (7%) eram prenhas.
Das 98 vacas com condicao moderada 28 (29%) mostraram distrofia dos ovários, 21 (21%) ficaram som sintomas de cio, 23 (23%) com cio normal e 26 (27%) eram prenhas. Das 97 vacas com condição boa ou melhor 9 (9%) mostraram distrofiados ovários, 18 (19%) ficaram sem sintomas de cio, 25 (26%) com cio normal e 45 (46%) eram prenhas. No rebanho leiteiro degeneração cística nao foi encontrado. A distrofia dos ovários foi tratado por 100 - 500 UI PMSC. Nas vacas com cio sem sintomas visiveis foi iniciado uma lise do corpo amarelo entre 10 e 17 dia do ciclo sexual. 56 e 80 horas depois da inoculação foram executadas duas inseminações artificiais. O sucesso do tratamento dependeu da condição das vacas.

DIE ABKLÄRUNG VON ZITZENSTENOSEN BEIM RIND MITTELS ULTRASCHALL

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EINLEITUNG

Zitzenstenosen können nach verschiedenen Kriterien gruppiert werden (4,5). Aufgrund ihrer Lokalisation lassen sie sich in vier Kategorien einteilen, nämlich in tiefe (Strichkanal), mittlere (Zitzenzisterne) und hohe (Zitzenbasis) Stenosen, sowie in Stenosen im Bereich der Fürstenbergschen Rosette.

Die Diagnostik umfasst folgende Massnahmen: Adspektion, Palpation, Überprüfung der Melkbarkeit, Sondierung mit einem Melkkatheter, röntgenologische Untersuchung und in Ausnahmefällen eine diagnostische Thelotomie (6,7). Das Röntgen ergibt gute Informationen, ist aber unter Praxisbedingungen aufwendig, verlangt besondere Schutzmassnahmen und ist bei Veränderungen im Bereich der Zitzenbasis aus technischen Gründen oft nicht realisierbar. Ziel der vorliegenden Arbeit war deshalb, abzuklären, ob die Darstellung der Zitze mittels Ultraschall bei der Diagnostik von Zitzenstenosen eine Alternative zum Röntgen darstellt.

MATERIAL UND METHODEN

Es wurden 65 Zitzen von 63 Kühen wegen Milchabflussstörungen nach folgenden Schema untersucht: Adspektion, Palpation, Probemelken, Sondierung mit einem Melkkatheter und Röntgen. Unabhängig von der röntgenologischen Untersuchung wurde zusätzlich eine Diagnose mittels Ultraschall gestellt. Zu jeder stenosierten Zitze wurde zum Vergleich auch die kontralaterale gesunde Zitze echographisch abgebildet.

Für die Ultraschalluntersuchungen wurden zwei Linearscanner der Firma Pie Data Medical verwendet. Der Scanner 560 ist mit einem 3,5 Megahertz-Schallkopf ausgestattet, der Scanner 450 mit einem solchen von 5 Megahertz (MHz). Die Anfertigung der Fotoaufnahmen erfolgte mit den Polaroid Schwarzweissfilmen 667 bzw. 611. Mit dem 3,5 MHz-Schallkopf wurden 36 Zitzen untersucht, mit den 5 MHz-Schallkopf deren 29.

Die Ultraschalluntersuchung wurde wie folgt durchgeführt:

Nach dem Auslösen des Milchejektionsreflexes (10-20 IE Oxytocin i.v.) und ev. einer leichten Sedierung (1,4- 3 mg Xylazin/100 kg KGW = 0,07-0,15 ml Rompun^{R}/100 kg KGW) wurde die mit einer desinfizierenden Seife (Vetedine^{R}) gewaschene Zitze in ein warmes Wasserbad getaucht. Als Wasserbehälter dienten modifizierte 1-Liter Plastikflaschen aus weichen Polyäthylen mit einem Bodendurchmesser von 8 cm. Der mit dem Gleitmittel Vetagel^{R} bestrichene Schallkopf wurde parallel zur Zitze auf die aussere Wand des Wasserbehälters gepresst. Bei Zitzen, die sich trotz Oxytocineinwirkung nicht oder nur unvollständig mit Milch füllten, wurde im Bereich des Fürstenbergschen Venenrings eine elastische Ligatur angelegt und hernach die Zitze mit physiologischer Kochsalzlösung prall gefüllt. Bei inkontinenten Zitzen verhinderte eine in den Strichkanal eingeführte verschlossene Zitzenkanüle das Ausfliessen der Milch bzw. der infundierten Kochsalzlösung.

RESULTATE

Bei den 65 untersuchten Zitzen wurden insgesamt 73 Stenosen diagnostiziert, da acht Zitzen an zwei Lokalisationen Veränderungen aufwiesen (Tabelle 1). Bei 44 Zitzen konnte die Diagnose durch eine Operation überprüft werden. In den übrigen Fällen wurde auf eine Operation verzichtet, da ein solcher Eingriff nicht erfolgversprechend schien.

Mittels Ultraschall konnten 68 Stenosen deutlich erkennbar dargestellt werden. Die fünf Veränderungen, die kein befriedigendes Resultat erbrachten, betrafen ausnahmslos Stenosen, die im Strichkanal lokalisiert waren.

Mit dem Röntgen konnten lediglich mittlere Stenosen in allen Fällen sichtbar gemacht werden. Die Darstellung des veränderten Strichkanals gelang bei drei der sechs im Strichkanal lokalisierten Stenosen. Schleimhautabrisse im Bereich der Fürstenbergschen Rosette waren in 25 von 26 Fällen erkennbar. Von den 14 hohen Stenosen liessen sich drei vollständig, fünf teilweise und sechs nicht auf dem Röntgenfilm festhalten.

Tabelle 1: Verteilung der Zitzenstenosen nach Lokalisationen und ihre Darstellbarkeit mit Ultraschall und Röntgen

Lokalisation der Stenose	Anzahl Zitzen	Erfolgreiche Darstellung mit Ultraschall	Röntgen
Strichkanal	6	1	3
Fürstenbergsche Rosette	26	26	25
Zitzenzisterne	27	27	27
Zitzenbasis	14	14	3
Total	73	68	58

Die Zitzenwand liess sich auf dem Ultraschallbild in drei Schichten einteilen. Die äussere Schicht hob sich als schmaler echodichter Streifen deutlich von der echoarmen mittleren Schicht ab. Die innere Schicht hingegen wies nur eine geringgradig stärkere Echodichte als die mittlere Schicht auf. Das Lumen der Zitzen- bzw. Euterzisterne war echofrei. Der Strichkanal stellte sich als echoarme, aber undeutlich abgegrenzte Zone dar. Über den Zustand des Strichkanals konnte daher nur in wenigen Fällen eine klare Aussage gemacht werden. Auf dem Röntgenbild hingegen war der Strichkanal meistens gut sichtbar.

Das Gerät mit dem 5 MHz-Schallkopf lieferte detailliertere Bilder als dasjenige mit dem 3,5 MHz-Schallkopf. Leichtgradige Schleimhautveränderungen konnten jedoch mit beiden Geräten nicht mit Sicherheit dargestellt werden.

Bei den vorliegenden Untersuchungen mit der beschriebenen Methode traten wiederholt Artefakte auf. In der Regel lagen die Artefakte jedoch ausserhalb des Zitzenlumens und führten deshalb nicht zu Fehlinterpretationen der Zitzenveränderungen.

DISKUSSION

Ultraschall stellt ein brauchbares Hilfsmittel bei der Diagnostik von Zitzenstenosen dar und kann daher als Alternativmethode zur röntgenologischen Untersuchung eingesetzt werden.

Das echographische Bild von unveränderten Zitzen entsprach Angaben aus der Literatur (1). Es zeigten sich der gleiche dreischichtige Aufbau der Zitzenwand sowie das echolose Lumen der Zitzen- und Drüsenzisterne. Blutgefässe nahe der Zitzenzisternenschleimhaut und im Bereich des Fürstenbergschen Venenrings waren ebenfalls häufig sichtbar. Der 5 MHz-Schallkopf war dem 3,5 MHz-Schallkopf bei der Darstellung dieser Strukturen überlegen.

Für eine befriedigende Darstellung einer Zitze mittels Ultraschall ist eine gute Füllung der Zitze mit Flüssigkeit von entscheidender Bedeutung. Wichtig ist auch eine gute Füllung des Zitzentauchbechers mit Wasser, da Luft die Ultraschallwellen reflektiert. Daher muss der Plastikbecher so zugeschnitten sein, dass sein oberer Rand lückenlos ans Eutergewebe gepresst werden kann und somit nirgends Wasser ausfliesst. Ausserdem ist es für die Untersuchung von hohen Stenosen von Vorteil, wenn die Becherwand lateral einige Zentimeter über den Fürstenbergschen Venenring hinaus nach proximal reicht.

Die Entstehungsweise von Artefakten bei Ultraschalluntersuchungen wurde verschiedentlich beschrieben (2,3). Zur Vermeidung von Fehlinterpretationen und zur Erkennung von Artefakten gilt es zwei Punkte zu beachten: Nur reproduzierbare Befunde dürfen gewertet werden und das Ergebnis der echographischen Untersuchung ist nicht isoliert, sondern immer unter Berücksichtigung der übrigen Befunde zu interpretieren.

Ein Vergleich der Echographie mit der röntgenologischen Untersuchung zeigt, dass beide Methoden sowohl Vor- als auch Nachteile aufweisen. Ultraschall weist gegenüber dem Röntgen folgende Vor- und Nachteile auf:

Vorteile:

- Es müssen weder für das Tier noch für den Untersucher besondere Schutzmassnahmen beachtet werden.
- Eine Belastung durch Röntgenstrahlen entfällt.
- Die Technik erlaubt, in kurzer Zeit beliebig viele Bilder in verschiedenen Ebenen festzuhalten.
- Die Bilder sind sofort beurteilbar. Dies erleichtert einen schnellen Entscheid hinsichtlich Prognose und Therapie.
- Veränderungen an der Zitzenbasis und in der Drüsenzisterne können gut dargestellt werden. Die Darstellbarkeit der Drüsenzisterne ist insbesondere bei Milchabflussstörungen nach Galtmastitiden und bei angeborenen Störungen von einiger Bedeutung.

Nachteile:

- Beschränktes Auflösungsvermögen, abhängig von der verwendeten Schallfrequenz.
- Der Strichkanal und geringgradige Läsionen der Zitzenzisternenschleimhaut lassen sich nur ungenügend darstellen.
- Artefakte.

Die mangelhafte Darstellung des Strichkanals und der feinen Schleimhautveränderungen ist jedoch von untergeordneter Bedeutung, da Strichkanalveränderungen aufgrund der Anamnese und der klinischen Befunde (Melkprobe, Sondierung mit einem Melkkatheter) mit ausreichender Sicherheit diagnostiziert werden können und geringgradige Veränderungen der Zisternenschleimhaut nur äusserst selten zu Störungen führen. Die Kenntnis von

Artefakten erlaubt es, sie als solche zu erkennen, zumal sie nur ausnahmsweise im Zitzenlumen auftreten.

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ZUSAMMENFASSUNG

Insgesamt 73 Zitzenstenosen von 63 Kühen wurden zusätzlich zur klinischen Untersuchung röntgenologisch und nach einer eigens entwickelten Ultraschall-Methode abgeklärt. Es gelangten zwei Linearschallköpfe mit Schallfrequenzen von 3,5 bzw. 5 MHz zum Einsatz. Die Resultate dieser beiden Verfahren wurden einander gegenübergestellt und mit den Operationsbefunden verglichen.

Die Ultraschalluntersuchung ergab in 68 Fällen, die röntgenologische Untersuchung in 58 Fällen eine korrekte Diagnose. Mittels Ultraschall konnte bei fünf Stenosen im Bereich des Strichkanals die Veränderung nicht eindeutig sichtbar gemacht werden. Bei der Darstellung des Strichkanals und der feinen Schleimhautveränderungen in der Zitzenzisterne war die Röntgentechnik überlegen. Hingegen konnten von 14 hohen Stenosen sonographisch alle, radiologisch aber nur deren drei dargestellt werden.

Mit der Ultraschall-Methode steht dem praktizierenden Tierarzt ein geeignetes Hilfsmittel zur Untersuchung von Zitzenstenosen zur Verfügung.

SUMMARY

Ultrasonography and radiography were used as an adjunct to clinical examination in the evaluation of 73 obstructed teats in 63 dairy cows. The images obtained with a 3,5 MHz and a 5 MHz linear array transducer were compared with radiographs and correlated with findings obtained at surgery.

Ultrasonography and radiography allowed a correct assessment of the lesions in 68 respectively 58 teats. In five cases the obstructive lesions in the teat canal were not clearly discernible when ultrasonography was used. Radiography was the method of choice for the evaluation of the teat canal and small mucosal folds in the milk cistern. Ultrasonography was the method of choice for the detection of obstructive lesions at the base of the teat; all 14 of these cases were assessed correctly using ultrasonography versus three of 14 when radiography was used.

Ultrasonography is a useful tool for the practician to evaluate obstructive teat lesions.

RESUME

En plus du diagnostique clinique, 73 cas de sténose du trayon furent examinés d'une part à l'aide de la radiographie et d'autre part avec une méthode ultrasonographique propre à l'auteur. Deux têtes linéaires présentant une longueur d'onde de 3,5 respectivement de 5 MHz furent employées. Les résultats de ces deux méthodes furent évalués entre eux et comparés aux constatations intraopératoires.

L'examen sonographique livra 68 diagnostiques exacts contre 58 pour l'examen radiologique. Dans cinq cas de sténose dans la région du canal du trayon, les ultrasons ne permirent pas de montrer parfaitement la lésion. Pour la mise en évidence du canal du trayon ainsi que des modifications fines de la muqueuse de la citerne, la radiographie s'avéra plus appropriée.

En revanche, les 14 cas de sténose proximale purent tous être mis en évidence sonographiquement, alors que seuls trois cas purent être documentés radiographiquement.

Avec la méthode ultrasonographique, le vétérinaire praticien dispose d'une aide précieuse pour l'examen des sténoses du trayon.

FREQUENCY OF *Eimeria* spp IN FAECES OF CATTLE IN TEODORO SAMPAIO CITY, BAHIA, BRAZIL.

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INTRODUCTION

Bovine coccidiosis has been registred in several places of the world (3,6,7,12). This disease occurs mainly in young animals (3,7), being responsible for high index of the animal morbidity and mortality. The losses it causes are relatively unknown in Brazil. The pathogenicity of *Eimeria* spp in the herd depends on several factors, such as: species, level of parasitism, resistance, age and nutritional state of the host, climate, type of husbandry and management. Many reports have been published in Brazil on the occurence of coccidian oocysts in faeces of cattle (1,2,4,8,11).

There is no information in the literature concerning species and frequency of coccidia in the cattle of Bahia. The object of this present study was to determine these species of *Eimeria* and their frequency.

MATERIALS AND METHODS

Faecal samples were collected from seventeen Holland-Zebu calves which were two to twelve months old. Samples were collected each month from July 1988 to June 1989 in a farm of Teodoro Sampaio city. Calves had free acess to water and to pasture of the *Brachiaria decumbens*. They were maintained in the same management adopted by the farm, but these calves had not received any coccidiostat treatments. When the calves were one year old they got substituted by other younger animals.

All animals were observed for signs of clinical coccidiosis.

Each faecal sample was examined for coccidian oocysts counting by means of Gordon & Whitlock technique (5) with salt solution. After that, five samples containing the highest numbers of oocysts per gram of faeces were incubated in a solution of 2.5% potassium dichromate at room temperature (25 - 30°C), at least for seven days. The samples were filtered on a screen and centrifugated with a Sheather's sugar solution in order to recover the coccidian oocysts. At least 100 sporulated

oocysts from each sample were identified by comparing the morphology and size of oocysts with the published descriptions (9,10).

The data of temperature, rainfall and humidity were obtained from the National Institute of Meteorology, 4th district.

RESULTS AND DISCUSSION

Coccidian oocysts occurred in 165 (80.8%) of 204 faecal samples of the cattle in Teodoro Sampaio, Bahia. In a way, the coccidial infection remained in a low level in these animals though it was constant during all months of this survey. The numbers of *Eimeria* oocysts per gram of faeces was usually below 600 in the samples, but the greatest number in a sample was 14.500 oocysts per gram of faeces. Carneiro *et al.*(1) also found in Goiania oocysts countings lower than 600 in faecal samples of 15 crossbred calves.

Average monthly numbers of coccidian oocysts per gram of faeces and temperature, rainfall and humidity data are presented in Figure 1.

The highest oocysts countings occurred in November, in the dry season it is suggested that the peak of the infection was caused by the rain fall in the same period or according to Carneiro *et al.*(1) when these calves are crowded in a little area and low pasture in the dry season, they may ingest a large number of infective oocysts. Significant numbers of coccidian oocysts in faeces of the calves were present in March and June in the rainy period, and it is probable that the humidity and rainfall were favourable in the development of these oocysts. Kasim & Al-Shawa(7) reported the incidence of coccidial infection was probable higher in the eastern, southern and western regions of Saudi Arabia because rainfall, humidity and intensive rearing methods were higher, so that habitats for the parasite were more plentiful, whereas the low incidence of infection recorded in the northern and central regions was probably due to the arid conditions existing in these two regions.

Eight different species of *Eimeria* were identified in faeces samples of the calves for the first time in Bahia State. They were in order of frequency: *E.bovis*, *E.auburnensis*, *E.ellipsoidalis*, *E.cylindrica*, *E.zuernii*, *E.subspherica*, *E.brasiliensis* and *E.bukidnonensis*.

The average monthly numbers of oocysts of the eight species of *Eimeria* are recorded in Table 1.

Eimeria bovis was the most frequent species in almost all seasons, except in the end of dry period and beginning of rainy period, whereas *E.auburnensis* was higher in these periods; in November, December and

February and *E.ellipsoidalis* was higher in January.

Eimeria cylindrica and *E.zuernii* occurred in a low level though they were present during almost all seasons. The frequency of *E.subspherica*, *E.brasiliensis* and *E.bukidnonensis* was consistently low during this experimental period. However, it was not possible to make relation between the various species of *Eimeria* with the meteorologic data.

In this study, *E.bovis* was the most common species. Although it was considered one of the most pathogenic species (7), no clinical case of coccidiosis was seen in any of the animals sampled during this survey. According to *Ernst et al.*(3) calves with the *E.bovis* oocysts countings as high as 45.000 OPG had faeces mildly diarrhetic without blood and tissue, so they concluded that many oocysts can be found in the faeces of cattle without the disease.

TABLE 1. Average monthly numbers of oocysts in faecal samples for each coccidian species of the calves in Teodoro Sampaio,Bahia.

Months	*Eimeria* species							
	bovis	aubur.	ellip.	cylind.	zuer.	bukid.	subs.	bras.
July/88	823	259	673	125	25	0	4	0
Aug.	640	529	19	22	31	0	0	0
Sept.	571	123	17	8	0	0	0	0
Oct.	613	438	62	20	8	0	0	0
Nov.	1.570	3.734	1.597	212	38	0	0	0
Dec.	99	216	14	57	3	0	2	0
Jan./89	674	37	685	34	5	0	26	0
Feb.	179	323	50	15	11	0	22	0
Mar.	1.490	54	301	281	155	5	4	0
Apr.	121	79	107	5	57	10	25	37
May.	803	76	75	6	83	0	0	7
Jun.	1.131	0	55	11	231	0	12	0

bovis = *E.bovis*
aubur.= *E.auburnensis*
ellip.= *E.ellipsoidalis*
cylind= *E.cylindrica*
zuer. = *E.zuernii*
bukid.= *E.bukidnonensis*
subs. = *E.subspherica*
bras. = *E.brasiliensis*

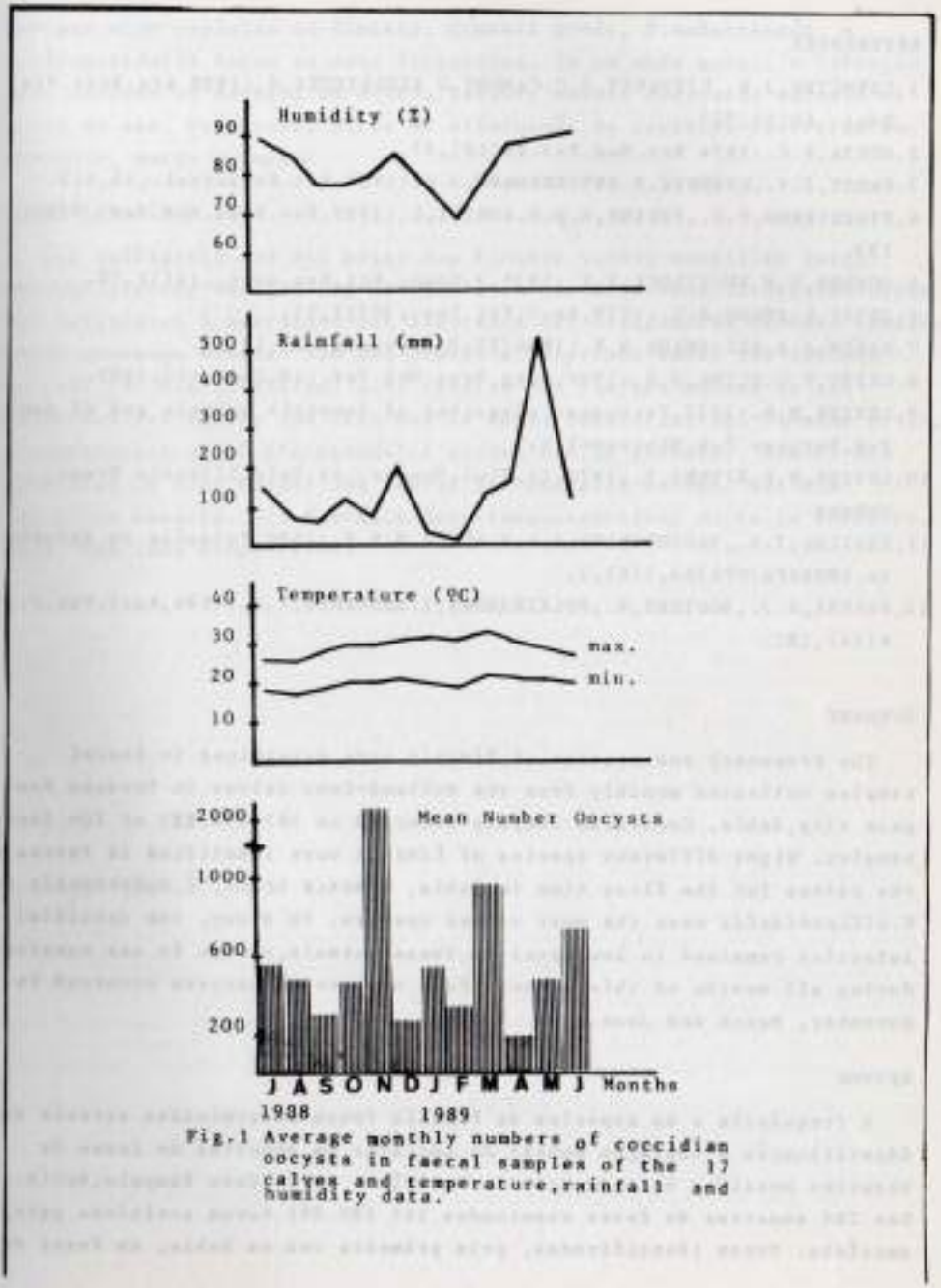

Fig.1 Average monthly numbers of coccidian oocysts in faecal samples of the 17 calves and temperature,rainfall and humidity data.

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SUMMARY

The frequency and species of *Eimeria* were determined in faecal samples collected monthly from the Holland-Zebu calves in Teodoro Sampaio city,Bahia. Coccidian oocysts occurred in 165 (80.8%) of 204 faecal samples. Eight different species of *Eimeria* were identified in faeces of the calves for the first time in Bahia. *Eimeria bovis*, *E.zuernensis* and *E.ellipsoidalis* were the most common species. In a way, the coccidial infection remained in low level in these animals,though it was constant during all months of this survey. Peak numbers of oocysts occurred in November, March and June.

RESUMO

A frequência e as espécies de *Eimeria* foram determinadas através da identificação e contagem mensal de oocistos em amostras de fezes de bezerros mestiços holando-zebu do Município de Teodoro Sampaio,Bahia. Das 204 amostras de fezes examinadas 165 (80.8%) foram positivas para o coccídio. Foram identificadas, pela primeira vez na Bahia, em fezes de

bovinos oito espécies de *Eimeria*. *Eimeria bovis*, *E.zuernensis* e *E.ellipsoidalis* foram as mais frequentes. De um modo geral, a infecção pelo coccídio se manteve em níveis baixos, embora constante durante os meses do ano. Os maiores picos de eliminação de oocistos ocorreram em novembro, março e junho.

ZUZAMMENFASSUG

Die Häufigkeit und die Arten von *Eimeria* wurden monatlich durch Identifizierung und Zählung der Oozisten von Kotproben durchgeführt,die von halbbluten Schwarzbunt-und Zeburasse der Stadgemeine Teodoro Sampaio Bahia gewonnen wurden. Von 204 geprüfte Kotproben waren 165 (80.8%) positiv für die Kokzidien. Acht Spezies von *Eimeria* wurden in den Kotproben des Rindes zum erst mal in Bahia identifiziert. *Eimeria bovis*, *E.zuernensis* und *E.ellipsoidalis* wurden haufig gefunden. Obwohl die Kokzidien in alle Monate des Jahres festgestellt wurden, war die Infektion niedrig. Die Maximale Oozystenausscheidung wurde im November, Marz und Juni eingetreten.

THE INFLUENCE OF THE PARATECT FLEX® BOLUS ON THE REPRODUCTIVE POTENTIAL OF SOME GASTROINTESTINAL NEMATODES IN CALVES.

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INTRODUCTION

The development of long acting devices, delivering continuous or pulse-like anthelmintic drugs to control parasitic gastroenteritis and in some cases parasitic bronchitis in cattle has proved to be very succesfull. One of the disadvantages is the permanent presence of the device or a part of it in the rumen/reticulum after the application. A recent breakthrough was made with the morantel sustained release trilaminate or MSRT (Paratect Flex Bolus; Pfizer Inc.) which does not incorporate metallic compounds and usually starts to disintegrate after a period of about 6 months. The release pattern has been described in detail (1, 2, 3). Recent publications have confirmed the success of this device in field conditions (4, 5, 6, 7, 8, 9, 10).
The present study aims to examine the effect of the MSRT on the reproductive potential of *Ostertagia ostertagi* and *Cooperia oncophora* female worms, surviving in calves from an artificial infection.

MATERIALS AND METHODS

Twenty-four one week old parasite-free male Friesian calves were shipped to the trial site. They were reared till three months of age under conditions that prevented nematode infection. They were allocated to two groups on basis of their weight. Calves of group 1 served as negative controls, calves of group 2 were treated with a MSRT on day-0 (13 September 1989). On day-0 all calves received a dose of 20,000 larvae of *O. ostertagi* and 40,000 larvae of *C. oncophora*. After 28 days three calves from each group were slaughtered and the remaining calves were challenged with the same infective dose as on day-0. This procedure was repeated on day-56 and day-84. On day-112 the remaining six calves were slaughtered.
The following parameters were used:
Egg-output: The negative status of the calves was checked 14 days before the start of the trial and on day-0. Individual rectal samples were taken from all calves from day-14 onwards. The daily faecal production was recorded from four calves during the whole experiment. A modified McMaster egg-count-technique was used to calculate the number of eggs per gram faeces (EPG) with a sensitivity of 25.
Larval identification: A larval culture of 25 grams of faeces of each individual rectal sample was made. Larvae were counted for an estimation of the number of larvae per gram faeces (LPG) and identified.
Pepsinogen: Starting on day minus 14 each week a blood sample was taken from each calf for an estimation of the pepsinogen level.
Post-mortem wormcounts: After slaughter worms were collected from the abomasum and the small intestine. Samples up to 1/50 th were taken, counted and worms identified to species, developmental stage and sex.

If present, the lenght of 50 males and females was measured and the number of eggs in utero counted.

RESULTS

In this paper only the results with regard to egg output and larval identification will be communicated.
Fig 1 shows the average egg output of the two groups.
The total egg output of the treated group was 278×10^6, of the control group 6.797×10^6. This results in a reduction in total egg output of 95.9% in favour of the MSRT-group.
On baxis of the percentages of larval types after culture the contribution of *O. ostertagi* and *C. oncophora* to the EPG was calculated. From these results it was clear that the reduction in egg-output of *O.ostertagi* was less than that of *C. oncophora*. In Fig 2 the egg-output of *O. ostertagi* is given and in Fig 3 that of *C. oncophora*.

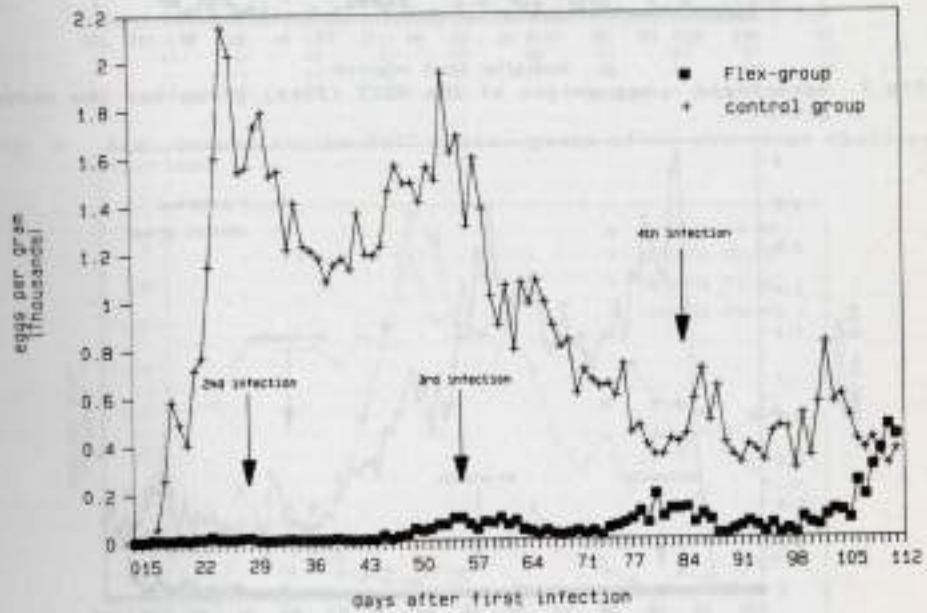

Fig 1. Egg output of the MSRT (Flex) group and the control group.

If one calculates the reduction of the *Ostertagia* - egg output and the *Cooperia* - egg output the following figures can be obtained:
- Reduction of the *Ostertagia* - egg output in the MRST group compared with the control group was 73.2 %.
- Reduction of the *Cooperia* - egg output in the MRST group compared with the control group was 99.3 %.

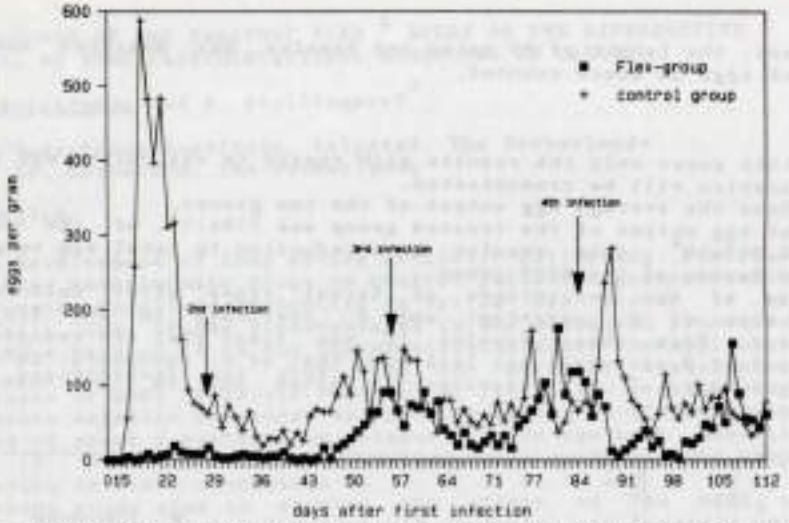

Fig 2. *Ostertagia* - egg output of the MRST (Flex) group and the control group.

Fig 3. *Cooperia* - egg output of the MRST (Flex) group and the control group.

The result of the challenge infections on day-28, day-56 and day-84 on the egg output is showed in Fig 4 for the MRST (Flex) group and in Fig 5 for the control group.

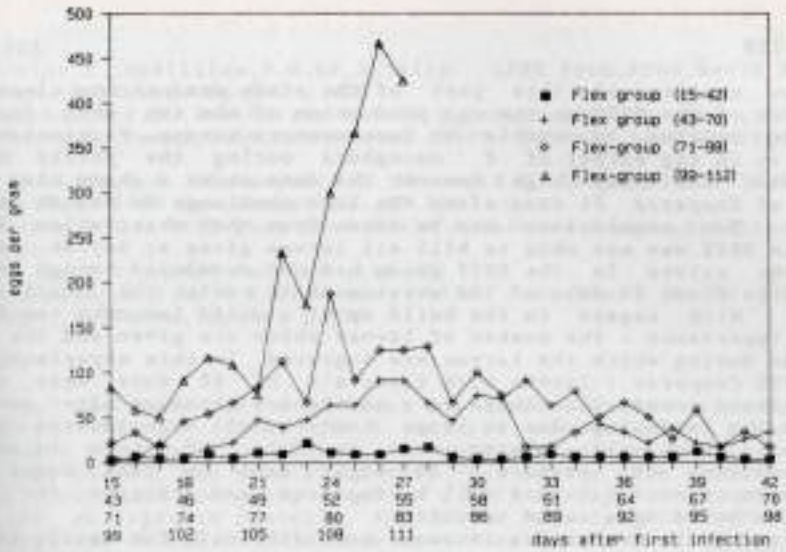

Fig 4. Egg output in the MRST (Flex) group after the three challenge infections.

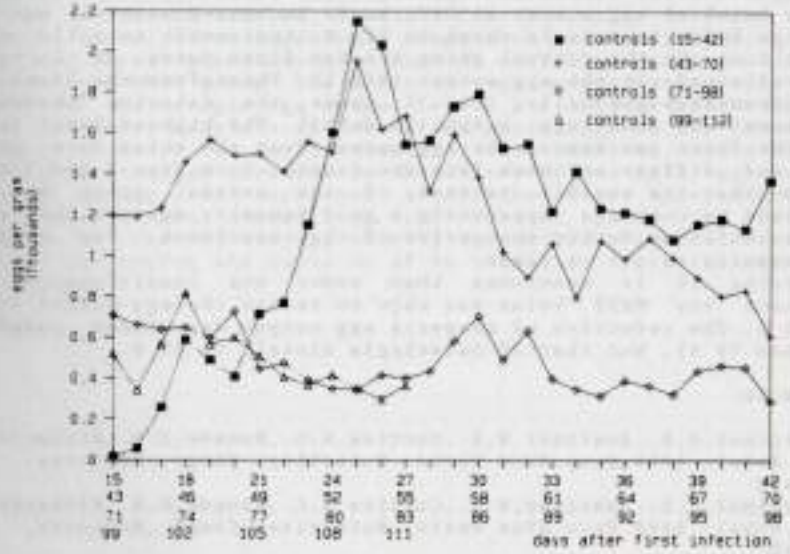

Fig 5. Egg output of the control group after the three challenge infections.

DISCUSSION

The results of this part of the study demonstrate clearly the influence of the MSRT on the egg production of the two most important nematode species in cattle in Northwestern Europe. Particularly the reduction in egg output of *C. oncophora* during the period of the experiment was very high. However the data shows a sharp rise in egg output of *Cooperia* 21 days after the last challenge on day-84 (Figs 1 and 3). Two conclusions can be drawn from that observation. Firstly that the MRST was not able to kill all larvae given at day-84. Secondly that the calves in the MRST group had not developed enough immunity during the first 84 days of the experiment to resist the challenge on day-84. With regard to the build up of a solid immunity two factors are of importance - the number of larvae which are given and the period of time during which the larvae are ingested. In this experiment doses of 40,000 *Cooperia* - larvae with intervals of 28 days were chosen. Under field conditions there is a continuous exposure after turn out. High in the beginning, due to large numbers of overwintered larvae, later declining till almost zero in July, if there is no other contamination of pasture. It might well be that under those circumstances enough larvae will be ingested and trigger the immune system to build up a solid immunity.
With regard to *Ostertagia* it seems more difficult for cattle to build up a solid immunity, expressed in a low or absent egg output. It is well known that older cattle harbour mainly abomasal worms. In this experiment the egg output of the MRST group was almost exclusively *Ostertagia* till around 90 days after the first infection (Figs 2 and 3). The level of egg output of *Ostertagia* increased with the number of challenge infections as is shown in Fig 4. Apparently no solid immunity was built up. In the control group the two first doses of larvae are well reflected in the egg output (Fig 1). Thereafter the level of egg output decreases gradually. Fig 3 shows the relation between the infections and the egg output in detail. The highest level is found after the first two doses. The egg output from the third dose is much lower and differs not much from the fourth. From Figs 2 and 3 one may conclude that the overall pattern of the control group is mainly determined by *Cooperia*. Apparently a good immunity against this species has been built up during the period of the experiment. For *Ostertagia* this immunity is not so clear.
Summarizing it is concluded that under the conditions of this experiment the MRST bolus was able to reduce the egg output for more than 95 %. The reduction of *Cooperia* egg output was almost complete (more than 99 %), but that of *Ostertagia* closely to 75 %.

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Summary

The influence of a morantel sustained release trilaminate bolus (Paratect Flex, Pfizer Inc.) on the reproductive potential of *Ostertagia ostertagi* and *Cooperia oncophora* female worms, as shown by their egg output, was studied. Twenty four calves were allocated to two groups. Calves of one group received a MRST at day-0, the other group remained as negative controls. All calves were infected with 20,000 *O. ostertagi* and 40,000 *C. oncophora* larvae at day-0, day-28, day-56, and day-84. Three calves of each group were slaughtered at day-28, day-56, day-84, and day-112. During the study the daily faecal egg output of four calves was collected to calculate the total egg production. Egg output of all individual calves was estimated on basis of daily rectally collected samples. From all faecal samples cultures were made to permit larval identification. Comparison of the results of egg counts and larval identification show an overall reduction of 95.8 % in favour of the MRST group. The *Cooperia* egg output was reduced for 99.3 % and the *Ostertagia* egg output for 73.2 %. Calves of the MRST group showed an almost complete absence of *Cooperia* eggs in the faeces up to 90 days. However no solid immunity was built up, since after that period *Cooperia* eggs appeared in the faeces. *Ostertagia* egg output in the MRST group was depressed after the first infection dose, but fluctuated with the others. In the control group the first dose resulted in the highest egg output, thereafter a gradual decrease was observed indicating the build up of an immunity, particularly against *C. oncophora*.

ENQUETE RELATIVE A LA DISTOMATOSE DANS LES CHEPTELS D'ENGRAISSEMENT BELGES ET DETERMINATION DE L'INTERET ECONOMIQUE DE L'UTILISATION D'UN DOUVICIDE SELECTIF

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INTRODUCTION

Partout en Europe, la production agricole Belge est bien représentée par les produits viandeux issus des cheptels bovins. Depuis que les unités d'engraissement ont pris une place non négligeable dans l'économie agricole, une attention toute particulière doit être apportée aux maladies susceptibles de réduire les bénéfices des engraisseurs.

Le concept de protection du jeune bétail par l'utilisation, à titre préventif, d'anthelmintiques gastro-intestinaux est maintenant clairement établi (9, 14). De telles substances médicamenteuses sont très largement utilisées lorsque les animaux entrent en station d'engraissement.

Cependant, les effets de la distomatose sur la marge bénéficiaire de l'engraissement n'ont jamais été déterminés chez des animaux culards engraissés en stabulation libre. A ce jour, les conséquences de cette pathologie ont été uniquement exprimées en données issues d'abattoirs (7, 8, 15) et les agents douvicides ne sont pas systématiquement utilisés dans les cheptels d'engraissement. En fait, la distomatose, naturellement contractée au cours de la période d'élevage, pourrait induire des effets subcliniques et des pertes insidieuses pour les engraisseurs.

Le but de cette investigation est de déterminer la fréquence de la distomatose dans les cheptels d'engraissement belges, d'évaluer, dans une unité d'engraissement sélectionnée, les conséquences économiques d'une telle affection chez les bovins culards de race Blanc Bleu Belge ainsi que l'efficience et les effets bénéfiques d'un traitement douvicide. En outre, le but de cette étude est de déterminer s'il est opportun de traiter chaque animal introduit dans un cheptel d'engraissement.

MATERIEL ET METHODE

A. Première partie de l'investigation

Animaux

Des prélèvements ont été réalisés, de manière aléatoire, dans des unités d'engraissement situées dans la partie sud de la Belgique. Entre janvier 1988 et Octobre 1989, 1513 taureaux de race Blanc Bleu Belge, âgés de 5 à 7 mois et pesant de 200 à 300 kg, ont fait l'objet de cette investigation. Ces animaux étaient répartis dans 35 unités d'engraissement et issus, via un marché, d'unités d'élevage localisées en Belgique mais non déterminées.

Prélèvements et analyses

Le jour de l'arrivée en station, des prélèvements de matières fécales ont été réalisés par fouiller rectal. La recherche d'oeufs de Fasciola hepatica fut immédiatement réalisée par la technique de flottaison au sulfate de zinc (4).

B. Deuxième partie de l'investigation

Animaux

30 taureaux culards de race Blanc Bleu Belge, pesant 365 ± 9 kg et âgés de 10 à 12 mois, furent achetés sur un marché et placés dans une unité d'engraissement sélectionnée. Ces animaux, cliniquement sains le jour de leur arrivée en station, n'ont présenté aucune symptomatologie clinique durant la période de l'essai. Durant toute cette période, les taureaux sont restés en stabulation.

Prélèvements et analyses

A sept reprises durant la période de l'essai, à savoir aux jours - 30 (arrivée en station d'engraissement), - 15, 0 (début de l'essai), + 15, + 35, + 45 et + 75, tous les animaux ont fait l'objet d'un examen coproscopique pour la mise en évidence de la distomatose.

Méthode de travail

L'essai proprement dit a débuté à la fin de la période préliminaire sus-mentionnée (jour 0). Sur base des examens coproscopiques, les 30 animaux furent subdivisés en animaux négatifs (groupe A ; n = 10) et positifs (n = 20) pour la distomatose. Par ordre décroissant de poids, les animaux positifs furent eux-mêmes classés par paires. Choisi aléatoirement, un taureau de chaque paire fit l'objet d'un traitement douvicide (groupe B ; n = 10), le second taureau de chaque paire étant considéré comme animal positif non traité (groupe C ; n = 10).

La substance douvicide choisie pour cet essai fut le nitroxinil (N-methylglucamine 4-cyano-2-iodo-6-nitrophenol)*, qui est la molécule douvicide la moins coûteuse parmi celles enregistrées sur le marché belge. Le nitroxinil a démontré son efficacité (11, 3). Au jour 0, ce produit fut injecté, par voie sous cutanée, à la dose de 13,5 mg/kg. A ce moment, les poids corporels moyens des trois groupes étaient identiques.

Durant toute la période d'expérimentation, les animaux reçurent un All-Mash comprenant des pulpes de betteraves, de l'escourgeon, de la farine de viande, du tourteau de soja, du tourteau de lin, du rebulet, des vitamines et des sels minéraux.

Traitement des résultats

Les gains quotidiens moyens furent déterminés sur base des poids aux jours 0, 35 et 75. Les performances, données sous forme "Moyenne ± Erreur Standard" furent comparées par une analyse de variance à un critère de classification.

Les pertes financières liées à la distomatose et l'intérêt financier du traitement furent comptabilisés sur base du prix moyen (FB/kg de Poids Vif) des taureaux culards et assimilés. Ce prix fut déterminé sur base de données récoltées par l'Office Belge de l'Economie et de l'Agriculture (O.B.E.A.) durant les 48 derniers mois.

RESULTATS

12,5 % des taureaux investigués furent positifs pour la distomatose. Ces taureaux étaient répartis dans 56,5 % des exploitations investiguées. Par ailleurs, le taux d'infestation, au sein même des exploitations, variait de 0 à 33,3 %.

Les résultats des examens coproscopiques réalisés dans le cadre de la seconde partie de cette investigation sont repris au tableau 1.

Tableau 1. Nombre de taureaux distomateux, dans les groupes A, B et C, aux jours -30 (arrivée en station d'engraissement), -15, 0 (début de l'essai), +15, +35, +45 et +75

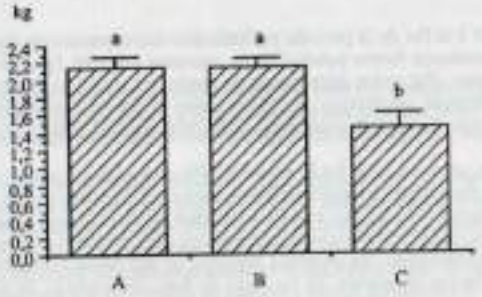
Jour	Groupe A	Groupe B	Groupe C
-30	0	10	10
-15	0	10	10
0	0	10	10
+15	0	0	10
+35	0	0	10
+45	0	2	10
+75	0	2	10

A : animaux négatifs pour la distomatose (n = 10),
B : animaux positifs et traités (n = 10),
C : animaux positifs et non traités (n = 10).

* nitroxinil (Dovenix ®) INSTITUT MERIEUX BENELUX S.A.

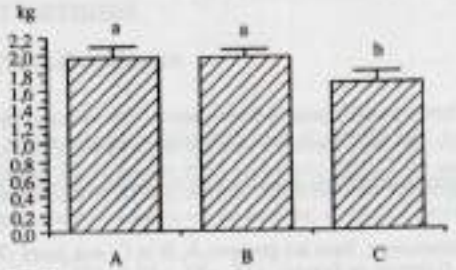
Les gains quotidiens moyens enregistrés pour les trois groupes sont comparés aux jours 35 (figure 1) et 75 (figure 2).

Figure 1. Comparaison des gains quotidiens moyens enregistrés dans les 3 groupes après 35 jours.



A : animaux négatifs pour la distomatose,
B : animaux positifs et traités,
C : animaux positifs et non traités.
a et b : résultat du traitement statistique du groupe en comparaison avec les autres groupes.
Si les lettres sont différentes, les deux groupes sont significativement différents.

Figure 2. Comparaison des gains quotidiens moyens enregistrés dans les 3 groupes après 75 jours.



Légende : voir figure 1.

Dans le groupe C, les gains quotidiens moyens furent significativement plus faibles que dans le groupe A. Par contre, il n'y eu aucune différence significative entre les performances zootechniques enregistrées dans les groupes B et A.

Les pertes financières dues à la distomatose et l'intérêt financier d'un traitement douvicide sont respectivement présentés au tableau 2. Toutes les données sont exprimées par taureau et par période de 75 jours.

Tableau 2. Pertes enregistrées suite à la distomatose, intérêt zootechnique et financier d'un traitement douvicide.

Taureaux distomateux	Unités	Pertes dues à la distomatose
Retard pondéral moyen	Kg	22,6
Prix moyen des taureaux B.B.B.culards et assimilés	Francs Belges / kg de croît	116,7
Manque à gagner moyen	Francs Belges	2637,4
Taureaux distomateux traités	**Unités**	**Intérêt zootechnique et financier du traitement.**
Supériorité pondérale moyenne	Kg	17,4
Prix moyen des taureaux B.B.B.culards et assimilés	Francs Belges / kg de croît	116,7
Supériorité moyenne liée au traitement	Francs Belges	2030,6

DISCUSSION

Les résultats de la première partie de notre investigation montre que la distomatose est un problème non négligeable dans les unités d'engraissement. Etant donné que notre enquête était basée sur des examens coproscopiques, la fréquence des individus positifs pour la distomatose (12,5 %) nous semble devoir être considérée comme une fréquence minimale. Nous devons dès lors penser que la fréquence de la distomatose pourrait être supérieure.

Chez les taureaux culards Blanc Bleu Belge, la distomatose induit une chute significative des performances zootechniques. Le déficit moyen des performances et les pertes financières consécutives, tels que présentés pour une période de 75 jours, démontre que cette pathologie induit un manque à gagner réel mais insidieux pour l'exploitant. Ces pertes sont, par ailleurs, sous-estimées puisqu'elles n'incluent pas le coût inhérent à la saisie des foies lors de l'abattage.

L'efficience du nitroxinil chez le taureau culard atteint de distomatose accroît, dans le cas d'unités d'engraissement suspectes, l'utilité d'un douvicide en période d'engraissement. Economiquement, il n'est pas concevable de réaliser un diagnostic sur chaque animal entrant dans une exploitation. Cependant et suivant les résultats obtenus, nous sommes à même de déterminer l'intérêt d'un traitement stratégique. Si nous considérons une unité d'engraissement où les taureaux pèsent en moyenne 300 kg à leur arrivée, le bénéfice financier résultant du traitement, en supposant que la fréquence des animaux positifs pour la distomatose soit de 10 %, sera 4,2 fois supérieur au coût du traitement de tous les animaux arrivés dans la station durant l'année.

Le fait que deux animaux soient, 45 jours après le traitement, à nouveau positifs pour la distomatose pourrait être expliqué par le spectre d'activité du nitroxinil plus exclusivement centré sur les derniers stades larvaires et sur le stade adulte de Fasciola hepatica (1, 13). Dès lors, nous pouvons considérer que ces taureaux étaient, au moment du traitement, infestés, de façon prépondérante, par des formes immatures de Fasciola hepatica. Durant toute la période de l'essai, ces deux animaux n'ont pas présenté de moindres performances zootechniques.

Les taureaux étant en stabulation libre durant l'engraissement, le cycle épidémiologique de la distomatose est interrompu dès que les animaux entrent en station. Dès lors, notre but n'était pas d'utiliser une substance douvicide qui puisse être candidate pour le contrôle de la distomatose. Cependant, il est important de noter que la réduction de la charge d'infestation et l'efficience pour combattre simultanément les formes immatures et mature de Fasciola hepatica sont supérieures avec d'autres douvicides tels que le triclabendazole (2, 5, 12, 10) et le diamphenetide (6). De plus amples études devraient être réalisées afin de savoir si de telles substances médicamenteuses, caractérisées par un pouvoir douvicide et un prix plus élevés, induisent, chez les taureaux culards Blanc Bleu Belge atteints de distomatose, une amélioration significativement plus grande des performances zootechniques.

En conclusion, la distomatose induit une réduction significative tant des performances que des bénéfices financiers. Ces effets insidieux de la distomatose sont anéantis par le traitement au nitroxinil. Le traitement de tout animal introduit dans une unité d'engraissement où la distomatose a été régulièrement mise en évidence doit être recommandé.

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RESUME

La fréquence de la distomatose dans les unités d'engraissement fut déterminée en réalisant, de façon aléatoire, des prélèvements de matières fécales sur 1513 taureaux Blanc Bleu Belge, âgés de 5 à 7 mois et pesant 200 à 300 kg. Les examens coproscopiques furent immédiatement réalisés.

12,5 % des taureaux investigués furent positifs pour la distomatose. Ces taureaux étaient répartis dans 56,5 % des exploitations investiguées. Par ailleurs, le taux d'infestation variait de 0 à 33,3 % au sein même des exploitations.

Afin d'évaluer les conséquences économiques de cette pathologie et les effets bénéfiques d'un traitement douvicide en engraissement du bétail culard, un essai, comprenant 30 taureaux culards de race Blanc Bleu Belge pesant 365 ± 9 Kg et âgés de 10 à 12 mois, fut réalisé dans une unité d'engraissement sélectionnée. Sur base des examens coproscopiques, les 30 animaux furent répartis en animaux négatifs (groupe A ; n = 10) et positifs pour la distomatose (n = 20). Ces derniers furent, pour moitié, traités (groupe B ; n = 10) et non traités (groupe C ; n = 10) au jour 0 de cet essai d'une durée globale de 75 jours.

Les performances zootechniques du groupe C (1,661 ± 0,140 kg) furent significativement inférieures à celles du groupe A (1,975 ± 0,120 kg). Nous n'avons, par ailleurs, noté aucune différence significative entre les performances des groupes B (1,960 ± 0,085 kg) et A. Les pertes financières, dues à la distomatose et calculées sur 75 jours d'engraissement, sont estimées à 2637 Francs Belges par taureau. Calculé sur la même période, l'intérêt financier du traitement s'évalue à 2031 Francs Belges par taureau distomateux traité. Si l'on considère une exploitation d'engraissement où le poids moyen des animaux, à l'entrée, est de 300 kg et la fréquence de la distomatose de 10 %, les bénéfices engendrés par le traitement sont 4,2 fois supérieurs au coût du traitement de tous les animaux entrant dans l'exploitation durant l'année.

En conclusion, la distomatose induit une réduction significative tant des performances que des bénéfices financiers. Ces effets insidieux sont anéantis par le traitement au nitroxinil. En définitive, le traitement de tout animal introduit dans une unité d'engraissement, où la distomatose a été régulièrement mise en évidence, est rentable.

ABSTRACT

The frequency of liver fluke disease in fattening units was determined by the analysis of random faeces samples issued from 1,513 Belgian White Blue bulls aged from 5 to 7 months and weighing from 200 to 300 kg. The presence or absence of fluke eggs in the faeces was immediately determined.

12.5 % of the investigated bulls were positive for liver fluke disease. These bulls were spread over 56.5 % of the investigated fattening units. Furthermore the infestation rate varied from 0 to 33.3 % inside the fattening units.

In order to assess the economic consequences of bovine fasciolasis in double-muscled cattle and the beneficial effects of a treatment against such a disease, a trial including 30 Belgian White Blue bulls, weighing 365±9 kg and aged from 10 to 12 months, was conducted in a selected fattening unit.

On the basis of faecal examinations, the 30 animals were subdivided in negative (group A; n=10) and positive animals (n=20) for fascioliasis, the latter being either treated (group B; n=10) or not (group C; n=10) on day 0 of this trial which was conducted during 75 days.

The daily body gains in group C (1.661 ± 0.140 kg) were significantly lower than those in group A (1.975 ± 0.120 kg). On the other hand there was no significant difference between the daily body gains registered in group B (1.960 ± 0.085 kg) and A. The financial loss, due to flukes and accounted on a 75 day-period, averaged 2,637 Belgian Francs per bull. Accounted on a similar period the treatment yielded a profit of about 2,031 Belgian Francs per bull. If we consider a fattening unit where bulls weigh 300 kg on average at their arrival and where the level of positive bulls for liver fluke disease reaches 10 %, the treatment shows a profit 4.2 times greater than the cost of the treatment of all the animals arriving at the station during one year. We can conclude that the liver fluke disease induces a significant reduction of both performances and profitability, that these hidden effects are countered by nitroxinil treatment and finally that the treatment of each animal incorporated in fattening units, where liver fluke disease has regularly been detected, is profitable.

ZUSAMMENFASSUNG

Die Frequenz von Leberegelkrankheitsfällen (Fasciola hepatica) in Mastbetrieben wurde an Hand von Fäkalien ermittelt, die 1.513 5 bis 7 monatiger Stiere der weiß-blauen belgischen Art mit einer durchschnittlichen Körpermasse von 200 bis 300 kg stichprobenweise entnommen wurden. Untersuchungen nach Leberegelwurmeiern wurden unmittelbar angestellt.

Bis 12,5 % der Stiere waren die Testergebnisse positiv. Diese Rinder waren auf 56,5 % der untersuchten Mastbetriebe verteilt. Außerdem schwankten die Infektionsquoten innerhalb der Betriebe selbst zwischen 0 und 33,3 %.

Um die wirtschaftlichen Folgen der Fasziolose beim Mastvieh und den positiven Einfluß von einer Behandlung dieser Krankheit einschätzen zu können, wurde in einem selektionierten Mastbetrieb ein Versuch durchgeführt, der 30 10-bis 12 monatige Rinder der weiß-blauen belgischen Art mit einem Körpergewicht von 365 ± 9 kg umfaßte. An der Hand der Fäkalienuntersuchungen nach Leberegelwurmeiern wurden die 30 Stiere in negative (A-Gruppe; n=10) und positive Tiere (n=20) eingeteilt. Am Tage 0 des insgesamt 75tägigen Versuchs wurde die eine Hälfte der positiven Stiere behandelt (B-Gruppe; n=10) und die andere nicht (C-Gruppe; n=10).

Die Rinder der C-Gruppe verzeichneten bedeutend niedrigere zootechnische Leistungen als diejenigen der A-Gruppe : 1,661 ± 0,140 kg anstatt 1,975 ± 0,120 kg.

Außerdem haben wir keinen bedeutenden Unterschied festgestellt zwischen den täglichen Gewichtszunahmen der B (1,960 ± 0,085 kg) und der A-Gruppe. Der durch Fasziolose verursachte Verdienstausfall über eine 75 tägige Mastzeit betrug ca. 2637 belgische Francs pro Stier. Über die gleiche Periode brachte die Behandlung einen Gewinn von ca. 2031 belgische Francs pro behandeltes Tier ein. Ein Beispielsfall : in einem Betrieb, in dem die Stiere am Anfang der Mast ein Körpergewicht von durchschnittlich 300 kg haben und wo die Fasziloserate 10 % beträgt, bringt die Behandlung einen Gewinn ein, der 4,2 mal höher liegt als die Kosten für die Behandlung von allen im Laufe eines Jahres im Betrieb eingestallten Tieren.

Aus dieser Untersuchung läßt sich ersehen, daß die Leberegelkrankheit einen bedeutenden zootechnischen Leistungsschwund als auch einen Verdienstausfall mit sich bringt. Diese schleichenden Auswirkungen werden durch die nitroxinil-Behandlung zunichte gemacht. Schließlich kann man sagen, daß es wirtschaftlich ist, jedes Tier zu behandeln, das in einem Mastbetrieb neu eingestallt wird, in dem die Leberegelkrankheit regelmäßig festgestellt wurde.

THE EFFICACY OF NITROXYNIL AGAINST DRUG RESISTANT STRAINS OF HAEMONCHUS CONTORTUS IN SHEEP.

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INTRODUCTION :

Although H. contortus resistance to anthelmintics is a distinctly regional problem, being far greater in the Southern Hemisphere, resistance to the benzimidazole anthelmintic group has also been reported from several European countries (2,3,7,8). In the Southern Hemisphere, following numerous reports of resistance to benzimidazoles, the appearance of multiple drug resistance in H. contortus strains has emerged in the last decade as a major threat to the sheep industry (1,4,5,12,13). This problem was highlighted when resistance to benzimidazole (fenbendazole), avermectin (ivermectin) and salicylanilide (closantel and rafoxanide) anthelmintics was reported in the South African White River Krtz strain (14,15,16).
The study described here was undertaken to assess the efficacy of nitroxynil against a benzimidazole resistant strain from the UK and against the White River Krtz strain from South Africa.

MATERIALS AND METHODS :

Experimental animals and their management :
In the first trial, nine (including three parasite naive controls) crossbred Suffolk-Scottish Blackface lambs, aged between seven and nine months, were matched for bodyweight and allocated into three groups. In the second trial, two experiments with groups of five lambs each were carried out with respectively twenty-five and ten crossbred Suffolk-Border Leicester lambs, aged between three and five months. In both trials, animals were reared on straw in concrete pens and fed a ration of hay and lamb finishing pellets ad libitum.
Experimental infections :
Each lamb was infected with a single oral dose of 10000 third stage larvae. In trial 1, the larval inoculum (L3) was obtained from the Central Veterinary Laboratory, Weybridge, its resistance to benzimidazoles having been confirmed (3,6). In trial 2, the original larval inoculum (L3) of the White River Krtz strain, imported from South Africa, was first passaged once in a worm free donor lamb without any further anthelmintic selection.
Anthelmintics :
Single doses of anthelmintics were administered 21 days after infection. Nitroxynil (DOVENIX, RHONE MERIEUX) was administered subcutaneously at its recommended dose rate of 10 mg/kg. Oral doses of ivermectin (ORAMEC; MERCK SHARP & DOHME) and fenbendazole (PANACUR; HOECHST) at their respective recommended dose rates of 0.2 and 5 mg/kg, and closantel (FLUKIVER; JANSSEN ANIMAL HEALTH) at both 5 and 10 mg/kg dose rates were also tested in trial 2.
Parameters measured
Faecal worm egg counts (EPG) were monitored daily using a modified McMaster method. All sheep were necropsied 28 days after infection and total worm counts calculated as recommended by Powers and others (11).

Statistical analysis :
In trial 1, no statistical analysis was possible because of the small size of the groups. In trial 2, worm counts were transformed using logarithms before analysis. A one-day analysis of variance followed by the Newman Keuls multiple range test was used to test for group differences in the 25 lambs study. In the other 10 lambs study, only two groups were compared and the analysis was undertaken using the unequal variance t-test. Logarithm transformed egg counts were used to undertake an analysis of variance and the two-factor repeated measures design. This provided tests for group X time interaction, group differences and time differences.

RESULTS

Trial 1 : The faecal egg output dropped to zero two to four days after treatment with nitroxynil and at necropsy no adult worms were found. In contrast, the faecal egg output of the untreated group continued to increase until necropsy when between 4 to 6 thousand of adult H. contortus were recovered from each lamb (Table 1).
Although no statistical analysis was possible, these results showed clear evidence of anthelmintic efficacy against adult worms.
Trial 2 : In the first experiment, the analysis of EPG group means over time (Table 2) indicated that groups produced significantly different patterns ($P<0.05$). First, the EPG patterns in the closantel and nitroxynil groups were similar, the egg count in both groups dropping sharply in the 2 days following treatment to remain steady at low levels until slaughter. In contrast, in the fenbendazole and ivermectin groups, the egg counts initially dropped slightly but only temporarily, rising again on day 2 and remaining high until slaughter. Finally, the egg count in the untreated control group remained at a high level showing evidence of some increase over time. Analysis of variance of total worm counts showed that the closantel and nitroxynil groups, with mean counts of 169 and 72 respectively, were not significantly different ($P<0.05$), both achieving, at 10 mg/kg, excellent control with over 99% reduction in worm numbers (Table 3). Similarly, the fenbendazole, ivermectin and untreated control groups with respective mean worm burdens of 3704, 2896 and 5738 were not significantly different. However, the closantel and nitroxynil groups were significantly different ($P<0.05$) from the fenbendazole, ivermectin and untreated control groups. In the second experiment, total worm counts shown in Table 3 in both groups were not significantly different ($P<0.05$).

TABLE 1 : Effect of nitroxynil (10 mg/kg) on egg output and worm count in TRIAL 1 (UK benzimidazole resistant strain)

TREATMENT GROUP	SHEEP NUMBER	EGG OUTPUT UNTIL SLAUGHTER				NUMBER OF ADULT WORMS
		DAY21	DAY23	DAY25	DAY28	
NITROXYNIL (10 mg/kg)	1	21500	100	ND	ND*	0
	2	2500	ND	ND	ND	0
	3	550	ND	ND	ND	0
UNTREATED CONTROL	4	5800	6200	8000	16000	6000
	5	7300	5450	7550	9500	5240
	6	4250	4850	5700	5450	4200

* ND : None Detected

DISCUSSION :

Nitroxynil is a potent fasciolicidal drug, but it is also very active against blood sucking nematodes such as Haemonchus, Oesophagostomum and Bunostomum spp. (10,17). Green and others (5) have already described the efficacy of nitroxynil against a levamisole, morantel tartrate, benzimidazole and organophosphorus resistant strain of H. contortus, isolated in Australia. These present results demonstrate the high efficacy of nitroxynil when used at its minimum recommended dose rate of 10 mg/kg against adult stages of both European and South African drug resistant strains of H. contortus. In addition, these results demonstrate the high resistance of the White River Krtz strain to ivermectin, fenbendazole and closantel at dose levels commonly recommended and used in South Africa. However, closantel when used at 10 mg/kg was also highly effective.
Whether or not cross resistance between nitroxynil and salicylanilides is likely to develop is not known. But, although both drugs act by uncoupling oxydative phosphorylation (9), nitroxynil is a substituted monophenol and therefore has a very different chemical structure from salicylanilides (i.e. closantel). Consequently, the site and mode of action of nitroxynil on the oxydative phosphorylation may be different from those of closantel.
Finally, the present results indicate that nitroxynil may be recommended and used for rotational programmes with other drugs in order to control haemonchosis in sheep, especially when drug resistance is suspected.

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TABLE 2 : Means and Patterns of Faecal worm egg counts per gramme over time in TRIAL 2 (South African White River Krtz Strain)

	In Experiment 1							
	Days post treatment							Pattern type (P)
	0	1	2	3	4	5	6	7
Nitroxynil 10 mg/kg	12080	2390	310	305	560	460	415	490	Pa
Closantel 10 mg/kg	9420	2300	80	55	245	80	210	140	Pa
Ivermectin 0.2 mg/kg	11800	4960	6400	6620	5940	5920	8340	6750	Pb
Fenbendaz. 5 mg/kg	13780	5570	8040	9220	9940	7700	6120	6110	Pb
Untreated. Control	13460	13520	17250	14020	16480	13600	21580	15210	Pc
	In Experiment 2							
Closantel 5 mg/kg	850	1870	1790	2470	3560	4510	7810	7350	P1
Untreated Control	2850	2660	2680	3470	4550	4270	6050	6350	P2

The two experiments were analysed separately and pattern types with a common superscript were similar.

TABLE 3 : Efficacy of nitroxynil and other anthelmintics in TRIAL 2 (South African White River Krtz Strain)

	Mean Worm Burdens (range)	Percentage Reduction
In Experiment 1		
Nitroxynil 10 mg/kg	72 (5-215)	99.3a
Closantel 10 mg/kg	169 (0-760)	99.8a
Ivermectin 0.2 mg/kg	2896 (1935-3795)	48.5b
Fenbendaz. 5 mg/kg	3704 (2055-5370)	36.5b
Untreated Control	5738 (3100-7710)	0.0b
In Experiment 2		
Closantel 5 mg/kg	1732 (1385-2260)	0.0c
Untreated Control	1574 (180-2545)	0.0c

Geometric means were used to determine drug efficacy. Percentages of reduction of worm burdens with a common superscript do not differ significantly ($P<0.05$). The two experiments were analysed separately.

SUMMARY :
Anthelmintic resistance by H.contortus strains have emerged as a major threat to the sheep industry. In view of this, the efficacy of nitroxynil (DOVENIX, RHONE MERIEUX) was assessed in experimentally infected animals with two resistant strains: one originating from the UK and resistant to benzimidazoles, the other originating from South Africa and resistant to benzimidazoles, ivermectin and salicylanilides. Nitroxynil was 100% effective against the UK benzimidazole resistant strain. Nitroxynil and closantel at 10 mg/kg were 99% effective against the multiple resistant strain from South Africa whereas closantel and fenbendazole at 5 mg/kg and ivermectin at 0.2 mg/kg (their commonly recommended and used dose rates in South Africa), did not show any significant efficacy. These results indicate that nitroxynil may be recommended for the control of multiple drug resistant strains of H. contortus in sheep.

RESUME
La résistance aux anthelminthiques de souches d'H. contortus est devenue une contrainte majeure de l'élevage ovin. Face à ce problème, l'efficacité du nitroxynil (DOVENIX, RHONE MERIEUX) a été évaluée à l'aide d'infections expérimentales contre deux souches résistantes : l'une d'origine britannique et resistante aux benzimidazoles, l'autre d'origine Sud-Africaine et résistante aux benzimidazoles, à l'ivermectine et aux salicylanilides.
L'efficacité du nitroxynil contre la première souche a été de 100%. L'efficacité du nitroxynil et du closantel à 10 mg/kg contre la souche multi-résistante d'Afrique du Sud a été de 99%. Par contre, le closantel et le fenbendazole à 5 mg/kg et l'ivermectine à 0.2 mg/kg (leurs doses respectivement recommandées et utilisées en Afrique du Sud), n'ont pas montré d'activité significative. Ces résultats confirment que le nitroxynil devrait être utilisé pour le contrôle des souches résistantes d'H. contortus des ovins.

RESUMEN
La resistencia en contra de cepas de H. contortus ha surgido como una de las principales amenazas en contra de la industria ovina. Debido a esto, el presente estudio se diseño con el objeto de evaluar la eficacia de nitroxynil (DOVENIX, RHONE MERIEUX) mediante infecciones experimentales con 2 cepas resistentes: una originaria del Reino Unido resistente a benzimidazoles y otra originaria de Sudafrica y resistente a benzimidazoles, ivermectina y salicylanilides.
La efectividad del nitroxynil fue del 100% en contra de la cepa del Reino Unido. Nitroxynil y closantel a dosis de 10 mg/kg fueron efectivas en un 99% en contra de la cepa Sudafricana mientras que closantel y fenbendazole a dosis de 5 mg/kg e ivermectina a dosis de 0.2 mg/kg (las dosis por lo común usadas en Sudafrica) no mostraron eficacia significativa. Estos resultados indican que nitroxynil puede ser usado para el control de cepas de H. contortus de ovinos que muestren multiple resistencia hacia drogas.

AVALIAÇÃO DOS PROTOZOÁRIOS CILIADOS NO RÚMEN DE BÚFALO E BOVINO.

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INTRODUÇÃO

Os microrganismos do rúmen, bactérias, protozoários e fungos, desempenham papel fundamental na nutrição de ruminantes. Os protozoários são responsáveis por grande extensão na produção de amônia ruminal e no metabolismo bacteriano e dietético, podendo conter entre 10-40% do total de nitrogênio no rúmen (14).

Nos últimos anos têm sido publicados trabalhos sobre a ocorrência de diferentes, famílias, gêneros e espécies de protozoários ciliados no rúmen de diversas espécies de ruminantes (3). Na Indonésia, Imai, 1985 (6) identificou 45 espécies de protozoários em bovinos e 37 em búfalos, sendo 30 espécies comuns em ambos animais. O número médio por animal foi de 7,8 x 10^4/ml de líquido ruminal e 1,5 x 10^4/ml no bovino e búfalo, respectivamente. Pant & Roy, 1970(11) encontraram número total de protozoários médio no rúmen de búfalo e bovino de 21,5 x 10^4/ml, obtendo contagem mais baixa cinco horas após a alimentação. Imai & Ogimoto, 1984(7) observaram menor número de ciliados no rúmen de búfalo (0,7 x 10^4/ml) que de zebuíno (7,1 x 10^4/ml), na Tailândia. Dehority, 1979(1), no Brasil, identificou 49 espécies diferentes de protozoários ciliados no rúmen de búfalos, sendo oito descritos pela primeira vez. O número médio de ciliados foi de 22,9 x 10^4/ml para búfalos e 26,4 x 10^4/ml para bovinos.

Segundo Dehority & Orpin, 1987(4) há uma variação considerável na concentração de protozoários e número de espécies por animal entre búfalos e bovinos alocados em várias regiões geográficas do mundo. Entre as espécies de ruminantes, concentrações e números de espécies de protozoários por animal tendem a ser mais altas no bovino zebuíno.

Há uma carência acentuada de pesquisas em diversas áreas do conhecimento da espécie bubalina. Com isso, muitos dados obtidos com bovinos são extrapolados para os búfalos e podem não representar a realidade, uma vez que são espécies diferentes e com hábitos peculiares.

O presente trabalho teve por objetivo estudar comparativamente, a fauna ruminal de bovino e búfalo, através da contagem diferencial dos principais gêneros de protozoários.

MATERIAL E MÉTODOS

Duas espécies de ruminantes foram comparadas, utilizando-se um búfalo (**Bubalus bubalis**) da raça Mediterrâneo e um bovino mestiço da raça Flamenga com zebuíno (**Bos taurus** x **Bos indicus**), adultos, pesando 504 e 443 kg, respectivamente. Os animais foram canulados no rúmen e mantidos sob as mesmas condições de manejo e alimentação em baias individuais com bebedouro automático, durante 35 dias. A alimentação foi fornecida, "ad libitum", duas vezes ao dia (8:00 e 17:00 hs) durante a fase de adaptação. Nos últimos dois dias do experimento, iniciou-se a fase de coleta do líquido ruminal às 6:00 horas, sendo oferecida a primeira alimentação, que prosseguiu-se a cada 3 horas, a medida que os animais consumiam a ração, de forma a não faltar alimentos no cocho. A ração

foi composta de feno de capim coast cross (**Cynodon dactylon**), milho em grãos moídos e farelo de soja, mantendo-se a proporção volumoso: concentrado de 70:30, com 12% de proteína bruta na matéria seca.

As amostras do líquido ruminal foram coletados de hora em hora a partir das 6:00 horas (To = antes da alimentação) até as 12:00 hs depois a cada 3 horas, até a meia noite, totalizando 22 amostras de cada animal, durante os dois dias consecutivos. As coletas foram feitas do saco ventral do rúmen, com o uso de uma bomba de vácuo manual, adaptada para tal finalidade, sendo retirados cerca de 100 ml de líquido ruminal por amostra, contendo também pequenas partículas sólidas. Imediatamente após as coletas, determinava-se o pH, através de medidor de pH digital. Após as amostras serem homogeneizadas, coletava-se alíquota de 10 ml, colocando-se em frascos de vidro, devidamente identificados, contendo igual volume de solução de formalina (1:2). Posteriormente, as amostras foram preparadas e procedeu-se a identificação e contagem dos principais gêneros de protozoários, em microscópio (100 x) conforme técnicas descritas por Dehority, 1987(2) e Ogimoto & Imai, 1981(10). Para cada amostra foram realizadas duas contagens, sendo utilizadas, as médias, para análise estatística dos dados.

RESULTADOS E DISCUSSÃO

As concentrações médias diária total e genérica dos protozoários ciliados no rúmen de búfalo e bovino podem ser vistas na Tabela 1. Houve diferenças significativas para a maioria dos gêneros estudados, exceto para o **Diplodinium** spp., **Ostracodinium** spp. e **Eudiplodinium** spp. O búfalo apresentou menor concentração total de ciliados, **Entodinium** spp., **Epidinium** spp., **Isotricha** spp. que o bovino. Somente **Dasytricha** spp. apareceu em maior número no búfalo, e a espécie **Elytroplastron bubali** só foi observada no bubalino. No geral, estes achados estão de acordo com vários pesquisadores que verificaram uma menor concentração de ciliados em búfalos (6, 7, 13). Entretanto, outros pesquisadores não observavam diferenças significativas entre as duas espécies (11, 1, 8). O pH no rúmen foi significativamente ($P < 0,01$) maior no búfalo (6,28) que no bovino (5,98), mas não houve correlação com a concentração de ciliados no rúmen. A distribuição percentual dos gêneros mostrou predominância de **Entodinium** spp e **Diplodinium** spp. em ambas espécies de animais, enquanto que o gênero **Epidinium** spp foi observado em uma porcentagem bem maior no bovino (11,4%) que no búfalo (3,9%). (Tabela 1).

Tabela 1. Concentrações médias de protozoários ciliados por ml de líquido ruminal (x 10^4) e distribuição genérica (%).

Protozoários	Búfalo	(%)	Bovino	(%)	Teste F
Total	$12,6^a$		$19,4^b$		*
Entodinium spp.	$7,1^a$	56,8	$12,6^b$	65,1	*
Diplodinium spp.	3,5	27,7	3,2	16,5	NS
Epidinium spp.	$0,5^A$	3,9	$2,2^B$	11,4	**
Isotricha spp.	$0,2^A$	1,2	$0,7^B$	3,9	**
Dasytricha spp.	$0,8^a$	6,6	$0,4^b$	1,8	*
Ostracodinium spp.	0,1	0,9	0,1	0,6	NS
Elytroplastron bubali	$0,2^A$	1,4	$0,0^B$	0,0	**
Eudiplodinium spp.	0,2	1,3	0,1	0,8	NS
pH	$6,28^A$		$5,98^B$		**

Marcada variação diurna tem sido notada na concentração de protozoários no rúmen em diversas espécies (12, 15, 9, 5). De fato, no búfalo, ciclo diurno aparentemente ocorreu com a maior parte dos protozoários estudados, principalmente, no número total, **Entodinium** spp. e **Diplodinium** spp., apresentando números elevados de protozoários antes da primeira alimentação, declinando em seguida, atingindo menores valores à noite (Tabela 2). Não houve observação aparente do ciclo diurno, mas notou-se ligeira queda do número de protozoários a partir do tempo inicial de coleta, com o bovino.

Tabela 2. Número total de protozoários, **Entodinium** spp., **Diplodinium** spp. nos diversos horários de coleta do líquido ruminal (x 10^4/ml).

Horário de coleta	Total		Entodinium		Diplodinium	
	Búfalo	Bovino	Búfalo	Bovino	Búfalo	Bovino
6:00	22,1	24,7	13,6	16,4	6,0	4,3
7:00	21,2	27,7	11,9	16,7	6,3	4,2
8:00	16,3	21,4	9,4	13,4	4,2	4,0
9:00	13,9	15,7	7,6	9,6	3,8	2,8
10:00	12,5	17,5	6,7	10,6	3,9	3,3
11:00	9,9	15,6	6,4	24,7	2,3	2,8
12:00	9,9	15,9	5,2	10,2	2,9	2,6
15:00	9,3	17,3	5,1	10,7	2,7	3,2
18:00	9,8	14,7	5,1	9,4	2,8	2,5
21:00	6,7	15,6	3,7	9,4	1,8	3,1
0:00	6,3	11,8	3,6	7,7	1,9	2,3

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RESUMO

O presente trabalho foi desenvolvido no Campus de pirassununga da Universidade de São Paulo, utilizando-se um búfalo da raça Mediterrâneo e um bovino mestiço Flamengo, adultos, canulados no rúmen, pesando 504 e 443 kg, respectivamente. Os animais foram alimentados "ad libitum" durante 35 dias com uma mesma ração constituido de feno de capim coast cross, milho em grãos moídos e farelo de soja, na proporção de 70:30 de volumoso e concentrado, com 12% de proteína bruta na matéria seca. Nos últimos dois dias, foram colhidas amostras do líquido ruminal, via fístula, de hora em hora das 6:00 às 12:00 hs., e depois, a cada 3 hs. até a meia-noite, totalizando 22 amostras por animal. O búfalo apresentou significativamente ($P < 0,05$) menor número total de protozoários (12,6 x x 10^4/ml) que bovinos (19,4 x 10^4/ml); também de **Entodinium** spp.; **Epidinium** spp. e **Isotricha** spp. e maior concentração de **Dasytricha** spp. A espécie **Elytroplastron bubali** só foi identificada no búfalo. Não houve diferenças significativas entre as espécies animal no número de **Diplodinium** spp., **Ostracodinium** spp. e **Eudiplodinium** spp. O pH no líquido ruminal do búfalo foi significativamente ($P < 0,01$) maior (6,28) que o bovino (5,98). Os dados obtidos ao longo do dia com o búfalo, mostraram uma queda gradual na concentração da maioria dos gêneros estudados, com números mais baixos obtidos nas coletas noturnas, fato de menor visualização no bovino. Os resultados sugerem a existência de um ciclo diurno de protozoários ciliados no rumen de búfalo, no Brasil e que observações são requeridas ao longo do dia todo.

SUMMARY

"AVALIATION OF RUMEN CILIATE PROTOZOA IN BUFFALO AND CATTLE".

An adult fistulated male Mediterraneo buffalo and a male crossbred (Flamengo x Zebu) bull weighing 504 and 443 kg, respectively, were fed with coast cross grass hay (**Cynodon dactylon**), corn grain and soybean meal for 35 days. The roughage: concentrate ratio was 70:30 with 12 % crude protein in dry matter. During the last two days, were collected 22 rumen liquid (RL) samples each animal (6:00 A.M. to 12:00 P.M.).

The ciliate protozoa were identified under microscope. Total protozoa countes were significantely ($P < 0,05$) lower in buffalo (12.6 x 10^4/ml RL) than cattle (19.4 x 10^4/ml RL); also with **Entodinium** spp., **Epidinium** spp. and **Isotricha** spp. The genus **Dasytricha** spp. was higher ($P < 0,05$) in buffalo. There was no significant difference in **Diplodinium** spp., **Ostracodinium** spp. and **Eudiplodinium** spp. counts in RL of buffalo and cattle. The specie **Elytroplastron bubali** was not found in cattle. The pH was significantely ($P < 0,01$) higher in buffalo (6.28) than cattle (5,98). The results suggest that there is a diurnal concentration of ciliate protozoa in the rumen of water buffalo in Brazil, and that observations are required over the whole of the 24 h period.

ZUSAMMENFASSUNG

"AUSWERTUNG DER PROTOZOEN IM PANSEN DES BÜFFELS UND DES RINDES"

Diese Arbeit wurde im "Campus de Pirassununga" der Universität São Paulo vervollständigt und es wurden dazu ein Büffel der Mitterlmeerrasse und ein Kreuzungstier der Flamengo-rasse benützt. Beide waren erwachsen, besassen Fisteln un wogen jeweils 504 kg und 443 kg. Die Tiere wurden "ad libitum" während 35 Tage mit gleichem Futter ernährt, das aus "coast-cross" (**Cynodon dactylon**), gemahlene Maiskörner und Sojamehl bestand. Das Verhältniss Volumös-Krasftufutter war 70:30 mit 12% Brutto Eiweissinhalt. In den letzten zwei Tagen wurden Muster der Pansen flüssigkeit durch die Fistel erworben. Sie wurden stündlich von 6:00 bis 12:00, und später jed drei Stunden bis Mitternacht gesammelt. Im ganzen wurden 22 Muster je Tier gewonnen. Der Büffel wies Beachtungsweise ($P < 0,05$) weniger Protozoen ($12,0 \times 10^4$/ml) auf, als Rinder ($19,4 \times 10^4$/ml): wie auch **Entodinium** spp., **Epidinium** spp. und **Isotricha** spp. Anderseits wurden grössere Konzentrattionen von **Dasytricha** spp. gefunden und **Elytroplastron bubali** waren nur im Büffel gegenwärtig. Es gab keine beachtungswerte Unterschiede zwischen den Tieren im Zusammenhang der **Diplodinium** spp., **Ostracodinium** spp. und **Eudiplodinium** spp. Der pH - Wert in der Pansenflüssingkeit des Büffels war bemerkenswerterweise ($P < 0,01$) grösaer (6,28) als jenen vom Rind (5,98). Die, während des Tages, aufgenommene Daten des Büffels, zeigten eine Sinkung der Konzentration der meisten studierten Gattungen, mit wenigern Zahlen in den nachtlichen Kolekten. Dies aber wurde nicht bei dem Rind beobachtet, ausser bei der Gattung **Isotricha** spp.

PHARMACOKINETICS OF MORANTEL TARTRATE RELEASE FROM AN INTRARUMINAL ANTHELMINTIC BOLUS, THE PARATECT FLEX® BOLUS, IN CATTLE

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INTRODUCTION

Morantel (1,4,5,6-tetrahydro-1-methyl-2-trans-2-methyl-2-thienyl-vinyl-pyrimidine) is the methyl ester analogue of pyrantel and is formulated as the tartrate salt for the control of gastrointestinal nematode infections in domestic animals.

Recent advances in drug delivery systems technology have resulted in the development of a number of controlled release intraruminal boluses for the administration of anthelmintics to cattle. A new non-metal morantel tartrate sustained release trilaminate bolus (Paratect Flex® bolus, Pfizer Inc.) has now been developed. It is designed to give season-long protection against gastrointestinal nematodes. The device consists of a rolled-up morantel tartrate sandwich tied with a tape which is unfolded on contact with the ruminal fluid (7). Once it comes into contact with ruminal fluid, the drug is released through the impregnated semi-permeable membranes at a continuous level for up to 90 days (7). The purpose of this study was to establish the pharmacokinetic and compartmental distribution of morantel tartrate after the administration of the aforementioned bolus to cattle.

MATERIALS AND METHODS

Experimental animals and their management

Six parasite-free Holstein steers were surgically fitted with permanent cannulae in the rumen, in the abomasum and in the ileum. Surgery was performed under general anesthesia. Atropine sulfate was given to inhibit salivation and gastrointestinal motility. The cannulae were inserted in the dorsal sack of the rumen, in the pyloric region of the abomasum, and at the distal region of the ileum as described by Komarek (5,6). A period of approximately 4 to 6 weeks was allowed before starting the pharmacokinetic trial.

The animals were housed in individual pens in an indoor cattle facility. They were handled and observed daily to ensure their good health. Good quality hay and water were given ad libitum.

Treatment and sampling procedure

A morantel tartrate sustained release trilaminate bolus (Paratect Flex®) was given orally to each of the cannulated animals by using a special gun. The animals were observed for few hours post-treatment to ensure that the bolus was not expelled.

Samples of ruminal, abomasal and ileal fluid (via cannulae), jugular vein blood and rectal feces were taken at each sampling time. Samples of approximately 10 ml (or g) were collected into vials labelled with the animal number, sampling time and type of sample. Blood samples were taken in sterile Vacutainer tubes containing heparin, cooled on ice and centrifuged. Plasma was removed into a labelled plastic vial. Samples of ruminal, abomasal and ileal fluids, feces and plasma were stored at -20°C until analysed.

Samples from each experimental compartment were obtained on days -3, 1, 4, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98.

Analytical procedures

Extraction - Basically, the extraction procedure was as described by Bioanalytical Research Ltd. (2). An aliquot of plasma, ruminal, abomasal, intestinal and fecal samples (5 ml or 5 g each) was spiked with 5 µg of pyrantel tartrate as internal standard. The spiked sample was made alkaline with ammonium hydroxide solution, extracted with chloroform, centrifuged and the chloroform layer filtered. The filtrate was made acidic with sulfuric acid, extracted, centrifuged and the acidic aqueous layer taken, neutralised and analysed by HPLC.

Drug analysis - Experimental and spiked samples were analysed for morantel tartrate by high performance liquid chromatography (HPLC). Fifty microliters of each extracted sample were injected by an autosampler to an LKB Bromma HPLC system (LKB, Bromma, Sweden) with a Bondex 10 µm C_{18} reverse phase column (Phenomenex, CA, USA). The HPLC mobile phase was acetonitrile/ammonium acetate buffer, pH 3.5 (28:72); each sample was isocratically run for 8 minutes with a flow rate of 1.3 ml/min. Morantel and pyrantel (internal standard) were determined at 318 nm with an LKB spectral variable wavelength absorbance detector.

Identification of each analyte was undertaken by comparison with the retention time of pure reference standards (morantel tartrate, standard # 9A302-02QCS, 57.8% as base; pyrantel tartrate, lot # OD153-18QCS-10, 57.6% as base, Pfizer Ltd.), which were also used to prepare standard solutions to establish calibration curves for each analyte in either fluid or tissue. The approximate retention times were: 3.97 minutes for pyrantel tartrate and 5.64 minutes for morantel tartrate.

Unknown concentrations were calculated by comparison of peak area of morantel tartrate with the peak area for the internal standard using the computer analysis program Nelson Analytical (model 2600, version 3.1, Nelson Analytical Inc., CA, USA) on a compatible AT computer (Packard Bell, Canada).

Aliquots of cattle plasma, feces and ruminal, abomasal and intestinal fluids were spiked with known amounts of morantel tartrate in a range of 0.025 to 5 µg/ml (or µg/g), extracted and then analysed by HPLC (triplicate determinations) in order to establish the percentage of recovery for each sample compartment.

RESULTS

The detection limit and percentage of recovery for morantel in each fluid ranged from 0.020 to 0.050 µg/ml and from 87 to 98%, respectively.

Morantel tartrate was not detectable in plasma at any time post-administration in any of the animals in this trial. The mean (± SEM) concentrations of morantel tartrate in ruminal, abomasal and ileal fluids and feces are summarized in Table 1.

TABLE 1. Mean concentrations of morantel tartrate in ruminal, abomasal and ileal fluids (µg/ml) and feces (µg/g) after the administration of a morantel sustained release trilaminate bolus to cattle

Days Post Treatment	Ruminal Fluid (µg/ml)	Abomasal Fluid (µg/ml)	Ileal Fluid (µg/ml)	Feces (µg/g)
-3	0.00	0.00	0.00	0.00
1	3.27±0.48	2.90±0.59	2.86±0.42	11.45±0.72
4	1.34±0.24	1.62±0.40	0.94±0.25	7.20±0.54
7	0.74±0.24	1.23±0.32	1.04±0.16	4.11±0.26
10	0.72±0.23	0.95±0.17	1.04±0.18	4.14±0.43
14	0.88±0.13	1.36±0.20	1.35±0.24	5.57±0.78
21	0.92±0.15	1.62±0.20	1.49±0.14	4.52±0.70
28	0.71±0.08	1.43±0.17	1.36±0.09	4.88±0.37
35	0.92±0.15	1.10±0.22	1.11±0.05	4.65±0.70
42	0.59±0.05	1.19±0.09	1.49±0.11	4.80±0.49
49	0.54±0.06	1.03±0.09	1.20±0.19	5.35±0.74
56	0.69±0.22	1.02±0.09	1.04±0.15	4.85±0.47
63	0.47±0.06	0.91±0.05	1.31±0.25	4.96±1.43
70	0.87±0.07	1.10±0.23	1.20±0.10	5.84±1.35
77	0.58±0.09	1.20±0.20	1.30±0.17	4.93±0.74
84	0.57±0.18	1.20±0.07	1.13±0.26	5.16±0.43
91	0.40±0.04	1.20±0.17	0.93±0.22	4.03±0.28
98	0.38±0.03	1.06±0.11	0.98±0.11	3.68±0.27

Data are presented as means±S.E.M.
ns = no sample available

DISCUSSION

Morantel tartrate was detected in ruminal, abomasal, ileal and fecal samples from day 1 to 98 after the administration of a morantel sustained release bolus to cattle. The morantel tartrate pharmacokinetic pattern was similar in each gastrointestinal compartment. An early Cmax was obtained at day 1 post-treatment. Then, the levels dropped to reach the steady-state level between 7 and 10 days post-treatment in each gastrointestinal compartment. This

morantel steady-state level was maintained over approximately 84-91 days in feces and ruminal fluid and over 98 days in abomasal and ileal fluids.

The morantel concentration was 2-fold higher in the abomasum and ileum than in the rumen. This may be attributable to a higher dilution of the compound in the ruminal fluid due to water absorption by the omasum. These results are consistent with those obtained by Alvinerie & Fioramonti (1) after the administration of a single dose of morantel to a cow. Water absorption by the large intestine may explain the higher levels of morantel in feces compared with other gastrointestinal compartments.

Morantel tartrate was not detectable in plasma at any time after the administration of this long-acting bolus in any of the animals of this trial. Earlier radiotracer studies in cattle using either tritium or ^{14}carbon-labeled morantel showed that while only 14 to 17% of a single dose (10 mg/kg) was recovered in the urine, 68% was recovered in the feces (4). This low percentage of dose measured in urine as total radioactivity included a number of polar metabolites and only traces of unchanged parent drug (4). These results may indicate both a low gastrointestinal absorption and first-pass metabolism of morantel.

The release rate of the sustained release bolus used in this trial has been shown to be 100 mg/day (150 mg/day for the first 10 days post-administration) (3), which represents a low dose rate compared with the 10 mg/kg single morantel dose used in the above mentioned studies. This difference in dose rate may contribute to the inability to detect morantel in plasma in this trial.

In conclusion, this pharmacokinetic study has established, for the first time, the compartmental distribution of morantel released from a sustained release bolus. The in vivo drug release profile of this trilaminate bolus has been confirmed. Steady-state levels for 91 to 98 days are important for parasite control.

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SUMMARY

Six Holstein calves (125-150 kg) had permanent in-dwelling fistulae surgically inserted into the rumen, abomasum and terminal ileum. After 4-6 weeks of recovery, they were each given a morantel sustained release trilaminate bolus (Paratect Flex® bolus) orally with a bolus applicator. Samples of jugular blood plasma and contents from the rumen, abomasum, ileum and fecal samples were taken on days -3, 1, 4, 7, 10, 14 and weekly up to day 98 post-bolus administration. Morantel was estimated by HPLC after extraction and clean-up. Although morantel was not detected in plasma at any time after the bolus administration, high concentrations were found in ruminal, abomasal and ileal fluids and feces over 98 days post-treatment. Morantel concentrations in feces were markedly higher than those in ruminal, abomasal or intestinal fluids. In each compartment, the steady-state level of morantel concentration was achieved between 7 and 10 days post-treatment and was maintained for 91-98 days post-treatment.

RESUMEN

Seis terneros Holstein machos (125-150 kg) fueron preparados con cánulas en el rumen, abomaso y porción terminal del ileón. Cuatro a 6 semanas posteriores a la cirugía los animales fueron tratados oralmente con un bolo de liberación lenta (Paratect Flex® bolus) usando un lanzabolos especialmente diseñado. Muestras de sangre yugular, materia fecal y fluidos ruminal, abomasal y ileal fueron obtenidas los días -3, 1, 4, 7, 10, 14 y así, semanalmente hasta 98 días post-tratamiento. Morantel fue determinado por cromatografía líquida, previa a una extracción de las muestras con diferentes solventes. Aunque morantel no fue encontrado en plasma, elevados niveles del compuesto fueron detectados en heces y en los fluidos ruminal, abomasal e ileal durante 98 días posteriores al tratamiento. Las concentraciones de morantel en heces fueron marcadamente mayores que aquellas en rumen, abomaso e ileón. Un nivel constante de concentración de morantel fue alcanzado entre 7 y 10 días post-tratamiento en todos los compartimentos y, mantenido durante 91-98 días post-tratamiento.

RESUME

Des cannules ont été implantées chirurgicalement, de façon permanente, dans le rumen, l'abomasum et la partie distale de l'iléum, chez six veaux Holstein. Après un temps de rémission de 4 à 6 semaines, les animaux ont reçu oralement un bolus trilaminé à décharge soutenue de tartrate de morantel (Paratect Flex® bolus). Des échantillons de plasma sanguin (veine jugulaire), de contenu ruminal, abomasal et iléal ainsi que des échantillons fécaux ont été prélevés aux jours -3, 1, 4, 7, 10, 14 et hebdomadairement jusqu'au 98ième jour suivant l'administration du bolus. Suite à l'extraction, la concentration de morantel fut déterminée par chromatographie liquide (HPLC). En aucun temps le morantel ne fut décelé dans le plasma. Des concentrations élevées de morantel ont été observées dans les liquides ruminal, abomasal et iléal ainsi que dans les fécès jusqu'à 98 jours après le traitement. Les concentrations de morantel dans les fécès se sont révélées beaucoup plus élevées que celles observées pour les autres compartiments. Un niveau constant de concentration de morantel fut atteint 7-10 jours après le traitement et maintenu ainsi jusqu'au 91-98ième jour.

AÇÃO DO ENXOFRE ELEMENTAR NO CONTROLE DO CARRAPATO BOVINO

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INTRODUÇÃO

Os prejuízos múltiplos causados pelo carrapato, são muito grandes.

GONZALES[4] afirma que cada carrapata em seu papel espoliativo ingere até 3 ml de sangue em toda a sua vida. Segundo FLECHTMANN[2], uma carrapata ingere de 0,5 a 2 ml de sangue por dia, regorgitando o excesso.

Visando o combate ao carrapato bovino, o enxofre é desde longa data citado por pesquisadores como supostamente dotado de certa eficácia.

Já em 1892 no Texas, EUA, M. FRANCIS[3] investigou essa possibilidade através da ministração de enxofre à ração sem contudo ter encontrado qualquer vantagem.

Mais recentemente, COSTA[1] em seus trabalhos no município de Castanhal, Estado do Amazonas, Brasil, admite que a porcentagem de 2% de flor de enxofre na mistura mineral, durante um ano aproximadamente, seja a forma eficaz de se controlar o parasitismo por Boophilus microplus em bovinos.

Na Austrália, UTECH & WHARTON[5] em 1972, verificaram que com a administração de até 3% de enxofre no sal mineral de bovinos havia a diminuição no número de carrapatos, sem entretanto ser esta diminuição significante estatisticamente.

Verificamos que apesar da literatura a respeito ser bastante vasta, nenhum dos trabalhos compulsados preenchia os 3 quesitos seguintes, simultaneamente.

1. Medida exata da quantidade diária de enxofre ingerida pelos animais.

2. Separação dos lotes testemunho e tratado, de forma que as larvas oriundas de animais do lote tratado só parasitassem animais tratados e vice-versa.

3. Tempo de duração do experimento suficientemente grande para várias gerações de carrapatos que parasitaram animais que ingeriram enxofre, sofram sua ação deletéria.

O presente trabalho teve como finalidade o estudo do binômio enxofre x carrapato, visando uma possível ação terapêutica do enxofre no combate a esse ácaro que infesta os bovinos.

MATERIAL E MÉTODOS

O experimento foi realizado de maio de 1986 a maio de 1990 na Estação Experimental de Zootecnia de São José do Rio Preto - SP, com temperatura e umidade relativa de ar médias, respectivamente 22,5ºC e 72%.

Para o experimento, utilizou-se de 32 bezerras da raça Santa Gertrudis, com idade variando entre 13 e 17 meses e peso médio 256± 49 kg que foram divididas em 4 lotes homogêneos.

Os animais dos lotes denominados A, B, C e D contidos no mesmo pasto, eram presos diariamente a fim de receberem 500g/cabeça de fubá de milho que era acrescido de 5, 10 ou 15g/cabeça de enxofre elementar, respectivamente para os lotes B, C e D. Os animais do lote A (testemunho), receberam apenas fubá de milho.

A mineralização "ad libitum" foi feita através da administração de sais totalmente isentos de radicais sulfatados.

No primeiro dia de experimento, todas as bezerras foram banhadas com carrapaticida à base de amitraz.

Semanalmente foram contadas todas as carrapatas ingurgitadas e semi-ingurgitadas que parasitavam o lado esquerdo do animal. A esta etapa, denominamos de fase 1.

Depois de 2 anos de coleta de dados na fase 1, o pasto foi dividido em 4, ficando 1 lote de bovinos em cada piquete, permanecendo aí por um ano, constituindo-se na fase 2.

Em seguida, foi feita rotação de piquetes(A com D e B com C), continuando-se com a coleta de dados por mais um ano, sendo este a fase 3.

Durante todo o transcorrer do trabalho, os animais foram banhados com carrapaticida apenas 2 vezes: no início das fases 1 e 3.

O delineamento estatístico empregado foi o de blocos ao acaso, com 4 tratamentos e 8 repetições nas fases 1 e 2, e 7 repetições na fase 3. Para o estudo dos efeitos dos tratamentos, a análise de variância foi feita levando-se em conta a regressão.

RESULTADOS

De junho de 1986 a maio de 1990, foram realizadas do lado esquerdo do animal 103 contagens na 1ª fase, 50 na 2ª fase e 49 na 3ª fase experimental.

Os resultados médios (nº de carrapatos/cabeça) contados do lado esquerdo do animal, obtidos por tratamento em cada fase experimental, são apresentados no quadro 1.

Tratamento	1ª fase (jun/86 a mai/88)	2ª fase (jun/88 a mai/89)	3ª fase (jun/89 a mai/90)
A (test)	234,2	154,4	198,0
B (5 g)	235,2	127,3	127,7
C (10 g)	159,5	107,9	99,8
D (15 g)	181,3	133,7	63,5

Quadro 1 - Número médio de carrapatas/cabeça, observado no lado esquerdo de vacas Santa Gertrudis, por tratamento e em cada fase experimental.

Através da análise estatística, foi observado que na 1ª fase experimental não ocorreram diferenças significativas entre os tratamentos ($P > 0,05$), no entanto foram verificados efeitos significativos, ao nível de 1% de probabilidade no componente quadrático por ocasião da 2ª fase e no componente linear na 3ª fase. Os gráficos das equações de regressão da 2ª e 3ª fases experimentais são apresentadas nas Figuras 1 e 2.

DISCUSSÃO

Verificamos que na 1ª fase experimental não foram observadas diferenças significativas ($P > 0,05$) entre os tratamentos, apesar dos animais do lote A (testemunho) e do lote B (5g), neste período, apresentarem maior número de carrapatas do que os demais. O fato de todos os animais do presente experimento permanecerem em apenas um pasto comum a todos, provavelmente colaborou para que ocorresse tal resultado.

Entretanto, por ocasião da 2ª fase experimental após a separação dos

lotes em piquetes distintos, o número médio de carrapatas variou segundo uma equação de segundo grau, em consequência da elevação dos níveis de enxofre (figura 1). Observando-se que o melhor tratamento neste período giraria ao redor de 9g/cab/dia.

Em nosso caso, a taxa de infestação média observada no tratamento D foi maior do que a apresentada pelos tratamentos B e C, devido, provavelmente, à diarréia profusa ocorrida nos meses de janeiro e fevereiro em dois (2) animais do lote D, o que alem de provocar a quebra de resistência destes animais, impediu a absorção do enxofre, fatos estes que favoreceram o parasitismo pelo carrapato.

Já na 3ª fase experimental, o número médio de carrapatas variou linearmente, diminuindo com a elevação das doses de enxofre (figura 2).

O fato de se verificar efeito significativo de tratamentos apenas na 2ª e 3ª fases do presente experimento, ocasião em que lotes de animais de um mesmo tratamento foram manejados em piquetes distintos, parece-nos demonstrar que a ação deletéria do enxofre sobre os carrapatos se processa através das gerações sucessivas dos mesmos.

CONCLUSÃO

Os resultados observados nos quatro anos de experimento, indicaram que o enxofre foi eficiente no combate ao carrapato bovino.

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RESUMO: AÇÃO DO ENXOFRE ELEMENTAR NO CONTROLE DO CARRAPATO BOVINO

Trinta e dois bovinos da raça Santa Gertrudis, do sexo feminino, com idade média de 15±2 meses, foram utilizados em um experimento em blocos casualizados, com 4 tratamentos e 08 repetições. Os animais colocados em um mesmo pasto, eram recolhidos diariamente em baias individuais onde recebiam 1 kg de rolão de milho, acrescido de zero, 5, 10 e 15 gramas de enxofre elementar, correspondente aos tratamentos A, B, C e D. Na mineralização foram excluídos do sal os radicais à base de enxofre, para os 4 lotes. Semanalmente foram contadas todas as carrapatas ingurgitadas e semi-ingurgitadas do lado esquerdo do animal. A esta etapa, denominamos de fase 1. Depois de 2 anos de coleta de dados na fase 1, o pasto foi dividido em 4, ficando um lote de bovinos em cada piquete, permanecendo aí por um ano, constituindo-se na fase 2. Em seguida, foi feita roteção de piquetes, continuando-se a coleta de dados por mais um ano, sendo esta a fase 3. Durante todo o transcorrer do trabalho, os animais foram banhados com carrapaticida apenas duas vezes: no início das fases 1 e 3. Neste experimento, utilizou-se a regressão na análise de variância, observando-se que na 1ª fase não ocorreram diferenças significativas entre os tratamentos, entretanto, verificaram-se efeitos significativos ao nível de 1% no componente quadrático por ocasião da 2ª fase e no componente linear na 3ª fase. Os resultados médios (nº de carrapatos/cabeça) do lado esquerdo do animal, obtidos durante cada fase, são os seguintes:

Tratamento	1ª fase (jun/86 a mai/88)	2ª fase (jun/88 a mai/89)	3ª fase (jun/89 a mai/90)
A (test.)	234,2	154,4	198,0
B (5 g)	235,2	127,3	127,7
C (10 g)	159,5	107,9	99,8
D (15 g)	181,3	133,7	63,5

Pelos resultados apresentados, concluimos que há efeito positivo de enxofre elementar no combate ao carrapato bovino.

SUMARY: ACTION OF SULPHUR IN CONTROL OF CATLLE TICK

Thirty-two 15±2 month old, female bovin of Santa Gertrudis breeders were placed in four randomized block design of eight animals each. Every day, the animals in a same pasture were gathered in stalls to eat 1 kg of sheaf of corn with zero, 5, 10 and 15 grams of elementar sulphur, corresponding to A, B, C and D treatments. The radical sulphur was taken from salt in mineralization for the groups of animals. Weekly, ingurgita te ticks from the left side of each animal were counted. This is phase 1. After 2 years collecting data in phase 1, the pasture was divided into four. Each group of bovin stayed in a pasture for one year. This is phase 2. Then, there was the rotation of pasture and data were collected for one more year. This is phase 3. During all the work, the animals were bathed with tick poison, only twice: in the beginning of 1 and 2. The regression in the analysis of variance was used in this experiment. No significative difference between the treatment took phase 1, neverthless sighificative effects could checked at level of 1% in quadratic component in phase 2 and at linear component in phase 3. The medium results (number of ticks per animal) on the left side of the animal, checked during each phase, are the following:

Treatment	Phase 1 (june/86 to may/88)	Phase 2 (june/88 to may/89)	Phase 3 (june/89 to may/90)
A (test)	234,2	154,4	198,0
B (5 g)	235,2	127,3	127,7
C (10 g)	159,5	107,9	99,8
D (15 g)	181,3	133,7	63,5

According to the results presented we can conclude that-there is positive effect of elementar sulphur in control of catlle tick.

RÉSUMÉ: ACTION DU SOUFFRE ELÉMENTAIRE POUR LE CONTRÔLE DU TIQUE BOVIN.

Trente et deux vaches de la race Santa Gertrudis, l'âge moyen 15±2 mois, ont été employées dans une expérience en groupe faite au hasard, avec quatre traitements et 8 répétitions. Les animaux placés dans une même pâture étaient regroupés tous les jours dans des barres individuelles où elles recevaient 1 kg. de maïs moulu, accrû de zéro, 5, 10 et 15 grammes de souffre élémentaire qui correspondaient aux traitements A, B, C et D. Lors de la minéralisation, les radicaux à la base du souffre ont été exclus du sel pour les 4 lots. À chaque semaine les tiques-femelles gonflées ou semi-gonflées trouvées du côté gauche de l'animal ont été comptées. Nous avons nommé cette étape de phase 1. Après deux ans de prise de données à la phase 1, la pâture a été partagée en 4 et nous avons mis un lot de bovins dans chaque clos, où ils sont restés pendant une année ce qui constitue la phase 2. Ensuite, chaque lot a été changé de clos et cette prise de données a été faite encore pendant une année, ce qui constitue la phase 3. Pendant le developpement du travail les animaux ont été baignés d'un poison à tiques seulement deux fois, au commencement des phases 1 et 2. Pour cette expérience, nous avons employé la régression dans l'analyse de variance en observant qu' à la 1ère phase, les différences significatives ne sont pas arrivées entre les traitements, cependant, nous avons observé des effets significatifs au niveau de 1% dans la composant quadratiques à l'occasion de la 2ème phase et dans le composant linéaire à la 3ème phase. Les résultats moyens (nombre de tiques-femelles/tête), du côté gauche de l'animal, obtenus pendant chaque phase sont les suivants:

Traitement	1ère phase (juin/86 à mai/88)	2ème phase (juin/88 à mai/89)	3ème phase (juin/89 à mai/90)
A (test)	234,2	154,4	198,0
B (5 g)	235,2	127,3	127,7
C (10 g)	159,5	107,9	99,8
D (15 g)	181,3	133,7	63,5

Par les résultats présentés, nous avons conclu qu'il y a un effet positif de souffre élémentaire pour combattre le tique bovin.

RIASSUNTO: AZIONE DELLO ZOLFO ELEMENTARE NEL CONTROLLO DEL CARRAPATO BOVINO

Trentadue bovini della razza Santa Gertrudis, del sesso femminile, all'età media di 15±2 mesi, sono stati utilizzati in un esperimento in blocchi casualizzati, con 4 trattamenti e 8 ripetizioni. Gli animali, collocati in uno stesso pascolo, erano raccolti giornalmente in tramezzi di stalla individuale dove ricevevano 1 kg di cruschello di miglio, accresciuto di zero, 5, 10 e 15 grammi di zolfo elementare, corrispondenti ai trattamenti A, B, C e D. Nell'atto de la mineralizzazione sono stati esclusi dal sale i radicali alla base dello zolfo, ai 4 lotti. Settimanalmente sono stati contati tutti i carrapati ingurgitati e semiingurgitati al fianco sinistro dell'animale. A questa tappa, abbiamo denominato fase 1. Dopo due anni di colleta di dati alla fase 1, il pascolo è stato diviso in 4, restando un lotto di bovini in ogni picchetto, rimanendovi per un anno, costituendosi la fase 2. Subito dopo, è stata fatta la rotazione dei picchetti, e si è continuata la colleta dei dati per un anno di più, essendo questa la fase 3. Durante tutto il trascorrere del lavoro, gli animali sono stati bagnati con carrapaticida solo due volte: all'inizio delle fasi 1 e 2. In questo esperimento, si è utilizzata la regressione nell'analisi di variante, osservandosi che alla 1ª fase non sono occorse differenze significative tra i trattamenti, intanto, si sono verificati degli affetti significativi al livello di 1% nel componente quadratico per occasione della 2ª fase e nel componente lineare nella 3ª fase. I risultati medi (nº di carrapato/capo), al fianco sinistro dell'animale, ottenuti durante ogni fase, sono i seguenti:

Trattamento	1ª fase (giu/86 a mag/88)	2ª fase (giu/88 a mag/89)	3ª fase (giu/89 a mag/90)
A (test)	234,2	154,4	198,0
B (5 g)	235,2	127,3	127,7
C (10 g)	159,5	107,9	99,8
D (15 g)	181,3	133,7	63,5

Conforme i risultati presentati, abbiamo concluso che c'è effetto positivo dello zolfo elementare nel combattimento al carrapato bovino.

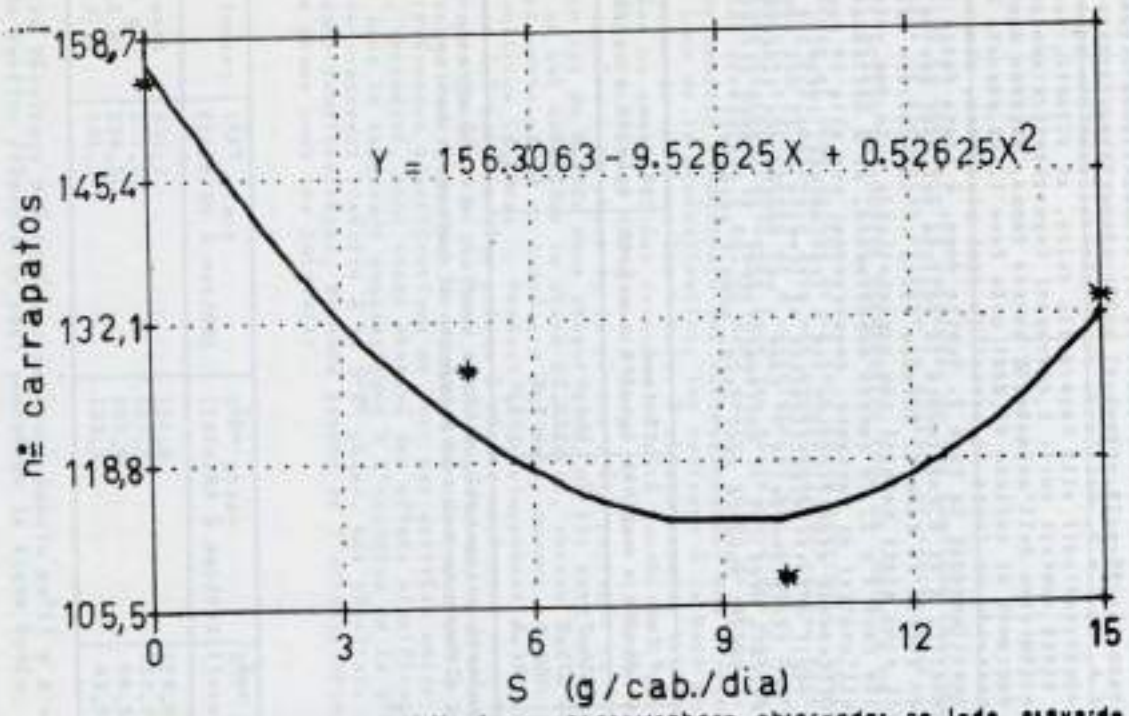

Figura 1 - Número médio de carrapatos/cabeça observados no lado esquerdo de vacas Santa Gertrudis, no período de jun/88 a mai/89 (2ª fase experimental), em função dos níveis de enxofre ingeridos diariamente pelos animais

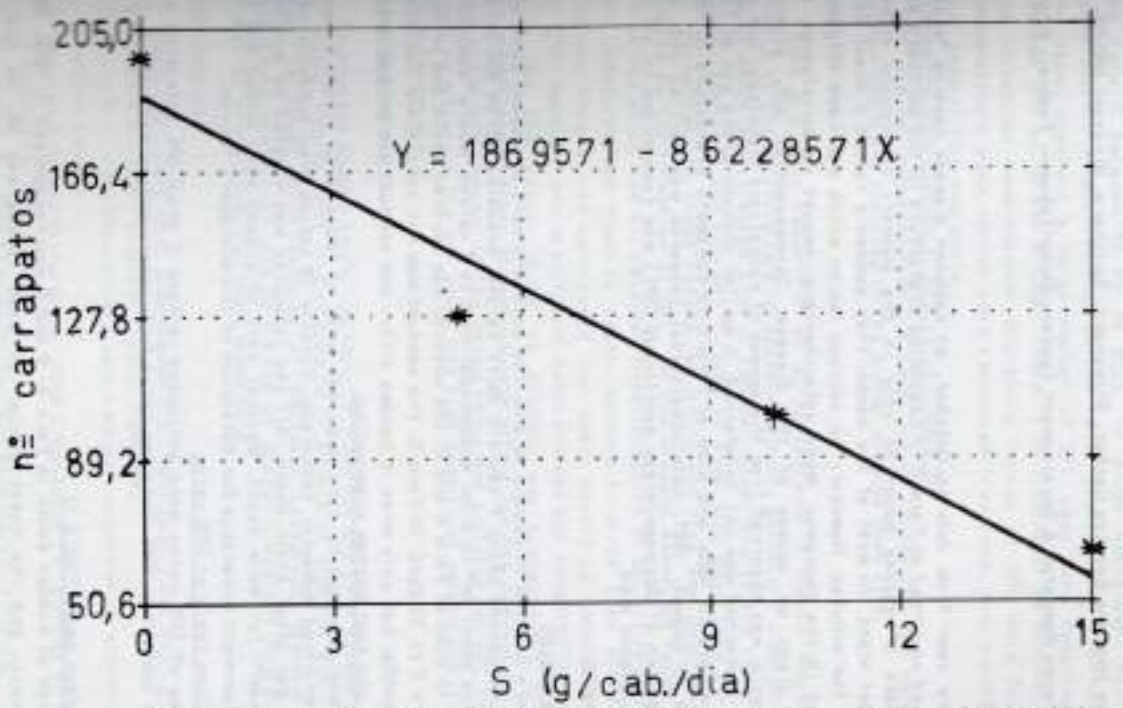

Figura 2 - Número médio de carrapatos/cabeça observados no lado esquerdo de vacas Santa Gertrudis, no período de jun/89 a mai/90 (3ª fase experimental), em função dos níveis de enxofre ingeridos diariamente pelos animais

OUTBREAK OF STRONGYLOIDIASIS CAUSING SUDDEN DEATH OF CALVES AT SOME FARMS IN JAPAN

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INTRODUCTION

Since 1978, every year from July to October, an unknown disease causing "sudden death" of calves had occurred at Farm A in Kagoshima prefecture in the southern part of Japan. The death of calves occurred only in the summer, previously without showing any clinical signs. In most of the cases, they suddenly raised a queer voice and died within a few minutes. However, no serious lesion which may have triggered death was observed in the necropsy. Many veterinarians, except parasitologist, set up a survey to clean up causes of the disease. Consequently, AIP, atypical interstitial pneumonia, was observed from the aspect of pathologic histology, but any specific pathogen concerned was not determined. A similar disease broke out at Farm B in Miyazaki prefecture in 1983, and also at Farm C in Kumamoto prefecture in 1987. For the first time in August, 1987, helminthological research was carried out, and then the hyperinfection of *Strongyloides papillosus* (SPL) was recognized in all the calves which had suddenly died.

MATERIALS AND METHODS

Farms and breeding system

Farm A, B and C are located in Kyushu Island, the southern part of Japan. Each of the above 3 farms introduced about 7-day-old dairy calves, breeding 200 to 300 calves in total. One pen was about 5m² in size, accommodating 5 to 7 calves, where "sawdust" was used as litter. In case of Farm A and B, the sawdust was layered about 5cm in depth, being renewed every 7 to 10 days. In Farm C, the sawdust was not completely replaced by new one for 3 months, but only when it became dirty, new sawdust was spread onto it.

Fecal examination and post mortem examination

Rectal content was examined to take eggs per gram of feces (EPG) within 6 hours after sampling by Modified McMaster technique. Totally 13 calves died suddenly were opened within one day after their death. All the intestine was treated by modified technique of Skerman[4] for worm count. Several part of organs were sliced and set into the Modified Baermann apparatus for detection of the migratory larvae.

Frequency distribution table of EPG value

All the 130 cases of EPG value were collected in Farm C when SPL infection was first recognized in September, 1987. Data was taken from calves before medication.

RESULTS

Outbreak of the sudden death(TABLE 1)

The first incidence of sudden death in Farm A, B and C was recognized in 1978, 1983 and 1987 respectively, and the total number of dead calves was 94, 43 and 15 respectively. Helminthological examination began in 1987, and the hyperinfection of SPL was recognized in the same year. Soon after the initial recognition of the hyperinfection of S. papillosus, the herd with some connection to the sudden death was totally medicated, which led to the complete termination of the incidence.

Postmortem examination (TABLE 2)

Age of calves which suddenly died in Farm A and B ranges between 119 and 144 days and Farm C between 46 to 76 days. In gross pathology, most of the cases had comparatively slight lesions. In the fecal examination, diarrhea was found only in one case, however, a large number of SPL eggs were detected in all cases. The maximum EPG value was 411,000. There is no finding of helminthes except SPL in fecal and intestinal examinations. Migratory larvae of SPL were found from all cases. Comparatively many larvae were recovered from lung, eye, mandibular muscle, trunk, four legs of limb and ankle.

Frequency distribution of EPG value (TABLE 3)

SPL egg was detected from 116 cases out of 130 (89.2%). EPG value of all 10 cases of sudden death was included in the higher classes than 11th.

DISCUSSIONS

There are many report[2,3] of *S. stercoralis* in human. However, there is a few report of Strongyloidiasis in domestic animal. Spindler[5] recognized sudden death in swine which was infected with *S. ransomi*. According to the report concerning SPL on sheep, goats[1,6] and calves[1,7], some of them died when they were severely affected by parasitism. In these repots, we could not find any description of sudden death caused by the infection of SPL. In the present paper, we found the sudden death of 13 calves which had all received hyperinfection of SPL. It is difficult to explain why SPL infection led them to death, especially since they showed just slight gross lesions. However, we considered that death should be induced by the hyperinfection of SPL. Because, any significant pathogen was not detected in the necropsy but SPL. Also the EPG value, 52,000 to 411,000, was tremendously high[7]. Ivermectin or Thiabendazole therapy showed obvious effect for the prevention of the sudden death. The whole environment in the above farms was suitable for SPL infection: sawdust was used as litter in the pen which had high density of calves and it was 25 to 30℃ in the room temperature.

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TABLE 1. Outbreak of "sudden death" of calves at three farms

Farm*1) breeding	A (S&D) 300 Freisian calves				B (YMNEC) 200 Freisian calves			C (YUNO) 300 Freigian calves		
Town, Prefecture	Yamagawa, Kagoshima				Kobayashi, Miyazaki			Minamata, Kumamoto		
Year/Month	July	Aug.	Sept.	Oct.	Aug.	Sept.	Oct.	July	Aug.	Sept.
1978	-	1	5		-	-	-	-	-	-
1979	-	4	16	2	-	-	-	-	-	-
1980	-	2	7	1	-	-	-	-	-	-
1981	-	2	6	1	-	-	-	-	-	-
1982	-	2	14		-	-	-	-	-	-
1983*2)	-	-	1	1	1	18	3	-	-	-
1984	-	1	3	1	1	8	1	-	-	-
1985	3	3	-	1	-	9	1	-	-	-
1986	2	-	-	-	- *3)	-	-	-	-	-
1987	2	10 *4)	1	-	1 *5)	-	-	1	4	10 *4)
1988	-	-	-	-	-	-	-	-	-	-
1989	-	-	-	2 *5)	-	-	-	-	-	-
Total	7	25	53	9	3	35	5	1	4	10
Ground total				94			43			15
Ground total										152

*1) ( ) Farm name: "sawdust" was used as litter in all farms.
*2) A vet. consultation, without helminthologist, set up survey to clean up causes of the disease.
*3) For the first time of helminthologycal survey had carried out on this disease.
*4) The first recognition of the hyperinfection of Strongyloides papillosus at the farm; subsequently the medication using Ivermectin or Thiabendazole done at the herd.
*5) These calves was not medicated, severe infection of S. papillosus in post mortem was recognized.

TABLE 2. Parasitological observations at postmortem of 13 calves died suddenly in three farms

	Farm	A		B	C									
	Calf No.	537	036	66	333	292	334	298	329*6)	327	529	678	315	330
	Month/Date in 1987	08/10	09/28	08/19	09/06	09/06	09/09	09/09	09/10	09/10	09/12	09/14	09/17	09/19
	Age in days	137	144	119	56	67	76	69	57	51	40	51	69	56
Gross pathology*1)	Scabbing of ankle/skin	?	-	++	-	-	-	-	+	++	-	+	+	-
	Lung (consolidation)	++	+++	-	+	++	+	-	+	-	-	-	++	-
	Lung (edema)	-	+	-	-	-	+	-	-	+	-	-	+	-
	Intestine(hyperemia)	-	-	+	-	-	-	-	-	-	-	+	+	+
Fecal examination	Consistency*3)	DRH	SFT	SFT	SFT	SFT	SFT	SLD	SLD	SLD	SLD	SLD	SLD	SLD
	EPG in hundred*4)	2199	1160	730	998	1720	638	890	254	780	1046	1162	4110	520
Adult worms	Worms in hundred*5)	640	410	1415	745	480	508	190	48	704	ND	540	1174	231
Migratory larvae of Strongyloides papillosus from 10g tissue of each organs*2)	Lung(normal)	42	0	0	10	95	24	8	122	13	6	1	4	2
	Lung(lesion)	ND*8)	0	ND	2	7	1	ND	300	2	ND	ND	0	0
	Myocardium	ND	0	0	1	1	0	0	0	0	ND	0	1	0
	Eye(orbit-tissue)	ND	0	0	0	8	5	0	54	1	0	4	24	4
	Tongue	ND	0	0	0	0	0	0	1	0	ND	0	0	0
	Mandibular muscle	45	0	1	0	1	0	0	8	0	ND	0	1	0
	Trunk muscle	10	0	0	0	5	0	14	33	4	3	0	1	0
	Fore-limb(left)	ND	0	36	6	0	0	1	64	3	1	0	0	0
	Fore-ankle(left)	ND	0	35	8	1	5	1	9	13	2	0	3	0
	Fore-limb(right)	ND	0	14	0	5	1	5	2	10	0	1	1	0
	Fore-ankle(right)	ND	0	9	3	1	1	4	7	2	8	0	1	0
	Hind-limb(left)	ND	0	5	0	0	12	3	24	1	1	0	1	0
	Hind-ankle(left)	ND	1	24	1	6	7	1	87	18	58	1	9	0
	Hind-limb(right)	ND	0	11	1	28	0	1	4	1	3	0	24	0
	Hind-ankle(right)	ND	4	46	5	1	8	0	16	11	19	6	9	2

*1) -:no lesion, +:slightly, ++:moderate, +++:severe; no clearly lesion was observed in the other organs.
*2) the other organs such as brain, liver, kidney and diaphragm were all negative.
*3) DRH:diarrhea, SFT:soft, SLD:solid
*4) EPG/100 of Strongyloides; the other kind of helminth egg was negative; coccidial oocyst was all negative except the calf A(537) showed OPG=4,400.
*5) (Number of adult worms)/100 of Strongyloids papillosus detected in the small intestine; the other kind of helminth was all negative.
*6) Calf died in one day after medication; EPG=132,800 revealed before the medication.
*7) The umbilical part was enlarged; 69 migratory larvae recovered from 10g of the part.
*8) ND:not done.

TABLE 3. Frequency distribution of EPG value of Strongyloides papillosus on pre-medicated 193 calves at farm C in September 1987

SUMMARIES

Recently, every year from July to October, totally 152 calves have died suddenly at three farms in the southern part of Japan where "sawdust" was used as litter. All of the calves died within a few minuets without showing any clinical signs before. In the necropsy of 13 cases, just a slight lesion was observed, however, they all had the hyperinfection of Strongyloides papillosus. They appeared to have not only the high EPG value (maximum=411,000) with many adult worms, but also the migrating larvae in the tissue such as lungs and muscles. These incidences stopped completely by the medication. We conclude that the sudden death mentioned above should be caused by the hyperinfection of Strongyloides papillosus.

Zusammenfassung

In den letzten Jahren starben immer zwischen Juli und Oktober insgesamt 152 Kälber in drei Bauernhöfen im südlichen Japan, in denen Sägespäne als Streu verwendet wurden. Alle Kälber starben innerhalb weniger Minuten ohne vorher irgendwelche Anzeichen von Krankheit aufgewiesen zu haben. Bei der Leichenschau von 13 Fällen wurden nur geringe Läsionen beobachtet. Alle waren jedoch stark mit Strongyloides papillosus infiziert. Nicht nur hatten sie einen sehr hohen EPG-Wert (Höchstwert = 411,000) mit vielen ausgewachsenen Würmern, sondern auch Larven in ihren Geweben, wie Lunge und Muskeln. Diese Todesfälle hörten mit der Gabe von Wurmmitteln völlig auf. Wir schließen daraus, daß die plötzlichen Todesfälle durch eine Überinfektion mit Strongyloides papillosus verursacht wurden.

Resumen

Recientemente, cada año desde julio a octubre, un total de 152 terneros murieron de repente en las 3 granjas en la parte sur del Japón, en que se utiliza el serrín como cebo. Todos estos terneros murieron de repente dentro de unos minutos sin mostrar ningún síntoma clínico antes. En la necropsia de 13 casos, se observó un poco de lesiones ligeras, pero todos los casos mostraron una hiperinfección de Strongyloides papillosus. O sea se detectaron no solamente un alto valor de EPG (valor máximo = 411.000) con muchos imagos, sino también las larvas en los organos tales como pulmón y músculos. Estas incidencias se pararon comopletamente por la medicación. Concluimos que la muerte repentina antes mencionada se debe a la hiperinfección de Strongyloides papillosus.

PERITONEAL AUTOGRAFTS DO NOT PREVENT TEAT CISTERN STENOSIS AFTER CIRCUMFERENTIAL MUCOSAL INJURY

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INTRODUCTION

Teat injuries in dairy cattle are an important cause of economic loss to the dairyman. Most injuries occur early in lactation when cows are entering peak production. While many injuries can be successfully managed with prompt veterinary care, injuries that result in extensive damage to the teat cistern mucosa are difficult to treat. Stricture of the teat cistern is a common consequence of granulation tissue formation and fibrosis following mucosal injuries. Blind attempts to enlarge the teat cistern by insertion of instruments through the papillary duct are often unsuccessful. Instruments used in this manner usually result in tearing rather than cutting of the underlying tissue, and may destroy normal tissue surrounding the area of the stricture. More fibrosis occurs as the surgical wounds heal.

Thelotomy (2,15,19,20) has been advocated to avoid the complications associated with blind attempts at treating teat strictures. By opening the teat, the surgeon can accurately assess the damage, and remove abnormal tissue without destroying adjacent structures. Thelotomy also allows elevation of mucosa for coverage of denuded areas, thus preventing excess granulation (7). In areas where a mucosal defect is too large to allow transposition of adjacent mucosa for coverage, it has been recommended that an indwelling tube be inserted into the teat cistern (1,6,7,8). The tubes may be removed after healing has occurred (6) or may be left in place indefinitely (1,7,8). However, indwelling tubes have been associated with many complications (1). The tubes are difficult to secure for long periods of time. When they are dislodged, they may migrate into the gland cistern. The ends of a mobile tube can damage the mucosal lining of the teat or the gland. Tubes coated with Dacron or Teflon felt have been used experimentally to decrease the incidence of tube migration (14). Other complications associated with indwelling tubes include: breakage of the tube, inadequate tube length to allow milking, increased or decreased milking time of the affected quarter, less milk production from the quarter, and an increased incidence of mastitis in quarters with tube implants (1).

Oral mucosal autografts to the teat cistern have been used experimentally (18) and clinically (3) to avoid complications associated with prolonged use of an indwelling tube. When these grafts are used, it is necessary to temporarily implant an indwelling tube to prevent teat stricture (18). After healing has occurred the tube can be removed and patency will be maintained (3,18).

Smooth oral mucosa is limited in cattle, and is contaminated by its normal microflora. Transplantation of oral mucosal autografts to the teat cistern could therefore potentially induce infection of the surgery site or mastitis. In contrast, cows have abundant peritoneum that is sterile under normal circumstances. The purpose of this study was to determine if peritoneum would serve as a suitable autograft after extensive, circumferential teat cistern mucosal injury.

MATERIALS AND METHODS

Experiment I

Two groups of four nonlactating Holstein cows were used. Secretions from all quarters of the udders were cultured for the presence of bacteria. All bacteria cultured (Staphylococcus [n=19] and Streptococcus [n=3]) were sensitive to erythromycin. These quarters were treated with a dry cow preparation of erythromycin

and recultured prior to inclusion in the study. All quarters were also cultured at the time of surgery.

Prior to surgery each cow was given an intramuscular injection of 22,000 IU/kg of procaine penicillin G. Each cow was given 5% glyceryl guaiacolate intravenously until signs of muscle relaxation were apparent. Anesthesia was then induced with thiamylal (5.5 mg/kg IV), followed by endotracheal intubation. Anesthesia was maintained with halothane in oxygen delivered by a mechanical ventilator. Blood gases were monitored during surgery, and respiratory rate, tidal volume, and intravenous fluid administration were altered as necessary to maintain blood gas values within acceptable ranges.

The cows were placed in dorsal recumbency. The udders, inguinal regions, and ventral abdomen were routinely prepared and draped for aseptic surgery. A 15 cm ventral midline incision through the linea alba was made midway between the umbilicus and sternum. Peritoneum was harvested from this site by bluntly elevating the tissue deep to the linea and internal sheath of the rectus abdominus muscle and excising it. After excision, the retroperitoneal fat and most of the external sheath of the rectus was removed from the peritoneum before its implantation. The abdominal incision was closed routinely.

Three teats on each cow were incised longitudinally on their cranial surface. The teat cistern mucosa was circumferentially removed from each operated teat for a length of 3 cm. One teat was then closed in two layers. The muscular wall of the teat was closed with 4-0 polyglactin 910 sutures in a simple continuous pattern. The skin was closed with interrupted sutures of 3-0 monofilament nylon. The second teat had a section of peritoneum, cut to fit the mucosal defect, sutured to the edges of the defect with interrupted 4-0 polyglactin 910 sutures. In addition, four additional interrupted sutures were placed through the peritoneum into the underlying wall of the teat in a diamond pattern. These sutures were placed to minimize dead space between the peritoneum and underlying teat wall. The teat was then closed in three layers. The edges of the peritoneum adjacent to the incision were closed with 4-0 polyglactin 910 in a simple continuous pattern. The remainder of the closure was identical to the first teat. The third operated teat was treated identically to the second, but also had an indwelling fenestrated chest tube implanted in its lumen. Tube size was matched to teat cistern diameter. The fenestrated end of the tube was placed in the teat cistern. The tubes extended from the distal end of the teat cistern into the gland cistern. Each group of four cows had the individual treatments rotated among the teats of the cows in that group.

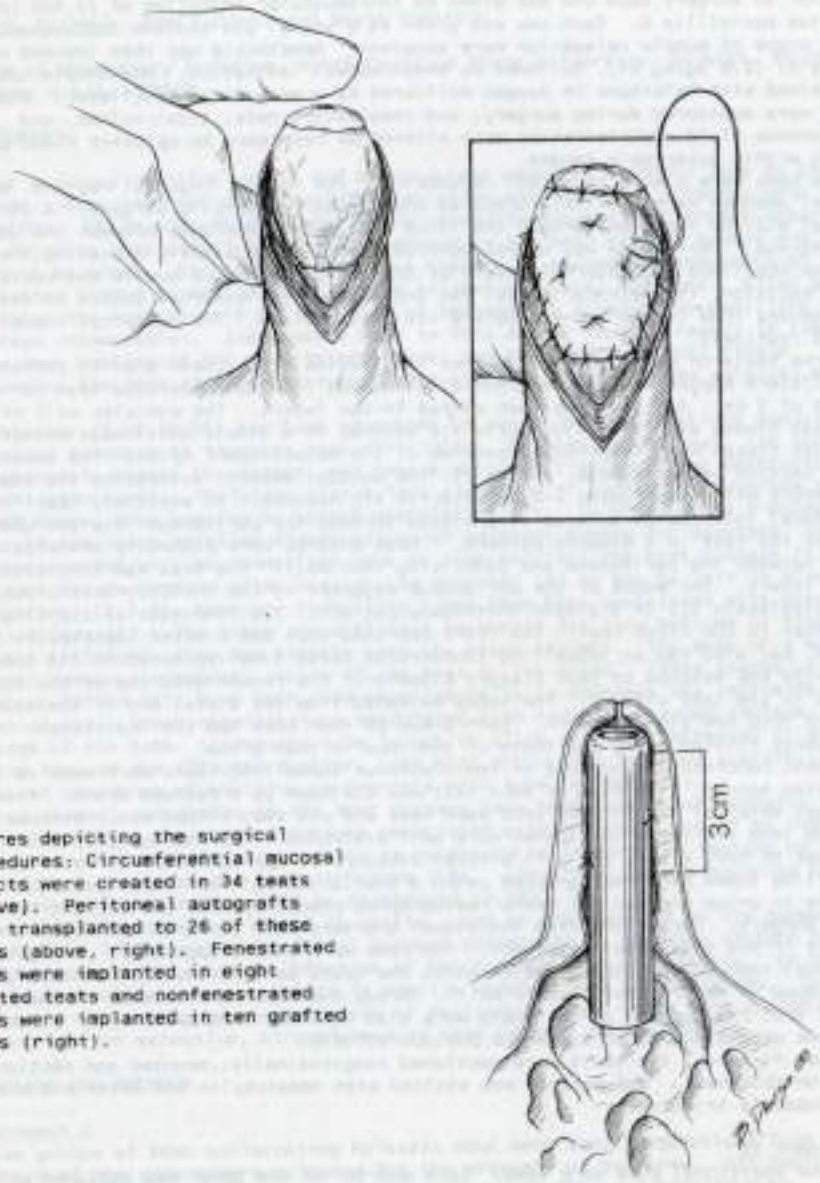
Double contrast radiographs of teats without indwelling tubes were made in the following manner. The base of each teat was occluded by a Penrose drain. Five ml of contrast material was infused into each teat and was then milked out. Air was then infused into the teats until they were well distended. The radiographic technique used was 60 KVP, 2 mAs, and a film-focal distance of approximately 75 cm. Teats with indwelling tubes were radiographed using a similar, single contrast technique.

Cows in group one had all teats radiographed preoperatively and one and two weeks after surgery. Secretions from individual quarters were cultured two weeks after surgery if the teat remained patent. The cows were then slaughtered. Slaughterhouse personnel removed the udders, after which the teats were harvested, examined grossly, and placed in neutral buffered formalin. Group two cows were treated similarly, except that radiographs of the teats were also obtained five weeks after surgery, and the cows were slaughtered six weeks postoperatively.

After fixation, the teats were sectioned longitudinally, mounted and sectioned at 6 micron thickness. One section was stained with hematoxylin and eosin and another with Masson's trichrome.

Experiment II

Three additional cows were used. Each quarter of the udder was cultured prior to surgery. Only quarters with no growth on initial culture were utilized for the study



Figures depicting the surgical procedures: Circumferential mucosal defects were created in 34 teats (above). Peritoneal autografts were transplanted to 26 of these teats (above, right). Fenestrated tubes were implanted in eight grafted teats and nonfenestrated tubes were implanted in ten grafted teats (right).

(n=10). Other quarters (n=2), had Streptococcus uberis cultured and were not treated. The anesthetic protocol was the same as described for Experiment I. The cows were placed in right lateral recuebency. The udder and left flank were prepared and draped for surgery. Peritoneum was harvested through a low left flank incision. Peritoneum harvested at this site appeared somewhat thicker than the ventral midline peritoneum, but did not have underlying adherent fibrous tissue.

The teats were incised, and 2 cm circumferential segments of mucosa were removed. These were replaced by peritoneum, as in Experiment I. After peritoneal implantation, nonfenestrated tubes were implanted, following the same protocol as in Experiment I. All cows were slaughtered two weeks after surgery. Teats were harvested and processed as described above.

RESULTS

Experiment I

No cows had systemic complications from the experimental procedure. Externally, all incisions appeared to heal without complications. However, all operated teats which did not have a tube implanted in the teat cistern showed radiographic evidence of partial obstruction one week after surgery, and complete obstruction by two weeks after surgery. In seven of eight teats with tube implants, filling defects were visible on contrast radiography of the teats by one week after surgery. The extent of the filling defects increased throughout the duration of the study. One cow dislodged the tube from the teat cistern between two and five weeks after surgery. At two weeks after surgery, this cow also had a filling defect within the tube, however at five weeks after surgery, the teat cistern was completely obliterated, and the tube was visible within the gland cistern.

Peritoneal tissue was not apparent on histologic examination of the teats. All teats had widespread granulation tissue formation with varying inflammatory response around implanted suture material.

Experiment II

While most of the ten teats with nonfenestrated tube implants remained patent, some of the tubes became obstructed by growth of granulation tissue down the sides of the tubes to their distal ends, and finally into the lumens. Histologic appearance was similar to cows of experiment one.

DISCUSSION

To serve as a suitable autograft, transplanted tissue should preserve function of the recipient site, with minimal dysfunction at the donor site. The grafted tissue may remain permanently, or may serve as a scaffold for ingrowth of tissue at the recipient site [4,5,17]. The tissue should prevent detrimental aspects of normal healing processes (ie: fibrosis and stricture formation).

Peritoneal grafts are used clinically to patch defects of the renal pelvis, ureters, and bladder in human surgery [10,17]. Peritoneal grafts have also been used experimentally to patch bulbar and palpebral conjuctival defects in rabbits [4,5], bile duct defects in dogs [11], and iliac vein [13] and aortic [16] defects in dogs. Peritoneum has also been used as an experimental material for heart valve replacement [9]. Peritoneal grafts provide a meshwork for migration of surrounding epithelial or mucosal cells [4,11,17]. They also act as a source of fibrinolytic activity. This fibrinolytic potential increases over baseline values after transplantation of peritoneal grafts to veins [13].

Despite the successful use of peritoneal grafts in the above situations, the results obtained in our circumferential teat mucosa injury model were unsatisfactory. Granulation tissue formation resulted in teat obstruction. Mesothelial cells were not identified in teats harvested two and six weeks after surgery in any of the cows. It is unknown why the peritoneum was lost after transplantation. Possible causes include ischemic necrosis, pressure necrosis, or loss of the graft due to suture

failure. Sutures were still present in the teats when they were harvested. Though the transplanted peritoneum has no direct vascular supply, it was sutured over the relatively rich submucosal vascular plexus of the teat. This vascular plexus has been sufficient for survival of oral mucosal grafts in the same location (3,18). Fenestrated tubes were selected for use in Part I to allow drainage of the potential space between the tube and the peritoneal graft. As granulation tissue grew through these fenestrations, nonfenestrated tubes were used in Experiment II, with no significant change in the final outcome. Mesothelial cells were not identified in teats and granulation tissue continued to proliferate around the tube. In some teats, this growth extended beyond the tube and obliterated the teat lumen.

Because we thought that the 3 cm circumferential defect used in Experiment I may have been too large for successful grafting, a 2 cm defect was used in Experiment II, however this also did not change the outcome.

A previous report (13) indicated that peritoneal grafts that include part of the internal rectus sheath undergo more fibrosis than those from parts of the abdomen that do not include fibrous tissue. Thus we selected a different peritoneal donor site for experiment two than in experiment one, however neither type of graft survived. While peritoneal grafts have been used successfully to patch defects in the canine bile duct (11), they are not successful if the defect is circumferential in nature (12). This suggests that peritoneal grafts may still have application in the repair of noncircumferential mucosal defects of the teat cistern, but further experiments would be needed to assess this possibility.

In conclusion, free peritoneal grafts did not prevent proliferative granulation tissue formation and teat obstruction after a circumferential teat cistern mucosal injury. Results suggest that only nonfenestrated tubes should be used in the treatment of teat cistern mucosal injury that cannot otherwise be repaired.

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SUMMARY

Circumferential 2 to 3 cm mucosal defects were created in 34 teat cisterns. Teats were treated by closure without peritoneal grafting (n=8), closure with peritoneal grafting (n=8), closure with peritoneal grafting and a fenestrated intraluminal tube (n=8), or closure with peritoneal grafting and a nonfenestrated intraluminal tube (n=10). Peritoneal grafts did not survive, nor did they provide a scaffold for ingrowth of mucosa. All teats became obstructed by granulation tissue except for some teats with nonfenestrated tube implants. Peritoneum was not a suitable autograft in this model of severe teat injury. The use of peritoneum in less severe injuries remains to be investigated.

RESUMEN

Lesiones de aproximadamente 2-3 cm de diametro fueron hechas en la mucosa de la cisterna en 34 tetas. Los diferentes tratamientos correctivos fueron: Sutura sin injerto alguno (n=8), sutura con injerto de peritineo (n=8), sutura con injerto de peritineo y ademas colocación de una cánula fenestrada en el lumen del canal (n=8) y sutura con injerto de peritineo y colocación de cánula no fenestrada en el lumen del canal (n=10). El injerto de tejido peritoneal no fué capáz de sobreviver o ayudar en el proceso de reparación en la mucosa. Todas las tetas desarrollaron tejido de granulacion causando obstrucción, con la excepción de unas pocas pertenecientes al grupo con canula no fenestrada. En este modelo de lesion severa el tejido peritoneal no resultò un autoimplante adecuado el uso de este tejido en lesiones menores esta por investigarse.

ZUSAMMENFASSUNG

Schleimhautdefekte im Umfang von 2-3 cm. wurden in 34 Zitzen-zisternen geschaffen. 8 Zitzen wurden durch Schlieszung ohne Transplantat von Peritoneum behandelt, 8 mit Transplantat von Peritoneum, 8 mit Transplantat von Peritoneum und eine Fensteranordnung mit einem Rohr, und weitere 10 mit Schlieszung und Transplantat und Rohr ohne Fensteranordnung. Die Transplantate vom Peritoneum überlebten nicht, und bildeten auch kein gerust für Schleinhautbildung. Alle Zitzen wurden von granulösem gewebe verstopft, ausgen manchen mit Röhre ohne Fensteranordnung. Das Peritoneum war ein ungeeignetes Autotransplantat in dissen Fällen von schweren Zitzen-Verletzungen. Ob das Peritoneum in Weniger schweren Fällen brauchbar ist musz noch untersucht werden.

SOMMAIRE

Les défauts muqueuses de 2-3 cm. en circonférence ont été fait dans 34 citernes des tétines. Tétines ont traité par clôture sans des greffes péritonaux (n=8), clôture avec des greffes péritonaux (n=8), clôture avec des greffes péritonaux et un tube intralumenal fenestré (n=8), ou clôture avec des greffes péritonaux et un tube intralumenal non-fenestré (n=10). Les greffes péritonaux ne sont pas vécu, et ils n'ont pas fait un échafaud pour croissance muqueuse. Toutes les tétines sont devenues obstrués par tissu de granulation sauf quelques tétines avec des tubes intralumenal non-fenestré. Péritoine n'ont été pas un autogreffe convenable dans cette modèle de blessure sévére de tétine. La utilité de péritoine pour les blessures moins sévére doit examiné.

ÜBER EINE ENZOOTISCH AUFTRETENDE AKTINOBAZILLOSE BEIM RIND

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EINLEITUNG

Aktinobazillose und Aktinomykose sind Erkrankungen, die beim Rind weltweit vorkommen und zu den seit vielen Jahren bekannten Leiden gehören. Sie können auch bei anderen Tierarten (Schwein, Schaf) und beim Menschen beobachtet werden und sind durch ein meist sporadisches Auftreten sowie durch einen chronischen Verlauf gekennzeichnet.

Ursprünglich mit dem einheitlichen Begriff als "Aktinomykose-Strahlpilzkrankheit" bezeichnet, sind ätiologisch und klinisch zwei unterschiedliche Formen zu unterscheiden (ROSENBERGER, 1978): Die Aktinomykose (auch Knochen-Aktinomykose genannt) wird vorwiegend durch das grampositive Bakterium Aktinomyces bovis hervorgerufen und befällt vorwiegend die Knochen. Besonders betroffen sind Ober- und Unterkiefer, von denen die Veränderungen jedoch nicht selten auch auf das umgebende Gewebe übergreifen (Maul- und Rachenschleimhaut, Lunge, Lymphknoten, Speicheldrüsen). Erreger der Aktinobazillose, der sog. "Weichteil-Aktinomykose" ist Aktinobacillus lignieresi. Die von ihm verursachten Veränderungen sind primär in den Lymphknoten, der Lunge, Muskulatur und Haut lokalisiert. Allerdings werden auch häufig Mischinfektionen mit beiden Erregern angetroffen (ROLLE und MAYR, 1984), ferner lassen sich vielfach weitere (Sekundär-)Keime wie Staphylococcus aureus und Corynebacterium pyogenes isolieren. Neben den beschriebenen Veränderungen können Abschwemmungen in anderen Organen (Kehlkopf, Lunge, Netzmagen, Euter, andere Weichteile) auftreten (ROSENBERGER, 1978).

In den letzten Jahren wird in Norddeutschland mit zunehmender Häufigkeit eine tumoröse Erkrankung von Rindern beobachtet, die aufgrund klinischer, morphologischer und mikrobiologischer Untersuchungen als Aktinobazillose zu diagnostizieren ist.

Die genannten Erreger sind alle obligate Bewohner der Schleimhäute des Rindes oder kommen ubiquitär vor. Die Infektion erfolgt in der Regel über kleine Wunden, die durch äußere mechanische Insulte von stechenden bzw. schneidenden Pflanzenteilen, Stroh- oder Getreidespelzen hervorgerufen werden. Bekannt als Prädilektionsstellen bzw. als primäre Eintrittspforten sind z.B. das sog. "Futterloch" der Zunge vor dem Zungenrückenwulst und die Zahnfächer (Zahnalveolen). Nach Hautläsionen sollen auch eine lymphogene und hämatogene Ausbreitung (Metastasierung) möglich sein. Die Widerstandsfähigkeit der Erreger in der Außenwelt wird in Schrifttum als gering angegeben. Eine Weitergabe der Erkrankung von Tier zu Tier erfolgt in allgemeinen nicht ("nicht kontagiöse Infektionskrankheit", ROLLE und MAYR, 1984). Die Symptome richten sich nach dem Sitz der Veränderungen und bestehen in Kaustörungen (Kieferknochen, Zunge,

Zahnalveolen), Nasenausfluß (Einbruch in Nasenhöhle) oder Atembeschwerden (Dyspnoe). Bei Befall der Zunge ragt diese aus der Mundhöhle heraus, es besteht vermehrte Salivation. Praktisch können (sekundär) alle Organe befallen werden, wodurch das klinische Bild sehr vielgestaltig wird. Das klinische Bild ist durch umschriebene, bisweilen auch diffuse Umfangsvermehrungen an den betroffenen Stellen, Knochenauftreibungen im Schädelbereich, Stenosengeräusche (Stridores) im Bereich der Atemwege, Bildung derber Knoten im Euterparenchym sowie durch ulzerativ-nekrotisierende Hautveränderungen gekennzeichnet. Die Veränderungen an der Haut bestehen in knotigen Neubildungen ("Granulome") von unterschiedlicher Form, Größe und Lokalisation. Sie können eine intakte, geschlossene Oberfläche, die in manchen Fällen Haarausfall zeigt oder eine zerklüftete, fistulös-ulzerierende Beschaffenheit aufweisen, wobei sich die Oberfläche pilz- bis blumenkohlartig über die Umgebung erhebt. Je nach der Lage sind regionale Lymphknoten und umgebendes Gewebe sowie die darunter liegenden Gewebe (Unterhaut, Fascien, Muskulatur) beteiligt. Fieber wird nur bei sekundärer bakterieller Eiterung beobachtet. Der Verlauf ist chronisch über Wochen oder Monate, Spontanheilungen kommen kaum vor.

Pathologisch-anatomisch sind die Veränderungen an den Knochen (bes. Kiefer) durch starke Auftreibungen gekennzeichnet, die nach innen oder außen aufbrechen können und von Höhlen durchsetzt sind, in denen sich ein weich-sulziges Gewebe befindet. Aus den granulomatös-eitrigen Prozessen entleert sich ein blutig-eitriges Sekret, das auch eintrocknen und Krusten bilden kann. Beim Sondieren wird oft Fistelbildung festgestellt. Im Inneren finden sich häufig Körnchen oder Granula von 1-2 mm Durchmesser, die als Drusen bezeichnet werden. Diese stecknadelkopfgroßen Gebilde sind weiß, gelblich, grau oder braun und bestehen bei mikroskopischer Untersuchung aus Bakterienfäden und einem peripheren Kranz kolbenförmiger Gebilde. Sie können zur Sicherung der Diagnose dienen (DAHME und WEISS, 1983).

Die Diagnose wird meistens aufgrund der klinischen Veränderungen und durch die histologische Untersuchung (Nachweis der Drusen) gestellt, während der direkte Erregernachweis nur selten gelingt.

Die Prognose der (Knochen-)Aktinomykose ist in fortgeschrittenen Fällen ungünstig. Die Aktinobazillose hat, insbesondere bei frühzeitigem Behandlungsbeginn, eine günstigere Prognose.

Die Therapie besteht primär in der weitestmöglichen Entfernung (Totalexstirpation) der chronisch-proliferativen Prozesse. Da die Erreger gut empfindlich gegenüber Antibiotika, Sulfonamiden und Jod sind, hat sich eine medikamentöse Kombinationstherapie eingebürgert, bei der zusätzlich zur chirurgischen Behandlung (DIETZ, 1958, SCHLEITER und FEDERWISCH, 1961) dreimal im Abstand von 5 Tagen intravenös Jodlösungen (1 g Jod, 12 g Kaliumjodid, 18 g Natriumjodid in 100 ml aqua dest.; davon 15 ml pro kg/KGW (verdünnt mit je 75 ml aqua dest.) und an den behandlungsfreien 4 Tagen jeweils Antibiotika (Penicillin 2 - 3 Mio. IE, Streptomycin 3 - 5 g, Tetrazyklin 5 g) intramuskulär oder subkutan appliziert werden. Lokal wird die Operationswunde ebenfalls vor Verschluß jodiert (Jodtinktur, Lugolsche Lösung). Häufig entwickelt sich im Anschluß an die Jodtherapie ein stark schuppendes (Jod)Exanthem, das in der Regel auch ohne weitere Behandlung wieder abklingt (SATTLER, 1968, REBHUHN und Mitarb.,

1988). Auch Aborte sind als Folge der Jodtherapie möglich. Eine wirksame Prophylaxe gegen das Abstoßen der Frucht ist dabei nicht möglich bzw. bekannt.

Während Aktinomykose und -bazillose beim Rind überwiegend als sporadische Erkrankungen auftreten und eine Kontagiosität weitgehend vermissen lassen, gibt es vereinzelte Hinweise auf bestandweise gehäuftes Auftreten. SAVEY und Mitarb. (1988) beschreiben eine Stall-Enzootie in Frankreich, bei der innerhalb von drei Jahren insgesamt 85 Mastbullen eines 600 Tiere umfassenden Bestandes an respiratorischen Störungen (Knotenbildung in Trachea oder Lunge) erkranken, starben oder geschlachtet werden mußten und als deren Erreger A. lignieresi erkannt wurde. Nach 3 Jahren blieben weitere Fälle spontan aus. Auf atypische Lokalisationsformen der Aktinobazillose weist SATTLER (1968) hin. Besonders Kniefaltenlymphknotenbereich ein- oder beidseitig, Haut einschließlich Haut der Gliedmaßen sowie der Bereich von Kniekehllymphknoten, Buglymphknoten und Haubenwand können Sitz derartiger Veränderungen sein. Beim Schwein ist ein enzootisches Vorkommen seit langem bekannt.

In eigenen Untersuchungen von enzootischer Aktinobazillose in Rinderbeständen Norddeutschlands wurden Epidemiologie, Ätiologie, klinische Erscheinungen, Diagnose und therapeutische Ansprechbarkeit der Veränderungen überprüft und sollen nachfolgend dargelegt werden.

EIGENE UNTERSUCHUNGEN

In den zurückliegenden Jahren wurden häufig Rinder vorgestellt oder in die Klinik eingewiesen, die an tumorösen, teils isolierten, teils multiplen, vielfach auch ulzerierenden Neubildungen im Bereich der Haut oder Unterhaut litten. Laut Anamnese wurde die Erkrankung vielfach bereits seit längerer Zeit oder an mehreren Tieren gleichzeitig im Bestand beobachtet. Ausgehend von derartig erkrankten Einzeltieren wurden daraufhin Bestandsbegehungen in den betroffenen Betrieben durchgeführt und Erhebungen über Dauer des Bestehens dieses Bestandsproblemes, Zahl der betroffenen Tiere, Verlauf der Erkrankung sowie über mögliche Ursachen vorgenommen. Die erkrankten Tiere wurden alle klinisch eingehend untersucht. Bei den stationär in die Klinik eingestellten Patienten wurden darüber hinaus wiederholt Blutuntersuchungen vorgenommen, Gewebeproben entnommen und untersucht* sowie Sekretproben (Eiter) gewonnen und auf ihren Keimgehalt analysiert.

Die Untersuchungen erstreckten sich auf 20 in die Klinik eingestellte und weiterhin auf insgesamt 85 aus 8 betroffenen Betrieben. In den betroffenen Beständen wurden in Gegenwart der Hoftierärzte** und der Tierbesitzer wiederholt Betriebsbegehungen vorgenommen, wobei die Bestandsanamnese erhoben, die Art der Fütterung, das zeitliche Erstauftreten (Weide/Stall, Jahreszeit) und die Art des Weidebewuchses überprüft sowie andere Auffälligkeiten im Betrieb registriert wurden.

*Die pathologisch-anatomischen und -histologischen Untersuchungen wurden im Veterinär-Pathologischen Institut und im Arbeitsbereich Pathologie der Tierärztlichen Ambulanz Schwarzenbek der Freien Universität Berlin vorgenommen, wofür wir herzlich danken.
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ERGEBNISSE

Insgesamt erstreckten sich die Untersuchungen auf 8 Betriebe, in denen es zu gehäuftem Auftreten von Aktinomykose (6 Betriebe) oder zu Erkrankungen nach (Weide-)Kontakt mit Tieren dieser Bestände (2 Betriebe) gekommen war. Eine Aufteilung der Erkrankungen auf die Jahre 1985 bis 1990 zeigt Tabelle 1. Für eine eingehende klinische Untersuchung standen 20 Tiere zur Verfügung. Signalement, Ausbreitung der Veränderungen am Tier, Art der angetroffenen Veränderungen sowie Art und Erfolg der Therapie können Tabelle 2 entnommen werden.

Die klinische Untersuchung der zur stationären Behandlung eingewiesenen 20 Rinder erbrachte in 11 Fällen eine Lokalisation der Veränderungen ausschließlich im Kopfbereich, überwiegend in der Gegend des Musculus masetericus. Bei drei Tieren waren die Neubildungen im Bereich der Schulter, bei Tieren an den Extremitäten zu finden. Zwei Rinder wiesen eine multiple Ausbreitung der Tumore einschließlich einzelner Organe (Sektionsbefund) auf (Tabelle 3). In 5 Fällen wurde auf eine Entnahme von Proben zu diagnostischen Zwecken verzichtet, weil der Befund eindeutig, bei einem gleichzeitig eingewiesenen weiteren Tier positiv oder die Veränderung dafür nicht geeignet war. Von 15 Tieren wurden dagegen Gewebeproben und Eiter bzw. Zelldetritus, einmal auch ein Punktat gewonnen und histologisch bzw. bakteriologisch untersucht. Das Allgemeinbefinden der Patienten war dabei ungestört oder nur geringgradig beeinträchtigt, und die klinische Untersuchung erbrachte nahezu ausnahmslos keine Hinweise auf anderweitige Organerkrankungen. In nahezu allen Fällen waren Körperlymphknoten beteiligt, wobei nicht geklärt werden konnte, ob es sich um primäre (Ausgangspunkt der Erkrankung) oder sekundäre Veränderungen handelte.

Die hämatologischen Untersuchungen, in denen das zelluläre Blutbild einschließlich Differentialblutbild, Hämatokrit, Hämoglobingehalt, Zahl der Erythrozyten und Leukozyten, ferner der Gehalt von GOT=AST, Gesamtbilirubin und Harnstoffgehalt im Serum, von Fall zu Fall auch Serumelektrolyte und weitere Parameter bestimmt wurden, meist wiederholt in zweiwöchigen Abständen, erbrachten keine charakteristischen Befunde.

Die pathologisch-histologischen Untersuchungen der Biopsien oder Punktate erbrachte 14mal das Vorliegen einer chronisch-eitrigen Entzündung, in der achtmal Drusen, die für das Vorliegen von Aktinobazillose sprachen, nachgewiesen werden konnten.

Mikrobiologische Untersuchungen*** wurden in 11 Fällen eingeleitet, der sichere Nachweis von Aktinobazillus lignieresi gelang nur einmal. Aufgrund aller vorliegenden Befunde wurde bei den 20 erkrankten Klinikpatienten 15mal die Diagnose "Aktinobazillose",

***Die mikrobiologischen Untersuchungen wurden dankenswerter Weise im Landesuntersuchungsinstitut für Lebensmittel, Arzneimittel und Tierseuchen (LAT) Berlin sowie im Institut für Hygiene und Infektionskrankheiten der Tiere der Justus-Liebig-Universität Gießen vorgenommen.

5mal "Aktinomykose" und zweimal keine eindeutige Diagnose gestellt (Tabelle 3).

Behandlung der Einzelerkrankungen

Bei der Therapie kann zum einen zwischen individueller Behandlung der erkrankten, in die Klinik eingestellten Tiere und der Bekämpfung der Erkrankung im Bestand unterschieden werden. Zum anderen ist zwischen operativen und chemotherapeutischen Maßnahmen zu trennen. Bei der Behandlung der erkrankten Tiere in der Klinik wurde immer versucht, die angetroffenen Veränderungen operativ zu entfernen. Zusätzlich wurden alle Tiere, die chirurgisch behandelt wurden, der Jodtherapie in der bei ROSENBERGER (1978) beschriebenen Weise unterzogen (siehe Einleitung). 14 Tiere wurden auf diese Art behandelt, die übrigen 11 erhielten ausschließlich eine chemotherapeutische Behandlung (Tabelle 2). Auf diese Weise konnten 16 von 20 Tieren geheilt werden, drei wurden wegen Unheilbarkeit geschlachtet, eines getötet. Eine nachträgliche Befragung der Tierbesitzer ergab nur bei einem der 15 geheilten Rinder ein Rezidiv, das anschließend erneut mittels Chemotherapie behandelt und geheilt wurde.

Bekämpfung der Bestandsenzootien

Die Untersuchungen in den betroffenen Beständen ergaben eine unterschiedliche Dauer des Bestehens der Erkrankung in den Betrieben, eine unterschiedliche Befallstärke und einen uneinheitlichen Verlauf (Tabelle 1). Auch hinsichtlich des Beginns der Erkrankung (Stall/Weide, Jahreszeit) bestanden keine verwertbaren aussagekräftigen Übereinstimmungen. Neuerkrankungen kamen vorwiegend im Spätherbst auf der Weide, aber auch während der Stallhaltungsperiode vor. Auffallend waren wiederholte Hinweise auf einen Weidebewuchs mit Sauergrasern und auf Überschwemmungsweiden. Die Stallhaltung bestand überwiegend aus Laufstallhaltung mit Liegeboxen. Für eine auffällige Häufung von Verletzungen gab es keine Hinweise, sie konnten jedoch auch nicht ausgeschlossen werden. In keiner der betroffenen Herde lag eine Begrenzung der Veränderungen auf eine bestimmte Lokalisation vor.

Die Bekämpfung des Bestandsproblems erfolgte auf dreierlei Weise:

1. Einweisung erkrankter Tiere zur Behandlung in die Klinik oder gleichartige Behandlung durch den Hoftierarzt
2. Schlachtung schwerstkranker Tiere, bei denen aufgrund der multiplen oder tiefgreifenden Veränderungen eine Behandlung nicht angebracht erschien.
3. Nachbehandlung der Tiergruppe sowie Behandlung leicht erkrankter Tiere mittels oraler Jodtherapie. Zu diesem Zwecke erhielten Einzeltiere oder alle Tiere einer Gruppe an 14 aufeinanderfolgenden Tagen jeweils 5-10g Jodkalium (Fa. Merck, Darmstadt) über das Kraftfutter

BESPRECHUNG DER ERGEBNISSE

Entgegen der weitverbreiteten Auffassung, daß es sich bei Aktinobazillose und Aktinomykose des Rindes um sporadisch auftretende, nicht kontagiöse Infektionskrankheiten handelt, gibt es vereinzelt Hinweise darauf, daß auch ein bestandweise gehäuftes Vorkommen der

Erkrankung (enzootische Erkrankung) möglich ist (SAVEY und Mitarb. 1988).

Die eigenen Untersuchungen in rinderhaltenden Beständen Schleswig-Holsteins (Norddeutschland) bestätigen diese Hinweise, indem bis zu 28 Erkrankungsfälle in einem Betrieb (auf 5 Jahre verteilt) bzw. bis zu neun Erkrankungen pro Jahr beobachtet werden konnten. Dabei gelang es nicht, Hinweise auf den Infektionsmodus und den Ausbreitungsweg zu gewinnen. Aufgrund der häufigen Mitbeteiligung von Körperlymphknoten muß jedoch eine lymphogene Ausbreitung im Tier nach vorheriger Mikroläsion vermutet werden. Bei frühzeitigem Erkennen der Erkrankung beim Einzeltier und sofortiger Behandlung ist die Prognose der Therapie günstig. Sie besteht in einer operativen Exstirpation ulzeröser bzw. tumoröser Veränderungen und einer mehrmaligen intravenösen Behandlung mit Jodlösungen in 4-5tägigen Intervallen und zwischenzeitlichen Antibiotikagaben. Zur Bestandssanierung ist die konsequente Entfernung aller erkrankten Tiere erforderlich. Während schwer und multipel erkrankte Rinder geschlachtet werden sollten, können in allen übrigen Fällen Einzelbehandlungen, möglichst nach vorheriger Separierung der betreffenden Tiere, erfolgen. Zusätzlich kann die Tiergruppe, in der Erkrankungsfälle aufgetreten sind, einer mehrtätigen oralen Jodkalium-Therapie unterzogen werden. Auf Stall- oder Weidewechsel bzw. andere hygienische Maßnahmen wurde in den hier beobachteten Betrieben verzichtet. Mit Ausnahme eines Rezidivs wird die Erkrankung in den von uns betreuten Betrieben derzeit als erloschen betrachtet.

ZUSAMMENFASSUNG

In 8 Betrieben Norddeutschlands wurde in den Jahren 1985 - 1989 ein gehäuftes Auftreten von Aktinobazillose beobachtet, wobei bis zu 28 Tiere pro Betrieb bzw. 9 pro Jahr erkrankten. Ursache und Infektionsmodus konnten nicht geklärt werden. Durch konsequente Behandlung der erkrankten Tiere und eine mehrtätige orale Zufütterung von Kaliumjodid (KJ) konnte die Enzootie gestoppt werden.

SUMMARY

On 8 farms in Northern Germany an increased occurence of actinobacillosis was observed during the years 1985-1989, whereby up to 28 animals per farm or 9 per year respectively got sick. The cause and an mode of infection could not be determined. By consequent treatment of the diseased animals and oral application of potassium iodide (PI) over several days the outbreak could be stopped.

RESUMO

Entre 1985 e 1989 se observou um aumento da incidência de actinobacilose bovina en oito campos de criaçôs no norte da Alemanha, nos quais até 28 animais por campo au 9 animais ao ans contraíram a enfermidade. A causa e via de transmissão não puderam ser esclarecidas. Através de manejo adequado dos animais afetados e tratamento oral com iodeto de potássio a enzootia póde ser controlada.

RESUMEN

Durante los años 1985 - 1989 se observo una mayor frecuencia de la actinobacilosis de bóvinos en ocho predios agricola-ganaderos de la región del norte de Alemania en los cuales hasta 28 animales por campo o 9 animales al año contrajeron la enfermedad. La causa y la vía de transmisión no pudo ser aclarada. Por medio de tratamiento adecuado de los animales afectados y tratamiento oral diario frecuente de yoduro de potasio, la enzootia pudo ser controlada.

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Tabelle 1 Erkrankungshäufigkeit in 8 Betrieben mit enzootischer Aktinobazillose der Rinder

Betrieb	Zahl der Rinder im Betrieb	erste Krankheitserscheinung	Zahl der untersuchten Tiere im Bestand insg.	davon Klinikpatienten	Aufteilung der Erkrankungen auf einzelne Jahre 1985	1986	1987	1988	1989	1990	total
I*	90	1984	6	3	24	5	6	7	6	1	29
II*	110	1984	1		+	+	+	+	3	+	3
III	150	1987	1		+	+	3	15	1	+	19
IV	70	1989	9	2	+	+	+	+	9***	+	9
V	180	1987	9	7	+	+	+	+	9	+	9
VI**	64	1989	1		+	+	+	+	1	+	1
VII**	48	1989	3		+	+	+	+	3	+	3
VIII****	250	1987	5	3*	+	+	4	6	5	3	16
8		total	37	15	24	5	13	28	39	4	91

Anmerkungen:
* Tiere der Betriebe I und II weiden gemeinsam
** Tiere der Betriebe VI und VII benutzen benachbarte Weiden
*** ein behandeltes Tier rezidivierte
**** Auswertung nicht abgeschlossen

Tabelle 2: Übersicht über die untersuchten Rinderpatienten. Wiedergabe von Signalement, Art und Ausbreitung der Veränderungen, Therapie und Therapieerfolg.

Tier/ Betrieb	Signalement m./w. Alter	Befund: Ausbreitung der Veränderungen am Tier isoliert	Befund: multipel	Therapie: Jod i.v.	Therapie: Operation	Erfolg: geheilt	Erfolg: geschl.	Erfolg: getötet
I	DSB, m, 1 Jahr	x		x	Teilresektion	x		
2	DSB, w, 1 3/4 Jahre	x		x	keine	x		
3	DSB, w, 3 1/2 Jahre	x		-			x	
4	DRB, w, 3 Monate	x		-	Gewebe abgetragen	x		
5	DRB, w, 1 1/2 Jahre	x		x		x		
6	DRB, w, 5 Jahre	x		x		x		
7	DRB, m, 2 Jahre	x		x			x	
8	DSB, w, 1 1/2 Jahre	x		x	Gewebeprobe	x		
I	DSB, w, 1 3/4 Jahre	x		x	Gewebeprobe	x		
I	DSB, w, 1 3/4 Jahre	x		x	Exstirpation	x		
I	DSB, w, 2 Jahre		x	x	Exstirpation	x		
V	DRB, w, 2 Jahre		x	x	Exstirpation	x		
V	DRB, w, 2 Jahre		x	x	Gew.p./Exst.	x		
IV	DSB, w, 2 Jahre		x	x	Gewebeprobe		x	
IV	DSB, w, 2 Jahre	x		x		x		
V	DRB, w, 2 Jahre		x		Gewebeprobe			x
V	DRB, w, 1 1/3 Jahre	x		x	Exstirpation	x		
V	DRB, w, 1 1/3 Jahre		x	x	Exstirpation	x		
V	DRB, w, 1 1/3 Jahre		x	x	Exstirpation	x		
V	DRB, w, 1 1/3 Jahre		x	x	Exstirpation	x		

Anmerkung: 1-8 = Einzeltiere
I-V = Tiere aus Problembeständen

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Tabelle 3: Wiedergabe der Lokalisation der angetroffenen Veränderungen sowie der pathologisch-histologischen und mikrobiologischen Befunde

Patienten lfd. Nr.	Lokalisation: Masseter-bereich	Lokalisation: Schulter-bereich	Lokalisation: Hinterglied-maße	histologisch chronisch eitrige Entzündung	Drusen	Diagnose: Actino-bacillose	Diagnose: Actino-mykose
1	x			x	x	x	
2	x					x	
3	x			x			x
4	x			x		x	
5	x					x	
6	x						x
7	x						x
8	x			x	x	x	x
9	x				x	x	
10		x		x	x	x	
11	x			x	x		x
12		x		x	x	x	
13			x			x	
14	Organe			x		V	
15	x	x	x	x	x	x	
16			x	x	x	x	
17			x	x		x	
18			x	x		x	
19			x	x		x	
20			x	x		x	
						x	
						x	

Anmerkung: 1 - 8 = Einzeltiere
9 - 20 = Tiere aus Problembeständen
V = Verdachtsdiagnose

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DIE BRÜCKENLAPPENPLASTIK ALS BEHANDLUNGSMETHODE EINER ZIRKULÄREN ZITZENSCHÄLWUNDE EINER KUH

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EINLEITUNG

Zitzenverletzungen sind beim kurativ tätigen Tierarzt ein sehr häufiger Anlaß, chirurgisch tätig zu werden. Für alle Wunden, bei denen genügend Haut zur Verfügung steht, werden verschiedene Nahttechniken zum Wundverschluß beschrieben.

Bei umfangreichen Hautdefekten an der Zitze ist oft eine einfache Naht nicht oder nur schwer möglich. Eine langwierige Heilung per secundam intentionem oder eine Amputation der Zitze ist unumgänglich.

Groote (1979) teilt die Zitzenwunden in verschiedene Kategorien

1. weniger komplizierte, nicht perforierende Wunden (60,2 % der Fälle).
2. a) komplizierte, nicht perforierende Wunden
   b) perforierende Wunden. (39,8 % der Fälle).

In 30,6 % der Fälle kamen Zitzenlappen und Triangelwunden sowie unregelmäßige Wunden zur Operation.

Die verzögerte Wundheilung kam nach Groote (1979) besonders oft bei nicht perforierenden Lappenwunden vor.

Auch Wigger und Martig (1985) stellten fest, daß Schälwunden signifikant schlechter heilten als Schnittwunden. Besonders hoch war in jener Gruppe der Anteil an Sekundärheilungen.

Beuche, et. al. (1986) begründeten die hohe Anzahl prognostisch ungünstig zu beurteilender Lappen- und Schälwunden, Zitzenquetschwunden und partieller oder totaler Zitzenabrisse im Überwiegen der Trittverletzungen. 21,3 % waren in ihren Untersuchungen Zitzenschälwunden.

Nach ihrer Beurteilung ist die Hauttransplantation vor allem bei größeren Schälwunden arbeitsaufwendig, erfordert intensive Nachbehandlung über längere Zeit und führt zu keiner wesentlichen Beschleunigung des Epithelisierungsprozesses. Sie habe daher in der Praxis kaum Eingang gefunden.

Bei Berücksichtigung anderer Techniken der plastischen Chirurgie kommt man zu einer wesentlich günstigeren Beurteilung der Heilungsaussicht per primam.

Die in der Humanmedizin verwendete Methode der Muffplastik oder der Stiel- bzw. Brückenlappenplastik am Rumpf zur Deckung von ausgedehnten Hautdefekten an der Hand kann ideal zur Defektdeckung an der Zitze angewendet werden. (Zoltan 1976).

Die Operationstechnik soll an der Zitze einer Kuh beschrieben werden.

FALLBERICHT

Patient

6 Jahre alte Schwarzbuntkuh "Adele", 450 kg schwer, 1 Woche nach der Geburt, Milchleistung ca. 25 l täglich. Der Milchentzug erfolgte maschinell. Die Geburt verlief normal, das Kalb zeigte sich gesund.

Vorbericht

Verletzung auf der Weide durch Stacheldraht.

Klinischer Befund

Allgemeinverhalten: ruhig, teilnahmslos. Schleimhäute: rosarot. Pulsfrequenz: 70/min. Atemfrequenz: 40/min. Innere Körpertemperatur: 39,0°C. Status chirurgicus.

Die rechte vordere Zitze wies eine zirkuläre Schälwunde auf. Der Hautdefekt erstreckte sich von der Zitzenbasis bis zur Zitzenspitze. Nur eine schmale Hautbrücke von ca. 5 mm blieb als Kontakt zwischen Basis und Spitze, welche in einer Größe eines 5-Groschen Stückes unversehrt geblieben war.

Diagnose

Nicht perforierende, nahezu zirkuläre Zitzenschälwunde rechts vorne.

Operation

- Analgesie und Sedierung: 500 mg Rompun (R) i.v., 20 ml Hostacain (R) an der Zitzenbasis entlang des Wundrandes.
- Reinigung und Desinfektion des Wundgebietes mit Polyvinyljodid.
- Auf Esmarchsche Blutleere wurde wegen des Umfanges des Defektes verzichtet.
- Wundtoilette, Auffrischen der Wundränder.
- Präparation von 2 Hautlappen an der Basis des Euters in der intakten Haut neben der Zitze.
- Aufklappen der Hautlappen.
- Einlegen der Zitze in die frisch geschaffene Wunde.
- Vernähen der Hautlappen mit Vicryl 2/o mit eingeschweißter scharfer Nadel mit einfachen Knopfnähten.
- Trockenstellen des Viertels mit 1 Mill. I.E. Benzylpenicillin-Procain und 1g Dihydrostreptomycin.
- Entfernen der Nähte nach 10 Tagen.
- 10 Tage nach Entfernen der Nähte Rückoperation:
- Präparation von 2 Hautlappen seitlich der reaktionslos eingewachsenen Zitze.
- Herunterklappen der Zitze, wobei besondere Bedeutung der Präparation der Zitzenbasis zukommt, um einen schiefen Stand der Zitze zu vermeiden, ohne die Zisterne zu eröffnen.
- Vernähen der abpräparierten Lappen an der Außenseite der Zitze.
- Schließung des Defektes an der Zitzenbasis.
- Entfernen der Nähte nach 10 Tagen.

Nachbehandlung

Die Wunde wurde täglich mit Polyvinyljodid gewaschen. Ein Euterschutznetz mit feuchter Kompresse sollte weitere Verletzungen und Verschmutzung verhindern. 20 Tage nach der Rückoperation wurde ein Milchkatheter 2 Tage lang eingesetzt und mit dem Milchentzug zuerst 3 Tage händisch, dann maschinell begonnen. Eine verminderte Milchleistung für diese Laktation wurde in Kauf genommen. Während der gesamten Behandlungsdauer konnten weder ein Auftreten von Mastitis noch Störungen im Milchabfluß oder Inkontinenz beobachtet werden.

Epikrise

Die beschriebene Methode scheint in der Praxis mit gutem Erfolg anwendbar zu sein. Beim vorliegenden Fall waren insgesamt 5 Hausbesuche

notwendig. Begünstigend für den guten Heilungsverlauf erwies sich außerdem der Umstand, daß Zisterne und Strichkanal bei der Verletzung unversehrt geblieben waren.

Als ein Vorteil der Methode kann auch angesehen werden, daß die eingenähte Zitze gegen schädigende Einflüsse aus der Umgebung weitgehend unempfindlich ist.

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ZUSAMMENFASSUNG

Bei einer Kuh wurde nach einer Zitzenverletzung die Schälwunde durch eine Hautplastik versorgt. Die verletzte Zitze wurde an die Euterbasis angelegt und in zwei dort abpräparierten Hautlappen eingenäht. Nach dem Anwachsen der Euterhaut an der Zitze erfolgte 10 Tage nach dem Entfernen der Nähte die Rückoperation und Deckung der Zitzenwunde mit Euterhaut. 30 Tage nach der Verletzung war die Heilung per primam abgeschlossen und die Melkfähigkeit wieder hergestellt.

SUMMARY

The transfer of cutanécus flaps as a method of treating a peeling wound injury of a teat of a cow.

A description of de surgical treatment of a peeling-injury at a cow's teat is given. As peeling wounds treated with conservative methods have a bad prognosis, in this case a tunnel plastic was used to protect the wound lesion under the skin of the udder by bending the teat upwards. In no stage of the treatment or afterwards the cow showed either a mastitis or an incontinence of the milk flow. A decrease in the milk production for the current milking period had to be accepted. Healing per priman could be obtained within 30 days after accident. This method seems to be a successful one in similar cases.

RESUMEN

Después de la lesión de una teta de una vaca la herida pelade fue curada por medio de una dermatoplastia. La teta herida fue doblada hacia la base de la ubre y ahí fijada por sutura dentro de dos colgajos de piel, que habían sido preparadas anteriormente. Después de pegarse la piel de la ubre a la teta, tras 10 días se sacaron los hilos de la sutura y se realizó la reoperación y la tapadura de la lesión de la teta con piel de la ubre. 30 días después de la lesión la cuaración estaba terminada y la capacidad para ordeñar restablecida.

RÉSUMÉ

Aprés une blessure au trayon chez une vache, la plaie qui pelait a été soignée au moyen d'une dermatoplastie. Le trayon blessé a été placé contre la base du pis et cousu dans deux lambeaux de peau qui y avaient été préparés. Aprés que la peau du pis a repoussé sur le trayon, dix jours aprés l'enlévement des points de suture a eu lieu la deuxiéme opération et le recouvrement de la plaie au trayon avec la peau du pis. Trento jours aprés la blessure la guérison per primam était terminée et la capacité de traite était restaurée.

Abb. 1: Zustand vor der Operation.

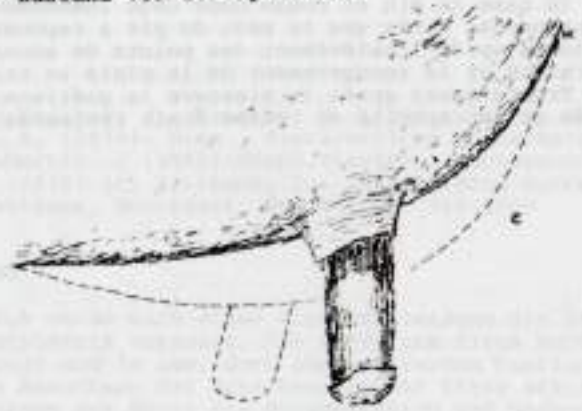


Abb. 2: Präparation der Hautlappen an der Euterbasis.

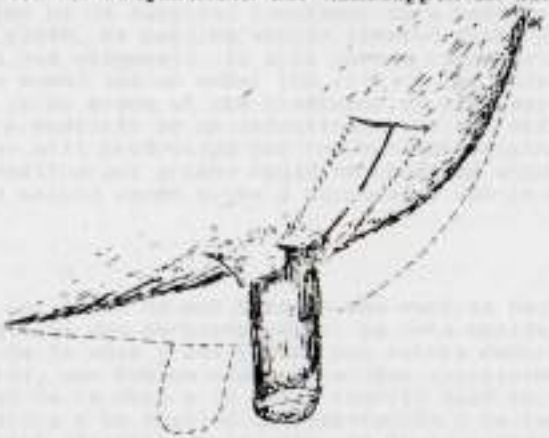


Abb. 3: Einlegen der Zitze in die frisch geschaffene Wunde.

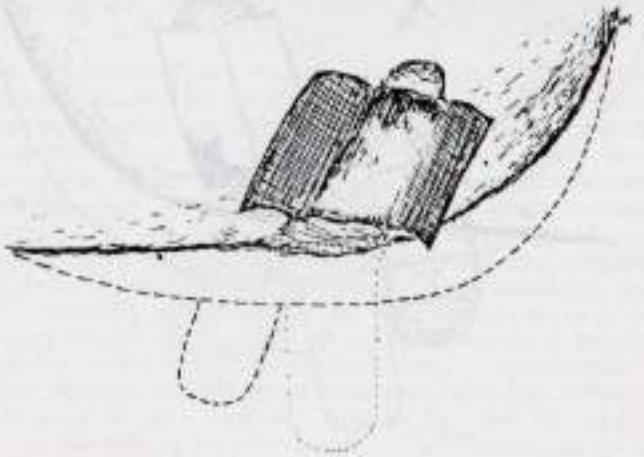


Abb. 4: Vernähen der Brückenlappenplastik.

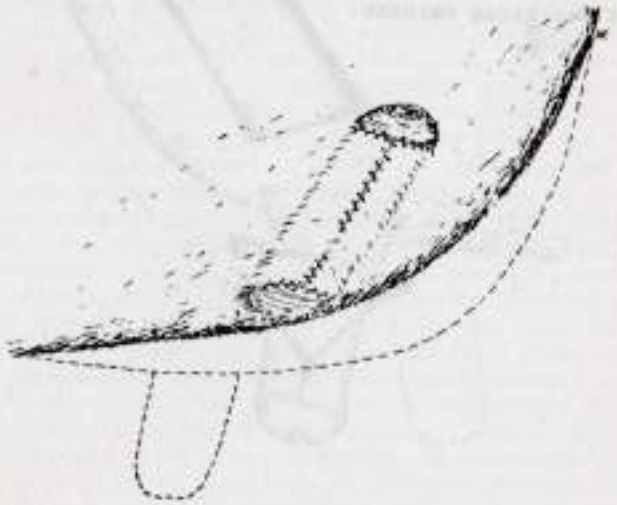


Abb. 5: Rückoperation

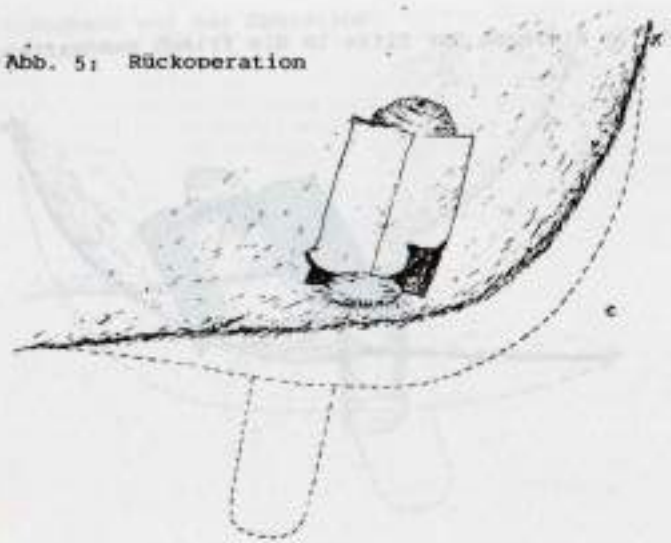


Abb. 6 Die transplantierte Haut wird auf der Zitze vernäht.

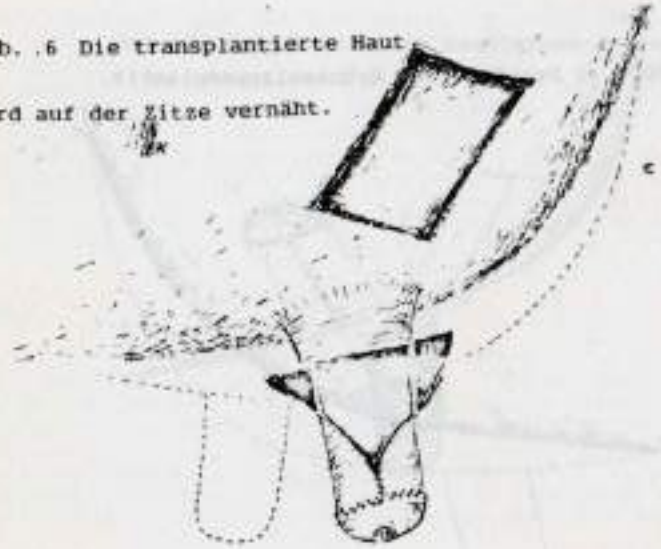
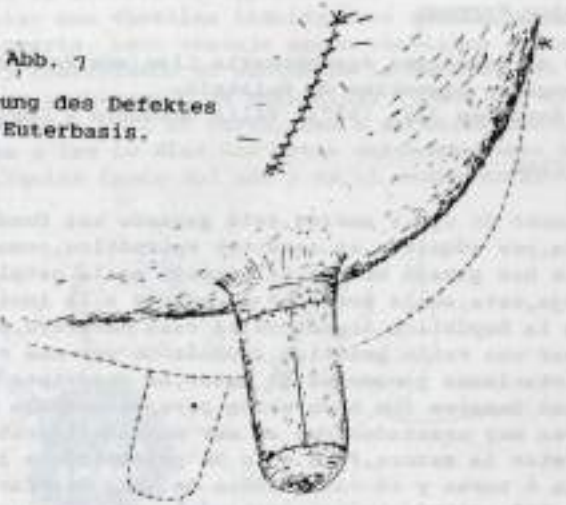


Abb. 7

Schließung des Defektes an der Euterbasis.



AUTOIMGERTO DE PIEL EN CARCINOMA DE OJO EN BOVINOS

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INTRODUCCION

El cancer de ojo y anexos,está ganando una fundamental importancia económica,por adquirir un caracter epizoótico,como las querato-conjuntivitis,que han ganado un amplio espacio en la patplogía animal.En el cancer de ojo,esta,suele producir la muerte o la inutilidad comercial del mismo.En la República Argentina,la raza Hereford,es muy propensa a sufrirla,por una razón genetica, además de ser una raza muy difundida en las explotaciones ganaderas.El autor,ha descripto una tecnica operatoria para estos tumores con todo exito,pero encontraba muchas dificultutades en tumores muy avanzados,que al ser muy proliferativos,faltaba piel para concretar la sutura.Para ello he practicado a la fecha,46 intervenciones;en 6 toros y 40 vacas todas de raza Hereford,utilizando un colgajo de piel colindante e ingertandolo en la solucion abierta en el lugar de extirpación del tumor.Este autoingerto,se efectúa sin ningun requisito especial, se hace en el medio rural, y el colgajo prende con toda facilidad y con un resultados que aseguran el exito.

MATERIALES Y METODOS

He operado 46 vacunos con tumores de ojos y anexos,con diagnostico reservado por la proliferación del mismo,donde utilizo la tecnica que ya he descripto anteriormente,el problema se presentaba cuando el tumor era muy grande.Para ello utilizo instrumental comun a estos tipos de cirugía cuidadosamente desinfectados, y se procede a la preparación del animal,sedando con clorhidrato de xilazina al 2%,a razon 1 cc. p/kg.v. procurando una buena sujeción,ya que generalmente se efectúa en el medio rural.Con el animal en decúbito lateral,efectuamos una muy buena desifección de la zona,especialmente en donde extraeré el colgajo,donde se debe depilar con suma precausión.No utilizo anestesia general.si,anestesia local por infiltración en el perimetro del colgajo,siendo suficiente para efectuar la intervención.(fig.1.).Extirpo el tumor de acuerdoa la tecnica del autor.Extirpado el tumor,efectúo dieresis con bisturie del area delimitada para utilizar el ingerto (fig.2).Una vez que he separadola piel que queda con un colgajo dependiente,embebo la submucosa o cara inferior del ingerto con una solución yodada.Cabe destacar,que la separacion de la piel se efectua por divulsión en el subcutaneo.Luego efectuo la confrontación del colgajo con la herida y se procede ala sutura no muy apretada,con hilo no absorvible, y con puntos simples.-El espacio abierto,que queda del colgajo,se sutura con puntos simples o puntos en U ,indistintamente.El colgajo dependiente,no se corta, se puede efectuar,una vez prendido el ingerto,ya que el nos mantiene

la irrigación necesaria,para los primeros días,en que se orgmniza el ingerto (fig.5).Finalizada la intervención,procuramos un buen vendaje,donde es conveniente pincelar con vaselina liquida para que la venda no se adhiera en demasia al ingerto, este vendaje puede cambiarse a los 3 o 4 días,evitando miasis y controlando el estado de la herida.Como tratamiento medico,inyecto una combinación de penicilina (5.000.000 U.I.), estreptomicina 2grs.repetidas cada 48 horas,unas 4 aplicaciones.Los puntos de sutura se retiran a los 10 días.Con estos cuidados, esta tecnica puede efectuarse en cualquier época del año y en el medio rural.-

Fig.1

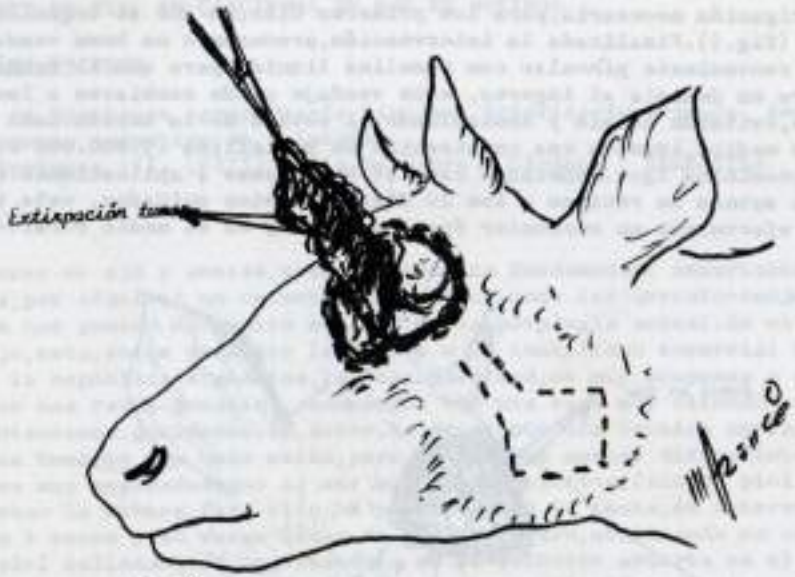

Fig. 2

Fig.3

Fig.5 Final

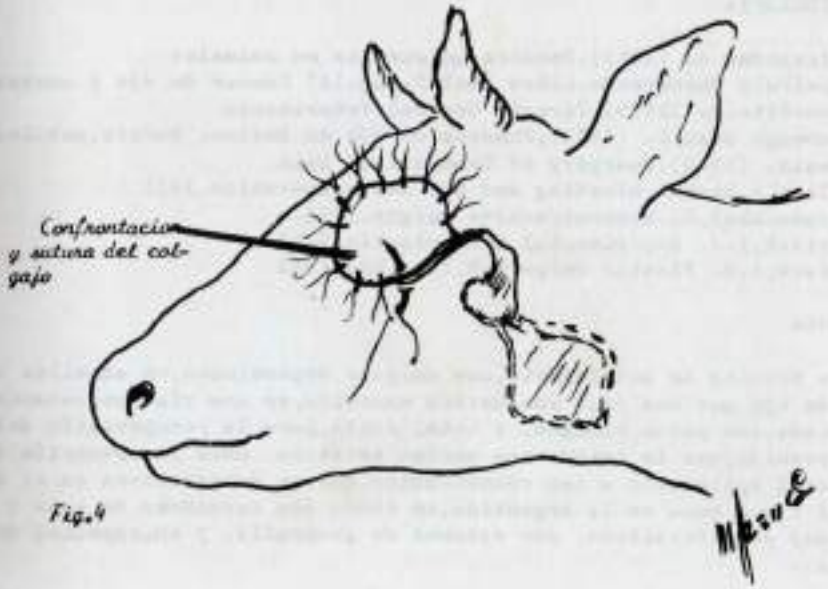

Fig.4

RESULTADOS

En los 46 vacunos operados,hemos comprobado la bondad del autoingerto,al prender sin ninguna dificultad,y que abre un camino casi nuevo enla curugía rural,en grandes animales,y en especial la efectuada en los canceres de ojo exuberantes.El resultado es muy optimista,ya que pudimos efectuar el seguimento a todos los operados,con un 100% de efectividad,y que hasta la fecha no ha habido problemas de recidivas,lógico suponer que el exito depende en mayor caso, en la extirpación total del tumor,sin dejar restos tumorados ni necrosados.No hemos comprobado infartos ganglionares,donde los parotídeos o submaxilares son los elegidos para la metástasis de estos tumores.Hay que tener en cuenta, que al cuarto días, cuando se efectua el cambio de vendaje,suele haber un olor,que nos hace pensar de inmediato,en una necrosis del ingerto, y en realidad se debe a la sangre que queda acumulada en el seno orbicular y que lógicamente se descompone. Tambien se puede apreciar un resecamiento del ingerto,cuando esto ocurre unto con cualquier pasta antibiotica y me despreocupo,ya que las retraciones son pasajeras y se deben al reordenamiento vascular.Una alternativa que ayuda,es que al suturar los bordes,mejora la posibilidad de confrontación,el tomar con la agujala parte muscular de la herida, ayudando la fijación.Hay que recordar, que las intervenciones efectuadas se lograron en el medio rural, consituaciones que deben tenerse en cuenta;polvo,inmovilidad a veces insegura,imprevistos,etc,que en animales de alto valor,al efectuarlo en clínica,no existirían riesgos alguno.

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RESUMEN

La tecnica de autoingerto,con colgajo dependiente,en aquellos tumores de ojo que nos deja una herida excesiva,es una vía interesante e indicada,con pocos riesgos, y total exito,para la recuperación del intervenido,que le brinda una optima estetica. Esta intervención tiene especial aplicación a los veterinarios que se desenvuelven en el medio rural y que como en la Argentina,es comun los carcinoma de ojos y anexos muy proliferativos, por razones de geografía, y en especial de manejos.-

RESUME

La technique d'autogreffe, avec de lambeau dépendant, dans ces tumeurs d'où il que nous laisse une blessure excesive; c'est une voie intéressante et bonne, avec peu de dangers et un succès plein, pour la recupération de l'intervenu. Ça lui donne une optime stétique. Cette intervention chirurgique a une spéciale application aux vétérinaires qui travaillent au moyen rural et que,comme en Argentine,ce sont fréquents les "carcinomas" des yeux et annexes très prolifératifs, par raisons de géographie, et particulièrement de maniements.

SUMMARY

The common surgical technique in the tomours of eyes generate an excesive wound or injury.The surgical tecnique of selfgraft (autograft),with dependent skin flap,is an interesting and accurate way.It has few dangers and total success. The recuperation of eye is complete and the esthetic is the best. The veterinarians,specially use, this surgical tecnique in the rural area.The "carcinomas" of eyes and annexed are very common and prolific in the Argentine by reasons of geography and special managements.

CRYOSURGICAL DEHORNING OF THE CALF

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INTRODUCTION

For over 35 years dehorned cattle have become an integral part of modern cattle farming. During this time numerous methods of dehorning have been described and put to use. For choosing a dehorning method we have found ROSENBERGER's division in 3 age groups very practicable. Calves up to 3 months of age are disbudded by hot cautery, caustic agents or ROBERTS' surgical removal of the horn button. Young cattle up to 6 or 7 months of age are dehorned with a dehorner (BARNES' Dehorning Gouge)(2) and older cattle using a steel wire to saw off the horn.

The above mentioned cauterization techniques have evolved to the point where the owner often dehorns the calves without any veterinary assistance. Although these methods in calves are simple and inexpensive they do have disadvantages. Particularly with caustic agents the amount applied is uncertain and hot cautery produces bothersome smoke and is also a fire hazard (3,5).

These disadvantages have caused farmers to return to the veterinarian for deborning calves. This in return prompted our clinic to develop a cold cauterization procedure which is simple, fast and inexpensive, as well as accurate in dosage. From the onset liquid nitrogen eliminates the smoke and fire hazard.

MATERIALS AND METHOD

For cryosurgical dehorning we use an open cryoprobe. This probe has a synthetic cover constructed to prohibit the liquid nitrogen from coming in contact with cells other than those to be destroyed. In addition, a cryospray instrument for liquid nitrogen, attaining 0,5 atmospheres of pressure when in use, and an adaptor between the probe and the cryospray apparatus are needed.

Prior to surgery the animal is sedated with Xylazine (Rompun^{R}) and the hair surrounding the horn bud is shorn. Liquid nitrogen is then sprayed onto the horn button and the neighbouring tissue until the skin is superficially covered with ice crystals and the nitrogen no longer vaporizes directly upon contact with the skin. The cryoprobe is then situated over the horn bud so that no liquid nitrogen droplets can escape from underneath the covering device (Fig.1). The pressure arising between the horn button and cryoprobe with cover can escape through a vent pipe, which can be extended with a rubber tube. The freezing cycle is continued in this manner for several

seconds until the tissue at the periphery of the cryoprobe cover is visibly coated with ice crystals (Fig.1). 2 to 3 week old calves are disbudded with this method.

Fig.1: Freezing cycle of the horn button with ice crystals visible outside of the cryoprobe cover

Fig.2: Frozen horn button in early thawing phase immediately after the freezing cycle has been terminated

RESULTS

Each disbudding takes between 20 to 30 seconds. The initial cooling of the immature horn and surrounding area with liquid nitrogen until ice crystals appear on the surface requires about 10 to 15 seconds. After the open cryoprobe is placed on the horn bud the continual spraying results in a subcutaneous temperature drop to -120°C in 8 to 15 seconds, measured under the epidermis of the horn.

By calves in which continual subcutaneous measurements recorded the temperature decrease, it was determined that, when the center of the horn button reaches -120°C, the diameter of the frozen cell group becomes greater than the diameter of the cryoprobe cover, whereby ice crystals become visible on the skin around the probe (Fig.1). At this time the surgeon can terminate the freezing process under the assumption that the horn bud has been cooled sufficiently.

The further immature horn growth and cornification has progressed the longer it takes until -120°C are achieved subcutaneously. The thawing phase is also dependent on the degree of cornification and takes approximately 3 to 5 minutes. Following the thawing phase the tissues surrounding the previously frozen cells are hyperaemic and slightly oedemic. No bleeding occurs.

In spite of sedation with xylazine the animals are in pain and demonstrate defensive behaviour during the freezing cycle. After sedation wears off the cryosurgically treated calf is clinically normal and begins ingesting food within 2 hours after surgery. In exceptional cases a slight itching was observed. To date no subsequent horn development has been seen.

However, at the present time no statistically documented statement can be made regarding the possible occurrence of stunted horns after using this technique.

DISCUSSION

For a better understanding of the variable effects of liquid nitrogen on living cells several biological fundamentals are worth mentioning :

Depending on the freezing method cells can be deep frozen at an optimal freezing rate and conserved until they are thawed to regain viability. These cryoconservative methods are well known for sperm and embryo conservation.
By changing the freezing modus so that the cells are cooled very rapidly to a low temperature and thawed slowly cell death results (4). An important factor which influences the freezing rate is the heat conductivity of the tissue to be treated. It is a known fact that bones have a low and parenchyma a high conductivity, in correlation with the water content of the tissues.

In order to inhibit horn development the cryosurgical technique must cause reliable destruction of those cells which induce horn growth.

With the described method a fast decrease in temperature of the cutaneous horn bud can be obtained so that within 15 seconds a temperature drop to -120°C can be measured in the subcutaneous tissue. However, the thawing phase is relatively fast, a return to body temperature being recorded in just a few minutes, due to the thermal increase resulting from the hyperaemia of the neighbouring tissues.

It may be possible to influence the thawing time by applying analgesics with vasoconstrictive properties to reduce the regional blood supply. For this reason, and in consideration of the pain and defensive behaviour exhibited by the calves inspite of sedation, further experiments are being performed to test the effects of such local anaesthetics.

We see less hazards in destroying the horn button with liquid nitrogen than with caustics. If liquid nitrogen comes in short contact with other areas of the skin the severe damages which may occur when using caustics need not be feared.

The fast cooling rate to -120°C in the subcutis and the clinical observation that the horn bud does not increase in size, leads us to believe that 2 - 3 week old calves can be reliably disbudded with this method. This would confirm the results obtained by BAER, KRANTZ and HEBER, who inhibited horn development in 100 calves by devitalizing horn buds using a different cryosurgical technique (5).

In contrast to surgical dehorning we and the owners see an advantage in this method, since no bleeding occurs.

Another positive aspect of cryosurgical dehorning opposed to burning is the lack of fire hazard and smoke.

Furthermore, no calf showed signs of disturbed general health or inappetenz in the post operative phase that could be attributed to the intervention.

Although this technique can be easily and quickly performed under field conditions and has the aforementioned advantages we would not introduce it into routine practice until statistical evaluation of the potential for stunted horn growth is available. The disadvantage of the described cold casterization, despite the low cost of the single treatment itself, is the high cost of an appropriate cryosurgical instrument. To decrease the investment costs it is desirable to extend its use to other therapeutic indications, such as in treating interdigital fibromas of cattle (6). Our clinic is examining further possibilities for practical application of cryotherapy in the bovine.

ACKNOWLEDGEMENTS

The author wishes to thank Professor Dr.med.vet. W. Gehring of the Ambulatory and Obstetrical Veterinary Clinic, Giessen for his assistance in performing this research. Mr. Winkler of the Erbe Company, Frankfurt/Main deserves particular mention for putting the cryospray instrument and a suitable apparatus for measuring tissue temperatures at our disposal.

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SUMMARY

A cryosurgical method for disbudding calves is presented. A specially constructed "open cryoprobe" is used to spray liquid nitrogen onto the horn button. The nitrogen comes out of a cryospray instrument, which can also be employed in other cryotherapeutic intervention in cattle.
In comparison with common cauterizing methods and surgical removal of the horn button the advantages of liquid nitrogen disbudding are its simple and rapid execution, the nonexistent fire and smoke hazard, as well as the lack of hemorrhage.

ZUSAMMENFASSUNG

Es wird eine Methode zur kryochirurgischen Enthornung des Kalbes vorgestellt. Bei diesem Verfahren wird mit einer besonders dafür geeigneten "offenen Kryosonde" flüssiger Stickstoff von außen auf die Hornanlagen aufgesprüht. Der Stickstoff kommt aus einem Sprühgerät, das auch für andere kryotherapeutische Eingriffe beim Rind eingesetzt werden kann.
Im Vergleich zu den bisher üblichen Ätz- und Brennverfahren, sowie der blutig-chirurgischen Enthornung zeichnet sich die kryochirurgische Methode dadurch aus, daß sie einfach und schnell durchführbar ist, keine lästige Rauchentwicklung und Brandgefahr entsteht und daß keine Blutung auftritt.

RESUMEN

Se presenta un método para el descornado crioquirúrgico del ternero. Mediante esta técnica se pulveriza nitrógeno liquido sobre los botones córneos con una "sonda criogénica abierta", especialmente diseñada para este uso. El nitrógeno sale de un aparato pulverizador, que puede también ser utilizado en otros tratamientos crioterapeuticos in vacuno.
En comparación con los métodos hasta ahora utilizados, como son la cauterización química y térmica y el descornado quirúrgico sangrante, el método crioquirúrgico destaca, porque es rápido y sencillo,
porque no se producen humos desagradables ni existe peligro de quemadura y
porque no se producen hemorragias.

RESUMO

O presente trabalho descreve um método criocirúrgico para a descorna de terneiros. Neste método utiliza-se una "criosonda aberta" apropriado para este fim, a qual pulveriza nitrogênio liquido sobre os cornos. O nitrogênio provêm de um aparelho pulverizador, o qual pode ses também utilizado em outras intervenções crioterapêuticas.
Em comparação com os métodos normais de cauterização este método oferece as seguintes vantagens :

1. é rápido e simples
2. evita desenvolvimento de fumaça ou o perigo de fogo
3. não ocorre hemorragia

A NEW APPROACH IN THE TREATMENT OF SEPTIC PHYSITIS IN YOUNG CATTLE

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INTRODUCTION

Septic physitis in young cattle is a frequently occurring phenomenon. Septic phyeitis is caused us ually by an haematogenous infection. Mostly the infectious agent involved is Corynebacterium pyogenes, but Escheria coli and Salmonella species are also isolated (van de Watering, Morgan, Kersjes 1976, Firth, Kersjes, Dik and Hagens 1987).

In mild cases treatment consists of rest and administration of antibiotics. In severely affected patients conservative treatment is not effective and surgery is indicated. An earlier study at the Clinic for Large Animal Surgery in Utrecht by Firth et al (1987) reveals that the outcome of surgical intervention in patients with osteomyelitis is reasonable. A disadvantage of the surgical treatment is the long recovery period.

It is known that osteomyelitis occurring after osteosynthesis of fractures can be positively influenced by better stabilization and by bone grafting (Waldvogel and Vasey 1980; Schmelzeisen and Kempf 1986; Eggers and Wolter 1986; Schmidt, Neikes and Zimmer 1987). Based on these findings patients with a severe septic physitis of the distal metacarpal or metatarsal bone have been treated by administration of antibiotics, curettage, autologous cancellous bone grafts and a walking cast for better stabilization. This paper describes the results of the new elements in the surgical procedure which includes the use of bone grafts and the application of a walking cast.

MATERIAL

A total of five male beef cattle, aged nine to eighteen months, and four female dairy cattle, aged eight to twentyfour months, were treated.

All these patients had been severely lame for a period of fourteen to twentyfour days and had been treated in practice by administration of antibiotics during a long period. Clinical examination revealed a very painful swelling at the level of the physis of the distal metacarpal or metatarsal bone (respectively one and eight patients). The radiographic signs of seven of the patients included a large, irregular radiolucent defect across the whole physis. In two cases the radiolucent defect extended only over a part of the physis. In some patients there was new periosteal bone formation and sharply or poorly defined sequestrum formation was often visibel. Extensive radiolucency across the whole physis was associated with a pathological fracture in a few cases.

METHOD

The patient is situated in lateral recumbency with the affected limb uppermost and is kept under general anaesthesia. The surgery is expedited by the use of two surgical teams. Cancellous bone is harvested by another surgeon while surgery of the physitis is performed. The cancellous bone grafts are collected from the tuber coxae and are for the time being kept in saline solution with ampicillin.

Surgery of the physitis is performed with the aid of an Esmarch's bandage with a tournequet to facilitate surgery. After routine preparation for surgery, a skin incision of not more than five centimeters is made over the site of the radiological lesion. The incision is continued down to the bone surface.

Then all soft necrotic bone is removed by a Brunn curette of appropriate size. The bone is curetted down to healthy tissue and fluoroscopic monitoring is used to check if the curettage of the affected bone has been complete. In 6 animals necrotic bone samples are collected into a sterile tube for bacteriological examination.

Following the debridement, the cavity in the physis is thoroughly flushed with a saline solution containing ampicillin.

The defects will be filled with the cancellous bone grafts. After filling the defect, the grafts are slightly tamped into place.

Closure of the wound is done in two layers; the subcutaneous tissue in a simple continuous pattern and the skin by simple interrupted sutures. Absorbable material is used in both sutures. Then the walking cast is applied for immobilization.

The walking cast is replaced by a Robert Jones bandage after three to four weeks in most patients. In heavy animals another plaster cast is used. Both the Robert Jones bandage and the second plaster cast will last for an additional two weeks.

Because of the uncertainty of acceptance of the cancellous bone grafts in a septic environment, antibiotics have been administered to the first patients for a long time, up to fifteen or even twenty days. In later patients administration of antibiotics was started before surgery and continued for five to ten days post-operatively.

RESULTS

Results were very good. All nine patients did recover completely. Some of the patients were still severely lame post-operatively, but this lameness gradually disappeared. The average casting period was four and a half weeks, varying from three to six weeks. In four of the 6 patients bacteriological culture was positive, Corynebacterium pyogenes being the infectious agent in all four cases.

DISCUSSION

Surgical treatment of septic physitis has been described by various authors (Delahanty 1952, Firth et al 1987 and Auer 1983). The results were beneficial but still improvement could be obtained.

A new element in the surgical method was bone grafting. Bone grafting was used to fill the cavity left by the curettage to eliminate the dead space and to speed resolution of bone infection. The treatment which has been described in this paper consists of a combination of therapies. Therefore it is difficult to assess the real impact of cancellous bone grafting itself. Nevertheless, the data

presented strongly suggest a positive influence on healing of septic physitis in cattle. Also no indication was obtained from our results for delayed grafting, four to 12 days after the initial debridement surgery, as described by Bardet, Hohn and Basinger (1983).

The second new element in the surgical method was the external coaptation. Whereas the integrety of the limb following curretage is in doubt, external coaptation is required to aid in achieving stability. Additionally, it has been repeated shown that a stable infected area heals faster than a unstable infected area. As external coaptation the walking cast has been used. It is questionable if the walking cast could be replaced by a normal cast. This might be possible, but in that case the carpus or tarsus should be immobilized. The advantages of the walking cast are twofold. Firstly it provides a better stabilization, and secondly it hampers the normal movements of the animal as little as possible by not including the carpal and tarsal joints in the cast.

Considering the economic aspects of the described surgical procedure, this treatment is restricted to valuable animals. Nevertheless, it was no problem to get patients. Current developed techniques like embryotransplantation do increase prices von cattle and owners are prepared to spend money on the treatment for these valuable animals.

The treatment of septis physitis is a therapeutic challenge. The final conclusion is that bone grafting in septic physitis helps in the fight against infection and clearly reduces the recovery period. Succesfull outcomes requires systemic approach with the subsequent stages of treatment: antibiotics, debridement, bone grafting and stabilisation.

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SUMMARY

Septic physitis in calves is a frequently occurring phenomenon. In mild cases treatment consists of rest and antibiotics. In severe patients conservative treatment is not successful and surgery is indicated. In this presentation the method of surgery with the use of cancellous bone grafts and the results of this treatment in nine calves with septic physitis of the distal metacarpal or metatarsal bone are discussed. The patient is situated in lateral recumbency and is kept under general anaesthesia. After curettage of the septic process, the defect is filled with cancellous bone out of the tuber coxae. After closure of the skin a "walking cast" is applied. Post-operatively antibiotics are given for 5-10 days parenterally. Four weeks after surgery the "walking cast" is replaced by a bandage in the light animals and by another cast in the heavy animals, both for a period of two weeks.
All treated patients did recover completely.
It is concluded that cancellous bone grafting in a septic process is of great help in the fight against infection.

RESUMAS

Uma terapia nova para tratamento da fisite séptica em bovinos.

Fisite séptica é uma doença que é frequentemente encontrada en bovinos. Nos casos ligeiros uma terapia consistindo dum tratamento com antibióticos e restriçao da liberdade de movimentos normalmente é suficiente, mas nos casos mais graves uma intervençao cirúrgica é indicada.
Discute-se um método cirúrgico, que é baseado na transplantaçao de spongiosa do osso, em nove bovinos com fisite séptica no metacarpo ou metatarso. O paciente é colocado no seu lado e é operado sob anestesia geral. O processo séptico é cuidadosamente extirpado para depois ser enchido pela spongiosa de osso que foi tirado do tuber coxae. Depois da sutura da pele a perna é imobilizada por meio dum "walking cast" (uma estrutura de aço na forma da letra "u" que é segurada a dois pregos que passam pela tíbia ou pelo rádio na qual a parte ferida da perna é pendurada para nao suportar o peso do corpo). Depois da operaçao, os animais sao tratados com drogas antibióticas durante 5 até 10 dias. Quatro semanas depois o "walking cast" é tirada e é substituido durante uns 15 dias por uma simples bandagem ou por um gesso nos animais mais pesados.
Todos os pacientes recuperaram, entao é concluido que a transplantaçao da spongiosa do osso seja uma ajuda considerável na luta contre a infecçao nestes casos.

RESUMEN

Un nuevo tratamiento para la epifisitis séptica en los miembros del bovino.

Epifisitis séptica es una afección frecuente en el bovino. En casos leves el tratamiento conservador, el cual consiste en reposo y antibioticos, esta indicado. En casos severos este tratamiento es insuficiente y por lo tanto reemplazado por el quirurgico, para el cual se realiza un transplante esponjoso autólogo. Los resultados obtenidos en nueve casos despues de una epifisitis septica del metacarpo o metatarso se discutiran.
El paciente se opera en decubito lateral bajo anestesia general. Despues de un curetaje prolijo del foco infeccioso se rellena el defecto óseo con tejido esponjoso autólogo, el cual se obtiene del tuber coxae. Luego de suturar la piel, se inmobiliza el miembro con un "walking cast". Los animales obtienen 5-10 dias post-operatorios antibioticos por via parenteral. Despues de 4 semanas se quita el "walking cast" y se lo reemplaza en animales livianos por un vendaje

sencillo, en animales pesados por un yeso; ambos por un lapso de dos semanas mas. Todos los pacientes tratados han curado.
Conclusion: el tejido esponjoso autólogo es adecuado para el tratamiento de infecciones óseas como las descriptas.

ZUSAMMENFASSUNG

Eine neue Behandlungsmethode septischer Epiphysenfugenveränderungen im Bereich der Gliedmaßen beim Rind.

Die Epiphysenfugeninfektion des Rindes im Bereich der Gliedmaßen ist keine seltene Krankheit. In leichten Fällen besteht die Therapie in Ruhe und Antibiotika. In schweren Fällen ist die konservative Therapie aber unzureichend, sodaß dann eine chirurgische Behandlung indiziert ist. Sie besteht in der autologen Spongiosatransplantation. Die Ergebnisse die bei der Behandlung von neun Rindern mit derartigen Epiphysenfugeninfektionen im Bereich des Metakarpus und Metatarsus erzielt wurden werden diskutiert.
Zur Operation wird der Patient in Allgemeinnarkose gelegt und in Seitenlage gebracht. Nach sorgfälltiger Kürettage des septischen Prozesses wird der Defekt mit autologer Spongiosa, die aus dem Tuber coxae entnommen wurde, ausgefüllt. Nach der Hautnaht wird die Extremität mit einem "Walking-cast" immobilisiert. Die Tiere bekommen 5-10 Tage postoperationem parenteral Antibiotika verabreicht. Vier Wochen postoperationem wird der "Walking-cast" bei leichten Tieren durch einen Verband und bei schweren Tieren durch einen Gipsverband, für weitere zwei Wochen, ersetzt.
Bei allen Patienten kam es zur Heilung.
Schlußfolgerung: Autologe Spongiosatransplantation ist gut geeignet zur Behandlung der Epiphysenfugeninfektion im Bereich der Gliedmaßen beim Rind.

A STUDY OF THE CLINICAL MANAGEMENT AND SURGICAL REPAIR OF ATRESIA COLI IN 110 NEONATAL CALVES

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INTRODUCTION

Atresia coli is a well recognized congenital defect in neonatal calves, with the spiral loop of the ascending colon (spiral colon) being the most commonly affected segment of intestine. Though the cause of intestinal atresia in calves is not well understood, there is growing evidence to suggest that the condition is not inherited (1,4,5,7,9). Until recently, the reported prognosis for surgical repair of atresia coli was poor (6,8); however, the successful management of atresia coli has improved with the short-term survival rate ranging from 43% - 71% (2,3). In this study, we describe the medical management and surgical technique used in calves with atresia coli admitted to referral institutions for surgical correction.

MATERIALS AND METHODS

We included calves admitted to the New York State College of Veterinary Medicine (65), and the University of Pennsylvania (2). Clinical, clinicopathologic, surgical and necropsy data were retrieved from the medical records. Long term follow-up information was obtained by direct communication with the owners after discharge from the hospital. For some of the analyses we combined data from the above-described calves with data from a previously reported and very similar population of calves (43) from the University of Guelph.

The chi-square of Fisher's exact test was used to compare the type of anastomosis performed and the experience of the attending surgeon with the calf short-term survival rate. The Wilcoxon rank sum test was used as a screening test to assess the relationship between the calf's short term survival rate and various clinical and laboratory parameters. Factors that were significantly ($P<0.10$) associated were evaluated simultaneously using logistic regression analysis.

RESULTS

Population A (Cornell University; University of Pennsylvania): The mean age of the 64 female and 3 male calves at the time of initial surgical correction was 3.2 days (range 1-8). History and clinical signs at the time of admission were consistent with those previously reported (2). Most calves had tachycardia and hyperpnea. Although they were dehydrated with hemoconcentration, they typically had low plasma protein concentrations. Acid base values varied widely, although many calves had a mild metabolic alkalosis.

Surgery was usually delayed until the calves had received appropriate preoperative medical support, including I.V. fluid therapy and antibiotics. In 23 calves, fresh bovine plasma or whole blood was administered to treat severe hypoproteinemia. General anesthesia was

induced and the calves were operated upon through the right paralumbar fossa, while positioned in left lateral recumbency. The site of atresia was confined to the spiral colon, but varied from the junction of the proximal and spiral loops of the ascending colon, to the junction of the spiral and distal loops of the ascending colon.

Intestinal contents proximal to the atresia site were removed by enterotomy in the apex of the cecum or in the proximal blind end of the spiral colon. In the 54 calves in which definitive surgical repair was attempted, intestinal continuity was established by anastomosis between the proximal segment of the spiral colon and the descending colon (n = 50) or the proximal and distal blind ends of the spiral colon (n = 4). The anastomosis was performed using either a side-to-side (n = 38) or end-to-side (n = 12) technique. Abdominal closure was routine.

Fifty-two calves survived through the end of surgical procedure. Of these 28 (54%) were discharged from the hospital. The long term survival rate of these calves was 28%. Calves with poorer hematologic and metabolic parameters were less likely to survive. Nonsurvivors typically had higher heart and respiratory rates, had higher PCV, and had metabolic alkalosis with superimposed metabolic acidosis and high anion gap.

Population A (Cornell University; University of Pennsylvania) and Population B (University of Guelph): When data from both populations were combined, no significant difference was observed between the survival rate and the year of presentation. Similarly, no significant differences were observed between the survival rate and the method of anastomosis used, or between the survival rate and the experience of the attending surgeon. Regression analysis revealed a poor correlation between various clinicopathologic parameters and survival, though the anion gap concentration was the most significant parameter ($p = .0513$). The optimal control point was found to be 24 mEq/l. Calves with an anion gap > 24 mEq/l had a lower survival rate than those with anion gap < 24 mEq/l.

DISCUSSION

Because survivors and nonsurvivors differed principally in their hematologic parameters, the condition of the calf at the time of presentation seemed to be the most important factor in determining a successful outcome. Therefore, we concluded that intense preoperative medical support is of critical importance, and only in unusual circumstances should surgical intervention be considered to be an emergency procedure.

Surprisingly, we found that the need for resection of the proximal blind end was not a significant factor, regardless of the type of anastomosis performed. However, we still recommend that union of the proximal blind end to the descending colon be used instead of uniting the proximal blind end to the distal blind end, because the former technique provides for a shorter distance of previously unused bowel through which digesta must pass.

Despite our attempts to identify variables which would be useful prognostic indicators, we found that the anion gap alone was the most useful predictor of survival.

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SUMMARY

The medical records of 110 calves presented to 3 referral hospitals with atresia of the spiral colon were reviewed and analyzed. Surgical treatment was completed in 94 calves. This consisted of enterotomy to allow meconium evacuation, resection of the proximal blind end (n = 68), and restoration of intestinal continuity by anastomosis of the proximal and distal blind ends, or of the proximal blind end to the descending colon. Of the 94 calves allowed to recover from surgery, 50% were discharged from the hospital. The anion gap alone (not in combination with other variables) was found to be the best predictor of short term survival. The best cut-off point was determined to be an anion gap of 24 mEq/l, with those calves with a higher anion gap being less likely to survive. The long term survival rate was 45%.

RESUMO

O arquivo médico de 110 bezerros apresentados a 3 hospitais com atresia do colon espiral foram revistos e analizados. Tratamento cirúrgico foi completado em 94 bezerros. Este consistiu em enterotomia para permitir mecônio evacuação, resecção do fundo cego proximal (n=68), e restauração da continuidade intestinal por anastonose dos fundos cegos proximal e distal ou do fundo cego proximal ao colon descendente. Dos 94 bezerros que se recuperaram da cirurgia, 50% foram dados alta. O gap aniônico por si (sem combinação com outras variáveis) revelou-se como melhor indicador de sobrevivência a curto prazo. O melhor ponto de prognóstico foi determinado por ser o gap aniônico de 24 mEq/l, onde os bezerros que apresentaram gap aniônico maior apresentaram chance menor de sobrevivência. O índice de sobrevivência a longo prazo foi 45%.

RÉSUMÉ

Les auteurs ont étudiés les dossier médicaux de 110 veaux présentés à trois différents hospitaux vétérinaires pour traitement d'une atrésie due colon. Le traitement chirugical fut complèté chez 94 veaux. Le traitement chirurgicale consistait d'une entérotomie pour évacier le méconium, d'une resection du segment intestinal proximal au site de l'atrésie (n=68), et de la restauration de la continuité intestinale par anastonose de la portion proximale et distale de l'atrésie ou par anastonose de la portion proximal de l'atrésie au colon descendant. Des 94 veaux soumis à la procedure chirurgicale, 47 ont reçus leur congé de l'hospital (survis à court terme). L'écart des anions, seul (sans autre variables), était le prédicteur de survis le plus précis (taux de survis à court terme). Un écart anionique de 24 mEq/l ou plus était associé avec un taux de survis très bas. Le taux de survis à long terme était de 45%.

EUTERGESUNDHEIT NACH ZITZENOPERATIONEN

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EINLEITUNG

Mit zunehmendem Leistungsdruck in der Milchwirtschaft kommt der tadellos funktionierenden Milchkuh immer mehr Bedeutung zu. Vom Tierarzt wird immer häufiger verlangt, Zitzen mit offenen oder, sehr wichtig, gedeckten Verletzungen zu behandeln und möglichst schnell wieder funktionenstüchtig zu machen. In vielen Fällen ist eine Operation die Therapie der Wahl. Der Erfolg einer operativen Behandlung von verletzten Zitzen wird durch folgende Parameter beurteilt:

+ die Melkbarkeit
+ die Eutergesundheit
+ die Nutzungsdauer der Kuh

Über die operative Wiederherstellung, die Wundheilung, die Melkbarkeit und die spätere Nutzung der Kuh nach erfolgter Operation von lädierten Zitzen liegen zahlreiche Arbeiten vor (1,2,4,5). Über die Eutergesundheit nach einer Zitzenoperation gibt es dagegen wenig Informationen (4). Die wenigen Arbeiten, welche darüber geschrieben wurden, beschränken sich ausschliesslich auf Euterviertel, deren dazugehörige Zitzen wegen einer Milchabflussstörung mit einem Zitzenmesser eröffnet wurden. Für den Landwirt lag bisher als Erfolgskriterium die Melkbarkeit im Vordergrund, die Eutergesundheit war für ihn eher von zweitrangigem Interesse. Dies scheint sich aber in der Schweiz seit der wesentlichen Verschärfung der Ablieferungsbestimmungen für die Verkehrsmilch im Dezember 1986 kontinuierlich zu ändern. In dieser Arbeit wurde versucht, folgende Fragen abzuklären:

1) Wie hoch ist der Anteil der Viertel mit ungenügender Eutergesundheit nach einer Zitzenoperation im dazugehörigen Viertel und in den Nachbarvierteln?
2) Bestehen Unterschiede hinsichtlich der Eutergesundheit nach verschiedenen Zitzenverletzungen und verschiedenen Therapieverfahren?
3) Welchen Einfluss haben Vorgeschichte und Komplikatonen während und nach der Operation auf die spätere Milchqualität?
4) Besteht ein Zusammenhang zwischen Melkbarkeit und Eutergesundheit?

MATERIAL UND METHODEN

Um diese Fragen zu klären, wurden sämtliche noch lebenden und laktierenden Kühe, welche zwischen 1978 und 1984 am Tierspital Zürich an der Zitze operiert wurden, im Bezug auf die Eutergesundheit und die Melkbarkeit überprüft. Die 139 kontrollierten Kühen wiesen folgende, in Tabelle 1 aufgeführten, Verletzungen auf. Zur Überprüfung der Eutergesundheit wurden von sämtlichen Tieren zweimal im Abstand von 14 Tagen Milchproben entnommen und der Zell- und Keimgehalt bestimmt. Als Grenze für schlechte bzw. gute Milchqualität im Bezug auf den Zellgehalt wurde gemäss Beschluss der International Dairy Federation, 1966, 500000 Zellen pro ml Milch angenommen.

Tabelle 1: Verteilung von verschiedenen Verletzungsarten bei 139 Kühen

Art der Verletzung	Anzahl Tiere / Zitzen		Bemerkungen
gedeckte Zitzenverletzung	71	75	1)
Perforation des Strichkanals	24	24	
Perforation der Zitzenzisterne	24	24	
nichtperforierende Querrisse und Schälwunden	20	20	2)

1) bei 19 Tieren "blinde" Erweiterung des Strichkanals
2) davon 19 Verletzungen als "kompliziert" beurteilt

RESULTATE

Von allen 143 untersuchten Vierteln waren, unabhängig von der Art der Verletzung zum Zeitpunkt der Untersuchung nur 65, d.h. 45% nach den festgesetzten Kriterien eutergesund (Tabelle 2).

Tabelle 2: Eutergesundheit der betroffenen Viertel frühstens sechs Monate nach erfolgter Zitzenoperation (n=143)

Eutergesundheit	Anzahl Viertel	
	n	%
gut	65	45
ungenügend	78	55

Bei den dazugehörigen nicht betroffenen Eutervierteln waren hingegen 64% eutergesund. Vergleicht man die Eutergesundheit der betroffenen Viertel mit der der nichtbetroffenen, so fällt auf, dass nur bei 15 Kühen (21%) alle 4 Viertel gesund waren. Die übrigen Kühe wiesen in mindestens einem (34.9%), zwei(17.8%), drei (15.5%) oder gar in allen vier Vierteln (11.6%) Anzeichen einer chronischen Mastitis auf. Im weiteren betrachteten wir die Eutergesundheit Monate bis Jahre nach einer Zitzenoperation in Abhängigkeit von der Art der Verletzung. Dabei wurde zwischen offenen und gedeckten Verletzungen unterschieden und beide Gruppen noch weiter nach Therapieverfahren (bei den Stenosen) und der Art der Lokalisation der Verletzung unterteilt (Tabelle 3, 4, 5 und 6).

Tabelle 3: Gedeckte Verletzungen

Eutergesundheit	Viertel	
	n	%
gut	29	38.7
ungenügend	46	61.3

Tabelle 4: Therapieverfahren

Abhängigkeit Eutergesundheit	Zitzen	Anzahl Viertel mit guter
Therapie:		
Thelotomie	56	21 (37.5%)
Hug	19	8 (42.1%)

Tabelle 5: Offene Verletzungen ohne Perforation

Eutergesundheit	Viertel	
	n	%
gut	8	40%
ungenügend	12	60%

Tabelle 6: Offene Verletzungen mit Perforation

Lokalisation der Perforation	n	Viertel mit ungenügender Eutergesundheit
Zisterne	24	10 (42%)
Strichkanal	24	11 (46%)

Trotz gewissen Unterschieden konnte entgegen unserer Erwartungen kein signifikanter Einfluss von Verletzungs- und Therapieart auf die Eutergesundheit Monate bis Jahre nach der Operation nachgewiesen werden. Hingegen konnten überraschend deutliche Signifikanzen bei der Betrachtung der Abhängigkeit der Eutergesundheit von der Ausgangslage, Abheilung und der Melkbarkeit gefunden werden.
Tiere, welche zum Zeitpunkt der Einlieferung ins Tierspital eine gute Eutergesundheit am betroffenen Viertel aufwiesen, zeigten auch später eine deutlich bessere Eutergesundheit als Kühe, welche schon mit Störungen der Eutergesundheit eingeliefert wurden (Tabelle 7). Ebenso deutlich fielen die Resultate bei der Betrachtung der Eutergesundheit nach der Zitzenoperation in Abhängigkeit vom Verlauf der Abheilungsphase aus (Tabelle 8). Nicht überraschend kamen die Resultate bei der Betrachtung der Abhängigkeit der Eutergesundheit von der Melkbarkeit vor allem bei den inkontinenten Kühen. Von 19 Kühen mit einer Inkontinentia lactis zeigten nur noch 2 (10.5%) eine ungestörte Eutergesundheit.

Tabelle 7: Eutergesundheit nach Zitzenoperationen in Abhängigkeit von der Eutergesundheit bei der Einlieferung

Verletzungsart	Eutergesundheit bei der Einlieferung		Eutergesundheit später	
			gut	ungenügend
Stenosen	gut	13 (19%)	8 (61.5%)	5 (38.5%)
	ungenügend	54 (81%)	17 (31.3%)	37 (68.5%)
Offene Verletzung				
	gut	32 (57%)	17 (53%)	15 (47%)
	ungenügend	24 (45%)	10 (42%)	14 (58%)

Tabelle 8: Eutergesundheit nach Zitzenoperationen in Abhängigkeit zum Verlauf der Abheilungsphase

Verlauf	Viertel/Zitzen	Anzahl Viertel mit ungenügender Eutergesundheit
komplikationslos	92	40 (45%)
mit Komplikation	37	21 (81%)

DISKUSSION

1. 55% der Kühe mit einer Zitzenverletzung wiesen unabhängig von der Art der Läsion nach der Operation im dazugehörigen Euterviertel eine ungenügende Eutergesundheit auf.
2. Nicht betroffene Viertel zeigten in 36% der Fälle Sekretstörungen.
3. Nur 26 von 129 Tieren mit einer Zitzenoperation erwiesen sich in allen vier Vierteln als eutergesund.
4. Folgende Faktoren haben einen signifikanten Einfluss auf die spätere Eutergesundheit:
   - Eutergesundheit zum Zeitpunkt der Operation
   - postoperative Komplikationen
   - Inkontinentia lactis
5. Hinsichtlich der Art der Läsion ergaben sich keine signifikanten Unterschiede. Trotzdem können folgende Verletzungsarten bezüglich späterer Eutergesundheit prognostisch als ungünstig bezeichnet werden:
- gedeckte Verletzungen, weil sie häufig während längerer Zeit und unsachgemäss vorbehandelt werden und somit vermehrt Sekretstörungen vor der Operation aufweisen.
- komplizierte, offene nicht perforierende Zitzenverletzungen (grossflächige Schälwunden, Querrisse im Bereich der Zitzenkuppe), weil sie vermehrt postoperativ Komplikationen nach sich ziehen.
- offene Zitzenverletzungen mit Perforation oder Ruptur des Strichkanals, weil sie einerseits vermehrt Komplikationen aufweisen und andererseits vermehrt zu Inkontinenz führen.

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ZUSAMMENFASSUNG

Bei 139 Kühen, welche zwischen 1978 und 1984 am Tierspital Zürich an einer Zitze operiert wurden, wurde durch zweimalige Milchuntersuchung die Eutergesundheit kontrolliert. Bei 75 Zitzen handelte es sich um gedeckte Verletzungen, die restlichen 68 wiesen eine offene Verletzung verschiedener Form und Ausdehnung auf. Folgende Resultate und Zusammenhänge konnten gefunden werden:
-nur 45% aller untersuchten Viertel waren eutergesund
-hinsichtlich der Art der Läsion (gedeckte/offen - perforierend/nichtperforierend ergaben sich keine signifikanten Unterschiede
-folgende Faktoren haben einen signifikanten Einfluss auf die spätere Eutergesundheit:
- Eutergesundheit zum Zeitpunkt der Operation
- postoperative Komplikation
- Inkontinentia lactis

ABSTRACT

A retrospective study was conducted on 139 Dairy cows which had had surgery between 1978 and 1984 at the University of Zürich Veterinary College. The condition of the respective quarters was assessed in terms of somatic cell count of two milk sampels collected two weeks apart several months to years after surgery. The reason for surgery was a teat lesion without an externally visible wound in 75 cows and a lesion associated with a visible wound in 68 cows.
Forty-five percent of the quarters examined had a satisfactory cell count (</=500000 per ml); the type of the lesion had no significant effect on the outcome. The following factors were found to have a significant longterm effect on udder health: the milk cell count at the time of surgery, the occurrence of post-operative complications and milk leakage due to teat duct injury.

RESUMEN

Entre 1978 y 1984, 139 vacas fueron operados de la ubre, en el hospital veterinario de Zürich. El estado de la ubre fue controlado dos veces mediante analisis de la leche:
- en 75 de los casos, se trataban de heridas cerradas.
- los otros casos presentaban heridas abiertas de diversos tramanos y formas.
Teniendo en cuenta los resultados obtenidos se llegaron a las concluciones siguientes:
- De los casos controlados, solo el 45% presentaban ubres sanas.
- no diferencia significante entre los diversos tipos de herida (cerrada/abierta, perforada / no-perforada)
- los factores siguientes tienen una influencia significante sobre el restablecimiento de la ubre:
  + el estado physiologico de la ubre al momento de la operation
  + complicationes post operationes
  + incontinentia lactis

RESUME

L'état de santé de la mamelle fut évalué à l'aide d'un double échantillon de lait chez 139 vaches opérées d'un trayon entre 1978 et 1984 à la clinique vétérinaire de Zurich. 75 cas consistaient en une lésion couverte du trayon, alors que les 64 autres présentaient une blessure ouverte de forme et d'étendue variables.
Les résultats se présentent comme suit:
- seuls 45% de tous les quartiers étaient sains
- aucune différence significative ne put être constatée en fonction du genre de lésion (couverte/ouverte - perforante/non perforante)
- les facteurs suivants influencèrent de manière significative la santé ultérieure de la mamelle:
  * état de santé de la mamelle au moment de l'opération
  * complication postopéatoire
  * Incontinentia lactis

ESTUDO SOMATOMÉTRICO EM BÚFALOS. I. PESO CORPÓREO, ALTURA NO GARROTE E NO SACRO E DISTÂNCIA DE RÓTULA A RÓTULA.

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INTRODUÇÃO

O Brasil possui hoje, segundo as estatísticas da Associação Brasileira de Criadores de Búfalos - ABCB, dois milhões de indivíduos distribuídos por todos os estados brasileiros. Na última década o crescimento populacional dos bubalinos tem atingido a taxa de 15% ao ano, valor esse aproximadamente seis vezes maior ao crescimento da população de bovinos. Isso, confere aos bubalinos a oportunidade de se constituirem numa opção na implantação dos sistemas de produção de leite e carne no complexo agropecuário do país.

Assim, constitui em objetivo do presente trabalho, avaliar as características do tipo, indicativas da habilidade de produção, tais como: peso corpóreo altura do garrote e sacro e distância de rótula a rótula, dos três principais grupos genéticos existentes no Estado de São Paulo.

MATERIAL E MÉTODOS

Foram utilizadas 50 fêmeas registradas de cada grupo genético (Jafarabadi, Murrah e Mediterrânea), todas com idade superiores a 40 meses e pertencentes a criatórios do Estado de São Paulo.

Os animais foram pesados e mensurados, utilizando-se uma bengala de medição e fita métrica. A análise dos dados foi efetuada pelo método dos quadrados-mínimos, tendo como causa de variação, os efeitos fixos dos grupos genéticos e regressão linear da idade. Para as diferenças entre médias utilizou-se o teste de Tukey.

RESULTADOS

A Tabela 1 apresenta as análises de variância do peso e das mensurações.

Tabela 1. Resumo das análises de variância.

Causa de variação	GL	Peso (kg)	Altura no Sacro (cm)	Altura do garrote (cm)	Distância de rótula a rótula (cm)
Raça	2	118551,46	318,21	273,18	468,10
Regressão linear da idade	1	129438,06	40,98	40,95	6,33
Resíduo	146	9176,71	39,21	46,27	73,14
Total	149				
Média		668,02±7,82	141,63±0,51	139,43±0,55	113,32±0,69
C.V. (%)		14,34	4,42	4,88	7,55

TABELA 1. Correlações simples e parciais entre as variáveis do tipo

	PESO	CCOR	ASAC	AGAR	CTOR	CABD	LTOR	LANC	CGAR	CCAN	ROTU	CCAB	LCAB
PESO		0,62	0,60	0,59	0,52	0,47	0,65	0,64	0,68	0,51	0,49	0,36	0,49
CCOR	0,51		0,67	0,68	0,43	0,55	0,51	0,69	0,60	0,54	0,39	0,55	0,49
ASAC	0,60	0,68		0,96	0,43	0,29	0,48	0,63	0,57	0,52	0,37	0,56	0,55
AGAR	0,59	0,69	0,96		0,47	0,30	0,51	0,66	0,58	0,49	0,43	0,56	0,54
CTOR	0,53	0,41	0,44	0,48		0,57	0,45	0,57	0,55	0,39	0,22	0,21	0,21
CABD	0,43	0,43	0,29	0,29	0,50		0,33	0,47	0,45	0,40	0,12^{ns}	0,01^{ns}	0,03
LTOR	0,63	0,47	0,57	0,58	0,47	0,30		0,64	0,55	0,29	0,50	0,47	0,58
LANC	0,64	0,69	0,67	0,69	0,56	0,44	0,65		0,64	0,53	0,35	0,45	0,55
CGAR	0,63	0,54	0,55	0,51	0,57	0,45	0,53	0,64		0,48	0,46	0,33	0,51
CCAN	0,50	0,48	0,48	0,45	0,39	0,40	0,26	0,52	0,41		0,24	0,25	0,31
ROTU	0,47	0,36	0,41	0,45	0,24	0,13^{ns}	0,46	0,33	0,43	0,22		0,25	0,41
CCAB	0,31	0,53	0,55	0,45	0,19*	-0,03^{ns}	0,49	0,44	0,29	0,22	0,24		0,58
LCAB	0,40	0,40	0,55	0,53	0,22	-0,01^{ns}	0,56	0,55	0,49	0,23	0,36	0,51	

ns = não significativo; * = > 0,16 (P≤0,05); > 0,21 (P≤0,01)

CCOR= Comprimento do corpo
ASAC= Altura sacro
AGAR= Altura garrote
CTOR= Circunferência torácica
CABD= Circunferência abdominal
LTOR= Largura torax
LANC= Largura da anca
CGAR= Comprimento da garupa
CCAN= Circunferência canela
ROTU= Distância rótula a rótula
CCAB= Comprimento da cabeça
LCAB= Largura da cabeça

de alojar grande quantidade de músculos no dianteiro, uma vez que essa medida é tomada sobre as paletas, apoiando a bengala de medição sobre o garrote. Essa medida mantém, uma estreita relação com o peso (r=0,51). Quanto ao CCOR, medida tomada da ponta da paleta até o Ísquio, numa linha diagonal e que engloba o CGAR também mantém uma correlação positiva com o peso, bem como com as características de produção de leite, conforme citam Mc Douvell[5] et alii para raça Jersey, Red Sindhi e seus mestiços e Javarkar & Johar[3] para a raça Murrah.

As medidas de altura tomadas no sacro e garrote (ASAC e AGAR), mostram-se correlacionadas significativamente com as demais medidas. O valor de r=0,96 entre as alturas, confirma a interdependência entre as mesmas, sendo que os bubalinos, principalmente, os da raça Mediterrânea demonstram uma equivalência entre as alturas de sacro e garrote, Cabrera[1].

Quanto às circunferências de torax (CTOR) e abdominal (CABD) e largura do torax (LTOR), somente as correlações entre CABD e ROTU, CCAB e LCAB, não foram significativas. As medidas de circunferências são grandemente influenciadas pelo estado alimentar e reprodutivo do animal e segundo Pavlina[6] et alii tendendo a aumentar até os 60 meses. Estas podem estimar a capacidade respiratória e digestiva dos animais e conjuntamente com as medidas de comprimento do corpo e de garupa estão relacionadas com as aptidões produtivas, Cockrill[2]. As correlações entre medidas corporais apresentam tendência de serem mais elevadas conforme aumenta a idade dos animais, Lee[4].

A largura de anca (LANC), mostrou-se correlacionada significativamente (p<0,01) com as demais medidas sendo os maiores valores com: CCOR (r=0,69), ASAC (r=0,67), AGAR (r=0,69), LTOR(r=0,65) e CGAR (r=0,41), tendo sido as medidas tomadas sobre bases ósseas, mostrando assim mais interdependências. As medidas de LANC e CGAR en

conjunto, apresentam correlações com as características reprodutivas, principalmente a facilidade de partos.

A CCAN, ROTU, CCAB e LCAB, foram as que apresentaram menores correlações em relação às demais medidas. A CCAN representa a ossatura, que para bubalinos é maior que em bovinos e apresentou maiores valores quando correlacionadas com peso (r=0,50) e LANC (r=0,52). A ROTU, ao contrário, não mostrou-se correlacionada com CABD (r=0,13), no entanto, foi maior em relação ao peso (r=0,47), uma vez que a ROTU representa o potencial de produção da musculatura do trazeiro refletindo diretamente no peso. O CCAB e a LCAB, apresentaram baixas correlações quando comparadas com as demais medidas, e são menos representativas para o tipo.

Os resultados do presente estudo para a caracterização das raças e do tipo dos bubalinos das raças Jafarabadi, Mediterrânea e Murrah, revelam que cada grupamento genético, possui características próprias diferenciadas entre si, e apontam valores que as diferem como de aptidão mista. Além disso, em todos os grupamentos observou-se a existência de uma interdependência entre as características do tipo.

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RESUMO

Entre os objetivos do presente projeto, inclui-se a descrição dos diferentes grupos genéticos de bubalinos, através da mensuração das características do tipo. Essas além de permitir descrição morfométrica dos animais permitem avaliar a proporcionalidade entre as regiões corporais. Além disso, também podem revelar as interrelações existentes entre as regiões, as quais resultam no equilíbrio exigido para descrever os tipos leiteiro e de corte. Foram mensuradas 150 fêmeas adultas registradas e com idades superiores 40 meses, sendo que as medidas tomadas foram: peso, alturas no garrote e sacro, comprimentos do corpo, cabeça e garupa, circunferências torácicas, abdominal e da canela e larguras da cabeça, anca e tórax. Os dados foram ajustados pelo método dos quadrados mínimos, para as causas de grupos genéticos e idade e determinadas as correlações simples e parciais entre as variáveis dependentes. os resultados revelaram a existência de uma interdependência positiva entre todas as características (P<0,01) tanto para as correlações simples como para as parciais, exceção feita entre a circunferência abdominal e o comprimento e largura de cabeça e a distância de rótula a rótula. Tais resultados permitem concluir a homogeneidade e a dependência existente entre as características dos três grupos raciais, garantindo assim, uma harmonia entre as principais regiões do corpo dos bubalinos.

SUMMARY

Among the objectives of this project, to included the description of differents genetics groups of buffaloes. This will permit to do the morfometric description of animal and permit to estimate the proporcionality among the body regions. More over, to can reveal too the intercorelations among regions that results in balance required to describe the dairy and beef type. If was used 150 females with more the 40 months of age, the traits mensured was: weight, wither and hip leight body, head and rumps lenght, chest, abdminal spindle circunference, head, rump and chest width and rotula and rotula distance. Descriptive traits were analyzed by least square methods. The results showed correlations positive among the traits, (P<.01), unless among abdominal circunference and head lenght and width and rotula and rotula distance. This results showed a homogeneity and dependence among traits in the three genetics groups of buffaloes.

SOMMAIRE

Entre les objectifs du ce projet on a mis la description de les differents groupes genétiques des buffles, a travers de la mensuration de les caracteristiques du type. Ces mensurations permetrent la description morfométrique chaque race et d'étuder la proporcionalité entre les differentes regions des corps des animaux. En outre, peuvent revéler les interelations existentes entre les regions du corps, resultant ainsi en equilibre exigé pour la description des types du lait et de la viande.

Pour la realization de c'etude on a mesuré 150 femelles adultes registrées, avec l'ages superieures a 40 mois, présentes a l'Expositions des animaux de São José do Rio Preto, Tietê, Araçatuba, Itapetininga, au Etat de São Paulo. Les trois groupes génétiques (Jafarabadi, Murrah et Mediterraneo) ont pris en égal number des individus. Les mensurations ont été: poids du corps, hauteur de

l'épaule et du sacrum, longueur du corps, tête et hanche, circunferences thoracique, abdominal et de la cannelle et largueur de la tête, hanche et thoracique. Les donnés ont été ajustés par le methode des carrés minimums.

Les results ont revelé l'existence d'une interdependence positive entre toutes les caracteristiques ($P<0,01$) autant pour les correlations simples comme pour les partieles, a l'exception entre la circunference abdominal et le longueur et largueur de la tête et la distance entre les rotules. Ces resultats permetrent concluir que il y a une homogénéité et dependence entre les caracteristiques des trois groups raciais.

EFFECTS OF STIMULATION OF FOLLICULAR ACTIVITY ON OESTROUS SYNCHRONIZATION WITH PROSTAGLANDIN

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INTRODUCTION

A limiting factor in use of prostaglandin (PG) $F_2\alpha$ or its analogues in oestrous synchronization programmes using artificial insemination (AI) is inability to predict time of oestrus (1,2). Fertility trials with $PGF_2\alpha$ in Zebu dairy cows and their crosses with Bos taurus breeds have seldom shown promising results since conception rates as low as 30% have been frequently reported (3,4,5). Several studies have shown that the response to $PGF_2\alpha$ depends on age of the corpus luteum, and therefore varies with the stage of the oestrous cycle at which it is given (5,6). Some of this variability is associated with ovarian follicular development. MacMillan and Henderson (7), Mark and others (8) have proposed that increase in the interval from $PGF_2\alpha$ injection to oestrus and improvement in precision of synchrony of oestrus may require synchronizing of both follicular development and luteolysis.

Crossbred cows are now very common in the tropics (Africa) and can be made to contribute more significantly towards milk production if only oestrous synchronization and artificial insemination could be improved (9). The success, however, depends mainly on accurate identification of onset of oestrus and its detection.

The aim of this study was to investigate the effects of stimulation of follicular activity on oestrous synchronization with $PGF_2\alpha$ and to analyse factors associated with conception in crossbred cows of the tropics.

MATERIALS AND METHODS

Study area

The study was conducted in ten farms within the practice area for the University Veterinary Clinic at Morogoro. Morogoro is 500-600m above sea level and lies along latitude 6° 50' South and longitude 37° 39' East. It receives over 800mm of rain spread over February to May and October to November. Temperatures are almost constant throughout the year, ranging from 27°C to 31°C at day time and not less than 14°C at night during the coolest months.

Animals

Animals used were crossbreeds of Friesian, Ayrshire and Jersey cows with indigenous Tanzania Short Horn Zebu. Cows were grazed in the mornings and evenings when temperatures were low, and were kept under shade and zero grazed in the afternoons when temperatures were high. In addition, cows were supplemented with concentrates at milking time according to standard nutrient requirements for dairy cattle. Morning milking was done between 06.00h and 08.00h and afternoon milking between 16.00h and 17.00h. Animals received regular veterinary care which included vaccinations, tick control and chemoprophylaxis against trypanosomiasis.

Field trials and laboratory analysis

52 cows with a healthy uterine environment and a fully grown corpus luteum palpable at rectal palpation and confirmed later by progesterone determination were selected (day 1 = day of selection) and randomly assigned into three groups. Sixteen cows in group 1 received intramuscular (IM) injection of 0.105µg gonadotropin releasing hormone (GnRH, Buserelin, Receptal(*), Hoechst Co., West Germany) on day 1 and 500µg cloprostenol (Estrumate(*), Imperial Chemical Industries, England), intravulvosubmucosally (IVSM) on day 8. Twenty cows in group 2 (control) received 500µg cloprostenol IVSM on day 1. Sixteen cows for group 3 received 0.105µg GnRH IM on day 1, 500µg cloprostenol IVSM on Day 8 and 0.105µg GnRH IM 8 days after insemination. Oestrus detection was done by the herdsman when the animals were at pasture and by a scientist when the animals were zero grazed. Oestrous signs which were observed and graded as absent = 1, weak/few = 2 and clearly exhibited = 3, included mucus discharge, oedematization of the vulva and mounting behaviour. Animals in oestrus within 3 days of $PGF_{2}\alpha$ treatment were inseminated upon detection of oestrus according to the am-pm insemination rule. Those animals not observed in oestrus within 3 days were also inseminated 72h and 96h after $PGF_{2}\alpha$ treatment. Starting two days before $PGF_{2}\alpha$ injection and ending two days after insemination, vagina mucus electrical resistance (VMER) using an ohmmeter (Brunstmessgeräte(*), Hauptner Co., West Germany) was determined once daily. Jugular blood (5ml) was collected on day 1, 8, day of insemination and day 19/20 into heparinized tubes and processed for progesterone analysis, and into plain tubes containing sodium fluoride and processed for glucose analysis. Plasma and serum samples harvested were frozen at -18°C until analysis. Glucose analysis was by glucose test kits (Merkost(*), Merck Co, West Germany). Progesterone was determined in plasma by radioimmunoassay testkits supplied by International Atomic Energy Agency (Seiberdorf, Austria).

Pregnancy diagnosis was done by rectal palpation in all cows 8 weeks after insemination.

RESULTS

All cows in group 1 and 3 had a palpable corpus luteum and a follicle ≤1cm at time of GnRH injection. Also all cows in the three groups had a palpable corpus luteum at time of $PGF_{2}\alpha$ administration and progesterone levels were >1ng/ml plasma. Several cows in group one and three, however, had large follicles greater than 1.5 cm at time of $PGF_{2}\alpha$ administration. The proportions of cows with large follicles were: 3 cows in group 1 with at least a follicle in the left ovary, 5 in the right and 8 in both ovaries; In group two (control with 20 animals), only 5 cows had some follicular activity, and among these, 4 were in the right ovary and one in the left; In group 3 (n = 16 cows), 3 cows had follicles in the left ovary, 9 in both ovaries and 4 in the right ovary.

The proportions of cows that showed oestrus within five days varied between the three groups. The median day following injection with $PGF_{2}\alpha$ at which oestrus was seen was day 3 in group 1 and 3, with ranges of two to six in group 1, and one to five in group 3. The median day for group 2 (control) was day 5, with ranges of two to nine days. In all animals exhibiting oestrus signs VMER values were between 25 and 35 ohms on day of oestrus.

The proportion of cows exhibiting clear oestrous signs (grade = 3) by day 3 after $PGF_{2}\alpha$ injection was 9 out 16 cows in group 1 and 8 out of 16 cows in group 3. Among the 14 animals not showing clear oestrous signs

(i.e grade = 2), five animals had palpable remnants of corpus luteum on the third day after $PGF_{2}\alpha$ injection, although progesterone levels ≤1ng/ml were recorded. Other 3 cows out of the 14 without clear oestrous signs had partial luteolysis with progesterone values remaining high (i.e. 2.2, 2.4 and 2.7ng/ml plasma). In group 2 (control), a total of three cows showed clear oestrous signs by day 3, five cows by day 4, eight by day 5 and nine by day 6. Among those with weak oestrous signs (n = 11 cows out of 20 cows) only six cows had progesterone values of less than 1ng/ml at oestrus. In all the three groups, the ovary (left or right) and number of follicles had no influence (P>0.1) on the intensity of the oestrous signs. Further details are shown in Text Table 1.

Also in all the three groups, cows which exhibited clear oestrous signs had glucose values >2.5nMol/litre (2.5nMol/l taken as borderline value with values greater than or equal to taken as indicative of positive energy balance). Among the cows which did not show clear oestrous signs three out of seven cows in group 1, four out of eight cows in group 3 and five out of seven cows in group 2 had glucose values of less than 2.5nMol per litre. Cows with a negative energy balance (glucose values ≤2.5nMol/l) showed on the average increase in the incidence of digital diseases. Among cows with clear oestrous signs and optimal glucose levels 2 out of 26 cows were lame. Among cows without clear oestrous signs but with optimal glucose levels two out five cows were lame whereas among cows without clear oestrous signs but with low glucose values lameness was diagnosed in ten out of 14 cows.

Pregnancy rates at 8 weeks after insemination tended to be lowest in Group 2 (control), followed by group 1. Retrospectively, 17 out 26 cows exhibiting clear oestrous signs at insemination were diagnosed pregnant at 8 weeks as compared to seven out 26 cows without clear oestrous signs. The difference between the groups being significant (P<0.01). Pregnancy rate was 56% in group 3 and 40% in group 1. Further details are recorded in Text Table 1.

Table 1. Effects of stimulating follicular activity on oestrous synchronization with prostaglandin and on fertility of Zebu crossbreds.

Group (n)	Number of cows with:		Mean P4* values			Oestrous grade			Conception Rates %
	Follicle	CL	A**	B***	C*	1	2	3	
one (n=16)	16	16	12.8 ±5.3	<1	-	-	7	9	43.7
two (n=20)	5	20	11.8 ±4.8	<1	-	3	8	9	40.0
three (n=16)	16	16	12.4 ±4.5	<1	9.8 ±6.9	-	8	8	56.0
Total	37	52				3	23	26	46.1

1 = Number of cows with follicles and corpus luteum detected per rectum on day of $PGF_{2}\alpha$ injection.
P4 = Progesterone values given as Mean±SEM ng/ml plasma.
A = P4 values on day of selection, B = on day of oestrus and C = on day of second GnRH injection after insemination.
Grades of oestrous signs 1= absent, 2 = weak & 3 = clearly present.

DISCUSSION

GnRH stimulate and regulate various stages of ovarian follicular growth in cattle (10). In this study, injection of GnRH in group 1 and 3 resulted into all cows developing follicles which were palpable per rectum on day 8, which was the day of cloprostenol injection. In comparison to group 2 (control), only five out of twenty cows had follicles greater than 1.5 cm. This indicates that stimulation of follicular activity in Zebu crossbred cows could be carried out successfully by use of GnRH. However, despite the presence of follicles on day of $PGF_2\alpha$ injection only 18 out of 32 animals showed clear oestrous signs. This indicates that the presence of GnRH induced follicular activity had little success in increasing intensity of oestrus. This information conforms to what is reported in literature and in agreement with Doble and Gupta (11). Nevertheless, in this study, oestrus occurred within five days after $PGF_2\alpha$ treatment in group 1 and 3. This was confirmed by the VMER values decreasing to minimum (25<35ohms). Thus the use of stimulation of follicular development prior to $PGF_2\alpha$ injection increases the number of animals synchronized within five days period. This also indicates that the common schedule of blind inseminations 72h and 96h after $PGF_2\alpha$ injection is worthwhile in the Zebu crosses only after stimulation of follicular development, though GnRH is expensive.

Some animals in group 1 and 3 treated with GnRH had corpora lutea detectable per rectum on day of oestrus and progesterone levels were above 1ng/ml. Most of these cows exhibited unclear oestrous signs. In literature occurrence of oestrus in presence of active corpus luteum is common during pregnancy and ovarian cystic degeneration (12).In this study no such animals became pregnant. Luteal dysfunction leading to inadequate progesterone production soon after conception could be a cause of high embryonic mortalities observed in the tropic (13, 14). However, as yet it is not clear whether progesterone supplementation could reduce embryonic mortality. In this study, injection of GnRH 8 days after breeding inproved pregnancy rates significantly ($P < 0.01$), but not to 100%. GnRH support of luteal function apparently increases progesterone production and it can be used to inprove fertility. Currently, however, GnRH is expensive and advantages obtained from increased pregnancy rates might be offset by the costs of GnRH.

Some animals in all the groups did not show clear oestrous signs. Most of the animals had digital problems suggesting that the animals were unable to graze properly. This wa substantiated by the low glucose levels in these animals. Indeed the results confirm that energy is most important not only for fertility but also for exhibition of oestrous signs.

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SUMMARY

To study the effects of stimulation of follicular activity on oestrous synchronization with $PGF_2\alpha$ 52 crossbred Zebu dairy cows were selected (day 1 = day of selection) randomly grouped and treated as follows: group 1 (n = 16 cows) GnRH on day 1, $PGF_2\alpha$ on day 8; group 2 (control, n = 20 cows) $PGF_2\alpha$ on day 1 and in group 3 (n = 16) GnRH on day 1, $PGF_2\alpha$ on day 8 and GnRH 8 days after insemination. Oestrus was detected visually and by determination of progesterone and vagina mucus electrical resistance. Glucose was determined to evaluate energy balance. The results showed that interval from PGF2α injection to oestrus was within 5 days when follicular activity was stimulated prior to PGF2α treatment. However, intensity of oestrous signs was not influenced by follicular activity. Lameness and pain was associated with poor oestrous signs and low glucose levels. It is concluded that stimulation of follicular activity followed by $PGF_2\alpha$ injection can improve oestrous synchronization allowing for inseminations to be done within five days of PGF2α treatment.

ZUSAMMENFASSUNG

In einen Feldversuch wurde die praktische Anwendung der Stimmulation von Follikeln mittels GnRH vor der Applikation von $PGF_2\alpha$ zur Brunstinduzierung bei 52 Zebu MilchkÜhen geprÜft. Die Behandlung bestand in einer Applikation eines GnRH am Tag 1 und eines $PGF_2\alpha$ Analoges am Tag 8 (Gruppe 1, n = 16 KÜhe), in einer einmaligen Applikation von $PGF_2\alpha$ Analoges am Tag 1 (Gruppe 2, kontrolle, n = KÜhe) oder in Verabreichung von GnRH am Tag 1, $PGF_2\alpha$ an Tag 8 und GnRH nochmals in Abstand von 8 Tagen nach einer Besamung (Grupp 3, n = 16 KÜhe). Zur Abklärung des Effektes von GnRH und $PGF_2\alpha$ wurden rektalen Ovardiagnose durchgefÜhrt. Progesterongehalt in Plasma wurde bestimmt. Als weitere Paraneter wurden Messungen des elektrischen Scheidenwiderstandes durchgefÜhrt. Glukosegehalt wurde bestimmt, un Hinweise auf den Energiestoffwechsel zu gewinnen. Die KÜhe wurden auf Grund aÜsseren Brunsterscheinungen besamt. Die KÜhe in Gruppe 1 und 3, die mit GnRH für einer Follikelnstimmurierung behandelt wurden, kommen in Brunst einer von 5 Tagen nach $PGF_2\alpha$ Applikation. Lahmheit und Schmerzen wurden besonders mit unzufriedigend Brunsterscheinungen und unzureichen Glukosegehalt verbinden. Aufgrund der vorliegender Ergebnisse kann die Eignung von GnRH Applikation vor $PGF_2\alpha$ Behandlung bestätigt werden.

RESUME

Pour étudier les effets de la stimulation de l'aectivité folliculaire sur la synchronisation oestrale avec $PGF_2\alpha$, 52 croisés de Zebu et vaches laitière étaient sélectionnés (jour 1 = jour de selection) groupés au hazard et traités comme suis: groupe 1 (n = 16 vaches), GnRH au jour 1, PGF2α au jour 8; Groupe 2 (temoin, n = 20 vaches) $PGF_2\alpha$ au jour 1, Groupe

3 (n = 16 vaches) GnRH au jour 1, $PGF_2\alpha$ au jour 8 , GnRH au jour 8 après insemination. L'oestrus était détecté visuellement et par détermination de progesterone et par résistance électrique du mucus vaginal. Le glucose était déterminé pour évaluer l'équilibre énergétique. Les résultats ont montré que l'intervale entre l'injection de $PGF_2\alpha$ et l'oestrus était d'au plus 5 jours quand l'activité folliculaire était stimulée avant traitement avec $PGF_2\alpha$. Mais l'intensité des signes d'oestrus n'était pas influencée par l'activite folliculaire. La boiterie et la douleur étaient associées avec les signes d'oestrus très peu marqués et le niveau bas de glucose. Nous pouvons conclure que la stimulation de l'activité folliculaire suivie de l'injection de $PGF_2\alpha$ peux ameliorer la synchronisation ostrale permettant de faire les inseminations dans les 5 jours qui suivent le traitement avec $PGF_2\alpha$.

EFEITOS DE RAÇÕES COM DIFERENTES NÍVEIS DE NITROGÊNIO DEGRADÁVEL NO RÚMEN SOBRE OS DESAPARECIMENTOS IN SITU DA MATÉRIA SECA, MATÉRIA ORGÂNICA, PROTEÍNA BRUTA E FIBRA EM DETERGENTE NEUTRO EM BÚFALOS.

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INTRODUÇÃO

O búfalo é reconhecido por técnicos e pecuaristas como um animal rústico, capaz de viver em locais onde não sobrevivem outras espécies, ou seja, sob pastejo de forragem de baixa qualidade e em regiões alagadas . Porém, há uma carência acentuada de dados de pesquisas sobre os parâmetros no rúmen quando estes são alimentados com os diversos tipos de rações.

A estimativa da quantidade de proteína do alimentos que é degradada pelos microrganismos no rúmen pode ser feita pela técnica de sacos de fibra artificial, com o uso de animais canulados no rúmen (7, 14, 12, 8). A adoção de um novo enfoque para calcular os requerimentos de proteína dos ruminantes pelo fracionamento da proteína da dieta em termos de proteína degradável no rúmen (PDR) e proteína não degradável no rúmen(PNDR) indica uma necessidade de reconsideração nas diferenças no crescimento em reposta aos vários suplementos de nitrogênio (10).

Nocek et alii, 1979 (9) estudando as degradabilidades do nitrogênio e materia seca de varios alimentos em bovinos alimentados com rações contendo três níveis de nitrogênio degradável no rúmen: NDR 30%; NDR 45% e NDR 60%, concluíram que quando ingredientes de definido NDR são combinados para formular ração de especifico NDR total, os resultados são variaveis. Posteriormente, Cummins et alii, 1983 (3) concluíram que a técnica de sacos de náilon in situ, parece produzir dados que permitem a formulação de rações com definidas degradabilidades do nitrogênio no rúmen, podendo ser usadas rotineiramente. Segundo Örskov, 1982 (11) uma vez que os microrganismos no rúmen necessitam de uma fonte de N degradavel e o ruminante hospedeiro requer proteína intacta para a digestão no intestino delgado, a degradabilidade da proteína no rúmen e as caracte - risticas dinâmicas envolvidas nesse processo desempenham papel central no novo sistema de avaliação dos requerimentos de proteína para os ruminantes.

O presente trabalho teve como objetivos: A) avaliar os efeitos da alimentação de búfalos com rações contendo diferentes níveis de nitrogênio degradável no rúmen, sobre os desaparecimentos da materia seca (MS), materia orgânica (MO), proteína bruta (PB) e fibra em detergente neutro (FDN), utilizando-se capim, milho e soja; B) determinar a concentração de amônia e valores de pH no líquido ruminal e C) estimar as degradabilidades da proteína desses alimentos no rúmen.

MATERIAL E MÉTODOS

Este trabalho foi conduzido no Campus de Pirassununga da Universidade de São Paulo, utilizando-se um experimento em Quadrado Latino (4 x 4),

com quatro búfalos (**Bubalus bubalis** L.), adultos, canulados no rúmen. Os tratamentos consistiram de quatro rações com diferentes níveis de nitrogênio degradável no rúmen (NDR): NDR 60%; NDR 80%; NDR 100% e NDR 120%. As rações foram calculadas a partir da quantidade de NDR suficiente para atender as exigências dos microrganismos para a máxima síntese de proteína microbiana (NDR 100%), utilizando-se o valor de 1,34 g N / MJ de energia metabolizável da ração, de acordo com o A.R.C., 1980 (1) e A.R.C., 1984 (2). Dessa forma, as rações foram acompanhadas por níveis crescentes de proteína bruta (8,7; 10,5; 12,3 e 14,3%). O volumoso utilizado foi o capim sempre verde (**Panicum maximum** Jacq var. gongyloides) cortado a campo, e o concentrado foi constituído de milho em grãos moídos e soja em grãos tostados e moídos. As composições das rações podem ser vistas na Tabela 1.

TABELA 1. Composições das rações consumidas com base na matéria seca.

Ingredientes	Rações (Tratamentos)			
	NDR 60%	NDR 80%	NDR 100%	NDR 120%
	(%)			
Capim sempre verde	70,0	70,4	70,3	69,7
Milho em grãos moídos	27,4	21,8	16,7	11,7
Soja em grãos tostados e moídos	2,6	7,8	13,0	18,6

Nos últimos dois dias de cada subperíodo foram introduzidos no rúmen de cada búfalo, via fístula, 35 sacos de náilon, feitos com tecido de paraquedas (10), compreendendo 3 ingredientes (capim sempre verde seco, milho em grãos e soja em grãos tostados e moídos), 2 repetições, 5 tempos de incubação no rúmen (3; 6; 12; 24 e 48 horas) e mais 5 brancos. Amostras do capim foram pré-secas a 65ºC por 72 horas em estufa e moídas em moinho Wiley com peneira de 1 mm, e os cereais em peneira de 2 mm. Foram colocadas entre 5 a 7 gramas de amostra em cada saco. Após retirados do rúmen, os sacos eram lavados um a um em água corrente, pré-secos, pesados e foram determinadas a MS, cinzas, e PB em todas as amostras e FDN apenas no capim. A solubilidade em água da proteína bruta foi determinada com sacos de náilon embebidos em água aquecida a 38ºC por 15 min (8) e utilizada para estimar a degradabilidade da proteína, através das fórmulas: $p = a + b(1 - e^{-ct})$; $p = a + bc/c+k$ (13). Para tanto, assumiu-se um taxa de "turnover" de 0,02/h. No último dia de cada subperíodo foram colhidas amostras do líquido ruminal, antes da primeira alimentação, uma, quatro e oito horas após, para determinações do pH, com o uso de medidor de pH digital, e da concentração de amônia (17).

RESULTADOS E DISCUSSÃO

Os búfalos apresentaram consumo médio de matéria seca de 76,92 g / $kg^{0,75}$ ou 1,7% do peso vivo, não sendo observadas diferenças significativas ($P > 0,05$) entre os tratamentos.

Não houve diferenças significativas ($P > 0,05$) entre os níveis de N degradável na ração nos desaparecimentos da MS, MO e PB do capim, milho e soja e FDN do capim, em qualquer tempo de permanência dos sacos de náilon no rúmen dos búfalos, exceto para a MS e MO do milho ($P < 0,05$), cujas média gerais por tratamento, mostraram um ligeiro aumento com a elevação do nível de N degradável na ração (Tabela 2). Estes resultados

concordam com Faria & Huber, 1984 (4) que também não verificaram efeitos significativos no desaparecimento da matéria seca entre três tipos de forragens (silagem de milho, feno de alfafa e feno de gramínea) em novilhos fistulados após incubações de sacos de dracon por 24; 48 e 72 horas, nem em função do teor de proteína (8,1; 11,3 e 13,3%), nem da concentração de energia da dieta.

O A.R.C., 1980 (1) apresenta dois valores (0,55 e 0,60) para a degradabilidade da proteína do milho no rúmen. Já, para a farinha de soja cita os valores de 0,60 e 0,83 para após 6 e 12 horas de incubação no rúmen respectivamente, determinados pela técnica de sacos de náilon, concluindo que o desaparecimento após 6 horas parece ser a medida mais apropriada para a degradabilidade. Os valores citados, são quase os mesmos encontrados no presente trabalho para a soja em grãos tostados, nos respectivos tempos de permanência no rúmen, que foram: 0,6034 e 0,8019 (Tabela 2). Os desaparecimentos da matéria seca e matéria orgânica no rúmen dos búfalos prosseguiram em diferentes taxas para os três alimentos, sendo mais lento com o capim sempre verde, seguido do milho e por último a soja. As degradabilidades estimadas da proteína no rúmen do capim sempre verde, milho em grãos e soja em grãos tostados, foram: 41%, 59% e 86%, respectivamente. Esses valores são muito próximos aos citados pelo A.R.C., 1980 (1) e que foram utilizados nos cálculos das rações no presente trabalho. Houve diferenças significativas entre os tratamentos ($P < 0,01$), na concentração de amônia no líquido ruminal, mostrando um efeito crescente nos valores médios de amônia com a elevação no NDR na ração (Tabela 3). Este efeito também foi verificado por vários pesquisadores (5, 15, 6). Não foram observadas diferenças significativas entre os tratamentos, nos valores de pH, cuja média foi 6,65. Este valor se encontra dentro da faixa de pH no rúmen considerada normal para a espécie bubalina, com vários tipos de alimentação (16), e ligeiramente abaixo dos valores de pH (6,98 e 7,03) encontrados em búfalos alimentados com 100% e 80% do requerimento de proteína digestível (PD) da mantença, respectivamente, por Pande & Shukla, 1981 (15), mas os dois níveis de PD na ração também não diferiram significativamente nos valores de pH.

TABELA 2. Desaparecimento da matéria seca, matéria orgânica e proteína bruta do capim, milho e soja e da fibra em detergente neutro do capim, em sacos de náilon no rúmen. (Médias gerais).

Tempo no rúmen (ha)	MS			MO			PB			FDN
	capim	milho	soja	capim	milho	soja	capim	milho	soja	capim
	(%)									
3	22,64	34,81	55,62	21,08	34,28	54,88	39,12	36,12	54,35	11,3
6	23,73	41,51	60,94	22,06	41,03	61,05	37,18	39,16	60,34	12,28
12	28,23	54,85	81,06	26,65	54,67	80,69	37,41	43,33	80,19	17,77
24	34,32	73,20	95,44	32,94	73,07	94,72	43,31	55,69	97,36	26,51
48	40,60	92,67	99,19	39,38	93,76	45,13	45,13	82,91	99,62	32,20

TABELA 3. Concentrações médias de amônia e valores de pH no líquido ruminal.

	NDR 60%	NDR 80%	NDR 100%	NDR 120%	Média
Amônia	7,20 a	12,14 b	18,59 c	19,42 c	14,34
pH	6,67	6,66	6,65	6,64	6,65

Médias seguidas por letras distintas diferem entre si pelo teste de Tukey ($P < 0,01$).

CONCLUSÕES

Nas condições experimentais desenvolvidas no presente trabalho, os resultados permitiram obter as seguintes conclusões:

1. Não houve influência de rações com quatro níveis de nitrogênio degradável no rúmen (60%, 80%, 100% e 20%) sobre o desaparecimento da proteína bruta do capim sempre verde, milho em grãos e soja em grãos tostados.

2. Rações com diferentes níveis de nitrogênio degradável no rúmen (NDR 60%, 80%, 100% e 120%), não influenciaram nos desaparecimentos da matéria seca e matéria orgânica no rúmen do capim sempre verde e soja em grãos tostados, mas houve diferenças significativas com o milho em grãos.

3. Não houve diferenças significativas no desaparecimento da fibra em detergente neutro do capim entre rações com quatro níveis de nitrogênio degradável no rúmen, 60%, 80%, 100% e 120%.

4. Rações com níveis crescentes de nitrogênio degradável no rúmen (NDR 60%, 80%, 100% e 120%) promoveram elevação nos teores de amônia no líquido ruminal até o nível de NDR 100% e não influenciaram o pH no rúmen.

5. Assumindo a taxa de turnover no rúmen de 0,02/h, as degradabilidades estimadas da proteína bruta do capim sempre verde, milho em grãos e soja em grãos tostados, no rúmen, foram: 41%, 59% e 86%, respectivamente.

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RESUMO

Foram formuladas quatro rações isoenergéticas contendo diferentes níveis de nitrogênio degradável no rúmen (NDR 60%, 80%, 100% e 120%). O capim sempre verde (**Panicum maximum** Jacq. var. gongyloides) cortado a campo foi utilizado como volumoso (70%) e o milho em grãos moídos e soja em grãos tostados e moídos serviram de concentrado (30%). Quatro búfalos canulados no rúmen, foram utilizados em um experimento em Quadrado Latino 4x4. Sacos de nailon contendo individualmente capim, milho e soja foram incubados no rúmen por 3, 6, 12, 24 e 48 horas. Coletas do líquido ruminal foram realizadas antes da alimentação, 1, 4 e 8 horas após para determinações do pH e amônia. Não houve diferenças significativas ($P > 0,05$) nos desaparecimentos da matéria seca (MS), matéria orgânica (MO) e proteína bruta (PB) nos alimentos estudados, exceto na MS e MO do milho, e nem na fibra em detergente neutro (FDN) do capim entre os níveis de NDR da dieta. As rações contendo níveis crescentes de NDR produziram elevação na concentração de amônia no líquido ruminal até o nível de NDR 100% ($P < 0,05$), mas não influenciaram o pH no rúmen. Assumindo a taxa de turnover de 0,02/h, as estimativas das degradabilidades da proteína bruta foram: 41%, 59% e 86% para o capim sempre verde, o milho em grãos e a soja em grãos tostados, respectivamente.

SUMMARY

"EFFECTS OF RATION WITH DIFFERENT LEVELS OF RUMEN DEGRADABLE NITROGEN ON IN SITU DISAPPERARANCE OF DRY MATTER, ORGANIC MATTER, CRUDE PROTEIN AND NEUTRAL DETERGENT FIBER IN BUFFALOES."

Four rations were formulated with different levels of rumen degradable nitrogen (RDN 60%, 80%, 100% and 120%) using Panicum grass (**Panicum maximum** Jacq var. gongyloides), corn grain and toasted soybeans in a 70:30 roughage: concentrate ratio. The four rations had increased levels of crude protein (8.7; 10.5; 12.3 and 14.3%) and were fed to rumen cannulated buffalo in a 4 x 4 latin square design. The grass was dried and each dietary component was placed individually in nylon bags and suspended in the rumen of each buffalo for desappearance determination

of dry matter (DM), organic matter (OM), crude protein (CP) and additionally, for the grass, neutral detergent fiber (NDF) and removed at intervals of 3, 6, 12 , 24 and 48 h. The buffaloes were fed twice daily , and each period was of 3 weeks duration. Samples were taken from the rumen for pH and ammonia concentration at 0, 1, 4 and 8 h after the morning feedings on day 21 of each period. The mean dry matter intake was 76.92 g/kg^{0,75} and no significant difference in DM, OM, CP or NDF degradation occurred with grass, toasted soybeans, and CP in corn, by dietary treatment, however significantly ($P<0,05$) higher DM and OM degradation of corn occurred at the higher RDN levels. Increasing RDN in the ration resulted in a significant ($P<0,01$) increased ruminal ammonia concentration (7.20, 12.14 and 18.59 mg NH3/100 ml rumen liquid) but not for RDN 120%(19.42), and did not influence pH (average 6.65). Assuming a turnover rate of 0.02/h, the "in situ" nylon bag technique yielded protein degradability on individual feedstuffs of 0.41 for Panicum grass, 0.59 for corn grain and 0.86 for toasted soybeans.

ZUSAMMENFASSUNG

"WIRKUNGEN DER FUTTERARTEN MIT VERSCHIEDENEN NIVEAUS DES IM PANSEN ABBAUBAREN STICKSTOFFES ÜDER DAS VERSCHWINDEN IN SITU DER TROCKENMASSE , DER ORGANISCHEN MASSE, BRUTTO EIWEISS UND FASERINHALT IN NEUTRALEN DETERGENTTEN, IM BUFFEL".

Es wurden vier isoenergetische Futterarten zusammengestellt, die verschiedene Niveaus des im Pansen abbaubaren Stickstoffes enthielten, in den Proportionen: 60, 80, 100 und 120%. Das Grüngras (**Panicum maximum** Jacq. var. gongyloides), im Feld geschnitten, wurde als Volumos (70%) benutzt, und gemahlene Maiskörner, wie auch gerostete und gemahlene Sojabohnen, wurden als Kraftutter gebraucht (30%). Vier Buffel wurden in einem Versuch verwendet, der dem Schema des Lateinischen Quadrates 4 x 4 entsprach. Kleine Nailonsäcke, die Gras, Mais oder Soja enthielten , wurden im Pansen 3, 6, 12, 24 und 48 Studen Incubationzeit gelassen . Pansenflussigkeit wurde vor der Verfütterung, und 1, 4 und 8 Stunden danach abgezapft, zur Bestimmung des pH-Werts und des Amoniakwertes. Es gab keinen beachtungswerten Unterschied ($P>0,05$) im verschwinden der Trockenmasse,der organischen Masse und dem Brutto Eiweiss in den untersuchten Elementen, ausser in der Trockenmasse und in der organischen Masse des Maises, wie auch nicht im Faserinhalt in Neutralen Detergentien des Grungrases, zwischen den Niveaus des im Pansen abbaubaren Stickstoffes der Diat. Die Futterarten, die steigende Werte des in Pansen abbaubaren Stickstoffes beinhalteten, produzierten die Erhöhung der Amoniakonzentration der Pansenflüssigkeit, bis zum Niveau vom 100% im Pansem abbaubaren Stickstoffes ($P< 0,05$), aber beeinflussten den pH-Wert des Pansen nicht. Der angenommene "turnover"-WErt von 0,02 / h ermoglichte die Bestimmung der abgebauten Brutto Eiweisswerte von 41 % fur das Grüngras, 59% für die gemahlene Maiskörner und 86% fur die gerösterte und gemahlene Sojabohnen.

PERFORMANCE PRODUTIVA E REPRODUTIVA DE BOVINOS LEITEIROS DA RAÇA GIR

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INTRODUÇÃO

A importância dos esforços dispendidos pelos homens pioneiros da seleção do Gir leiteiro pode ser avaliada, dizendo que o leite é o mais completo alimento natural, imprescindível à estrutura da alimentação humana para o desenvolvimento sócio-econômico. Em comparação aos outros alimentos de origem animal, a produção leiteira é, todavia, a mais difícil de alcançar elevada produtividade no mundo tropical.

Decorrida duas ou três décadas e algumas gerações de zebuínos Gir selecionados para a produção de leite, era igualmente indispensável fazer operação de recolhimento dos resultados dispersos em doze rebanhos de melhoramento, incluindo oficiais e privados para ter ampla visão unitária dos níveis de produção leiteira e de eficiência reprodutiva e outras partes essenciais.

Os animais da raça Gir, embora considerado por alguns autores pobres produtores de leite, ocupam o primeiro lugar, juntamente com a Sahival entre as raças zebuínas, seguindo-se os das raças Tharparkar, Red Sindhi, Hariana e outras. Para isto, não se leva em conta os vários fatores envolvidos, mas apenas as produções médias e máximas verificadas por (18), para animais da raça Red Sindhi, com 1.969,2 kg, (3), para um rebanho da raça Hariana, com 1.588,6 kg, (14), para a raça Tharparkar com 2.428,8 kg, na India. Já no Brasil, (27), (35) e (22) em rebanhos Gir, relataram, na ordem, as produções média por lactação de 2.832,0, 3.741,6, 3.027,7 e 2.715,0 kg.

Quanto a produção de gordura, no Brasil (24), (21) e (35), estudando, 1147, 79, 1333 e 871 lactações provenientes de rebanhos Gir, anunciaram, respectivamente, as seguintes produções e percentagem de gordura: 88,8 kg e 4,52%, 187,8 kg e 5,02%, 134,0 kg e 4,86% e 155,4 e 5,14%. Para o período seco, os estudos de (11), (13) e (20), para a raça Gir apresentaram, respectivamente as médias de : 195,10, 270,77, 176,30 e 231,90 dias. Para a duração da lactação os estudos de (26), na India, (23), (33) e (35) esses no Brasil relataram as médias de 327,7, 256,0, 278,1 e 298,7 dias respectivamente. Nas características reprodutivas, o período de serviço torna-se o principal fator de controle do intervalo entre partos. É essa variável que determina a eficiência reprodutiva dos bovinos, uma vez que o período de gestação tem pouca variação para a espécie. Sob condições de trópico, no Brasil, (9), revelaram a mais alta média do período de serviço, 364,0 dias, enquanto (33) obtiveram a mais baixa média, 110,5 dias. Isso mostra que o intervalo entre partos oscilou em média de 651,0 dias a 397,0 dias, tendo-se o período de gestação a duração média de 287,0 dias como revelou (20).

A idade por ocasião da primeira parição tem-se mostrado ocorrer em idade avançada 50,16 meses, como revelam os estudos de (15), no

Paquistão e Índia.

No Brasil (7), obtiveram 45,8 meses para a idade à primeira parição, porém não faltavam dados inferiores, 42,0 meses como os revelados por (30), (31) e (27). Porém, ainda no Brasil (20), (21) e (10), revelaram a média de 52,0 meses para animais da raça Gir exploradas para leite.

Constituiu-se em objetivo do presente estudo apresentar os parâmetros do desempenho produtivo e reprodutivo dos animais dessa raça, no Brasil tropical.

MATERIAL E MÉTODOS

Constitui-se em material do presente estudo, a avaliação de 8.274 lactações controladas de vacas da raça Gir, pertencentes a doze rebanhos dos Estados de São Paulo, Minas Gerais e Rio de Janeiro, das quais 7.661 foram determinadas a percentagem de gordura. Destas lactações foram possíveis identificar 4.438 observações pertencentes às características reprodutivas (intervalo entre partos, período de serviço e gestação e, de gestação e lactação simultâneas) e, 2.364 vacas com dados de nascimento e da primeira parição. Essas informações foram obtidas de 4.126 vacas acasaladas por 570 touros no período de 1948 a 1982, cujos controles foram homologados pela Associação Brasileira de Criadores de Zebú. No presente estudo, limitou-se a estimar os parâmetros médios e seus respectivos desvios padrões, das características produtivas e reprodutivas para avaliar a performance dos animais da raça Gir sob as condições de trópicos.

RESULTADOS

A Tabela 1, reune os valores médios do desempenho produtivo e reprodutivo de zebuínos leiteiros da raça Gir, explorados para leite sob condições de trópicos.

TABELA 1. Características produtivas e reprodutivas de vacas da raça Gir. Número de observações, média e desvio padrão.

Características	No. de Observações	Média	D.P
Produtivas:			
Prod. de leite obser. (kg)	8.274	2.513,00	930,93
Prod. de leite em 305d (kg)	8.274	2.508,59	669,29
Prod. de leite/dia lact. (kg)	8.274	8,38	2,30
Duração da lactação (dias)	8.274	296,75	65,54
Período seco	8.274	231,60	194,00
Prod. de gordura (kg)	7.661	125,88	52,06
Prod. de gord./em 305 dias (kg)	7.661	125,16	50,87
Prod. de gord./dia lact. (kg)	7.661	0,413	0,145
Percentagem de gordura (%)	7.661	4,91	0,53
Reprodutivas:			
Período de gestação (dias)	4.438	287,06	2,80
Período de serviço (dias)	4.438	244,16	140,25
Intervalo entre parto (dias)	4.438	531,49	140,01
Período gestando e lactando (dias)	4.438	72,12	106,01
Idade à primeira parição (dias)	2.364	1.430,36	256,00

Os valores médios obtidos para as carcterísticas produtivas colocam em destaque os animais da raça Gir no Brasil, quando comparados a outros zebuínos leiteiros existentes. A média obtida para a produção de leite é alta como as mencionadas por (27), (20) e (35). Para a duração de lactação a média de 296,75 dias, corrobora com os relatos de (6), (20) e outros. Para a produção e percentagem de gordura do leite, os resultados obtidos superam os relatos por (29) e outros. O período seco de 231,0 dias corrobora aos relatados por (4), (5), (19), (31) e (20) e outros.

Todavia a longa duração do período seco revela que as vacas Gir deste estudo só entraram em gestação nos dois últimos meses de lactação, indicando assim, a inabilidade da raça de desempenhar simultaneamente três atividades: manutenção, produção e reprodução o que lhe compromete o desempenho reprodutivo.

Para as características reprodutiva, o valor médio obtido para o período de gestação, confirma os achados da maioria dos pesquisadores de zebuínos, colocando-se entre o menor valor, 283,5 dias constatado por (25) e (16), o mais longo, 290,2 dias, por (30). Para o período de serviço, a média obtida é alta, revelando uma baixa eficiência reprodutiva, 68,72% para os animais estudados, sendo semelhante aos estudos de (9), (28), (12) e outros. Tal fato levou a obtenção de intervalo entre partos longo, 531,49 dias, porém, se acha no intervalo estabelecido por (1), para as raças zebuínas especializadas e se assemelha aos valores relatados por (9), (28), (20) e outros. Isso requer que se faça controles adequados dos fatores de meio e genético que interferem na variável, a fim de propiciarem maior eficiência reprodutiva para os animais da raça explorada para produção de leite.

A idade à primeira parição de 1430,36 dias, verificada na presente estudo é alto, porém se encontra de acordo com os achados de (15), (7), (2) e (20), além aos de outros estudos da raça Gir.

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RESUMO

Constitue em objetivo do presente estudo, avaliar os parâmetros das características produtivas e reprodutivas dos principais rebanhos leiteiros da raça Gir que há tempo vem sendo controlados. Utilizou-se dados de doze rebanhos distribuídos pelos Estados de São Paulo, Minas Gerais e Rio de Janeiro. Os dados parciais apresentaram os seguintes resultados para as características produtivas: com (8.274 obs.) Produ. de leite observ.= 2.513,00 ± 930,93 kg, prod. de leite em 305 dias= 2.508,59 ± 669,29 kg, prod. de leite/dia de lactação = 8,38±2,30 kg, dur. da lact. = 296,75±65,54 dias, per. seco = 231,60±194,00 dias, com (7.661 obs.) prod. de gord. = 125,88±52,06 kg, prod. de gord. em 305 dias = 125,16±50,87, prod. de gordura/dia de lact. 0,413±0,145 kg, percentagem de gordura (%) 4,91±0,53; características reprodutivas: com (4.438 obs.) per. de gest.= 287,06±2,80 dia, per. de serv.= 244,16 ±140,25 dias, intervalo entre parto= 531,49±140,01 dias, per. de gest. + lact. = 72,12±106,01 dias, idade à primeira parição = 1.430,36 ± 256,00 dias para 2.364 observações.

Concluiu-se dos resultados que os zebuínos da raça Gir podem ser utilizados como uma alternativa do sistema de produção de leite, à pasto, todavia o manejo reprodutivo deve ser melhorado em decorrência da baixa eficiência reprodutiva, em comparação com outras raças leiteiras.

SUMMARY

The purpose of this study were to avaliat the productive and reproductive traits of dairy Gir cows belongs to various herds of São Paulo, Minas Gerais and Rio de Janeiro States. The results showed that dairy Gir cows to can be exploited for milk production (2.513,00±930.93 kg or 8.381±2.30 kg/day) in pasture condition but it has however fell= reproductive efficiency (calving interval of 531.49 ± 140.01 day or 68,72%).

RESUMO

El proposito deste estudio fue de avaliar los parametro de las caracteristicas productivas y reproductivas de las principales fincas del ganado Gir de los Estado de São Paulo, Minas Gerais y Rio de Janeiro. Los resultados indicaram que las vacas Gir pueden ser utilizadas en el sistema de produccion de leche de las pasturas, todavia el manejo reproductive tiene de ser mejorado en decorencia de la baja eficiencia reproductive en comparacion con outras razas lecheras.

COMPARATIVE EVALUATION OF THERAPEUTIC TRIALS IN UREA INDUCED AMMONIA TOXICITY IN BUFFALO CALVES

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INTRODUCTION

The economic savings that occur due to feeding of non-protein-nitrogenous (NPN) substances has evoked a keen interest amongst dairy scientists all over the world. Though the research carried out in the last two decades has helped in better understanding of the principles involved in utilization of NPN in ruminants, but unjudicious use of urea by the feed manufacturers and other dietary errors has often led to ruminal alkalosis in the dairy animals maintained on high concentrate diets (4,5). Since there is paucity of information on the efficacy of various therapeutic regimen in buffaloes, therefore, the present therapeutic trials were undertaken in urea induced ammonia toxicity in growing buffalo calves.

MATERIALS AND METHODS

Experimental animals and their management

Fifteen, 1 - 1½ year old Murrah buffalo calves, reared together at the dairy farm of Punjab Agricultural University, weaned soon after birth were selected for experimental studies. Ten calves were included, two in each group comprising treatments (a) to (d), whereas five calves were included in treatment group (e). These calves were kept in the animal shed under good management conditions. The daily feeding schedule consisted of concentrate mixture (DCP, 14% & TDN, 70%), green fodder and dry roughages.

Induction of ammonia toxicity and sampling procedure

After an overnight fasting, rumen alkalosis was induced in calves by intra-ruminal feeding of urea at the rate of 1.25 g/kg body weight as a single dose. Rumen liquor and blood samples were drawn before induction and at 1, 2, 4, 24, 48 and 72 hrs after induction of urea toxicosis.

Therapeutic trials

The therapeutic trials comprising of different treatments were as under :

(a) Oral administration of acetic acid.
(b) a + parenteral fluid therapy (Normal saline) and 10% solution of magnesium sulphate (intravenous drip).
(c) a + parenteral administration of 5% solution of sodium glutamate in normal saline.
(d) c + parenteral administration of diazepam.
(e) d + evacuation of rumen contents
(f) a & evacuation of rumen contents at the terminal stages of urea toxicity with subsequent transplantation of fresh rumen cud.

In all the treatment groups, parenteral administration of liver tonics and oral administration of ruminotoric (Antimony potassium tartarate) was also carried out.

Treatment procedures

The oral administration of 5% acetic acid for neutralization of ruminal alkalinity was started at about 60 minutes and was repeated at 90 and 120 minutes after induction in groups (a) to (d) whereas a single oral administration of acetic acid was followed in groups (e) and (f). Parenteral fluid therapy for reduction of hyperammoniaemia was started at 60 minutes and was repeated after 60 minutes of the first infusion and 24 hours of induction. Parenteral administration of liver tonics for restoration of derranged liver functions started at 60 minutes was repeated at 24 and 48 hours. Fresh rumen cud

transplantation for restoration of ruminal microbial activity was carried out at the peak of convulsive phase followed by oral administration of ruminotoric and repeated at 24 and 48 hours in groups (e) and (f) only.

Rumen liquor analysis procedures

The physical characteristics, pH, qualitative microbial activity tests viz., sedimentation activity test (SAT), glucose fermentation test (GFT), methylene blue reduction test (MBRT), protozoal motility and concentration of rumen liquor were determined. The ruminal ammonia nitrogen (NH_3-N) and total volatile fatty acid concentrations were analysed as described elsewhere (6).

Blood analysis procedures

NH_3-N of venous blood was also determined by microdiffusion technique. Acid-base status comprising pH, actual bicarbonate (HCO_3), actual base excess (ABE) and gas dynamics comprising partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), oxygen saturation (SAT) and oxygen content (O_2CT) of venous blood were measured with Blood Gas Analyser (Radiometer, Copenhagen, Denmark) at 37°C. Venous carbonic acid (H_2CO_3) was calculated from the PCO_2 values and solubility coefficient of carbon dioxide (0.03) in the blood ($H_2CO_3 = PCO_2 \times 0.03$). The data were subjected to student's t-test.

RESULTS AND DISCUSSION

All the calves before induction of ammonia toxicity were bright, alert and active. However, following induction of urea toxicity, all the calves exhibited typical symptoms of ammonia toxicosis. Initially symptoms of nervousness, twitching of ears were apparent associated with increased salivation, frequent micturition with no clinical alterations in the physiological functions of cardiovascular and respiratory systems. This was followed by increase in the intensity of tremors particularly involving facial region and hind quarters associated with tympany, complete ruminal stasis and increase in respiratory rate. At this stage, treatment trials were instituted in all the groups (a) to (f). Survival of the animals was the response criterion. It was observed that none of the calves survived treated with therapeutic regimen undertaken in (a) to (d) groups. Acetic acid which was administered about 60 minutes after urea dosing in group (a) appeared to alleviate the toxicity symptoms for about 15 minutes, but afterwards the toxicity signs reappeared leading to recumbency which warranted readministration of acetic acid, even after oral administration of 5% acetic acid for the third time by 120 minutes after urea dosing, severe tetanic spasms were present within 45 to 60 minutes of acetic acid administration. Both the calves died within 200 minutes of urea dosing. It was thus observed that repeated administration of acetic acid was not efficacious in the advanced stages of urea toxicity (7). Similarly, parenteral administration of normal saline and 10% magnesium sulphate solution in group (b) and parenteral administration of 5% solution of sodium glutamate in normal saline in group (c) with oral administration of 5% acetic acid was also not effective in alleviating the signs of urea toxicity and thus failed to save the life of the calves. However, in treatment group (e), the severity of convulsions was reduced temporarily but still the death occurred due to toxic levels of systemic circulating ammonia. In group (d) also though the calves died, but it was observed that oral administration of 5 % acetic acid along with parenteral administration of diazepam and sodium glutamate solution prolonged the death time. The severity of convulsions was markedly improved but the convulsive phase reappeared even after repeating administration of diazepam two times with the onset of signs of muscular tetany. The death in group (d) probably occurred due to regurgitation of rumen contents by 270-300 minutes after urea administration though the convulsive attacks were arrested at that stage.

The results of the therapeutic trial undertaken in group (e) comprising 5 calves revealed that though oral administration of 5% acetic acid initially at the onset of convulsions brought the rumen liquor pH within normal range with reduction in the severity of convulsions but within 20-30 minutes of the initial treatment, convulsive symptoms became more intense warranting parenteral administration of diazepam and 5% solution of sodium glutamate which also checked the signs of muscular tetany temporarily

Table 1. Effect of urea induced ammonia toxicity and its treatment on ruminal microbial and biochemical changes in buffalo calves of groups (a) to (d) (Mean ± SE)

Time of sampling (hr)	SAT (min)	GFT (ml/h)	MBRT (min)	Ruminal pH	NH_3-N (mg %)	TVFA (mEq/L)
0	10.26±0.14	1.95±0.11	1.99±0.08	6.88±0.04	7.76±0.62	74.64±2.26
1	-	2.26±0.16	5.72±0.26*	8.25±0.07*	64.46±4.26*	51.68±3.82*
**						
2	-	1.45±0.08*	6.31±0.51*	7.01±0.03	53.51±3.82 *	53.44±4.36*
4	-	1.06±0.10*	9.26±0.81*	7.95±0.10*	76.52±4.45*	49.28±2.06*

Table 2. Effect of urea induced ammonia toxicity and its treatment on ruminal microbial and biochemical changes in buffalo calves of group (e) (Mean ± SE)

Time of sampling (hr)	SAT (min)	GFT (ml/h)	MBRT (min)	Ruminal pH	NH_3-N (mg %)	TVFA (mEq/L)
0	9.76±0.11	2.10±0.09	2.1±0.06	7.01±0.06	7.76±0.41	70.16±2.01
1	-	2.46±0.15	7.26±0.19*	8.33±0.08*	71.51±5.10*	53.72±2.96*
**						
2	-	1.52±0.11*	8.72±0.32*	8.21±0.08*	62.10±4.49*	51.09±3.33*
24	12.24±0.15*	1.89±0.11	1.86±0.11	6.82±0.04*	5.72±0.21*	23.33±2.55*
48	10.16±0.14	2.14±0.09	2.09±0.09	7.09±0.08	7.42±0.51	74.26±3.01

* Significant at P / 0.05, (-) Activity not detected, ** Treatment instituted 1 h after induction

for a period of about 30-45 minutes only. At this stage, evacuation of the rumen contents with transplantation of fresh cud was carried out at the peak of convulsive phase when the animals were showing signs of dyspnoea and hyperpnoea. At this stage, the rumen was flushed with fresh water.The animals with emptied rumen initially lay prostrate, exhibited severe symptoms of muscular tetany and were susceptible to loud noise. The calves become placid, depressed and appeared to be sleeping and refused to take feed and water. However, within next two hours, they were alert and appetite was partially restored within 6-8 hours after treatment.

In treatment group (f) single oral administration of acetic acid at the appearance of signs of muscular tetany also delayed the onset of convulsive symptoms but subsequently evacuation of the rumen contents has to be performed to save the life of the calves. Similar type of response was seen as in group (e) except that after evacuation in group (f), the convulsive period was prolonged and calves appeared to be more exhausted after the attack being unable to sit without support even after one hour and appeared to show no response to the external stimuli. Subsequently for next 1-2 hours, the calves appeared to be sleepy and placid. However, they were able to get up in next 3-4 hours but showed little interest in green fodder or grains when offered. Resumption of appetite was observed after 24 hr and was completely restored with clinical recovery in next 3 days (2,4). The microbial activity following induction of urea toxicity was markedly affected as indicated by significant alterations in SAT, GFT and MBRT values and significant decrease in protozoal concentration and motility (Table 1). However, with adaptation of therapeutic measures, the microbial activity was restored within 48 hours indicating revival of ruminal fermentation activity in the recovered animals following transplantation of fresh rumen cud in groups (e) and (f) (Table 2). Significant changes in qualitative microbial activity gradation tests could be related to the destruction of normal microflora particularly the absence of cellulolytic bacteria due to production of NH_3-N in toxic concentration in rumen liquor. The pH of rumen liquor which was initially 6.88 ± 0.04 in calves comprising groups (a) to (d) significantly increased to 8.25 ± 0.07 by 60 minutes. However, following oral administration of 5% acetic acid, the pH recorded at 120 minutes was 7.01 ± 0.03 which again significantly increased before the death of the calves. But in calves of the treatment group comprising (e) and (f), the rumen liquor pH, sharply increased by 60 minutes of induction, was temporarily arrested following oral administration of acetic acid. The rumen liquor pH again increased to a significent level at the time of evacuation of the rumen contents and was restored within normal range by 24 hrs in the recovered calves. Significant increase in rumen liquor pH following induction could be ascribed to significant increase in the ruminal NH_3-N concentration due to rapid microbial hydrolysis of urea (3,7).

The mean NH_3-N concentration of rumen liquor showed significant increase from the base values of 7.76 ± 0.62 to 64.46 ± 4.26 mg % at 60 minutes post induction of rumen alkalosis in calves of groups comprising (a) to (d), but declined rapidly when acetic acid was administered intra-ruminally. Afterwards ruminal NH_3-N concentration increased to a maximum value of 76.52 ± 4.45 mg % by 240 minutes (4 hrs) post induction in groups (a) to (d) (Table 1). However, in calves of group (e) initially NH_3-N concentration of rumen liquor rose sharply by 60 minutes followed by significant decline following acetic acid treatment but again the values increased to a significant level warranting evacuation of the rumen contents. The ruminal NH_3-N content recorded at 48 hr was 7.42 ± 0.51 mg per cent which was comparable with the base values of healthy calves (Table 2). NH_3-N concentration of venous blood showed a significant increase 60 minutes post induction in calves of groups (a) to (d) but subsequently declined following acetic acid treatment by 120 minutes but again rose to a significant level by 240 minutes which was associated with development of severe form of convulsions leading to death of calves. However, in calves of group (e) though similar pattern was observed upto 120 minutes but following evacuation of the rumen contents, the blood NH_3-N concentration declined sharply attaining normal values by 24 hrs post induction. This is comparable to findings of Bartley (2) and Davidovich et al (4).

An acid-base analysis of venous blood revealed that onset of rumen alkalosis was always associated with non-significant increase in pH in early stages (upto 60 minutes) indicating development of mild degree of metabolic alkalosis. This was associated with non-

Table 3. Effect of urea induced ammonia toxicity and its treatment on venous acid-base status and gas tensions in buffalo calves of groups (a) to (d) (Mean ± SE)

Time (hr)	pH	PCO_2 mmHg	PO_2 mmHg	HCO_3 mM/L	H_2CO_3 mM/L	ABE mM/L	SAT %	Hb gm %	NH_3-N mg %
0	7.375±0.01	44.72±0.65	37.50±0.79	25.81±0.66	1.343±0.02	2.04±0.77	66.78±1.32	10.77±0.28	0.39±0.01
1	7.439±0.02	36.55±0.92	35.18±1.10	28.64±0.71	1.084±0.03	4.92±0.46	65.22±2.04	10.41±0.33	0.76±0.02*
**									
2	7.406±0.01	33.81±1.36	35.60±1.33	24.06±1.04	1.026±0.06	1.78±1.01	64.82±1.77	9.84±0.62	0.51±0.04*
4	7.321±0.02	28.50±1.99	33.72±2.46	16.66±0.82	0.861±0.10	-8.33±1.62	48.20±2.89	10.97±0.50	1.08±0.06*

Table 4. Effect of urea induced ammonia toxicity and its treatment on venous acid-base status and gas tension in buffalo calves of group (e) (Mean ± SE)

Time (hr)	pH	PCO_2 mmHg	PO_2 mmHg	HCO_3 mM/L	H_2CO_3 mM/L	ABE mM/L	SAT %	Hb gm %	NH_3-N mg %
0	7.352±0.01	43.16±0.51	38.72±0.64	24.10±0.41	1.289±0.02	1.25±0.42	67.55±1.26	10.30±0.31	0.44±0.01
1	7.428±0.03	37.92±0.76	35.96±0.56	27.76±0.66	1.126±0.04	4.00±0.56	66.10±1.49	10.02±0.29	0.89±0.03*
**									
2	7.393±0.03	34.83±0.99	34.24±0.77	25.22±0.24	1.039±0.07	1.32±0.36	64.96±1.82	9.46±0.36	0.78±0.03*
4	7.368±0.02	37.08±1.01	34.61±1.01	20.94±1.02	1.121±0.10	-3.92±0.49	62.36±2.22	10.82±0.41	0.61±0.02*
24	7.325±0.02	42.64±0.88	35.50±1.42	24.87±0.92	1.268±0.08	0.65±0.92	60.76±1.77	10.99±0.39	0.47±0.01
48	7.389±0.02	46.19±0.91	38.11±0.59	25.52±0.49	1.362±0.05	2.08±0.32	64.15±2.01	10.46±0.28	0.42±0.01

* Significant at P < 0.05

** Treatment instituted 1 hr after induction

significant increase in actual bicarbonate, actual base excess and standard base excess (SBE). However, with the onset of symptoms of convulsions, recumbency and tachycardia, the acid-base alterations were significantly reversed as reflected by marked reduction in HCO_3, ABE and SBE indicating development of metabolic acidosis which persisted till the death of the calves Ahuja et al. (1). Similar pattern was observed in calves of groups (e) and (f). However, these acid-base alterations were rapidly restored by 24 hrs (Table 4) The results also revealed significant decrease in PCO_2 and H_2CO_3 contents which indicated development of respiratory alkalosis in urea intoxication which persisted till the death of the calves even after undertaking treatment in groups (a) to (d) (Table 3). However, gradual increase in PCO_2 and H_2CO_3 content of venous blood was observed in groups (e) and (f) and the values were restored within 48 hrs post induction (Table 4).

It was thus inferred that inspite of undertaking treatment, all the calves in groups (a) to (d), after exhibiting typical symptoms of ammonia toxicity, died though the period between adoptation of therapeutic measures and death of the calves varied. It was thus, concluded that acetic acid neutralizes the alkalinity of the rumen only for a short duration. Similarly, it was observed that following acetic acid treatment, blood NH_3-N concentration also showed a significant decrease. The relationship between rumen pH and absorption of ammonia or acetic acid could be explained to the fact that if rumen pH is low, the absorption of acid is rapid. Emptying the rumen of the animals showing toxic signs of urea toxicity resulted in rapid decrease in blood ammonia concentration and also restored the acid-base alterations within normal range. This is similar to the findings of Wood et al. (7) and Bartley et al. (2). It can be thus, concluded that evacuation of rumen contents at the peak of convulsive phase along with supportive treatment is highly effective in saving the life of dairy animals affected with acute ammonia toxicity as assessed on the basis of clinical findings and evaluation of biochemical alterations in rumen liquor and blood.

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SUMMARY

Studies were conducted to evaluate the efficacy of therapeutic trials undertaken in ammonia toxicity in buffalo calves experimentally induced by oral feeding of urea @ 1.25 g/kg body weight as a single dose. Each of the therapeutic trial was undertaken about 60 minutes after experimental induction of ammonia toxicity which was characterised by clinical symptoms of muscle tremors, rumen stasis, hyperaesthesia, convulsions and biochemical alterations in rumen viz., significant increase in rumen liquor pH, ammonia nitrogen and significant decline in total volatile fatty acid concentration with marked alterations in the values of qualitative microbial gradation tests. It was observed that oral administration of acetic acid alone only could alleviate the symptoms of ammonia toxicity temporarily followed by their revival which always proved fatal. Similarly, oral administration of acetic acid with parenteral administration of normal saline/5 per cent solution of sodium glutamate

and 10 per cent solution of magnesium sulphate were also not effective in saving the life of the animals. However, it was concluded that evacuation of the rumen contents at the peak of the convulsive phase along with supportive treatment is highly effective in saving the life of buffalo calves affected with ammonia toxicity as assessed on the basis of clinical findings and evaluation of biochemical alterations in rumen liquor and acid-base alterations in blood.

EFFECT OF EXCLUSIVE FEEDING OF PADDY STRAW ON MACRO AND MICRO ELEMENTS IN RUMEN LIQUOR, BLOOD, PLASMA AND TISSUES IN BUFFALO CALVES (BUBALUS BUBALIS)

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INTRODUCTION

Cereal straws viz., wheat, rice, maize and sorghum constitute major feed resources in Asia. Approximately, 39.5 per cent of total fibrous crop residues available in world are from wheat (20 %) and paddy (19.5 %) of which 62 per cent are available in Asia (6) and thus comprises major/sole feed constituent of the dairy animals especially during lean periods of green fodder availability. Although, there is an increasing experimental evidence that anomalies in macro and trace elements supply can influence growth, reproductive performance and immuno-competance of domestic animals (3), yet comprehensive information regarding their distribution in body fluids and tissues is lacking in ruminants and particularly so in buffaloes exclusively maintained on poor quality roughages. Hence, the present investigation was undertaken to study the status of macro and trace elements in rumen liquor, whole blood, blood plasma and tissues in buffalo-calves maintained on exclusive feeding of chaffed paddy straw for 92 days.

MATERIALS AND METHODS

Experimental animals and their management

Eight, 1-1½ year old local bred male healthy buffalo calves were procured from the dairy farmers selected on the basis of excellent bodily condition and lack of clinical signs of disease. All the calves were kept under observation for about a month and their faecal samples were examined for parasitic infection. The daily nutrients requirement of the calves was met by *ad libitum* feeding of green fodder (Barseem).

Sampling procedure

Rumen liquor and blood samples were collected simultaneously from each animal on three occasions in the morning hours before feeding to establish base values. Thereafter, six calves were chosen randomly and put on exclusive feeding of paddy straw for 92 days whereas remaining two calves served as healthy control. Samples were subsequently collected from both groups of calves periodically throughout the period of experimentation. Tissues of two calves which died at 48 and 90 days and of three, calves euthanised after 92 days of paddy straw feeding were collected and processed for mineral analysis. Two healthy control calves were also bled to death at the end of experimental study and their tissue pieces were also digested with triple acid for minerals' estimation. One gram each of paddy straw and succulent green fodder, berseem, were also processed for mineral estimations in a similar manner.

Analysis procedure

Sodium, potassium, calcium, magnesium, zinc, iron, copper, cobalt and manganese contents of rumen liquor, blood, plasma, tissues and feed samples were determined by an atomic absorption spectrophotometer with aqueous calibration standards.

RESULTS AND DISCUSSION

The mean concentration of copper in rumen liquor, whole blood and plasma before feeding of paddy straw was found to be 126.72 ± 9.96, 165.69 ± 9.50 and 152.06 ± 6.65 µg %, respectively. The copper content of rumen liquor showed a significant decline from 8th day which persisted throughout the period of experiment (Table 1). The decrease could be ascribed to low copper content of paddy straw (2.4 ppm) as compared to copper concentration of 14.00 ppm recorded on mineral analysis of green fodder fed to healthy controls. The whole blood and plasma copper concentration was significantly lower throughout the period of

Table 1. Effect of paddy straw induced alkaline indigestion on mineral concentration of rumen liquor in buffalo calves (Mean ± SE)

	Sampling time (days)										
	0	8	16	27	37	50	64	71	78	85	92
Cobalt (ug %)	0.718 ±0.032	0.550 ±0.088	0.750 ±0.108	0.650 ±0.125	0.775 ±0.085	0.800 ±0.103	0.580 ±0.111	0.700 ±0.057	0.650 ±0.125	0.800 ±0.020	0.775 ±0.025
Copper (ug %)	126.72 ± 9.96	48.48* ± 6.99	57.57* ±6.66	27.27* ±5.66	60.60* ±5.53	72.72* ±8.13	63.63* ±5.24	63.63* ±11.73	56.81* ±5.71	50.90* ±6.80	40.90* ±4.54
Iron (ug %)	2192.26 ±133.86	2641.46 ±220.76	2030.00 ±164.77	1860.00 ±266.17	2165.00 ±331.32	2210.00 ±212.95	1780.00 ±182.07	1900.00 ±109.54	1705.00 ±124.09	1280.00 ±206.51	1475.00 ±270.95
Zinc (ug %)	319.98 ±19.15	305.20 ±27.87	318.75 ±31.41	193.75* ±29.18	320.00 ±13.24	240.62* ±20.17	229.68 ±36.83	237.50 ±33.36	215.62* ±18.13	227.50* ±21.70	195.83* ±22.91
Manganese (ug %)	322.58 ±23.11	1172.10* ±108.65	1160.94* ±218.17	773.25* ±95.09	1508.18* ±149.16	1549.84* ±181.33	1273.20* ± 80.54	1508.18* ±241.64	1473.18* ±264.42	1108.22* ±157.14	891.57* ±116.55
Calcium (mg %)	6.48 ±0.45	4.07* ±0.57	4.91* ±0.28	3.95* ±0.40	5.59 ±0.33	4.72* ±0.38	-	-	4.82* ±0.38	4.09* ±0.46	5.27 ±0.99
Magnesium (mg %)	8.37 ±0.38	6.55 ±0.73	10.04 ±1.53	7.90 ±0.37	-	9.55 ±1.49	8.32 ±1.33	-	8.05 ±0.64	8.73 ±1.08	7.93 ±1.03
Sodium (mEq/L)	99.31 ±3.30	93.23 ±6.58	102.89 ± 8.06	102.60 ± 6.71	85.74 ±5.68	94.20 ±9.39	90.14 ±2.35		97.39 ±9.34	97.24 ±6.09	97.46 ±8.98
Potassium (mEq/L)	26.88 ±1.58	21.19 ±3.25	33.23 ±3.87	28.92 ±1.50	31.79 ±3.19	35.04 ±4.20	32.82 ±1.91	46.92 ± 10.79	-	27.48 ±3.16	32.30 ±3.75

*Significant at P < 0.05 (-) Not analysed

Table 2. Effect of paddy straw induced alkaline indigestion on blood and plasma mineral concentrations in buffalo calves (Mean ± SE)

	Sampling time (days)										
	0	8	16	27	37	50	64	71	78	85	92
Blood											
Copper (ug %)	165.69 ± 9.50	129.07 ±20.63	115.14* ±10.42	93.93* ±7.67	67.83* ±7.52	99.99* ±9.57	105.44* ±13.00	81.81* ±10.90	87.26* ±9.82	81.81* ±5.14	74.53* ±3.98
Iron (ug %)	3810.20 ±118.85	4243.33 ±152.97	3137.50 ±244.75	3775.00 ±117.74	3966.50 ±165.70	4190.00 ±362.76	4005.00 ±132.85	4225.00 ±110.39	4120.00 ±145.00	3975.00 ±153.90	3433.33 ±466.88
Zinc (ug %)	377.48 ±18.09	421.25 ±27.62	278.12* ±26.25	258.75* ±12.57	233.69 ±15.67	313.54 ±12.09	332.5 ±18.14	275.00* ±24.60	263.75 ±32.37	225.00 ±14.49	-
Manganese (ug %)	49.74 ±2.18	46.84 ±5.09	37.50 ±4.16	58.34 ± 19.00	50.01 ±4.30	59.95 ±6.94	52.00 ±4.28	50.01 ±7.45	54.17 ±7.98	66.68 ±17.48	58.34 ±4.81
Calcium (mg %)	9.57 ±0.39	8.11 ±0.37	8.16 ±0.53	7.75* ±0.64	7.50 ±0.45	7.83 ±0.47	9.07 ±0.26	8.45 ±0.24	8.95 ±0.25	7.65* ±0.47	8.95 ±0.81
Plasma											
Copper (ug %)	152.06 ± 6.65	121.23 ± 6.06	123.65 ±15.64	115.17 ±24.24	78.80* ± 10.14	100.01* ± 6.21	103.04* ± 2.12	101.83* ±17.81	92.73* ± 14.77	72.73* ±7.42	99.82* ±5.63
Iron (ug %)	634.89 ±42.66	1130.00 ±104.70	293.75* ±76.63	365.00 ±45.13	554.16 ±56.79	483.33 ±66.97	583.33 ±72.24	445.05* ±49.66	391.66* ±72.64	391.66* ±71.20	493.75 ±46.06
Zinc (ug %)	244.5 ±15.94	41.14 ± 22.22	159.90* ±25.24	183.84 ±16.78	135.93* ±17.63	184.29 ±16.97	257.29 ±12.01	149.37* ± 9.07	147.50* ±29.28	153.00* ±14.89	129.68* ± 2.99
Manganese (ug %)	50.73 ±2.09	68.96 ±8.72	58.34 ± 12.36	44.45 ± 11.11	55.56 ±5.12	48.62 ±4.52	54.17 ±7.98	53.34 ±3.33	46.67 ±8.16	47.92 ±8.59	54.17 ±7.98
Calcium (mg %)	8.41 ±0.25	8.37 ±0.90	8.73 ±0.82	8.85 ±0.85	7.66 ±0.97	8.94 ±0.26	9.11 ±0.77	8.93 ±0.64	9.33 ±0.88	8.99 ±0.78	8.88 ±0.31
Magnesium (mg %)	2.79 ±0.08	3.14 ±0.17	3.56 ±0.46	3.48* ±0.14	3.77* ±0.15	3.46* ±0.20	4.36* ±0.61	3.26 ±0.52	3.60* ±0.16	3.79* ±0.34	3.88* ±0.33

*Significant at P < 0.05 (-) Not analysed

paddy straw feeding, which could be attributed to decreased availability of this element for absorption. The development of hypocupraemia was further confirmed from estimation of copper in liver (8786.87 µg/100 g) as compared to control value of 12544.0 mg/100 g of fresh tissues and decreased activity of cytochrome oxidase and monoamine oxidase in hepatocytes as observed histoenzymologically (5).

The zinc content was lower in rumen liquor, when calves were maintained on paddy straw which could be essentially ascribed to lower level of this element in paddy straw (2.1 ppm) as compared to zinc content of 25.0 ppm recorded on mineral analysis of green fodder. The plasma and blood zinc contents showed a decline (Table 2) after registering an initial rise. The initial increase was probably due to development of dehydration, the effect of which was later over masked by its decreased content in blood. The observed decline might be due to decreased availability and decreased absorption because of villous atrophy observed in the small intestinal mucosa on histopathological examination as described elsewhere (5). The mean concentration of zinc was well below the control values in all the tissues collected for mineral analysis, the decline being significant in liver (3106.79 ug/100 g) as compared to its value of 3600.00 ug/100 g recorded in liver of healthy controls. Low zinc content in testicular tissues (2143.61 ug/100 g) observed in the present study as compared to healthy controls (2650 ug/100 g of fresh tissue) might be responsible for the atrophy of seminiferous tubules and vacuolar degeneration of the cells lining the seminiferous tubules which might lead to impairment of reproductive functions in male ruminants as reported earlier (2,7) in zinc deficient male goats and cattle (Table 3).

The mean concentration of calcium in rumen liquor declined significantly from 4th day of the experiment and persisted during the entire period of experimental study. The average blood calcium concentration showed a declining trend following exclusive feeding of paddy straw whereas plasma calcium concentration showed non-significant alterations throughout the period of study. The calcium contents of various tissues were within normal limits as cited earlier (2). The non observance of decline in plasma calcium content despite its decreased availability could be explained as per the observations of Duncan (1) who reported that within wide limits the retention of calcium appeared to depend more on the stage of life cycle and state of animal reserves than the intake. A significant decline in calcium concentration of rumen liquor was essentially because of lower calcium content of paddy straw (0.13 %) as compared to value of 1.19 % recorded on mineral analysis of the green fodder fed to healthy control animals.

A drastic and significant rise in the manganese concentration of rumen liquor was recorded in buffalo calves fed paddy straw which could be ascribed to high manganese content of paddy straw (227 ppm) as compared to base value of 46 ppm recorded on mineral analysis of rumen liquor of healthy calves fed green fodder. The blood manganese content tended to be apparently higher at some instances whereas plasma manganese(Table 2) content was unaltered throughout the experimental trial. However, average concentration of manganese increased in rumen, reticulum, omasum, abomasum, liver, gall bladder, (Table3) kidneys, small intestine, large intestine, lungs, testes, skin, skeletal muscles and hair. The iron content of rumen liquor declined in buffalo calves exclusively maintained on paddy straw which was probably ascribed to lower iron content of paddy straw (110 ppm) as compared to value of 600 ppm observed on mineral analysis of green fodder. The mean plasma iron content also showed a decline after 12th day of induction. Similar findings have been reported by Pandey (4) in cow calves exclusively fed wheat straw for 67 days. The apparent decline in plasma iron could be ascribed to its decreased availability in rumen liquor which also accounted for its decreased concentration in all the tissues analysed for mineral estimation. The developing iron deficiency might be responsible for microcytic and hypochromic anaemia as evinced on haematological analysis in the present study. The mean blood iron content showed initially higher values during first 12 days of induction but subsequent analysis did not reveal any definite pattern. The higher blood iron content might be due to dehydration as observed clinically which probably overmasked the effect of developing iron deficiency in the present investigation.

The average magnesium, sodium, potassium and cobalt contents of rumen liquor (Table1) did not vary significantly. Similarly, non-significant alterations were observed in sodium,

Table 3. Effect of paddy straw induced alkaline indigestion on mineral concentration of tissues in buffalo calves (Mean)

Parameters		Abomasum	Rumen	Liver	Kidney	Heart	Small intestine	Lungs	Testis	Skin	Skeletal muscle	Hair
Copper (ug/100g) of fresh tissue	C	145.00	110.0	12544.0	265.00	365.0	123.00	150.00	125.00	80.00	95.00	420.0
	E	150.46	121.76	8786.87	226.04	409.07	204.22	208.15	151.42	121.13	102.42	454.90
Manganese (ug/100 g of fresh tissue)	C	140.00	130.00	320.00	175.00	120.00	140.00	135.00	130.00	125.00	110.00	330.00
	E	1083.29	58.28	1049.95	475.55	444.29	666.64	383.31	611.08	583.31	229.15	2565.45
Zinc (ug/100 g of fresh tissue)	C	3200.00	3425.00	3500.00	2500.00	2900.00	2775.00	2725.00	2650.00	1350.00	4250.00	5005.00
	E	2246.99	2893.15	3106.79	2140.55	2088.54	2514.21	1660.40	2143.61	1290.11	3195.34	3860.33
Iron (ug/100 g of fresh tissue)	C	8562.50	5312.5	27125.00	20312.50	11062.60	7500.00	25312.50	8625.00	6500.00	10000.00	9100.00
	E	3441.66	4875.00	14300.60	3437.5	8000.00	5218.75	15500.00	4291.66	6496.25	4350.00	7312.50
Calcium mg/100 g of fresh tissue	C*	-	-	10-30	6-20	8-25	13-15	10-25	-	6-20	5-14	-
	E	13.22	15.41	9.72	19.90	9.86	8.70	12.54	9.02	17.50	8.33	167.70
Magnesium mg/100 g of fresh tissue	C*	-	-	20-25	15-20	15-18	11-12	6-16	-	6-10	20-30	-
	E	16.47	14.81	20.15	19.88	14.54	13.86	12.93	-	11.98	23.09	56.96

C-Mean tissue mineral concentration of control calves.
C*-Mineral tissue concentration (range) cited by Georgievskii et al. (1982)
E- Mean mineral tissue concentration in paddy straw induced alkaline indigestion

potassium and cobalt contents of plasma as well as blood. However, the mean plasma magnesium concentration maintained an upward trend. The average magnesium content of liver, kidneys, heart, intestine, lungs, skin and skeletal muscles was within normal limits cited by Georgievskii et al. (2).

The results of the experimental trial reveal that exclusive feeding of paddy straw leads to zinc, copper and iron deficiency. The results also suggests that high manganese content of paddy straw may be another antinutritional factor. The alterations in macro and micro elements in body fluids and tissues are thus correlated with the mineral status of the paddy straw which may markedly affect the functional status of various organs as supported by histopathological and histoenzymological findings in the present investigation.

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SUMMARY

Studies were undertaken to assess the status of macro and micro elements in rumen liquor, whole blood, plasma and tissues in 6 buffalo-calves maintained on exclusive feeding of paddy straw for 92 days. The mineral profile of rumen liquor revealed that mean concentration of copper, zinc and calcium showed a significant decline whereas a drastic and significant rise in the manganese concentration was recorded. The mean whole blood and plasma copper and zinc concentration was significantly lower throughout the period of induction of rumen alkalosis. However, non-significant variation was observed in sodium, potassium, magnesium, cobalt and calcium contents of rumen liquor, blood and plasma. The mineral analysis of tissues revealed that mean concentration of manganese increased dramatically in various organs which was associated with significant decline in copper and zinc contents. Thus exclusive feeding of paddy straw resulted in marked alterations in mineral status of body fluids and tissues which may interfere with the functional status of various organs as supported by histopathological and histoenzymological findings.

PRODUCCION DE LECHE EN LA REPUBLICA MEXICANA

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INTRODUCCION

La producción de leche en América Latina estimada en 1988 fue de 35 millones de toneladas lo que representa el 8% de la producción mundial, con una disponibilidad de 120 litros/año.

De acuerdo a los reportes estadísticos en América Latina existen alrededor de 35 millones de vacas especializadas que representan el 15% del rebaño mundial. El promedio de producción es de 1,000 litros que corresponden al 48% de la media mundial (1,960 litros).

Factores que inciden en la falta de productividad.

a) Clima.- Alta temperatura media y elevada humedad relativa de las zonas tropicales y subtropicales.

b) Condiciones sanitarias.- Enfermedades infecciosas altas parasitosis (externos e internos).

c) Deficiencias nutricionales.- Deficiente manejo de pastizales, falta de tecnología en la conservación de forrajes.

d) Falta de adaptabilidad de razas lecheras para zonas tropicales, y

e) Ausencia de políticas lecheras definidas.

En México, la producción de leche no escapa a los efectos de la crisis económica - por la que atraviesan muchos países de Latinoamérica, como lo muestra la grave disminución en el ritmo del crecimiento de la producción lechera anual que de 1980 al presente ha sido de 1.2% mientras que la población crece en un 3.1% el crecimiento de producción láctea en la década anterior (1970-1980) fue de un 4.2% anual, y se estima que para esta (1980-1990) será de menos del 3.0% anual.

De acuerdo a las informaciones de fuentes oficiales la producción de leche en México en el presente es de cerca de los 7,500 millones de litros, cantidad insuficiente - para atender la demanda de una población de cerca de los 80 millones de habitantes, y que requiere para su consumo un total de 9,000 millones de litros de leche (deficit de 1,500 millones de litros).

México para el año 2,000, tendrá más de 100 millones de habitantes, de los cuales más del 50% serán menores de 15 años, población joven a la que habrá que ofrecer, alimentos como la leche para ser integrada a las dietas alimenticias especialmente en las primeras etapas de su vida, pero la insuficiente producción primaria y sus altos costos son una barrera para su oferta y por lo tanto para su consumo.

La problemática de la actividad lechera en México la podemos resumir en los siguientes puntos:

Problemática de la Actividad Lechera en México.

- Inadecuada política de precios.
- Vigencia de un Reglamento Sanitario de la Leche, incongruente con la Realidad.
- Aumento de los Costos de los Insumos
- Dependencia del exterior
- Falta de Apoyos Institucionales y Servicios en forma Coordinada a los productores lecheros en general (créditos oportunos con intereses preferenciales, asistencia técnica, etc.)
- Inadecuada y en ocasiones falta de organización integral de los productores a nivel Nacional y Regional para la venta de sus productos y adquisición de los insumos.

Estructura Productiva de la Ganadería Lechera.

La producción de leche en México proviene de 2 sistemas de producción: la especia-

lizada la que aporta el 70% de la producción y la no especializada que contribuye con el 30% restante estos sistemas por su nivel de desarrollo tecnológico se dividen a su vez en subsistemas.

Lo anterior está condicionado por las características geográficas, disponibilidad de capital y tenencia de la tierra aspectos que definen y determinan particularidades en la estructura productiva.

Ganadería Especializada.

Este sistema se integra por 3 Subsistemas.

a) Estabulado altamente especializado, el que se caracteriza por poseer un alto grado de tecnología e inversión; el ganado es de razas puras y el destino de la producción es la pasteurización mostrando gran dependencia del exterior en lo que se refiere a reemplazo de vaquillas, material genético, maquinaria y equipo.

b) Semi-estabulado, se caracteriza por tener un nivel de tecnología medio, el ganado que predomina es de razas puras en sus diversos grados, la alimentación del ganado es en base a pastoreo con suplementación de forrajes y esquilmos agrícolas que genera el propio productor.

En épocas de estiaje es poco común el uso de técnicas de conservación de forrajes, la comercialización de la leche es como (Leche bronca) o cruda y para la elaboración de derivados artesanales (cajetas, queso, etc). Aunque también una parte se destina a la pasteurización.

c) Traspatio suburbano, posee un nivel de tecnificación deficiente, el ganado se maneja en confinamiento, existiendo problemas de retiro de desechos sólidos, la alimentación no está bien definida ya que depende de la disponibilidad de forrajes en el mercado y sus productos agrícolas o agroindustriales, además no cuenta con recría propia y no existe integración y organización de los productores; Se destina a la venta "Leche bronca" y para la elaboración de derivados de leche (quesos, etc).

Ganadería no Especializada.

Este sistema se divide en dos subsistemas:

a) Doble propósito.- Se caracteriza por tener un grado de tecnificación bajo, la producción está orientada a la obtención de carne y leche, existiendo una marcada estacionalidad en esta última, la alimentación se realiza a base de pastoreo con escasa suplementación concentrados, existe un deficiente sistema de acopio, conservación y comercialización de la leche, el destino de la misma es su venta como leche bronca y para la elaboración artesanal de derivados, no existe integración de los productores, estos últimos desarrollan sus propios reemplazos y cuentan con bajos costos de mano de obra directa.

b) Pastoreo Familiar.- Posee un nivel tecnológico bajo, la alimentación se basa en el pastoreo con escasa suplementación de subproductos agrícolas, la explotación animal es fundamentalmente para la economía familiar por lo cual existe un alto nivel de autoconsumo, sin embargo pequeñas cantidades se destinan a la comercialización ya sea como leche bronca o bien para la elaboración artesanal de quesos.

Como punto aparte, cabe hacer mención de la existencia de otro sistema de explotación, el cual se denomina de producción de material genético. Este sistema tiene características peculiares, ya que la producción de leche pasa a segundo término, cuenta con un alto nivel de tecnificación y se especializa en la producción de material genético como semen, becerras y vaquillas al primer parto.

Localización Geográfica de la Producción.

Geográficamente la producción lechera puede dividirse en regiones, las cuales poseen ciertas características climáticas y fisiográficas.

De esta manera el País se divide en:

a) Región Árida y semiárida.- Se localiza en el norte del País, abarcando los estados de Baja California Norte, Baja California Sur, Coahuila, Aguascalientes, Durango, Nuevo León, Sonora, Zacatecas y San Luis Potosí, existe una marcada dependencia del exterior, debido a que es la que realiza las mayores importaciones de pié de cría, semen, maquinaria y equipo e inclusive la asistencia técnica de producción de forrajes. Los tipos de

explotación que prevalecen son el estabulado, de pastoreo familiar y en menor proporción del semiestabulado.

Durante 1986 esta región aportó el 29.0% de la producción nacional y participó con el 22.2% del inventario.

b) Región Centro Templado t Montañoso.- Se ubica en la parte central del país, comprendiendo los estados de Guanajuato, México, Michoacán, Puebla, Querétaro, Tlaxcala, Distrito Federal, Morelos, Nayarit, Colima, Sinaloa, Hidalgo y Jalisco. Esta región es la más importante en cuanto a la producción de leche, sin embargo, existe un rezago tecnológico en cuanto a prácticas productivas. Por otra parte se caracteriza porque en ella se explotan diferentes sistemas de explotación. Esta región contribuyó con el 54.5% de la producción nacional durante 1986, y con el 42.1% del inventario.

c) Región del Trópico Húmedo y Seco.- Comprende los estados de Guerrero, Oaxaca, Chiapas, Veracruz, Tabasco, Yucatán, Quintana Roo, Campeche y Tamaulipas, así como parte de los estados de San Luis Potosí e Hidalgo en el área de la Huasteca.

Esta región es la que presenta los índices de productividad más bajos por unidad animal, debido a la calidad genética del ganado, así como a la escasa asistencia técnica en aspectos nutricionales, reproductivas y sanitarios.

Por otra parte se caracteriza por la explotación de ganado de doble propósito, siendo el pastoreo la forma de alimentación tradicional por lo que en temporada de lluvias aumenta marcadamente la producción.

En 1986 solo aportó el 16.5% de la producción nacional, y contó con el 35.7% del inventario.

Comercialización.

La fase de comercialización depende de una serie de factores entre los que se encuentran el sistema de explotación el grado de integración de los productores, el precio, la infraestructura básica con que cuenta, así como la calidad y volumen de leche disponible que tiene relación directa con la producción.

Las explotaciones especializadas en la producción de elevados volúmenes de leche la destinan principalmente a la pasteurización, son generalmente productores integrados horizontal y verticalmente que entregan su producto a la planta de la que son socios. Los costos de recolección y transporte pueden ser absorbidos por la planta industrial o bien se descuenta una prima de transporte al productor primario y a la vez se le otorga un sobre precio por la calidad de producto.

En la ganadería no especializada, cuyo volumen de producción es reducido e insestable, el destino de la leche es fundamentalmente para su venta como leche bronca, elaboración de derivados y un volumen mínimo se dirige a la pasteurización.

Es en este sector donde se presenta una mayor diversidad de agentes e intermediarios privados y públicos que intervienen en el proceso de comercialización.

Por lo que se refiere al destino de la leche, esta última está en función del precio, la oferta, la ubicación de los centros de producción, consumo y demanda. Existen productores que entregan la leche a recolectores o a la planta industrial o industrialización o bien venden al público en forma directa, así como a acopiadores e intermediarios.

La leche con cuentas bacterianas altas se destina fundamentalmente a procesos industriales, mientras que la mayor calidad se dirige a la pasteurización, los derivados o al consumo directo. Cabe hacer mención que el control sanitario que ejerce el estado es más estricto para la leche que se destina a la pasteurización; en lo que respecta a la leche que se vende bronca o se destina a la elaboración de derivados artesanales, dicho control es relativo o casi nulo.

Otro elemento básico que condiciona el destino de la producción es el sistema de precios, la política de precios mínimos al productor y máximos al consumidor establecido por zonas geográficas y calidad sanitaria del producto, tiene ingerencia sobre la leche pasteurizada e industrializada donde el control es lineal hasta el consumo.

Recientemente los ajustes en los precios pagados al productor se han efectuado tomando en cuenta la relación costo-precio-utilidad, sin embargo, la falta de adecuación al incremento en los precios de los insumos requeridos en la actividad ha provocado, sobre todo en los períodos de baja producción la falta de suministros para el sector in-

dustrial y las plantas pasteurizadoras.

En lo referente a la leche bronca, su venta se determina en base a la preferencia del consumidor y a la relación entre la demanda, lo que ha dado por resultado un precio que fluctúa entre 10 y 60% superior al establecido para la leche que se destina a la pasteurización.

En cuanto a los agentes comercializadores, se distinguen dos tipos el privado y el público; siendo el primero el que predomina en la cadena producción-consumo.

Por lo que se refiere a las importaciones de leche en polvo, CONASUPO es el canal oficial que se encarga de realizarlas y posteriormente se distribuyen a organismos públicos, y a la industria privada a través del régimen de concurrencia que es dictaminado por la Comisión Nacional para el Fomento de la Producción y el Aprovechamiento de la Leche, A.C.

Distribución.

La producción nacional se encamina a cuatro destinos, aproximadamente el 46.20% se utiliza para el consumo directo, 20.94%, en la pasteurización, 23.47% a la elaboración de derivados lácteos y 9.39% se destina para obtener leches procesadas.

Los agentes que intervienen en la distribución de la leche y sus derivados son privados y públicos.

Por lo que respecta a los agentes privados existen gran variedad, de esta manera se encuentran agentes que se dedican a satisfacer las necesidades de los sectores urbanos de mayores recursos, por otra parte se encuentran un gran número de comercios tradicionales que atienden al sector suburbano y rural, de lo que se deduce que coexisten formas mercantiles muy desarrolladas y estructuras antiguas y tradicionales.

Consumo.

En 1986 la disponibilidad nacional de leche fue de 8,236.2 millones de litros de los cuales 84.1% correspondieron a la producción nacional y 15.9% a las improtaciones.

Es importante resaltar que el crecimiento de la producción láctea ha sido menor al de la población consumidora, de lo que se puede deducir que el consumo per-cápita ha disminuido en los últimos años.

Aunado a lo anterior, es conveniente señalar que el pueblo mexicano no cuenta con una buena educación nutricional, por lo que frecuentemente desvía su consumo hacia productos de muy baja calidad alimenticia.

Generación de Empleos.

La actividad lechera ha sido un medio generador de empleo e ingresos es decir, ha servido como medio de sustento, tanto para las explotaciones altamente tecnificadas en donde se ocupa personal remunerado, como para aquellos subsistemas en donde la explotación animales fundamental para la economía familiar.

En 1986 la actividad generó empleo a 4.5 millones de personas ocupadas desde la producción de forrajes hasta la distribución de leche y sus derivados e industrias conexas.

En lo referente al sector industrial, este dió empleo a aproximadamente 200,000 personas.

Objetivos y Estrategias de la Actividad lechera.

- Mejorar los niveles de empleo e ingresos de ejidatarios y pequeños productores pecuarios.
- Elevar la productividad y eficacia de las unidades de producción.
- Aumentar la producción nacional de proteína de origen animal, para apoyar la soberanía alimentaria.
- Desarrollar la ganadería familiar y consolidad la tecnificada, principalmente ejidal y comunal.
- Incorporar organizadamente a los productores pecuarios a la dinámica de crecimiento del país.

Respecto a las estrategias que se plantean, son:

- Regionalizar el desarrollo ganadero, en función de los recursos y su óptima utilización; de la preservación de las condiciones ecológicas, de las especies y razas así como de los tipos de productores.
- En materia de producción de carne bovina y leche, éstas se fomentarán principalmente mediante el aprovechamiento óptimo de praderas naturales e inducidas, dando prioridad a los agostaderos subutilizados en los ejidos.

En este contexto se promoverá la ganadería de doble propósito carne-leche, dando énfasis en las zonas tropicales.

- Paralelamente se apoyará la consolidación de la ganadería genética, insumos, maquinaria y equipo, para lo cual se le dará un fuerte impulso a la investigación básica y aplicada, y se apoyará la realización de acuerdos internacionales que aseguren una verdadera transferencia tecnológica, acorde a los requerimientos nacionales y a la disponibilidad de recursos.
- Se promoverá la investigación aplicada, acorde con las características de la ganadería lechera, asimismo se intensificará la capacitación técnica necesaria que permita asegurar una aplicación integral de los métodos de producción más adecuados.
- El transporte, se impulsará la construcción de caminos rurales, dando prioridad a la conservación oportuna y buscando que se utilicen especificaciones apropiados y bajo costo.
- El establecimiento de vías de comunicación en general deberá contemplar las actuales zonas productoras, así como las potenciales a efecto de lograr un desarrollo regional armónico e integrado.
- La regularización de la tenencia de la tierra, se llevará a cabo considerando los aspectos productivos, como la óptima y racional utilización del recurso, y los relativos a los factores sociales.
- Se otorgará seguridad en la tenencia de la tierra que permita una mayor tecnificación de los predios ganaderos.
- El consumo rural deberá ser inducido mediante campañas publicitarias que especifiquen las cualidades nutricionales de productos con mayor contenido vitamínico y proteíco; así como de las bondades que éstos proporcionan para tener un desarrollo sano, físico y mental.
- Racionalizar las importaciones a las estrictamente necesarias y que coadyuven al desarrollo integral que espera.
- Lo anterior, deberá coadyuvar en el corto plazo, a recobrar la ventaja perdida de tener una balanza comercial agropecuaria favorable.
- En materia de empleo, se buscará consolidar y crear nuevas fuentes permanentes y remunerativas, acordes con la disponibilidad de recursos regionales, mano de obra y capital, mediante su incorporación a actividades productivas y de servicios que incidan en el desarrollo integral del médio rural.

ANEXO I

LOCALIZACION GEOGRAFICA	PRODUCCION	INVENTARIO
A	29%	22.2%
B	54.5%	42.1%
C	100.0%	100.0%

ANEXO II

DISTRIBUCION

Consumo Directo	46.20
Pasteurización	20.94
Elaboración de Derivados	23.47
Leches procesadas	9.39
	100.00%

Se expone el estado de la producción lechera en México.

La actividad lechera en México, se enfrenta en la actualidad a una problemática muy especial como resultado de la crisis económica.

La situacion se puede resumir en un déficit de 1,500 millones de litros de leche — anuales, un aumento en el costo de insumos, una tecnificación insuficiente, un sistema deficiente de comercialización y una marcada tendencia a la reducción del consumo per - cápita.

Ante esta situación, se plantean diversas estrategias, tendientes al desarrollo de - la industria lechera.

The status of the dairy production in Mexico is exposed.

The mexican dairy industry is presently facing a critical period resulting from the general economic depression. The situation can be summarized as a 1.500 millions liters of milk shortage per year as a result of the great increase in the production costs, in suficient technification and a deficient commercial system together with a marked trend to decrease per-capita consumption.

In the presence of this situation, several approaches to improve the national dairy industry are presented.

On expose le etat de la production laitière au Mexique.

La activitié laitière du méxique se expose actuelement dans une problematique très spécial comme résultat de la crise economique.

La situation a eté résume pour un déficit de 1,500 millions de litres pour chaque - année, une augmentation des prix de la matierre prime, une tecnique insuffisant, un deficit du systeme de sa commercialisation et une tendence a la reduction de la consumation per-capita.

Devant cette situation on propose divers stratégies orientes au devéloppement de le industrie laitière.

HERD HEALTH PROBLEMS. THE EPIDEMIOLOGICAL APPROACH.

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INTRODUCTION

Modern approach to herd medicine involves collection and evaluation of data. A good data should include records of clinical cases, as well as that based on routine clinical examinations and laboratory tests. Data are recorded and processed, active care for detection of any fall from preset targets is carried out through statistical monthly reports. Integrated computer programs are designed to provide ongoing monitoring of herd performance which is compared to targets of performance. When falls from targets are detected, epidemiologic study designs are being used to explain the fall from targets. The procedure involves the evaluation of the statistical associations between the traits and any known contributing factors, so that the impact of management on health and performance is determined and herd health, preventive and control programs are carried out.

While the aspects of monitoring and statistical reports have been extensively dealt with in the new developing field of herd health (1), studies describing clinical applications of quantitative epidemiological techniques are rare (7). The present study describes the use of a computer program based on individual cow records for epidemiological evaluation under common practice conditions.

MATERIALS AND METHODS

Study population.

Data are from the author's routine practice on eight Israeli Holstein herds (250 to 400) cows each. These herds are characterized by high annual milk yield (8500 to 10000 kg/cow) and heavy feeding. Cows are kept in groups according to yield, dry cows are kept in a separate group. Rations basically conform to NRC recommendations. All herds are artificially inseminated. Heifers and cows are inseminated 70 to 90, and 50 to 80 days from calving respectively.

Data collection and storage.

Farms are visited five times weekly while routine examinations are carried out once a week. Cows that calve are presented for routine examination 5 to 12 d postpartum when the state of the uterus is evaluated. All cows with unobserved heat are presented for a rectal examination 60 to 90 d postpartum. Inseminated cows are examined for pregnancy 40 to 47 d from insemination. Data are recorded on individual cards for each cow. Although some of the data are collected on microcomputers on the farms, a veterinary program is run as part of the routine service given and covers all aspects of herd health. The data are recorded on DBASE softwear in two files. One file is based on individual cow data (Table 1) and contains the information about calving and reproductive traits (a record per lactation). The second file deals with populations, and stores information on herd basis. Additional information such as monthly milk recordings, are obtained from the farm microcomputer, when needed.

A monitoring monthly report is issued to each farm. The report details the information for the running calender month and for the cumulative period. Actual performance of the cows is compared with targets of performance. Shortfalls are further investigated using the

epidemiological programs described. The chapter presented here (Table 2) is that dealing with reproduction. Herds with rate of cows OPEN GREATER THAN 150 d of more than 30.0% are further investigated. Heifers and cows are analyzed separately.

Table 1: Parturition file: Structure of Database.

Field	Field name	Description	Type
1	FARM	NUMBER OF FARM	N
2	CALVING	CALVING DATE	DATE
3	NUMBER	COW NUMBER	C
4	PARITY	NUMBER OF PARITY	N
5	DAYDRY	NUMBER OF DAY DRY	N
6	DEXA	INDUCED CALVING	L
7	TWIN	TWIN CALVING	N
8	STILL	STILLBIRTH	N
9	MF	MILK FEVER	L
10	PRO	PROLAPSED UTERUS	L
11	LDA	DISPLACED ABOMASUM	L
12	RP	RETAINED PLACENTA	L
13	MET	PRIMARY METRITIS	L
14	KET	KETOSIS	N
15	ACID	ACIDOSIS	N
16	MAST	MASTITIS	L
17	RESNUM	CULLING	N
18	ANEST1	NON-OBSERVED HEAT	L
19	ANEST2	INACTIVE OVARIES	L
20	1ST	CONCEIVED FROM 1ST	L
21	CONCEIVED	DATE CONCEIVED	DATE
22	REPEAT	REPEAT BREEDER	L

Type: N=Numeric, C=Character, L=Logical, Date=Date,

Table 2: Monitoring Report. (Reproduction).
(Farm 7 December 1988)

4. Reproduction.

	1st calf heifers		Cows	
Calving period:	Month	Year	Month	Year
a. Total	16	84	21	132
b. % anestrus	43.8	38.1	66.7	59.1
c. % inactive ovaries	6.3	6.0	19.0	19.7
d. % 1st service C.R.	81.3	47.0	45.0	28.0
e. % days open >150	6.3	28.0	33.3	37.8
	heifers		Cows	
Running month:	Month	Year	Month	Year
f. -ve in preg check				
1) n checked	12	158	14	237
2) % negative	0.0	10.1	28.6	27.0
g. % aborted				
1) abortions	1.2	2.7	0.0	0.4
2) return to heat	0.0	9.4	0.8	5.5

Epidemiological designs.

These designs aim to identify and quantify any possible associations between the traits examined and known possible factors which can explain the fall from targets. The following designs will be presently

described:
1. The relative contribution of various factors to **OPEN GREATER THAN 150 d.**
2. The association between **ANESTRUS** and **HIGH MILK YIELD** (representing a relative shortage of energy)

Variables definitions:
1. **TWIN** = any cow with a multiple birth.
2. **RP** = any cow of a single calving with a history of retained placenta of more than 18 h.
3. **MET** = any cow of a single calving with a history of primary metritis (without previous retaining the placenta) as diagnosed by a routine examination 5 to 12 d post partum.
4. **KET** = any cow with ketonuria of more than 1.5 mmol/l with no previous history of **TWIN, RP, MET.**
5. **ANESTRUS** = any cow presented for examination 60 to 90 d postpartum because of unobserved heat with no previous history of twin, RP, MET, KET.
6. **REPEAT BREEDER** = any open cow reaching 150 d postpartum with at least 3 inseminations and with no previous history of **TWIN, RP, MET, ANESTRUS, KET.**
7. **OPEN GREATER THAN 150 d** = any open cow kept for breeding reaching 150 d postpartum.

Factors responsible for "open greater than 150 d"

The relative contribution of each factor to the trait is evaluated as follows: the **INCIDENCE RATES** for the trait in **ALL** and **"NORMAL"** COWS are calculated. The contribution of each factor to rate is than calculated by subtracting the theoretical from the observed values of **DAYS OPEN** for each factor. The contribution of "other" (undefined) factors can now be calculated by substracting the sum contribution of all known factors from the total rate of **DAYS OPEN.** Statistical signification of the difference between theoretical and observed frequencies are than calculated using a Chi-square test with 1 d.f.

Association between anestrus and a high milk yield.

This computation enables evaluation of possible factors responsible to the traits examined. In the example presented, milk yield in the beginning of the lactation is used to simulate a negative energy balance in the preservice period. Cases and controls are matched on the trait examined to parity and order of calving. The first 3 monthly milk recordings are interpolated for 3.5% FCM. The highest two out of the first three monthly corrected records for cases and controls respectively are averaged. The averages for each case and control are compared and the higher one scores. Equal averages draw. A Mantel Haenszel test (3) is carried out. The statistical signification of the results are presented.

Table 3. Contribution to OPEN GREATER THAN 150 d.
Farm # 3 01/01/88-31/12/88

	Heifers			Cows		
n	82			119		
% Open	28.0			37.8		
	IR	Contribution	x^2	IR	Contribution	x^2
TWIN	2.4	-0.4	2.6	10.1	2.2	0.0
RP	2.4	0.8	0.0	13.4	4.2	3.4
MET	34.1	6.0	1.6	7.6	0.6	0.0
KET	0.0	0.0	***	1.7	0.4	0.0
ANEST	20.7	3.5	2.0	37.0	6.7	2.7
RB	7.3	7.3		4.2	4.2	
OTHERS	32.9	10.9		26.1	19.4	

RESULTS

The monitoring report of farm 3 for 1988 is presented in Table 2. The rates of cows with DAYS OPEN are high for both heifers and cows (37.9% and 39.9% resp.). Quantitavely **ANESTRUS** are responsible for 3.5% and 6.7% of the heifers and cows open greater than 150 d postpartum respectively (Table 3). Cows with high milk yield (Table 4), showed a greater risk for **ANESTRUS** compared to low yielders (R=4.5, p<0.05).

Table 4. Association of REPEAT BREEDERS with HIGH MILK YIELD
Farm # 3 01/01/88-31/12/88

with factor		without factor		
n	% positive	n	% positive	"R"
23	73.9	23	26.1	8.0**

"R" = Risk of a cow with high yield before service to be anestrus compares to her low yielder counterpart.
** p<0.01

DISCUSSION

Monitoring reports serve as an alarm for any fall from targets and as such should be short, concise, engulph all aspects of herd health and issued at regular times (5,6). Herd size is a limiting factor for both statistical and epidemiological reports, attempts to use those tools in small sized herds covering short periods often prove futile. It is evident from the results that in few cases tendencies alone could be pointed out while statistical signification is hard to establish. Most recorded health reports are based on various data banks, information from all sources must be collected and processed to one program. This limitation is also valid in the present study where milk recordings must be brought in from the farm data base.

Multivariate analytic techniques which reduce a large mount of descriptive data into multivariate relationships must be employed. These relationships can be further explained by causal studies The use of a multiple regression technique might be more proper but lack the ability to present the results quantitavely.

The chapter of reproduction presented relates both to the period of calving and to the current one. The index picked to monitor for an existing reproduction problem is that of "% cows with days open greater than 150 d". This choice evoides the problem associated with that of "days open" when culled barren cows cannot be averaged on their true value. When intervention is called for, narrowing down the field of investigation often proves essential if results are to be obtained (2). This selection process enables the clinician to concentrate both his efforts and resources, in clinical and laboratory investigations at most promising directions. The next step is therefore to try and quantitate the factors responsible for "cows open greater than 150 d". In the method presented, only the first diagnosis for each case is taken into account, its relative contribution to the trait is evaluated. Determinants used, must be both of a previously established association with the trait studied and routinely diagnosed and recorded. All unknown factors are grouped together under "others". It is assumed that each open cow with a known factor examined, have two components for her infertility. While one is common to all cows (that of "normal" ones), the second is that brought about by the trait. The relative contributio. of each factor to the

trait, is therefore the difference between the observed and the theoretical (normal) values.

The example used for a further epidemiological investigation presented deals with a herd problem of anestrus. Structured case-control studies are used to establish the disease determinants as suggested before (7). Anestrus was associated with a negative energy balance before (4). It can be postulated similarly, that high yielders kept at the same feeding group will be in a relative shortage of energy compared to their low yielders counterparts. Milk yield after calving could therefore be used to indicate a relative shortage of energy. Repeat Breeding was found the present example to be associated with high milk yield in the first three month of the lactation.

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SUMMARY

Applied clinical epidemiological methods in an integrated program of herd health are described. These include the data base used to store the data, part of the monitoring report dealing with reproduction, and programs of epidemiological evaluation. Anestrus is used as an example for the methodology applied. Alarming levels of "% cows open greater than 150 d" when monitored, are further evaluated for the relative contribution of various possible factors to the trait. The effect of underfeeding before service on the rate of anestrus is further investigated using the data base. The merits and limitations of such tools as part of the integrated herd health programs are discussed.

SUMARIO

Se describen los metodos clinicos y epidemiologicos aplicados en un programa de sanidao de rodeo. Incluye la data base usado para almacenar los datos, parte del seguimiento del proceso de reproduccion y programas de evaluacion epidemiologica. El anestro es usado como ejemplo de la metodologia aplicada. Niveles alarmantes de "% de vacas vacias mas de 150 dias" que se siguen, son posteriormente evaluadas en su aporte como variable posible de la caracteristica. El efecto de subalimentacion antes del servicio en el nivel de anestro es posteriormente investigado usando la data base. Se discuten los meritos y limitaciones de estos medios en un programma de sanidad de rodeo.

ZUSAMMENFASSUNG

Es werden anwendbare klinische und epidemiologische methoden in einem integrierten program beschreiben. Diese enthalten die grundaten die zum aufbewaren der daten benutzt werden, ein teil des aufgezeichneten berichts handelt von reproduktions verfolgung und programme über epidemiologische evaluation. Der anestrus wird als beispiel der angewändeten methoden benätzt. Alarmierende werte von "% kühen die über 150 tage leer sind" werden verfolget, und später als möglicher faktor für diese eigenscaft bewertet. Es wird weiterhin der effekt de unterernährung vor der paarung im anestrum mit diesen grunddaten erforscht. Es werden die vorteile und auch grenzen dieser hilfsmitiel eines herde gesundheits program diskutiert.

EFEITO DA SUPLEMENTAÇÃO DE GORDURA PARA VACAS LEITEIRAS

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INTRODUÇÃO

O atendimento das necessidades energéticas de vacas leiteiras de alta produção é um dos problemas que mais preocupam os nutricionistas. Com o grande desenvolvimento da genética, no campo da produção de leite, a crescente demanda de energia tem sido atendida pelo fornecimento de altos níveis de concentrado nas rações. Porém, a ingestão de grandes quantidades de carboidratos de fácil fermentação, principalmente amido, tem ocasionado freqüentemente alterações de ordem digestiva, principalmente acidose ruminal, além de provocar diminuição da porcentagem de gordura no leite e redução da digestibilidade da fibra bruta. O grande desafio é elevar o nível energético das rações, sem alterar o metabolismo ruminal e conseqüentemente, reduzir o déficit energético das vacas leiteiras.

A suplementação de gordura nas rações de vacas de alta produção, além de aumentar a densidade energética da dieta, eleva a produção de leite, durante a fase inicial da lactação, quando aumenta a necessidade de ingestão de energia (5,6).

Dados recentes (2) evidenciaram que a persistência de lactação pode ser aumentada com adição de lipídios, mesmo durante a fase de declínio da produção de leite.

O presente trabalho teve o objetivo de avaliar o efeito de gordura animal cristalizada, para vacas em lactação.

MATERIAL E MÉTODOS

Para avaliar o efeito da suplementação de gordura na eficiência produtiva de vacas leiteiras, foram utilizadas 20 vacas da raça Holandesa, no início da 2ª e 3ª lactações, separadas em dois lotes de 10 animais, de acordo com a produção da lactação anterior. Os animais permaneceram confinados (free stalls), durante um período de 150 dias, com duas ordenhas, tendo à disposição água, concentrado e volumoso. O leite A (tratado) teve como fonte de lipídio, gordura animal (sebo) processado com sais de cálcios e o lote B (testemunha) teve como fonte de lipídio, gordura vegetal. As rações A e B, tiveram respectivamente a seguinte composição: silagem de milho 50%; milho triturado 15,9 e 16,3%; farelo de trigo 10%; farelo de algodão 9,5%; melaço 8%; fosfato bicálcico 0,7%; bicarbonato de sódio 0,7% e microminerais 0,5%. O lote A teve na sua composição de ração 4,4% de gordura animal cristalizada e o lote B 4% de gordura vegetal. A análise química das rações A e B (1) revelou a seguinte composição, com base na matéria seca: PB%: 18,28 e 18,21; FDA%: 21,5 e 21,3%; FDN%: 32,8 e 32,6; EE%: 6 e 5; Ca%: 1,04 e 1,02%; P%: 0,55 e 0,53. O consumo de ração e a produção de leite individual foi mensurada diariamente e as amostras de leite de cada animal foram coletadas 3 vezes por semana para análise química. Os resultados foram analisados estatisticamente através da análise de variância e as diferenças entre médias foram analisadas pelo teste T, com uma probabilidade de 5% (4).

RESULTADOS E DISCUSSÃO

De acordo com a Tabela 1, verifica-se que o lote A (gordura animal cristalizada) apresentou um consumo estatisticamente diferentes ($P < 0,05$) com base na matéria seca da ração (21,5 x 22,7 kg) 1,2 kg a menos do que o lote B e uma produção de leite/dia de (25,3 x 23,4 kg) 1,9 kg a mais do que o lote B, estatisticamente diferente. Provavelmente a redução do consumo está diretamente relacionado com o melhor aproveitamento energético da ração, uma vez que a gordura animal, processada com sais de cálcio (5) apresenta menor efeito inibidor sobre a digestibilidade da fibra, do que a gordura vegetal, além do mais, permite uma menor biohidrogenação dos ácidos graxos pelos microorganismos do rumen e um melhor aproveitamento energético da ração, pela liberação lenta de energia (by pass) para a síntese do leite, a nível de glândula mamária, uma vez que o uso de lipídios esterificados redirecionam a repartição de nutrientes a partir do tecido adiposo (3). A análise da composição química do leite não mostrou diferença significativa entre os lotes (2).

TABELA 1. Valores médios do consumo de ração, produção e composição do leite de vacas consumindo gordura (animal e vegetal).

	LOTE A	LOTE B
Produção de Leite (kg/dia)	25,3[a]	23,4[b]
Consumo Matéria Seca (kg/dia)	21,5[a]	22,7[b]
Consumo % PV	3,67	3,76
Composição Química Leite, %		
gordura	3,38	3,36
proteína	3,06	3,08
caseína	2,20	2,21
lactose	4,60	4,65
sólidos não gordurosos	8,60	8,67
sólidos totais	12,00	12,01

Médias com letras diferentes, diferem estatisticamente ($P < 0,05$)

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RESUMO

Efeito da Suplementação de Gordura para Vacas Leiteiras.

Para avaliar o valor nutricional da gordura na eficiência produtiva de vacas leiteiras, foram utilizadas 20 vacas da raça Holandesa, no início da 2ª e 3ª lactações, separadas em dois lotes, de acordo com a produção das lactações anteriores. As rações para os lotes A e B tiveram respectivamente a seguinte composição com base na matéria seca: PB: 18,28% e 18,21%; FDA: 21,5% e 21,3%; FDN: 32,8% e 32,6%; EE: 6% e 5%; Ca: 1,04% e 1,02%; P: 0,55% e 0,53%. O lote A foi suplementado com 4,4% de gordura animal cristalizada com sais de cálcio e o lote B com 4% de gordura vegetal. O lote A apresentou um consumo de ração, na base de matéria seca de (21,5 x 22,7 kg) 1,2 kg a menos do que o lote B e uma produção de leite/dia (25,3 x 23,4 kg) de 1,9 kg a mais do que o lote B.

SUMMARY

Effect of Fat Supplementation on Milking Dairy Cows.

Twenty Holstein milking cows were used to evaluate the nutritional value of fat supplementation on the productive efficiency. Animals were divided into two groups, treatment and control, at the beginning of the 2nd and 3rd lactation according to previous milk productions. Ration composition for treatment and control cows were: 18.28, 18.41% crude protein; 21.5, 21.3% ADF; 32.8, 32.6% NDF; 6.0, 5.0% EE; 1.04, 1.02% Ca and .55, .53% P, respectively. Treatment cows were supplemented with 4.4% of animal fat cristalized with calcium salts and control ones with 4% of vegetal fat. The dry matter intake of treatment cows was 1.2 kg lower (P ✓ .5) and tehe milk production 1.9 kg greater (P .5) than control cows.

RESUMEN

Efecto de la Suplementación de Grasa para las Vacas de Leche.

Para evaluar el efecto de la grasa en las vacas de leche se utilizaron 20 vacas doe la raza Holstein-Friesian, en inicio de la 2ª e 3ª lactation, separadas de acordo com la producción de la leche. Los tratamientos A e B fueron respectivamente: PC: 18,28% e 18,41%; FDA:21,5% e 21,3%, FDN: 32,8% e 32,6%; EE: 6% e 5%; Ca: 1,04% e 1,02%; P: 0,55% e 0,54%. Los grupos A y B tuvieran respectivamente la suplementación de 4.4% e 4% de grasa aninal e vegetal. El grupo A tuevo um consumo de ración (MS) de 1,2 kg a menos do que el grupo B y una produción de leche/dia de 1,9 kg a más do que el grupo B.

EFEITO DO USO DO MILHO E DA AVEIA NO CRESCIMENTO DE VITELOS DO NASCIMENTO AO DESMAME E DESTA FASE AOS 6 MESES

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INTRODUÇÃO

A produção de vitelos, como qualquer outra actividade zootécnica, é determinada por objectivos económicos.

Este facto determina que se dedique atenção as todas as soluções que contribuam para uma diminuição dos custos de produção ou para uma diluição destes, através de performances produtivas melhoradas.

Entre as soluções, em produção animal e nomeadamente em ruminantes, que contribuem para a diminuição dos custos de produção podem destacar-se as reduções no consumo do leite de substituição, o privilegiar do consumo de alimentos sólidos e ainda outras soluções que contribuam para a poupança de mão de obra ou simplificação do maneio.

Dentro dos alimentos sólidos, o concentrado constitui a fracção mais cara, pelo que se justifica procurar soluções de menor custo, nomeadamente reduzindo as quantidades administradas e procurando matérias primas de mais baixo preço que permitam bons crescimentos.

A aveia, justifica-se por poder contribuir para a diminuição da importação de outros cereais, nomeadamente milho, e por se constatar que em Portugal existem condições naturais favoráveis para a sua produção (1,3,4,7,9). A utilização da aveia em vitelos, poderá ainda justificar-se por se reconhecer a possibilidade do seu uso em explorações leiteiras, nomeadamente no que se refere às produções obtidas com vacas adultas (8).

OBJECTIVOS

Comparação do efeito de dois cereais (milho e aveia), no desempenho produtivo de vitelos, dos 8 dias aos 2 meses de idade e em fases ulteriores do crescimento dos animais.

Valorização da aveia como substituinte de outros cereais, determinando os limites máximos da sua utilização em substituição do milho.

METODOLOGIA

Este ensaio foi realizado em duas fases distintas (1ª fase, do nascimento ao desmame e a 2ª fase, do nascimento aos 6 meses de idade).

Para o efeito recorreu-se a 30 vitelos (machos e fêmeas), de raça Frísia,os quais foram divididos em três grupos.

1ª FASE: Os animais receberam um total de 189 litros de leite de substituição, uma vez ao dia.

A cada um dos grupos foram administrados três concentrados "ad libitum": 100 % milho, 50% milho+50% aveia e 100% aveia suplementados com bagaço de soja.

A composição dos concentrados utilizados encontra-se apresentada no Quadro 1.

QUADRO 1. Composição do concentrado por 100 kg de alimento

	Grupos		
	I	II	III
	Milho	Milho+Aveia	Aveia
Milho	65.0	32.5	-
Aveia	-	32.5	65.0
Bagaço de soja	31.9	31.9	31.9
Fosfato bicálcico	1.0	1.0	1.0
Cloreto de sódio	1.0	1.0	1.0
Saninix vitelos	0.1	0.1	0.1
Bentonite	0.5	0.5	0.5

O alimento grosseiro (feno) e a água foram igualmente distribuídos "ad libitum".

Este regime prolongou-se até às 8 semanas de idade que coincidiu com o desmame.

2ª FASE - 5 animais de cada um dos referidos grupos continuaram a receber os respectivos concentrados, feno e água "ad libitum", no sentido de continuar a identificar quaisquer diferenças no desempenho dos animais submetidos aos diferentes regimes.

RESULTADOS

Os resultados obtidos no presente ensaio encontram-se apresentados no Quadro 2.

Na 1ª fase não se observaram diferenças significativas no que se refere à ingestão total de M.S., de feno, concentrado e leite de substituição, embora se tenha constatado uma tendência para valores mais elevados no grupo com 100% de aveia. No que se refere ao desempenho produtivo (ganho de peso vivo e eficiência alimentar) também não se verificaram diferenças significativas entre tratamentos. No entanto, observou-se uma tendência para valores mais elevados no regime com 100% de milho.

Na 2ª fase, constatou-se que o grupo com 100% milho ingeriu mais M.S. que o que recebeu o concentrado com 50% de aveia e 50% de milho. O regime 100% de aveia ficou numa posição intermédia não tendo exibido diferenças significativas em relação aos dois primeiros. No que se refere ao desempenho produtivo (ganho de peso vivo e eficiência alimentar), o grupo com 100% de milho apresentou valores mais altos do que o que havia recebido o concentrado com 100% de aveia.

QUADRO 2. Ingestão, Peso Vivo, Ganho de Peso Vivo e Eficiência Alimentar nas duas fases e nos três tratamentos estudados.

		100% M	50% M + 50% A	100% A
Do início da experiência até ao desmame (49 dias)	INGESTÃO TOTAL DE M.S. (kg):			
	leite de substituição	24.77	24.77	24.77
	concentrado	23.76	20.03	24.30
	feno	.45	.43	.51
	total	48.98	45.23	49.58
	PESO VIVO (Kg):			
	no início da exper.	42.60	42.40	42.30
	ao desmame	68.50	65.50	67.70
	GANHO DE PESO VIVO (Kg/dia)	.548	.479	.542
	EFICIENCIA ALIMENTAR (Kg M.S./Kg G.P.V.)	1.89	1.96	1.95
Do desmame até aos 6 meses (112 dias)	INGESTÃO TOTAL DE M.S.(Kg):			
	concentrado	392.95	359.30	375.04
	feno	14.14	9.73	10.38
	total	407.09a	369.03b	385.42ab
	PESO VIVO AOS 6 MESES (Kg)	200.00	179.80	181.20
	GANHO DE PESO VIVO(Kg/dia)	1.181a	1.065ab	1.020b
	EFICIENCIA ALIMENTAR (Kg M.S./Kg G.P.V.)	3.10b	3.23b	3.40a

Valores acompanhados por letras diferentes em asterisco, revelam-se significativamente diferentes (P<0.05)

DISCUSSÃO

Na fase I o leite foi totalmente ingerido pelos vitelos. A ingestão de M.S. de concentrado e de feno não foram significativamente diferentes embora se verificasse uma ligeira superioridade dos grupos Milho e Aveia em relação ao grupo Milho+Aveia.

No que se refere aos Pesos Vivos e GMD, as diferenças observadas também não se revelaram significativas o que seria de esperar atendendo à semelhança de ingestão entre os grupos. Os crescimentos médios que oscilaram entre os 0.48 kg e 0.55 kg são considerados apreciáveis (10). Estes crescimentos poderão ser atribuídos à boa qualidade dos regimes lácteo e concentrado, já que as ingestões de feno foram muito baixas.

A eficiência de utilização dos alimentos, considerada com base no índice de conversão, foi também idêntica para os três tratamentos. Esta situação resulta obviamente da ausência de diferenças entre ingestão e variação de peso.

Considerando a fase II, que teve lugar entre a 9ª e 24ª semanas de idade dos animais, a ingestão total de M.S. do grupo Milho foi significativamente superior ao grupo Milho+Aveia, tendo o grupo Aveia assumido uma posição intermédia em relação aos primeiros, não sendo diferente de nenhum deles.

A ausência de diferenças de ingestão entre os grupos Milho e Aveia e as menores ingestões do grupo Milho+Aveia são difíceis de explicar, com base em qualquer reação aos tratamentos experimentais, dado que os casos extremos (100% milho e 100% aveia) se apresentaram idênticos. Assim, a interpretação desta situação poderá ser feita com base na diferença de peso entre os três grupos. Os animais do grupo Milho+Aveia eram mais pequenos e portanto os seus limites de ingestão foram mais baixos.

Considerando os ganhos médios de peso vivo diário, ao longo desta fase II, observou-se que o grupo Milho apresentou crescimentos significativamente mais elevados que o da Aveia, tendo o grupo Milho+Aveia exibido crescimentos intermédios e idênticos aos dois primeiros.

Estes factos associados aos da ingestão permitem verificar que os grupos Milho e Aveia, que exibiram consumos semelhantes de M.S., apresentaram taxas de crescimento diferentes.

A presente situação poderá resultar da maior riqueza energética do milho em relação à aveia, facto que é divulgado pela bibliografia (2,5,6). Esta interpretação parece ser reforçada pelo facto do regime Milho+Aveia se colocar em posição intermédia em relação aos grupos Milho e Aveia.

A eficiência de utilização dos alimentos apresentou algumas diferenças, que se afiguram óbvias, face aos resultados da ingestão e ganho de peso.

O grupo Aveia apresentou índices de conversão mais elevados o que traduz as ingestões relativamente elevadas e o baixo crescimento dos animais. Os grupos Milho e Milho+Aveia exibiram índices de conversão mais baixos do que o grupo Aveia e semelhantes entre si. No que se refere ao grupo Milho+Aveia, esta situação traduz o facto da ingestão ter sido mais baixa e os ganhos de peso terem sido mais elevados.

CONCLUSÕES

Os resultados obtidos, associados às potencialidades do País para a produção de aveia, levam-nos a concluir do eventual interesse da maior utilização deste cereal em dietas para vitelos.

Esta indicação é ainda reforçada pela observação de que o desempenho produtivo de vacas leiteiras adultas é idêntico com este cereal, ou outros mais dependentes da importação.

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RESUMO

O ensaio, com 30 vitelos machos e fêmeas, decorreu em duas fases - a primeira do nascimento ao desmame, realizado às 8 semanas, a segunda do desmame aos seis meses de idade. Teve como objectivo comparar o efeito de dois cereais (milho e aveia) no desempenho produtivo de vitelos.

Na primeira fase os animais foram divididos em três grupos, recebendo um total de 189 litros de leite de substituição uma vez ao dia. A cada um dos grupos foram administrados três concentrados "ad libitum": 100% milho (M), 50% milho+50% aveia (M+A) e 100% aveia (A) suplementados com bagaço de soja. O alimento grosseiro (feno) e a água foram igualmente administrados "ad libitum". Para os grupos M, M+A e A obtiveram-se respectivamente: ingestão total (Kg MS) - 48.98, 45.23 e 49.58; GMD (Kg/dia) - 0.548, e 0.469 e 0.542; índice de conversão (Kg MS/Kg PV) - 1.89, 1.96 e 1.95.

Na segunda fase, 5 animais de cada um dos referidos grupos continuaram a receber os respectivos concentrados, feno e água "ad libitum". A ingestão total de MS (Kg) do grupo M (407.09) foi significativamente superior ao grupo M+A (369.03), tendo o grupo A (385.42) assumido uma posição intermédia em relação aos primeiros não sendo diferente de nenhum deles. Considerando os GMD, o grupo M apresentou crescimentos significativamente mais elevados (1.181) que o grupo A (1.020), tendo o grupo M+A (1.065) exibido crescimentos intermédios e idênticos aos dois primeiros. O grupo A apresentou um índice de conversão mais elevado (3.40) o que traduz as ingestões relativamente elevadas e o baixo crescimento dos animais; os grupos M e M+A, apresentaram índices de conversão mais baixos, respectivamente 3.10 e 3.23, que o grupo A e semelhantes entre si.

RESUMÉ

EFFET de L'UTILIZATION DU MAIS ET DE L'AVOINE DANS LA CROISSANCE DES VEAUX DES LA NAISSANCE JUSQU'AU SÉVRAGE ET DES LE SÉVRAGE JUSQU'À L'ÂGE DE SIX MOIS

L'essai, avec trente veaux mâles et femelles, a été réalisé en deux phases - la première dès la naissance jusqu' au sévrage, effectué à 8 semaines et la deuxième dès le sévrage jusqu' à l'âge de 6 mois. L'objectif de l'essai a été de confronter l'effet de deux céréales (mais et avoine) sur le comportement produtif des veaux.

Dans la 1ère phase les animaux ont été partagés en 3 groupes, recevant chacun 189 litres de lait de remplacement, une fois par jour, du forrage (foin) et de l'eau "ad libitum". On a aussi administré, "ad libitum", à chacun des groupes trois concentrés d'initiation: 100% mais (M), 50% mais +50% avoine (M+A) et 100% avoine(A) supplementés avec du tourteaux de soja. On a obtenu, respectivement pour les groupes M, M+A et A: ingestion total (Kg M.S.) - 48.98, 45.23 et 49.58; gain moyen journalier (Kg/jour) - 0.548, 0.469 et 0.542; éficacité alimentaire (Kg M.S./Kg gain de poid vif) - 1.89, 1.96 et 1.95.

Dans la 2ème phase, 5 animaux de chaque groupe ont été mantenus en essai recevant les mêmes concentrés, du foin et de l'eau"ad libitum". L'ingestion totale de M.S. (kg) du groupe M (407.09) a été significativement supérieure au groupe M+A (369.03), mais pour le groupe A (385.42) on a obtenu une valeur intermediaire par rapport aux autres groupes. En considérant le gain moyen journalier, le groupe M (1.181) a montré des croissances significativement supérieures au groupe A (1.020) et le groupe M+A (1.065) a montré des croissances intermediaires et non différentes des groupes M et A. En ce qui concerne l'éficacité alimentaire on a obtenu pour le groupe A la valeur la plus élevée (3.40), les groupes M et M+A ont montré une éficacité alimentaire plus bas et semblable de 3.10 et 3.23, respectivement.

SUMMARY

THE EFFECT OF COMPARING MAIZE AND OATS IN FEEDSTUFFS FOR CALVES FROM BIRTH TO WEANING AND FROM WEANING TO SIX MONTHS OF AGE.

The aim of the present trial was to estimate the effect of using two different cereals (maize and oats) on the performance of calves. The experiment was carried out in two different periods with black and white calves, both males and females.

In period 1 (from birth to weaning), animals were allocated to three different treatments. All calves were allowed a total amount of 189 liters of milk replacer, water and hay ad libitum. Group M received a concentrate ration mainly consisted of maize; group M+O received a concentrate in which 50% of maize was replaced by oats; finally group O received a ration mainly consisted by oats. Protein content of concentrates was constant for all groups which was achieved by using soya as a N supplement. Total Dry Matter intake for groups M, M+O and O was 48.98, 45.23 and 49.50 Kg respectively. Daily liveweight gains in the same order were 0.548, 0.469 and 0.542 Kg. Conversion rate (Kg D.M./Kg LW) was 1.89, 1.96 and 1.95 respectively for groups M, M+O and O.

In period 2, from weaning (8 weeks) up to 6 months of age, calves in the mentionated groups were switched into a system in which concentrates and hay were fed ad libitum. Total Dry Matter intake of group M (407.09 Kg) was significantly higher than group M+O. Considering daily liveweight gains, group M (1.181 Kg/day) showed significantly higher values than group O (1.020). In this case group M+O showed intermediary values between M and O. Group O showed also a relatively high conversion rate (3.40) when compared with similar values found for groups M (3.10) and M+O (3.23). This situation reflect a lower feed efficiency of group O in relation to the others.

OBSERVACIONES DE LOS EFECTOS DE UN ADITIVO ANTIBIOTICO EN LA ALIMENTACION DE BOVINOS.

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INTRODUCCION

En Chile existe una amplia gama de antibióticos para ser usados en alimentación animal y desde hace algunos años se ha desarrollado una marcada difusión sobre el uso de ellos como promotores de crecimiento en raciones para bovinos. Considerando la importancia que este tipo de aditivos ha adquirido y su creciente incorporación en el curso de la producción bovina, hemos concebido este estudio con fines de comprobar e investigar el efecto del Flavofosfolipol (Flavomycin 40, Laboratorio Hoechst de Chile), en raciones para novillos de engorda, vaquillas de reposición y terneros lactantes.

El Flavofosfolipol es un antibiótico fosfoglicolipídico formado por un grupo de estreptomicitos, también es conocido como Flavomycin, Baber mycin, Flavocorn o Moenomycin (8). El mecanismo de acción antibiótica del Flavofosfolipol se basa en la inhibición de la biosíntesis de las paredes celulares de la bacteria. Es utilizado exclusivamente en la alimentación animal como promotor del crecimiento y mejorador de la conversión alimentaria (8,9).

El aumento de la eficiencia se asocia en animales monogástricos a un aumento de la capacidad de digestión de los sustratos energéticos y de las proteínas ya que reduce el grosor de la pared intestinal, aumentando la absorción de los nutrientes (13). Flavofosfolipol en rumiantes aumenta significativamente la cantidad de ácidos grasos volátiles (AGV) en el rumen y también tiene efecto sobre la proporción de los diferentes AGV, decrece los porcentajes de ácido acético y butírico y se produce un aumento en la proporción del ácido propiónico (12), sin embargo otros autores indican que el Flavofosfolipol no alteraría la fermentación ruminal y su actividad estaría centrada en el intestino delgado posiblemente aumentando la absorción de nutrientes en un efecto similar al encontrado en monogástricos (1,10).

Trabajos publicados indican que el Flavofosfolipol mejora el aumento de peso diario en terneros y novillos en engorda en relación a animales que no reciben el antibiótico (2,3,4,5,6,7,12). Conociendo estos antecedentes se diseñó una serie de experimentos realizados en la Estación Experimental Maipo (Temuco, Chile) con el fin de determinar la influencia del Flavofosfolipol en el consumo, aumento de peso y eficiencia alimentaria en bovinos de diferentes edades y sometidos a distintos regimenes de alimentación.

MATERIAL Y METODOS

Experimento 1

Dieciseis terneros frisones machos y hembras nacidos en otoño fue

ron utilizados para este ensayo. La alimentación se basó en 5 litros de leche entera, concentrado starter y heno desde los 5 días de edad hasta los 90 días.

Fueron distribuidos al azar en dos grupos, 4 machos y 4 hembras, uno de los grupos recibió 16 mg. de Flavofosfolipol por animal al día disuelto en la leche. Los pesajes fueron realizados cada 15 días y el ensayo duró 90 días.

Experimento 2

Se utilizaron 16 vaquillas frisonas de 6 a 8 meses de edad y con pesos promedios de 210 kgs. Las vaquillas se mantuvieron estabuladas y recibieron diariamente una dieta constituída por subproductos agrícolas de origen vegetal; paja de cebada, hoja y corona de remolacha azucarera y afrecho de raps. La ración completa contenía 11,8% de Proteína Total (PT) y 2,5 Mcal/kg de Materia Seca (MS). Los animales se distribuyeron en dos grupos al azar y un grupo recibió diariamente por animal 40 mg. de Flavofosfolipol. Los pesajes se realizaron cada 15 días, durante los 90 días que duró el experimento. No se midió en este ensayo el consumo de MS.

Experimento 3

Veintiún novillos de la raza Hereford de 1 a 2 años de edad y con un peso promedio de 360 kgs. fueron distribuidos en tres grupos al azar. Todos los animales recibieron un anabólico al comienzo del ensayo y su alimentación consistió en afrecho de raps, coseta seca de remolacha azucarera y ensilaje de maíz. La ración final contenía 14,77% de P.T. y 2,6 Mcal/kg de M.S. El ensayo se realizó en los meses de invierno y la distribución de los grupos fué la siguiente: Grupo A (n=7) Control sin antibiótico; Grupo B (n=7) Flavofosfolipol 30 mg por animal al día Grupo C (n=7) Flavofosfolipol 60 mg.por animal al día.

El ensayo tuvo una duración de 90 días y los pesajes se realizaron cada 15 días.

RESULTADOS

La influencia del Flavofosfolipol en la ganancia de peso en terneros se observa en el cuadro Nº 1. Los terneros que recibieron 16 mg. / día del antibiótico ganaron 0.08 kg./día más que los que no lo recibieron ($p > 0.05$). No se observan diferencias estadísticas significativas en el consumo de concentrado inicial y heno. La eficiencia de conversión se vió mejorada de 2.14 a 1.90, al adicionar 16 mg. de Flavofosfolipol al día por ternero ($p > 0.05$).

El cuadro Nº 2 muestra el efecto del antibiótico en la ganancia de peso diaria de vaquillas, los animales que reciben 40 mg. de Flavofosfolipol al día tuvieron un aumento de peso de 1.12 kg./día en comparación al grupo control que obtiene una ganacia diaria de 0.98 kg.($p > 0.05$).

El efecto del Flavofosfolipol en el aumento de peso y conversión alimentaria en novillos Hereford está indicado en el cuadro Nº 3. Las ganacias de peso diario de los animales que recibieron el aditivo fué igual o superior a los novillos que no lo recibieron. Este parámetro presentó una gran dispersión y el análisis estadístico no reveló diferencias significativas ($p > 0.05$). El consumo diario de alimento expresado en kg. de materia seca disminuye a medida que aumenta la dosis de Flavofosfolipol logrando el menor consumo 8,20 kg. de M.S., al administrar una dosis de 60 mg. por animal al día, el mayor consumo se obtuvo en el grupo que no recibió el antibiótico (8,81 kg. de M.S. por animal

CUADRO Nº 1 Influencia del Flavosfolipol en la ganancia de peso, consumo de alimentos y eficiencia de conversión en terneros.

	CONTROL	FLAVOFOSFOLIPOL
Nº de animales.	8	8
Días de ensayo.	85	85
Ganancia de peso diaria.	0.68a	0.76b
Consumo concentrado (kg. de M.S.)	73.80a	70.70a
Consumo heno (kg. de M.S.)	50.01a	52.08a
Eficiencia de conversión (Kg. de M.S./kg. de peso).	2.14a	1.90a

(a,b) Letras diferentes en una línea indican diferencias estadísticamente significativas ($p > 0.05$).

CUADRO Nº 2 Influencia del Flavofosfolipol sobre el aumento de peso en vaquillas frisonas de reemplazo.

	CONTROL	FLAVOFOSFOLIPOL
Nº de animales.	8	8
Días de ensayo.	90	90
Peso inicial (kg)	211	207
Peso final (kg)	300	308
Ganancia diaria (kg)	0.98a	1.12b

(a,b) Letras diferentes en una línea indican diferencias estadísticas significativas ($p > 0.05$).

CUADRO Nº 3 Efecto del Flavofosfolipol sobre la ganancia de peso y consumo de alimentos en novillos Hereford.

	CONTROL	FLAVOFOSFOLIPOL 30 mg.	60 mg.
Nº de animales.	7	7	7
Días de ensayo.	70	70	70
Peso inicial (kg)	361	360	361
Peso final (kg)	451	460	451
Ganancia diaria (kg)	1.29a	1.43a	1.29a
Consumo diario Materia seca (kg)	8.81a	8.55b	8.20c
Conversión alimenticia kg/kg de M.S.	6.83a	5.98b	6.36c

al día) ($p > 0.05$).

La conversión alimenticia, medida como Kg. de materia seca consumida en relación a kgs. de aumento de peso vivo, demostró que el grupo de animales que recibió 30 mg./animal/día de Flavofosfolipol tienen la mayor conversión (5,98 kg.de M.S. por kg. de aumento de peso) en comparación con el grupo control (6.83 kg. de M.S. por Kg. de aumento de peso). El grupo que recibió los 60 mg. de Flavofosfolipol al día demuestra una conversión de 6,36 kg. de M.S. por Kg. de aumento de peso diario. La eficiencia de conversión difiere significativamente ($p > 0.05$) entre los tratamientos.

DISCUSION

En terneros frisones el Flavofosfolipol produjo una ganancia de peso adicional de 0.08 kg/día lo que equivales a un 18% por sobre el grupo control, este resultado es significativo (p 0.05) y concuerda con otros autores que al utilizar terneros machos y hembras overo negro alimentados con austituto, heno y concentrado obtienen diferencias significativas al adicionar Flavofosfolipol en dosis de 16 mg/ternero al día (5,6).

Los terneros tratados con el antibiótico tuvieron una ganancia diaria mayor enel período de 5 a 35 días que en los períodos siguientes, esto concuerda con los resultados de Díaz (6) que señala que el efecto de los antibióticos sobre el crecimiento de terneros es mayor durante las primeras semanas de vida.

En el consumo de concentrado y heno no hubo diferencias significativas entre los grupos pero la eficiencia en un 11% en los terneros que reciben Flavofosfolipol, resultado concordante con otros trabajos (6,8).

En el ensayo realizado en vaquillas las que recibieron en la ración 40 mg./día de Flavofosfolipol obtuvieron un aumento de peso diario significativamente más alto que el grupo que no recibe el antibiótico. Esta diferencia probablemente se produjo al contener la ración un alto porcentaje de fibra, hecho que ha sido demostrado en otras experiencias (8,12).

Varios autores (7,9,12) concuerdan que la adición de Flavofosfolipol es beneficiosa al aumentar la ganacia de peso en novillos. Los resultados de este ensayo, indican que el Flavofosfolipol no afectó significativamente la ganancia diaria de peso en los novillos Hereford, pero es posible destacar que la incorporación del Flavofosfolipol determinó para ganancias de peso iguales o mayores que el control, una disminución en la cantidad de alimento consumido, siendo estos resultados estadísticamente significativos ($p > 0.05$).

Los novillos que recibieron 30 mg. al día de Flavofosfolipol en el alimento demostraron ser más eficientes en la conversión de kgs. de alimento en kgs. de peso. El grupo B que recibió 30 mg de Flavofosfolipol por animal al día, fué un 12,5% más eficiente que el grupo control y un 6% más eficiente que los animales del grupo C, estas diferencias entre los grupos fueron estadisticamente significativas ($p > 0.05$).

Estos resultados son superiores a trabajos de otros autores quienes señalan un aumento entre un 3,5% y un 5,5% en la eficiencia alimenticia al administrar Flavofosfolipol en la alimentación de novillos (7,8,12).

A partir de los resultados obtenidos en el presente trabajo se puede concluir:

- La adición de Flavofosfolipol produjo un mejoramiento estadisticamente significativo ($p > 0.05$) en la conversión del alimento, este efecto es más notorio en dosis de 30 mg./ animal/día en novillos y 16 mg/animal/día en terneros lactantes.

- La adición de Flavofosfolipol disminuye el consumo de alimento en relación a los testigos, efecto que es directamente proporcional a la dosis incorporada y estadisticamente significativa ($p > 0.05$).
- El efecto del Flavofosfolipol sobre la ganancia de peso fué significativa en los terneros y vaquillas (16 y 40 mg/animal/día). En novillos (30 mg/animal/día) hubo un aumento de 10.85% pero este efecto no fué significativo.

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RESUMEN

Con el propósito de evaluar el efecto del antibiótico Flavofosfolipol (Flavomycin 40, Laboratorio Hoechst de Chile) en la ganacia de peso y consumo de alimentos en bovinos, se realizaron a partir del año 1986 experiencias con grupos de animales de diferentes edades y sometidos a distintos regimenes de alimentación en la Estación Experimental Maipo de la Universidad de la Frontera en Temuco, Chile.

En terneros frisones criados en un sistema artificial se obtienen aumentos de peso hasta los 90 días de 0.68 kg./día para los terneros testigos y de 0,76 kg./día para los animales que recibieron 16 mg. de Flavofosfolipol al día ($p > 0.05$). El grupo de terneros que recibió el antibiótico demostró una mejor eficiencia alimenticia que el grupo control ($p > 0.05$).

En vaquillas frisonas de 210 kg. de peso y alimentadas con una mezcla de subproductos agrícolas de origen vegetal, se obtuvo un aumento de peso diario de 0.98 kg. promedio para los 90 días de ensayo en las vaquillas controles y de 1.12 kg/día para los animales que recibieron 40 mg. de Flavofosfolipol ($p > 0.05$).

En novillos Hereford de 360 kg. no se observó diferencias significativas en el aumento de peso diario al adicionar Flavofosfolipol en la dieta, sin embargo en el consumo diario de materia seca se vió disminuido en los novillos que recibieron el antibiótico lo que determinó para estos animales una mejor eficiencia alimenticia ($p > 0.05$).

SUMMARY
In order to evaluate the antibiotic Flavophospholipol (Flavomycin 40 by Hoechst,Chile) in weight gain and food intake of cattle, experiments were started in 1986 with groups of animals of different ages and feeding systems at the Experimental Station "Maipo" of Universidad de la Frontera in Temuco, Chile. Friesian calves raised on an artificial system gained weight up to the age of 90 days at .68 kg/day for control calves,and .76 kg/day for animals receiving 16 mg of Flavophospholipol per day ($p > 0.05$). The group of calves receiving the antibiotic showed a better feeding efficiency than the control group ($p > 0.05$). With Friesian heifers weighing 210 kg. and fed with a mixture of agricultural subproducts of vegetable origin a weight gain of .98 kg daily was obtained for the control animals and 1.12 kg. daily for those receiving 40 mg of Flavophospholipol for the 90 days of the experiment. ($p > 0.05$). With 18-month old Hereford steers weighing 360 kilos on a semiintensive feeding system consisting of corn silage no significant differences were observed in the daily weight gain when Flavophospholipol was added to the diet. However, the daily feed intake of dry matter was less for the steers which received the antibiotic and this determined for these animals a better feed efficiency ($p > 0.05$).

RESUMO
Com o proposito de evaluar o efeito do antibiotico Flavofosfolipol (Flavomycin 40,Laboratorio Hoechst do Chile) na ganancia de peso e consumo de alimentos em bovinos, realizaron-se a partir do año 1986 experiencias com grupos de animais de diferentes edades e sometidos a diferentes regimenes de alimentacao na Estacao Experimental Maipo da Universidade de la Frontera, Temuco, Chile. Em bezerros "frisones" criados num sistema artificial obtiveram-se aumentos de peso ate os 90 días de 0.68 kg/día para os terneros controles e, de 0.76 kg/día para os animais que recibieron 16 mg. de Flavofosfolipol por día ($p > 0.05$). Ogrupo de terneros que recibio o antibiotico mostrou uma melhor eficiencia alimenticia que o grupo controle ($p > 0.05$). Em novilhas frisonas de 210 kg. de peso e alimentadas com uma misture de subproductos agrícolas de origen vegetal, obtiveram-se um aumento de peso diario de 0.98 kg. promedio para los 90 días de ensaio nas vaquinhas controles e de 1.12 kg/día para os animais que reciberam 40 mg. de Flavofosfolipol ($p > 0.05$). Em novilhas Hereford de 18 meses de edade e com pesos de 360 kg. num regime semiintensivo com alimentacao a base de silagem de milho nao observou-se diferencas significativas no aumento de peso diario quando adicionous-seFlavofosfolipol na dieta. Porem o consumo diario de materia seca diminuio nos novilhos que reciberam o antibiotico,determinando uma melhor eficiencia alimenticia destes animais ($p > 0.05$).

ZUSAMMENFASSUNG
Um die Wirkung des Antibiotikum Flavophospholipol (Flavomycin 40, Hoechst,Chile) bei der Gewichtszunahme und Futterverbrauch von Rindern zu bewertun,sind ab 1986 versuche mit Gruppen von Rindern verschiedenen Alters unter verschiedenen Fütterungssystemen auf der Experimentalfarm "Maipo" der Universidad de la Frontera in Temuco, Chile,durchgeführt worden. Bei Friesenkälbern, die unter einen künstlichen System aufgezogen waren,wurden bis zum Alter von 90 Tagen Gewichtszunahmen von 0.68 kilo/Tag bei Kontrollkälbern und 0.76 kilo/Tag für die Tiere, die 16 mg Flavophospholipol pro Tag bekamen,festgestellt ($p > 0.05$). Die Gruppe Kälber die das Antibiotikum bekamen zeigten eine bessere Fütterungsleistung als die Kontrollgruppe. Bei 210 kilo wiegenden Friesen-Jungkühen die mit einer Mischung landwirtschaftlicher Nebenprodukten pflanzlicher Herkunft gefüttert waren,wurde in den 90 Tagen des Experiments eine tägliche Gewichtszunahme von durchschnittlich 0,98 kilo bei den Kontrollkühen und 1,12 kilo täglich bei den Tieren die 40 mg Flavophospholipol bekamen,erreicht ($p > 0.05$). Bei 18 Monat alten,360 kilo wiegenden Hereford Bullen,unter einen semi-intensiven System mit Fütterung aus Maissilage,wurden keine bedeutenden Unterschiede in der täglichen Gewichtszunahme mit einer Futtermugabe von Flavophospholipol beobachtet. Der tägliche Verbrauch von Trockenstoff verminderte sich aber bei den Bullen die das Antibiotikum bekamen,was für diese Tiere eine bessere Fütterungsleistung bedeutete. ($p > 0.05$).

DISTRIBUIÇÃO MUSCULAR EM BOVINOS DE RAÇAS NACIONAIS - INFLUÊNCIA DA PERCENTAGEM DE GORDURA NA CARCAÇA E DA RAÇA

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INTRODUÇÃO

Embora em termos práticos diferenças na distribuição muscular possam ter importância, dado que partes da carcaça têm um valor comercial superior a outras, a maioria dos trabalhos realizados sobre o assunto levaram à conclusão que este factor não tem tanta importância quanto muitas vezes se considera (5).

O desenvolvimento muscular processa-se durante o crescimento como resposta a necessidades funcionais sendo regulado por principios uniformes (5). Assim, o desenvolvimento pré-natal dos músculos que permiten ao animal deslocar-se e mamar é de extrema importância nas possibilidades de sobrevivência do recém nascido. Contrariamente, outros músculos com funções menos importantes nesta fase completaram apenas uma pequena parte do seu crescimento total. Entre os dois extremos, o espectro de desenvolvimento muscular reflecte a sequência de prioridades que permiten a sobrevivência e o crescimento normal do aninal (3). Os diversos músculos desenvolven-se assim a velocidades diferentes havendo, à medida que o animal cresce, alterações nas proporções que cada um representa relativamente à musculatura total. Os estímulos e necanismos que dão origem àquelas diferentes velocidades de crescimento parecem estar mais relacionados com o património genético, e principalmente no periodo pré-natal, e com necessidades funcionais após o nascimento (4). As alturas em que se dão as alterações dos ritmos de crescimento dos diferentes músculos e, consequentemente, da percentagem que estes representam relativamente ao total da musculatura, não está bem esclarecida. Contudo, vários autores têm referido que as principais modificações ocorrem durante as primeiras fases do desenvolvimento pós-natal (4) (6), até que o animal dobre o peso da sua massa muscular (7) e antes de ele se tornar comercialmente importante (5), ficando a distribuição muscular depois desta fase praticamente constante. Dentro da mesma espécie e para graus de naturidade idênticos as proporções que representam os diferentes músculos são semelhantes (16) (8) (6) (20). Apesar de se terem encontrado, tanto em bovinos como em ovinos (13) (2) (5) (18) (20) (9) (15) (18), diferenças significativas entre raças na distribuição muscular, nomeadamente nas peças nobres da carcaça, os autores de um modo geral concluiram que essas diferenças poderão ser reflexo de possiveis graus de maturidade diferentes e que quando questionandas comercialmente não são importantes.

MATERIAL E MÉTODOS

Os pormenores relativos à engorda e ao abate dos animais utilizados descreveram-se detalhadamente noutro trabalho (11). Resumidamente, com o objectivo de comparar e caracterizar as raças bovinas nacionais em termos de indicadores produtivos do crescimento e das características da carcaça, submeteram-se ao mesmo regime alimentar desde aproximadamente os 275 Kg de peso vivo 28 machos inteiros de cinco raças nacionais (6 Alentejanos, 5 Arouqueses, 6 Maroneses, 6 Mertolengos e 5 Mirandeses). Para

cada raça estipularam-se 2 pesos de abate (45 e 55% do peso adulto da raça, estimado teoricamente). Dividiu-se a meia carcaça direita de cada animal em 13 peças comerciais (acém comprido, acém redondo, rosbife, alcatra e perna, lombinho, cachaço, pá, chambão anterior, chambão posterior, maçã do peito, prego do peito, aba das costelas e aba grossa) e separou-se cada peça em músculo, gordura subcutânea e intermuscular e osso. Considerou-se peso da meia carcaça a soma dos três tecidos nas 13 peças, dos depósitos adiposos renal e pélvico, torácico e testicular, do rim e do resíduo da dissecção. As percentagens dos principais tecidos calcularam-se em relação ao peso da meia carcaça. Neste trabalho os autores verificaram que os valores das características da carcaça não mostraram as tendências esperadas num processo de crescimento normal (4) (17) (1) e admitiram quatro possíveis razões para os seus resultados: 1) uma diferença muito pequena entre os dois pesos de abate e/ou, 2) uma variabilidade muito grande em cada raça e/ou, 3) uma ingestão insuficiente em termos quantitativos e/ou qualitativos e/ou, 4) uma determinação incorrecta do peso adulto das raças.

No presente trabalho procurou-se avaliar o efeito do estadio de maturidade e da raça na distribuição muscular com os objectivos de caracterizar nestes termos as carcaças mais comercializadas no País e de esclarecer a razão dos resultados obtidos anteriormente (11). A percentagem de músculo em cada peça calculou-se relativamente ao peso de músculo da meia carcaça. Para avaliar o efeito do estadio de maturidade na distribuição muscular e admitindo que os pesos adultos não foram correctamente estimados no trabalho anterior, dado terem-se verificado diferenças entre as cinco raças na percentagem de gordura na carcaça (11), formaram-se dois grupos, G1 - 10/15% e G2 - 15/20% de gordura na carcaça (incluindo a gordura dissecada nas peças e os depósitos renal e pélvico, torácico e testicular), independentemente do peso de abate dos animais. Estes grupos correspondem respectivamente às classes 2 e 3 da classificação comercial que se utiliza no País (12). O efeito da raça analizou-se dentro de cada um dos referidos grupos e incluiu para G1 as raças Alentejana, Arouquesa, Mertolenga e Mirandesa e para G2 as raças Alentejana, Arouquesa, Maronesa e Mirandesa. Em ambos os casos, os resultados analizaram-se estatisticamente por análise de variância.

RESULTADOS

QUADRO 1. Pesos vivos médios ao abate e percentagens de músculo e de gordura dos animais incluídos nos grupos 1 e 2.

	Grupo 1	Grupo 2	Significância
PV ao abate (Kg)	505	480	NS
Músculo (%)	68.9	65.3	**
Gordura (%)	14.1	17.6	***

Nível de significância ** p ≤ 0.01 *** p ≤ 0.001

QUADRO 2. Pesos vivos médios ao abate e percentagens de músculo e de gordura das raças incluídas no Grupo 1.

	Alent.	Arouq.	Mert.	Mirand.	Significância
PV ao abate (Kg)	548	493	469	547	--
Músculo (%)	67.8	69.1	69.8	68.0	NS
Gordura (%)	14.6	14.2	13.7	14.2	NS

QUADRO 3. Pesos vivos médios ao abate e percentagens de músculo e de gordura das raças incluídas no grupo 2.

	Alent.	Arouq.	Maron.	Mirand.	Significância
PV ao abate (Kg)	514	477	465	473	--
Músculo (%)	64.0	69.2	65.0	64.3	NS
Gordura (%)	17.5	16.5	18.0	17.4	NS

QUADRO 4. Efeito da percentagem de gordura na carcaça como expressão de estádio de maturidade na distribuição muscular.

	Grupo 1	Grupo 2	Significância
Acém comprido %	10.6	10.5	NS
Acém redondo %	4.0	3.9	NS
Rosbife %	4.6	4.8	*
Alcatra e perna %	30.0	29.4	NS
Lombinho %	2.7	2.8	NS
Pá %	10.7	11.2	NS
Cachaço %	14.2	14.2	NS
Chambão anterior %	3.8	4.0	NS
Chambão posterior %	2.1	2.2	NS
Maçã do peito %	2.9	2.7	NS
Prego do peito %	3.0	3.2	NS
Aba das costelas %	6.0	5.7	NS
Aba grossa %	5.4	5.5	NS

Nível de significância * p ≤ 0.05

QUADRO 5. Efeito da raça na distribuição muscular - Grupo 1.

	Alent.	Arouq.	Mert.	Mirand.	Significância
Acém comprido %	10.5	10.8	10.8	10.3	NS
Acém redondo %	3.8	4.0	4.0	4.1	NS
Rosbife %	4.5 a	4.4 a	4.8 c	4.6 b	**
Alcatra e perna %	30.2 b	28.7 a	29.9 b	31.2 c	*
Lombinho %	2.7	2.8	2.7	2.6	NS
Pá %	10.7	10.8	10.8	10.7	NS
Cachaço %	14.6	14.6	13.7	14.1	NS
Chambão anterior %	3.9	3.6	3.6	4.0	NS
Chambão posterior %	2.0	2.1	2.0	2.2	NS
Maçã do peito %	2.5	2.9	3.0	2.9	NS
Prego do peito %	2.8	2.9	3.1	3.2	NS
Aba das costelas %	6.3	6.8	6.0	5.0	NS
Aba grossa %	5.3	5.5	5.5	5.4	NS

Nível de significância * p ≤ 0.05 ** p ≤ 0.01

QUADRO 6. Efeito da raça na distribuição muscular - Grupo 2.

	Alent.	Arouq.	Maron.	Mirand.	Significância
Acém comprido %	10.2	11.4	10.6	9.8	NS
Acém redondo %	3.7	4.4	3.9	3.8	NS
Rosbife %	5.0	4.5	4.7	5.0	NS
Alcatra e perna %	31.0	27.0	28.1	31.5	NS
Lombinho %	2.6	2.8	2.8	2.9	NS
Pá %	11.0	11.3	11.7	10.2	NS
Cachaço %	12.5	14.6	15.2	13.8	NS
Chanbão anterior %	4.1	4.5	3.7	4.1	NS
Chanbão posterior %	2.2	1.9	2.2	2.4	NS
Maçã do peito %	2.5	3.1	2.7	2.7	NS
Prego do peito %	3.8	2.4	3.2	3.1	NS
Aba das costelas %	5.6	6.6	5.4	5.6	NS
Aba grossa %	5.7	5.5	5.6	5.1	NS

DISCUSSÃO E CONCLUSÕES

A divisão dos animais por grupos de percentagens de gordura (G1 e G2) independentemente do seu peso de abate levou a que para as raças Mertolenga e Maronesa apenas existissem animais para um dos grupos e que para as outras etnias existissem animais dos dois pesos de abate em G1 e em G2. Isto significa uma grande variabilidade individual já que todos os animais foram submetidos ao mesmo regime alimentar e que a diferença entre os dois pesos de abate era, em cada raça, de cerca de 100 Kg de peso vivo.

Entre os grupos G1 e G2 não se verificaram alterações importantes na distribuição muscular. Apenas a percentagem que o rosbife representa relativamente à musculatura total aumentou significativamente (0.2%) de G1 para G2. Estes resultados estão de acordo com a ideia de que os grandes ajustamentos musculares se verificam mais cedo do que o período de crescimento que se considerou no presente trabalho (cerca de 12 a 18 meses de idade) (7). Pode-se assim afirmar que entre carcaças com 14.1 e 17.6% de gordura na carcaça não há praticamente alterações em termos de distribuição muscular. Considerando como músculo das peças nobres da carcaça aquele que está incluído no acém redondo, no acém comprido, no rosbife, na alcatra e perna, no lombinho e na pá (12) verifica-se que entre os dois grupos de percentagem de gordura não houve alteração da percentagem que o músculo naquele grupo de peças representa relativamente ao total (G1 - 62.6, G2 - 62.6). Estes valores são ligeiramente diferentes dos fornecidos pela estiva comercial de carcaças R2 e R3 (59.1 e 55.0, respectivamente) (12). No entanto consideraram-se neste caso carcaças com valores mais dispares de percentagens de gordura em cada classe, ou seja, 11.8% para R2 e 17.4% para R3, não há referência à inclusão do lombinho nos cálculos e há ainda que referir a subjectividade do método de estiva comercial utilizado em ambos os casos no que diz respeito à quantidade de gordura intermuscular a renover em cada peça (14). Sendo nos dois grupos considerados no presente trabalho a percentagem de músculo nas peças nobres semelhante, teria interesse contabilizar em termos de peso e economicamente as alterações entre G1 e G2. Esta contabilização não é no entanto possível pois a já referida variabilidade individual levou a que a média dos pesos vivos ao abate entre os dois grupos não diferisse significativamente. Deste modo pode-se considerar que o grupo 1 se revelou vantajoso já que engloba carcaças com uma gama de percentagens de gordura apontadas como ideais para o mercado consumidor português (10), com maior percentagem de músculo e com uma distribuição muscular

não desfavorável relativamente ao segundo grupo.

Verificaram-se entre as raças incluídas no grupo 1 diferenças de distribuição muscular nalgumas peças nobres da carcaça (rosbife e alcatra e perna). Diversos autores referem, em bovinos, diferenças pequenas mas estatisticamente significativas entre raças (13)(2)(18). Tal como no presente caso estas diferenças não podem ser consideradas, pela sua ordem de grandeza, comercialmente importantes.

Em resumo, não há, no conjunto das raças bovinas consideradas, diferenças importantes em termos de distribuição muscular entre os graus de maturidade considerados (percentagens de gordura na carcaça). Há contudo diferenças pequenas, mas estatisticamente significativas, entre raças quando comparadas no mesmo estadio de desenvolvimento.

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SUMÁRIO

Utilizaram-se 28 carcaças de bovinos machos inteiros de 5 raças Portuguesas (Alentejana, Arouquesa, Maronesa, Mertolenga e Mirandesa) para avaliar os efeitos da percentagem de gordura como expressão do estadio de maturidade e de raça na distribuição muscular.

Para avaliar a influência da percentagem de gordura formaram-se 2 grupos (G1 - 10/15% e G2 - 15/20% de gordura na carcaça) independentemente do peso de abate dos animais (2 pesos de abate por raça com uma diferença de cerca de 100 Kg). Verificou-se que para as raças Mertolenga e Maronesa apenas existiam animais para um dos grupos e que para as outras estas existiam animais dos dois pesos de abate em G1 e em G2, o que significa uma grande variabilidade individual. Entre G1 e G2 não houve alterações importantes na distribuição muscular. O grupo G1 revelou-se vantajoso englobando carcaças com uma gama de percentagens de gordura apontado como desejável para o mercado Português, com uma percentagem de músculo superior (p≤0.01) e com uma distribuição muscular não desfavorável relativamente a G2.

As diferenças que se manifestaram entre raças na distribuição muscular não podem, pela sua ordem de grandeza, ser consideradas comercialmente importantes.

SUMARIO

Se utilizaron 28 canales de bovinos machos enteros de cinco razas Portuguesas (Alentejana, Arouquesa, Maronesa, Mertolenga y Mirandesa) con el objetivo de evaluar los efectos del porcentaje de grasa, como expresión del grado de desarrollo, y de la raza en la distribución muscular.

Para evaluar la influencia del porcentage de grasa se formarón dos grupos (G1 - 10/15% y G2 - 15/20% de grasa en la canal) independientemente del peso al sacrificio de los animales (2 pesos al sacrificio por raza con una diferencia de cerca de 100 Kg). Se verifico que para las razas Mertolenga y Maronesa apenas existian canales para uno de los grupos y que para las otras razas existian animales de los dos pesos al sacrificio en los grupos G1 y G2, lo que significa una gran variabilidad individual. Se constato que entre los dos grupos no hubieron alteraciones importantes en la distribución muscular. El grupo G1 revelá ventajas, englobando canales con un porcentaje de grasa deseable para el mercado Portugues, con un porcentaje de musculo superior (p≤0.01) y con una distribución muscular no desfavorable relativamente a G2.

Las diferencias encontradas entre razas en la distribución muscular no pueden, por su orden de grandeza, ser consideradas comercialmente importantes.

SUMMARY

Twenty eight carcasses of entire males belonging to 5 Portuguese breeds (Alentejana, Arouquesa, Maronesa, Mertolenga and Mirandesa) were utilized to evaluate the effects of fat percentage expressing degree of maturity and of breed in muscle distribution.

To evaluate the effects of fat percentage 2 groups were formed (G1 - 10/15% and G2 - 15/20% of total fat in carcass), without taking into consideration the animals slaughter weight (2 slaughter weights per breed differing in about 100 Kg live weight). It was verified that for the breeds Mertolenga and Maronesa there were only carcasses for one of the groups and that for the other breeds there were in each group animals from both slaughter weights. This means that there was a big variability within breed. There were no important differences between G1 and G2 in muscle distribution. Group 1 can be considered a better one because it includes carcasses with a fat percentage ideal for the portuguese market demands, with a superior muscle percentage and with a muscle distribution not worst than Group 2.

The differences in muscle distribution verified between breeds in G1 are little ones and can not be considered commercialy important.

AVALIAÇÃO ECONÔMICA NA SUPLEMENTAÇÃO MINERAL EM BOVINOS DE ENGORDA

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INTRODUÇÃO

Devido a grande dúvida sobre a utilização de sal mineralizado ou mesmo sal comum para bovinos de engorda e, considerando os diferentes meios de administração aos animais, resolvemos iniciar um teste na Fazenda Vista Bonita, município de Barretos, SP, propriedade da Agro-Pecuária CFM Ltda.

É rotina no Brasil o fornecimento de sal comum ao gado. Na empresa Agro-Pecuária CFM Ltda temos como norma a suplementação de todos os animais durante todo o ano utilizando sal mineral concentrado misturado ao sal comum, na porporção 1:2 para o gado de cria e 1:3 para o gado de engorda.

Considerando informações da EMBRAPA através do Centro Nacional de Pesquisa de Gado de Corte em Campo Grande, MS; Costa et alii (1982) chegaram a conclusão de que a alternativa mais econômica na suplementação aos bovinos de corte foi o sal comum com uso restringido durante as secas; Souza et alii (1979, 1980, 1981, 1982, 1983, 1985, 1986, 1987) apontam diversas deficiências minerais nas pastagens brasileiras principalmente de sódio, fósforo e zinco, nas regiões centro-oeste e norte, assim como, deficiências nos tecidos animais comprometendo a produtividade. Mesmo assim, houve grandes variações nas deficiências dos elementos minerais de acordo com as regiões e capins utilizados assim como, variações na resposta econômica sobre a suplementação mineral no ganho de peso dos animais (Souza et al agosto de 1985 x Souza et alii março de 1983).

Outros pesquisadores como Beno Pott et alii (1984) concluíram que apenas a suplementação mineral não é suficiente para melhorar o desempenho reprodutivo de bovinos de corte, sendo o fator limitante a estacionalidade das pastagens e consequente falta de proteína.

Jardim et alii (1962) relata teor de fósforo na região de Barretos ao redor de 0,15% da matéria seca, chegando a 0,17% nas análises encontradas pelo Instituto Biológico de São Paulo (N=0,18% para P). Segundo O Biológico 50(4):71-84 a região de Barretos apresentou: cobalto = 0,09ppm (N=0,1ppm), zinco = 26ppm (N=30 ppm), os outros elementos como cálcio, magnésio, manganês e ferro estavam na faixa de normalidade ou acima.

Dayrell et alii (1972, 1973) determinaram deficiências minerais em soro sanguíneo de bovinos mantidos em criação extensiva em região de cerrado (Brasília); Morris (1980) aponta a deficiência de sódio ocorrendo mais em pastagens tropicais e menos em zonas temperadas, devido as espécies tropicais acumularem menos sódio que as temperadas. Morris et alii (1980) encontra um consumo de sal de 27g/vaca/dia na primeira semana da suplementação e uma variação entre os animais de 0 à 65g nos períodos semanais posteriores, na Califórnia (E.U.A.).

Como pode se notar existe grande controvérsia no assunto com diferentes resultados e conclusões, deixando uma falta de definição gerando uma grande especulação no mercado de sais minerais.

Acredita-se que as misturas minerais sejam vantajosas para engorda durante o período das águas e não durante as secas. Este fato se deve à falta de volumoso (carbohidratos e proteínas) durante o inverno, não sendo necessários minerais para corrigir qualquer deficiência, pela pró

pria falta do alimento. Em contraposição quando há alimento volumoso em abundância, qualquer deficiência mineral poderia prejudicar o aproveitamento máximo da conversão alimentar. Neste trabalho procurou-se mostrar os efeitos de quatro (4) tratamentos distintos em um período das secas e um período das águas nos anos de 1987/1988.

MATERIAL E MÉTODOS

Em 24.04.87 foram trazidos para o curral 335 bois magros nascidos entre agosto e outubro de 1985 recriados em 2 lotes, dos quais foram refugadas 15 cabeças, deixando um lote padrão de 320 animais, os quais foram divididos em 4 lotes de 80 cabeças cada, todos da raça nelore, pareando animais de pesos semelhantes entre si a fim de obter um peso final médio aproximadamente igual em todos os lotes. Foram utilizadas balanças eletrônicas da Filizola e os animais foram pesados sem jejum.

Os pesos médios seguintes foram obtidos:
Lote 1 = 339,2kg, lote 2 = 339,0kg, lote 3 = 339,2kg, lote 4 = 339,7kg.
Todos os animais receberam o número respectivo do seu lote, marcado a ferro quente na garupa e em seguida voltaram para seus pastos originais.

Os tratamentos foram iniciados em 07.05.87:
Lote 1 = suplementação com Ivafós e Rumisal-mix (macro-elementos separados dos micro-elementos) do laboratório IVA.
Lote 2 = testemunho (só pasto e água).
Lote 3 = suplementação com o sal mineralizado 65 da Purina.
Lote 4 = suplementação com sal comum.

Fórmula dos produtos (por kg pronto para uso):

Purina 65		Ivafós		Rumisal-mix	
Cálcio	100g	Cálcio	131,00g	Ferro	9,00g
Fósforo	65g	Fósforo	168,99g	Cobre	11,11g
Magnésio	1.500mg	Enxofre	2,49g	Cobalto	0,88g
Enxofre	5.000mg	Nitrogênio		Manganês	0,44g
Cobalto	100mg	Não proteíco	11,30g	Iodo	0,33g
Cobre	360mg	Magnésio	100,00mg	Zinco	0,44g
Ferro	3.000mg	Zinco	400,00mg	Sal Comum	977,80mg
Manganês	660mg	Proteína	19,76g		
Zinco	900mg	Carbohidratos	49,00g		
Iodo	240mg				
Selênio	2,5mg				
Sódio	156g				
Cloro	238g				

Foram utilizados 4 pastos de capim colonião semelhantes entre si tentando diminuir o efeito do pasto, com sistema de rotação quinzenal dos lotes em teste nos primeiros 60 dias e mensal nos dias subsequentes até o término da experiência.

Pasto Açudinho: Colonião formado em 1984 com área de 80ha(1,0 U.A./ha durante o experimento).
Pasto Zé da Silva: Colonião formado em 1984 com área de 73ha(1,09 U.A./ha durante o experimento).
Pasto Barreirinho 3: Colonião formado em 1983 com área de 64ha(1,25 U.A./ha durante o experimento).
Pasto Barreirinho 4: Colonião formado em 1983 com área de 58ha(1,38 U.A./ha durante o experimento).

Todos os animais receberam os seguintes tratamentos de rotina:
Junho/87: Vacinação contra Aftosa e Ripercol injetável (16ml cada).
Outubro/87: Vacinação contra Aftosa + Ripercol injetável (16ml cada) + pulverização com o carapaticida Ultimate (piretroide da Smith Kline).
Fevereiro/88: Vacinação contra Aftosa e Ripercol injetável (20ml cada).

Todos os cochos para sal mineral são cobertos, com 3 metros de comprimento e 30cm de largura e acesso dos 2 lados(7,5cm p/cabeça), sendo razoável para bom o estado das pastagens nos 4 pastos escolhidos e todos com boas aguadas.

RESULTADOS E DISCUSÕES

Tabela 1

	Lote 1 IVA Peso(kg)/Pasto	Lote 2 TESTEMUNHO Peso(kg)/Pasto	Lote 3 PURINA 65 Peso(kg)/Pasto	Lote 4 SAL COMUM Peso(kg)/Pasto
24.04.87	339,2 / ?	339,0 / ?	339,2 / ?	339,7 / ?
07.05.87	345,0 / A+Z	342,6 / B4+B3	335,8 / Z+A	331,8 /B3-B4
08.06.87	359,6 / B3+B4	351,9 / Z+A	-	-
09.06.87	-	-	359,8 / B4+B3	352,4 / A+Z
08.07.87	351,9 / A	354,2 / B4	-	-
09.07.87	-	-	353,3 / Z	356,5 / B3
06.08.87	351,9 / Z	335,2 / B3	-	-
07.08.87	-	-	346,4 / A	342,7 / B4
14.09.87	334,6 / B3	320,1 / Z	-	-
15.09.87	-	-	331,0 / B4	320,3 / A
14.10.87	339,8 / B4	332,4 / A	-	-
15.10.87	-	-	331,5 / B3	332,8 / Z
16.11.87	356,9 / A	355,4 / B4	-	-
17.11.87	-	-	364,1 / Z	365,9 / B3
16.12.87	402,4 / Z	375,1 / B3	-	-
17.12.87	-	-	400,1 / A	386,4 / B4
19.01.88	425,0 / B3	404,3 / Z	-	-
20.01.88	-	-	427,3 / B4	410,8 / A
11.02.88	428,3 / B4	411,6 / A	-	-
12.02.88	-	-	430,1 / B3	428,8 / Z
07.03.88	436,1	422,9	-	-
08.03.88	-	-	446,6	444,8

A = Açudinho Z = Zé da Silva B3 = Barreirinho 3 B4 = Barreirinho 4

A partir do dia 08.06.87 resolvemos fazer as pesagens de 2 lotes por dia assim como, a inversão de pastos juntamente com a mistura de sal mineral. Trabalhando com um menor número de animais temos maior confiabilidade nos pesos obtidos, utilizando desta forma um período máximo de três horas no tempo de pesagem para 160 animais.

Considerando para efeito da pesquisa uma adaptação ao sistema de rotação e mudança de minerais o período de 07.05 a 08.07.87, temos um tempo de análise de 242 dias compreendidos entre 08.07.87 a 07.03.88 para os lotes de 1 e 2 e, entre 09.07.87 a 08.03.88 para os lotes 3 e 4.

Neste período de 242 dias tivemos os seguintes ganhos de pesos adicionais: Lote 1 = + 84,2kg (IVA)
Lote 2 = + 68,7kg (Testemunho)
Lote 3 = + 93,3kg (Purina)
Lote 4 = + 88,3kg (Sal comum)

Considerando o ganho de peso obtido no período pelos animais em cada pasto:
Açudinho = + 122,1kg = 0,504kg/animal/dia (1,0 UA/ha)
Zé da Silva = + 98,7kg = 0,408kg/animal/dia (1,09 UA/ha)
Barreirinho 3 = + 78,5kg = 0,324kg/animal/dia (1,25 UA/ha)
Barreirinho 4 = + 30,9kg = 0,128kg/animal/dia (1,38 UA/ha)

O ideal seria que todos os lotes tivessem ficado 60,5 dias em cada pasto para completar os 242 dias do período mas, como isto não é possível na prática, houve uma variação de 53 dias no mínimo e 73 dias no máximo para cada lote. Este efeito sendo corrigido pelo ganho determinado em ca-

da pasto, devemos utilizar as seguintes correções:
Lote 1 = 84,2 - 1,3kg = 82,9kg
Lote 2 = 68,7 + 1,3kg = 70,0kg
Lote 3 = 93,3 - 3,2kg = 90,1kg
Lote 4 = 88,3 + 3,6kg = 91,9kg

No período de 242 dias foram consumidos:
Lote 1 = 325kg de Rumisal-mix + 75kg de Ivafós = 20,6g/cabeça/dia
Lote 2 = Testemunho
Lote 3 = 450kg de sal mineralizado Purina 65 = 23,2g/cabeça/dia
Lote 4 = 250kg de sal comum do nordeste = 12,9g/cabeça/dia

No período total compreendido entre 07-08.05.87 a 07-08.03.88 (305 dias) foram consumidos:
Lote 1 = 472kg de Rumisal-mix + 90kg de Ivafós (23,0g/cabeça/dia)
Lote 2 = Testemunho
Lote 3 = 650kg de Purina 65 (26,6g/cabeça/dia)
Lote 4 = 339kg de sal comum (13,9g/cabeça/dia)

Interessante notar que o período de menor consumo pelos animais foi durante o final das secas e início das águas (meses de setembro e outubro).

Outro fato curioso foi a diminuição do consumo dos macro-elementos (Ivafós) durante as águas, talvez devido à falta de sal comum na mistura.

O custo dos produtos na data (09.05.88) foi o seguinte:
Rumisal-mix (25kg) = Cz$ 1.154,00 + frete (IVA-promoção)
Ivafós (25kg) = Cz$ 3.200,00 (Cafealta-Coop.dos Cafeicultores da Alta Araraquarense-São José do Rio Preto)
Purina 65 (25kg) = Cz$ 1.100,00 (Cafealta-Coop.dos Cafeicultores da Alta Araraquarense-São José do Rio Preto)
Sal comum Ema(25kg) = Cz$ 280,00 (Cafealta-Coop.dos Cafeicultores da Alta Araraquarense-São José do Rio Preto)

De acordo com o período analisado de 242 dias temos em relação ao Lote Testemunho:
Lote 1 = + 12,9kg/cabeça x 80 = 1.032kg de peso vivo x0,52=536,6kg/carne
Lote 3 = + 20,1kg/cabeça x 80 = 1.608kg de peso vivo x0,52=836,1kg/carne
Lote 4 = + 21,9kg/cabeça x 80 = 1.752kg de peso vivo x0,52=911,0kg/carne

Ao preço de Cz$ 2.000,00 a arroba de carne no mesmo prazo para pagamento dos produtos minerais temos um adicional:
Lote 1 = 536,6kg : 15 = 35,77 arrobas x 2.000,00 = Cz$ 71.546,00 com uma despesa de 325kg de Rumisal-mix : 25 = 13 sacos x Cz$ 1.154,00 = Cz$ 15.002,00 + 75kg de Ivafós = 3 sacos x Cz$ 3.200,00 = Cz$ 9.600,00, totalizando Cz$ 24.602,00, portanto dando uma vantagem de Cz$ 46.944,00.
Lote 3 = 836,1kg : 15 = 55,74 arrobas x Cz$ 2.000,00 = Cz$ 111.480,00 com uma despesa de 450kg de Purina 65 = 18 sacos x Cz$ 1.100,00 = Cz$ 19.800,00, portanto dando uma vantagem de Cz$ 91.680,00.
Lote 4 = 911,0kg : 15 = 60,73 arrobas x Cz$ 2.000,00 = Cz$ 121.466,00 com uma despesa de 250kg de sal comum = 10 sacos x Cz$ 280,00 = Cz$ 2.800,00, portanto dando uma vantagem de Cz$ 118.666,00.

CONCLUSÕES

1. Fica evidente que nesta experiência o uso do sal comum para animais de engorda foi mais vantajoso que a suplementação com misturas minerais mais completas.

2. Não houve vantagens em separar os macro e micro-elementos, considerando que os animais pudessem escolher mais cálcio e fósforo quando desejassem, parecendo ser o fator limitante a falta de sal comum.

3. Houve leve diminuição no consumo de sal comum e sal mineralizado durante as secas, exceto para a mistura Ivafós provavelmente por conter

proteína e carbohidratos, sendo mais evidente nos meses de setembro e outubro.

4. A menor perda de peso sem correção durante as secas foi para o lote 1(Ivafós),não havendo vantagem em relação ao testemunho para lotes 3 e 4.

5. O maior ganho de peso sem correção durante as águas foi para o lote 3(Purina 65),havendo vantagens em relação ao testemunho para todos os lotes.

6. A influência do fator pasto neste tipo de experiência é bastante considerável, ficando claro que são necessários outros testes escolhendo pastos mais semelhantes entre si e com a mesma área pois a lotação também influenciou demais.

7. O nível de consumo da mistura mineral neste experimento (23,0 a 26,6g/cabeça/dia) não é muito diferente do consumo normal em regime extensivo nas condições brasileiras (25 a 30g/cabeça/dia), o que limita o efeito pelo baixo nível de ingestão dos elementos com prováveis deficiências nas pastagens, principalmente, em regiões onde as deficiências encontradas são pequenas, como provavelmente neste caso, com Panicum maximum na região de Barretos,SP. O lote 1 consumiu 0,62g de fósforo/cabeça/dia e lote 3 consumiu 1,73g de fósforo/cabeça/dia.

8. Para complementar a informação da influência dos pastos em relação aos tratamentos, torna-se necessário neste tipo de pesquisa as análises bromatológicas das pastagens no transcorrer do experimento.

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RESUMO

Na Fazenda Vista Bonita, propriedade da Agro-Pecuária CFM Ltda, município de Barretos,SP, foram analisados 4 tratamentos com 320 bois magros, sendo 80 animais por lote, em sistema de rodízio quinzenal por 60 dias e mensal por mais 245 dias, em pastagens semelhantes de Panicum maximum.

Os tratamentos foram fornecidos em cocho coberto (7,5cm /animal):
Lote 1 = suplementação com macro e micro-elementos separados.
Lote 2 = testemunho.
Lote 3 = suplementação com sal mineralizado pronto para uso.
Lote 4 = sal comum.

Após o período considerado de análise chegou-se aos seguintes ganhos de peso por lote: Lote 1=+82,9kg, Lote 2=+70,0kg, Lote 3=+90,1kg e Lote 4=+91,9kg.

Neste experimento concluímos que houve vantagem econômica para o uso de sal comum aos animais de engorda. O baixo nível de consumo das misturas minerais em regime extensivo(25g/cabeça/dia) e a limitada deficiência regional podem ser fatores limitantes à mineralização para gado de engorda.

SUMMARY

On Vista Bonita farm, property of Agro-Pecuária CFM Ltda, located in Barretos,São Paulo State,Brasil, 4 treatments were analysed with 320 thin steers, 80 animals per lot, for60 days in a fortnightly rotation and 245 days in a monthly rotation in similar pastures of Panicum maximum.

The treatments were supplied in a covered trough(7,5cm/animal)
Lot 1 = macro and micro elements separated.
Lot 2 = control (no additions).
Lot 3 = salt + mineral ready for use.
Lot 4 = ordinary salt.

After the trial period of analyses the following weight-gains per lot were found: Lot 1=+82,9kg, Lot 2=+70,0kg, Lot 3=+90,1kg and Lot 4=+91,9kg

In this trial we concluded that there is an economic advantage using the ordinary salt for fattening animals. The low consumption level of minerals in extensive conditions in Brasil(25g/head/day) and the low mineral deficiency on the local region of Barretos,SP, can be the limiting factors to minerals suppliers for fattening cattle on grass.

RESUMEN

En la estancia Vista Bonita, perteneciente a Agro-Pecuária CFM Ltda, municipalidad de Barretos,estado de São Paulo,Brasil, 4 tratamientos con 320 novillos magros fueron analizados, siendo 80 animales por lote, en sistema de rodezno quincenal por 60 dias e mensual por otros 245 dias,en pasturas semejantes de Panicum maximum.

Los tratamientos fueron realizados en comedero cobierto.
Lote 1 = suplementación con macro e micro elementos separados.
Lote 2 = lote para base de comparación.
Lote 3 = suplementación con sal mineralizada pronta para uso.
Lote 4 = sal común.

Después del período considerado de analize, se llego a los siguientes aumentos de peso por lote: Lote 1=+82,9kg, Lote 2=+70,0kg, Lote 3=+90,1kg Lote 4=+91,9kg.

Con este experimento concluimos que hubo una mejora de rendimiento económico con el uso del sal común en los animales de engorde. El bajo nível de consumo de las mixturas minerales en régimen extensivo (25g/cabeza/dia) y la limitada deficiencia regional puéden ser factores limitativos a mineralización para el ganado de engorde.

EVALUATION OF ANIMAL HEALTH IN BEEF PRODUCTION IN RELATION TO HOUSING AND FEEDING SYSTEMS

"The Götala husbandry project"

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In Sweden the specialized beef production is based on groups of bull calves collected from dairy herds. The calves are usually reared in a wellcoming unit for two months and thereafter in a fattening unit. The most common type of building for this production is an insulated building with slatted floor in the pens. Problems with pneumonia occur regularly either during the period in the wellcoming unit or during the first month in the fattening unit. The environment in the conventional fattening unit may contribute to the problems with respiratory diseases. Furthermore the insulated building is relatively expensive. In order to evaluate the influence of the housing system on the animal health and production a three year study was conducted. A conventional building and an uninsulated building was used for the fattening period. Two different feeding regimes were also compared and in the uninsulated building two stocking densities in the pens were studied.

The study was performed as a joint project with the Swedish University of Agricultural Sciences; the department of Animal Nutrition and Management and its Western District of Animal Research, the Department of Animal Hygiene and the Animal Health Service.This part of the study covers the results concerning animal health.

Materials and Methods

Animals: 10 groups of 60 or 90 male calves of the Swedish Red and White Breed, were purchased during a three year period. After a period of 10 weeks in a wellcoming barn the calves were divided into two groups for fattening. Each group was then introduced into its designated building where the animals were reared until slaughter.

Buildings: An insulated house with boxes with slatted floor was used together with an unisolated house with strawbedding in a lying area and a concrete passage in front of the feed bunk. The ventilation was controlled in the insulated building and the air volume was 9 cubic metres per animal. The uninsulated building was ventilated through on open ridge and the air volume was about 40 cubic metres per animal.
In the uninsulated building there was either 11 or 15 animals per pen while there was 11 animals per pen in the insulated building.

Feeding and production system: Seven of the ten groups were fed concentrates ad lib and a restricted amount (0.5 kg/animal) of hay daily. The hay was after one month substituted with straw. The groups were sent to slaughter either at a live weight of 220 kg (bull calves) or 430 kg (bulls). The rearing period in the fattening unit being 90 and 240 days respectively. Three groups were fed silage ad lib (silage bulls) and 1 kg concentrates per 100 kg live weight daily. They were sent to slaughter at a live weight of 460 kg after a rearing period in the fattening unit of 320 days.

The design of the study was that the first group was reared as bulls, the second as silage bulls followed by a group of bull calves and so on. The number of animals and groups are given in the table below.

Cathegory	No of batches	No of animals	Age at slaughter
Bull calves	3	254	7 months
Bulls	4	316	12 "
Silage bulls	3	165	15 "

Animal health: All treatments in the two housing systems were individually recorded. On animals sent to sanitary slaughter or animals found dead autopsies were performed. At slaughter all animals were examined for pathological lesions in the lungs, heart and liver. Pneumonic lesions and liver abscesses were recorded according to severity of the reactions. A 3-graded scale was used (mild, moderate and severe macroscopic reaction). The rumens of bulls and silage bulls were examined for lesions in the mucous membrane such as parakeratosis and rose buds formulations.

Results
Comparing the animal health in the two housing systems the preliminary results indicate that there was no difference in the incidence of pneumonia. In the insulated building 2.4 % of all animals were treated as compared to 3.5 % in the uninsulated building. The only difference in disease incidence observed between the housing systems was that 15.7 % of all animals in the uninsulated building had to be treated against interdigital phlegmon in contrast to 6.1 % of the animals on slatted floor. The incidance of clinical pneumonia was 3.6 % in the groups fed concentrates ad lib (bull calves and bulls together) versus 0.5 % in the silage fed bulls. Differences between the feeding regimes were also observed concerning interdigital plegmon. In the groups fed concentrates ad lib 9.1 % of the calves and 18.7 % of the bulls had to be treated in contrast to 2.2 % of the silage-fed bulls.
Looking at the lesions observed at slaughter no differences was recorded comparing the housing systems. The different feeding regimes and ages at slaughter gave the following results concerning pneumonic lesions.

	Pneumonic lesions %		
Cathegory	grade 1	grade 2	grade 3
Bull calves	25.8	17.5	8.3
Bulls	21.5	9.8	3.8
Silage bulls	16.7	5.4	0.5

Different stadges of leverabscesses were observed in the groups fed concentrates ad lib. Multiple lever abscesses was recorded in 17.0 % of the bull calves and 25.9 % of the bulls. In the groups of silagefed bulls only one case with a single leverabscess was seen. Feeding bulls with concentrates ad lib also resulted in frequently observed pathological reactions in the rumen. Rosebudformations and parakeratoses was seen in 50.3 % and 27.4 % of the bulls. Amongst the silagefed bulls these reactions were rarely observed (1.2 and 1.8 %).

Discussion
The disease incidences during the fattening period was not influenced by the housing system with the exception of a higher incidence of interdigital plegmon in animals kept in pens with strawbedding and concrete floor. This may partly be explained by the fact that the concrete floor was only cleaned three times a week. As expected the feeding of concentrates ad lib resultat in a high incidence of liverabscesses and reactions in the rumen mucosa. However the concentrates ad lib fed animals also had higher incidences of clinical diseases as well as pneumonic lesions at slaughter as compared to the incidences found in the silage fed bulls. The underlying reasons for these results need further investigations.

ZUSAMMENFASSUNG

Der Einfluss von 2 verschiedenen Haltungsformen sowie 2 Fütterungssystemen auf den Gesundheitszustand bei Schlachtrindern wurde in einer 3 Jahre lang dauernden Untersuchung ausgewertet. Ein isolierter Stall mit Spaltenbodenversehenen Boxen wurde mit einem nicht isolierten Stall mit Stroh boxen, verglichen. Zwei verschiedene Fütterungssysteme wurden in den Ställen angewendet; Kraftfutter ad lib. und c a 0.5 Kg Raufutter pro Kalb und Tag, bzw. Silage ad lib. und 1 kg Kraftfutter pro 100 Kg Lebendmasse und Tag. Keine Unterschiede lagen in Hinblick auf klinischer Erkrankung zwischen den beiden Aufstallungsformen vor, abgesehen von einem häufigeren Vorliegen von interdigitaler Phlegmone bei der Einstreuhaltung. Die klinische Erkrankungshäufigkeit war bei den mit Silage gefütterten Gruppen geringer, wie auch das Vorliegen von pathologischen Veränderungen bei der Schlachtung. Pneumonische Veränderungen , Leberabscesse sowie Reaktionen in der Pansenschleimhaut waren häufiger vertreten bei den mit Kraftfutter ad lib. gefütterten Gruppen. Der Befund zeigt eine erhöhte Infektionsanfälligkeit bei diesen Tieren.

SUMMARY

The effect of two different housing systems and two different feeding regimes on the animal health was evaluated during a three year period. An isulated building with boxes with slatted floor was compared with an unisulated building with boxes with strawbedding. Two different feeding regimes were used in both barns: concentrates ad lib and 0.5 kg roughage/calf and day or grass-silage ad lib and 1 kg concentrate/100 kg live weight and day. There was no significant difference in diseases incidence in the two housing systens except a higher incidence of interdigital phlegmon in bulls from the uninsulated building with strawbedding. The number of treatments against pneumonía and interdigital phlegmon was lower in the groups of bulls fed silage ad lib. Pneumonic lesions, liver abscesses and reactions in the rumen were more frequent in the groups fed concentrates ad lib in contrast to the silage-fed bulls. The present investigation thus indicates an increased susceptibility to infections of the bulls, fed concentrates ad lib.

RESUMEN

Se evaluan aqui los resultados obtenidos en la salud de grupos de bovinos sometidos a dos sistemas de estabulación y a dos diferentes regimes de alimentación. El tiempo de estudio fue de tres anos. Diez grupos de terneros SRB (Rojo sueco) de 60-90 dias de edad, fueron despues de haber permanecido dos meses en un establo inicial, agrupados al azae en dos grupos. Un grupo fué criado en corrales con piso de madera "entretabldo" en establos y el otro en establos con lecho de paja y un pasaje de concreto atraz de la tarima de alimentación. Dos diferentes regimenes de alimentación fueron aplicados en ombos establos: uno con concentrado ad lib y aproximadamente 0,5 kg de alimento grueso por ternero/dia y el otro con ensilado de pasto ad lib y 1 kg de concentrado por 100 kg de peso vivo/dia. Durante el periodo de estudio, se registraron individualmente todo tipo de tratamiento. Al momento de sacrifico lasreses fueron examinadas para observar cambios patalogicos. No se observaron diferencias significantes en la incidencia de enfermedades a excepción de una mayor incidencia de flegmones interdigitales en animales proventientes de los establos no aislados y con lecho de paja. El número de tratamientos contra pneumonia y flegmon interdigital fué mas bajo en animales alimentados con ensilaje ad lib. Lesiones pneumonicas, abcesos hepaticos y reacciones en el rumen fueron mas frecuentes en grupos alimentados con concentrados ad liben contraste con los grupos alimentados con ensilaje. La presente investigación indica un aumento de susceptibilidad a infecciones en animales allimentados ad lib.

BOVINE SOMATOTROPIN, ROLE OF THE VETERINARIAN IN THE MODERN MANAGEMENT OF DAIRY PRODUCTION IN WESTERN AND EASTERN EUROPE

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1. INTRODUCTION

Bovine somatotropin has been presented on several occasions at national and World Buiatrics Congresses as a potential new technology which could increase the role of the veterinarian in the modern management of dairy production: British Cattle Veterinary Assoc. 1987 (1) Soc. Française de Buiatrie 1989 (2) XV World Buiatrics Congress, Palma de Majorca 1988 (3).

Since 1985 (BCVA UK) (4) and 1986 (AFT France) (5)(6) when the first interesting results were presented, a lot of work has been carried out in Europe with BST products. In 1988 the EEC Commission organised a seminar (7)(8) on the status of somatotropin confirming its animal and human safety, its efficacy in the dairy cow and also on its minimal impact on the dairy structures in the EEC.

Recently a symposium was organised by Monsanto on the subject "Sometribove, where do we stand?" (9). Its conclusions were clear: BST is safe, efficacious and the best benefit is obtained when applied strategically according to the management needs either under quotas or in countries without milk quotas. At this seminar the important role of the dairy practitioner was also stressed (10)(11).

2. THE VETERINARIAN IN MODERN DAIRY MANAGEMENT

As pointed out during the World Buiatrics Congress in Palma de Majorca (3) the dairy practitioner is becoming more and more involved in preventive medicine and less and less in individual treatment.

Our US colleagues speak about "Production Medicine" (12). To achieve and sustain high milk production the veterinarian must maintain cow health by providing a programme that addresses "the whole picture". The primary objective of this programme is to prevent disease rather than treat it. The second greatest concern is to maintain optimum body condition. The goal is to improve all aspects of health and productivity on the farm, and extensive use of records to monitor results is a key in achieving this goal.

3. SAFETY AND EFFICACY OF BST

The safety and efficacy of sometribove have been demonstrated in Europe both in clinical trials (3-5 consecutive lactations) under the conditions which are more likely to produce possible secondary effects and under short term field trials involving more than 3,000 cows in more than 30 different herds (2)(13)(14)(15).

4. OPTIMAL USES OF SOMETRIBOVE IN EEC COUNTRIES

Because of the existence of a production ceiling (quota) in the EEC and the economic advantages of maintaining the herd size in order to obtain the largest possible number of calves. It is expected that BST will be used only under defined circumstances to get the optimum benefit.

Some of those circumstances may be summarized under the slogan "IST" (Individual, Selective, Tactical) here are some examples:

- supplementation only during the grazing season, without added concentrates. This is an economic method of producing milk, provided that there is sufficient grass of good quality,
- supplementation when the price of milk is high. Altering the seasonal pattern of milk production can be obtained by boosting milk yield during the summer trough when, as in the UK, milk price is increased with a significant premium.
- supplementation in order to reach the quota level at the end of the season; compensation during winter and spring of the deficit. The use of BST as a management tool can rectify this position by raising milk yield substantially in two weeks. No other management aid can achieve this effect.
- maintenance of milk deliveries to the dairy in case of illness in the herd. For example, if there is mastitis or lameness in a certain number of animals, treatment can be given to other animals to maintain milk deliveries.

Figure 1 (2)

Increase of milk production and feed consumption related to somatotropin treatment

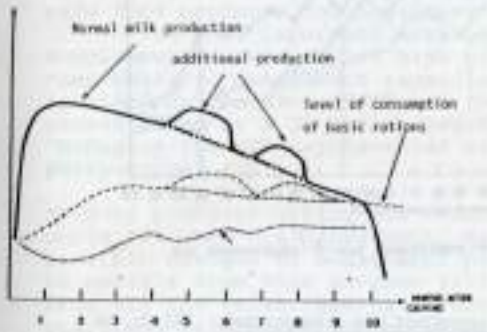


Table 1. INFLUENCE OF STAGE OF LACTATION ON RESPONSE TO SOMETRIBOVE (17)

4.0 % Fat Corrected Milk Response
12 Treatment Weeks

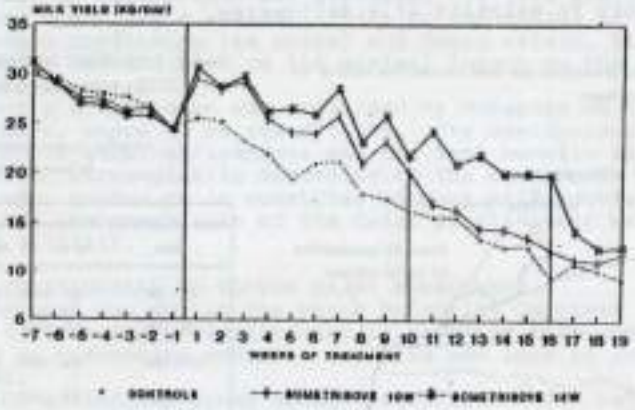
Stage of Lactation Days	Primiparous Kg/day (kl)	Multiparous Kg/day (kl)	Overall Kg/day (kl)
57- 98	2.9 (48)	3.5 (104)	3.3 (150)
99-140	3.0 (48)	4.1 (146)	3.8 (194)
141-182	4.3 (67)	5.0 (138)	4.8 (205)
183-224	3.7 (40)	4.2 (88)	4.0 (128)
Overall	3.5 (194)	4.2 (478)	4.0 (681)

Milk production can be increased very rapidly and then return to normal with no negative effect or "feed-back" after BST treatment is stopped (Fig 1). To confirm those interesting uses commercial trials have been conducted in the UK (16) and in France (17)(18). Average increases in milk production were 4.25 kg/cow/day (4% FCM) in 20 herds in France and 4.0 kg/cow/day in 10 trials in the UK under a wide variety of conditions. Some important conclusions can be drawn from those commercial farm trials:

1) The increases are in absolute values (expressed in kg) and not in relative terms (%) since results are similar across breeds and independent of the level of production before supplementation started.
2) Variability of individual milk production is relatively high and above average results are repeatedly obtained in herds with the best management related to the nutrition, animal health and the level of care and attention.

3) Stage of lactation and milk production. The stage of lactation in which cows commenced supplementation with sometribove does appear to affect the magnitude of the lactation response: when supplementation starts after day 100 of lactation cows tended to respond better to sometribove than cows which received first injection in the period days 57-100; this relationship may be related to an improved nutrient balance (energy or protein) with stage of lactation (table 1). Fig (2) represents the milk yields obtained in one UK trial in 2 groups one supplemented 10 weeks, one other supplemented 16 weeks; diet was pasture bufferfed with total mixed ration: overall response was 4.9 kg 4% FCM more per day. But most interesting is the fact that this supplementation is making the curve much more even.

Figure 2 (19): Milk Response in a UK Pasture Trial



4) Feeding systems and milk production response. With an adequate plane of nutrition the administration of sometribove has increased milk yield substantially. These increases have been recorded in animals grazing pasture - av +4.9 kg (2); housed cows receiving concentrates either at a flat rate - av +3.4 kg (16)(17) or according to yield - av +5.8 kg (16)(17) or when offered total mixed rations - av +3.8 kg (16)(17)(20). This plane of nutrition is a reflection of both the energy and protein concentration of the ration. If feeding management is inadequate the response will be small, however the cows will remain healthy (2). In a recent trial cows were fed either an adequate or a low plane of nutrition, and reponsed with, +5.3 or +1.5 kg/milk/day respectively (21). This variation in response should however not be seen as a failure of BST but one of its strongest points providing a built-in safety mechanism.

5. OPTIMAL USES OF SOMETRIBOVE IN EAST EUROPEAN COUNTRIES
The purpose of the field trials performed in East European countries such as CSSR and USSR was to confirm the efficacy

and safety on local breeds - Friesian-Bohemian, mixed Baltic and under local nutritional conditions.

A high proportion of cows are in large sized state and co-op farms and diets for cows are based mainly on grain and roughage together with only a moderate amount of concentrates. Economical results in CSSR were excellent: 1.39-4.98 kg/day more milk and this over a period of 20 weeks (22)(24). Supplemented multiparous cows turned out to graze spring pasture increased milk yield by more than 4 kg daily (22). Safety was also confirmed in all these trials (22). Preliminary results from ongoing USSR trials with a mixed Baltic breed indicate milk yield increases of 3.1 and 3.7 kg/cow/day in 2 full lactation experiments involving 100 first calf heifers and 120 mature cows respectively (23).

6. MANAGING THE BST SUPPLEMENT COWS; ROLE OF THE VETERINARIAN IN THE COMPLEMENTATION OF PROGRAMMES INVOLVING THE USE OF BST. In EEC member countries, BST will be available under veterinary prescription.

Thus the veterinary profession will play a crucial role in helping farmers obtain the optimum benefits from BST appropriate to their circumstances. Let us not forget that the aim of practitioners already working in dairy production and preventive medicine programmes is to help farmers produce safe food products for consumers at the lowest cost.

"The most important message concerning managing BST supplemented cows is that high producing cows require the same comprehensive management regardless of whether that production is aided by exogenous BST" was the introduction of a recent presentation by a US bovine practitioner, J.W. Ferry. "Managing the BST supplemented treated herd - a veterinarian's perspective" (12).

A very likely scenario in Western Europe will be the up to date producer asking his veterinarian how the use of BST could fit into his management, and seeking advice about possible changes to management practices that would allow him to benefit from this advance (11).
From all the trial results I referred to earlier some practical guidelines for optimum BST reponse can be stated:
1) Initiate treatment between day 120 and 180 post partum.
2) Treat for a minimum of 10 weeks and up to 20 weeks.
3) Treat only healthy cows in good body condition.
4) Treat the middle 70-80% of the herd, i.e. not the highest producing cows - they may already be feed and management limited - and not the lowest - there are probably other reasons why they are the lowest.
5) Provide the treated cows with enough, good quality feed anticipating 4-5 kg higher milk production.

The veterinarian's advice in selecting the cows for BST supplementation will no doubt be invaluable to the farmer.

One important point to stress in relation to adequate feeding is to maintain optimum body condition. Cows that calve thin do not have the body reserves to sustain high production.

And as every herd has cows that never carry much condition, using BST to individualise treatment, has big advantages versus other tools which increase milk production overall in the herd. The veterinarian together with the farmer can decide not to involve in a BST programme cows that lose weight for other reasons, such as a foot injury; those cows can simply be taken off treatment until their condition improves.

CONCLUSIONS

In conclusion I would like to quote Dr. D. Pepper, a veterinarian who was involved in monitoring trials in the UK and who recently made the following statement (11): "When BST formulations do receive product licences the veterinary surgeon will welcome this advance in management manipulation that genetic engineering has allowed, as far as the safety, efficacy and welfare aspects of it are concerned.

He will need reassurance that the sales of such milk and milk products will not suffer through ill-informed minority groups wielding inordinate influence thus affecting his clients' and his own livelihood. He will also need a back-up programme unprecedented in agricultural veterinary medicine. But if he receives this reassurance and back-up, he is likely to look forward to the controlled use of a product which will allow him to enhance his own involvement in the maintenance and improvement of his client's profit margins, without compromising the health of the cattle which is his primary concern."

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Summary

BST: Role of the veterinarian in the modern management of dairy production in West- and East-Europe

Bovine Somatotropin, the first veterinary medicine produced by Biotechnology, has been tested in a lot of field conditions, and this in countries with or without milk quotas.

Data of these field trials in France, UK and East-Europe, involving more than 1,500 animals and about 30 different herds, resulted in a lot of practical examples which optimise milk production and which demonstate the key role of the veterinarian.

Résumé

BST: Rôle des vétérinaires dans la production laitière moderne en Europe de l'Ouest et de l'Est

La somatotropine bovine, le premier médicament vétérinaire produit par la biotechnologie, a été testée dans de nombreuses conditions de terrain et cela dans des pays ayant ou n'ayant pas de quotas laitiers.

A partir des données de ces essais terrains en France, Grande Bretagne et en Europe de l'Est (englobant plus de 1500 animaux et environ 30 fermes différentes) on a pu déduire un grand nombre d'exemples pratiques qui optimalisent la production laitière et qui démontrent le rôle important du vétérinaire.

Zusammenfassung

BST: Die Rolle der VeterinÄrmedizin in der modernen Milcherzeugungswirtschaft in West- und Osteuropa.

BST (engl. Bovine Somatotropine = Rindersomatotropin) wurde als erstes Erzeugnis von Biotechnology im Bereich der Veterinärmedikation unter einer Vielzahl unterschiedlicher Rahmenbedingungen, und zwar in Ländern mit und ohne Milchquotenregelung, getestet.

Das Datenmaterial, das im Rahmen der in Frankreich, Grossbritannien und Osteuropa durchgeführten Feldversuche an über 1.500 Tieren aus ca. 30 underschiedlichen Herden gewonnen wurde, führte zu einer Vielzahl praktischer Beispiele dafür, wie eine Optimierung der Milcherzeugung erzielt werden kann und welche Schlüsselrolle heute der Veterinärmedizin zukommt.

OCORRÊNCIA DO DESLOCAMENTO DO ABOMASO EM BOVINOS, CRIADOS NO ESTADO DE SÃO PAULO - CASUÍSTICA DO PERÍODO DE 1977 A 1986.

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INTRODUÇÃO

O deslocamento do abomaso é uma ectopia que acomete os ruminantes, e, entre estes, principalmente os bovinos (6). As suas duas formas clínicas características - deslocamento do abomaso para a esquerda e deslocamento do abomaso para a direita, são descritas em várias raças de bovinos criados intensivamente, sempre com gradativo aumento de suas incidências(6). A primeira descrição da ocorrência do deslocamento do abomaso para a esquerda foi feita na Inglaterra, em 1950(1); e a seguir nos Estados Unidos da América: relatando que a enfermidade já estava caracterizada desde 1948(10). Na Alemanha a ocorrência desta doença foi afirmada em 1953(11), destacando-se maior frequência na região norte e que na Clínica de Bovinos de Hannover, no período de 1954-56 diagnosticaram-se 8 casos clínicos; doze casos em 1958 e nos 3 anos subsequentes 77 casos clínicos(6, 12). O complexo nosológico conhecido por deslocamento do abomaso para a direita inclui a dilatação do abomaso com seu deslocamento à direita e deslocamento com torção à direita ou à esquerda(6). A ocorrência da torção do abomaso, destacando um posicionamento do abomaso à direita do rume, foi descrita em bezerro ao final do século passado (5) e em ovino em 1914(9). Os primeiros casos desta gastropatia em bovinos adultos foram detectados em 1928 e 1930(8, 13). Na Dinamarca, no período entre 1940 e 1942, observou-se que até 7,6% das mortes de vacas eram consequentes ao deslocamento do abomaso para a direita, com ou sem torção(7). Neste país em 1950 caracterizaram-se as formas clínicas do deslocamento abomasal para a direita, dando-se destaque ao diagnóstico clínico.

No Brasil há referência afirmando ter sido, em 1960, o deslocamento do abomaso para a direita, observado durante a realização de uma laparotomia(7). A seguir, em 1966, (2, 3) ressaltou-se a necessidade do aperfeiçoamento dos métodos semiológicos e dos conhecimentos sobre a etiopatogenia desta gastropatia, pois era iminente sua ocorrência nos rebanhos leiteiros de alta produção submetidos a manejo intensivo(2, 3).

Na década de 80 aparecem alguns relatos pessoais ou em congressos afirmando a ocorrência das várias formas clínicas do deslocamento do abomaso em bovinos criados no Brasil. Todavia não existem trabalhos baseados em casuística com número substancial de observações; assim sendo, julga-se oportuna a descrição dos presentes 12 casos clínicos, observados em vacas leiteiras criadas em São Paulo, caracterizando o quadro sintomático e destacando normas de diagnóstico e tratamento.

MATERIAL E MÉTODOS

Animais utilizados

Os relatos apresentados estão baseados em casuística obtida no período 1977-1986 no setor de Clínica de Bovinos do Hospital Veterinário da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, sob a supervisão do Departamento de Clínica Médica. No referido setor e no mencionado período, foram internados 437 bovinos, pertencentes a granjas leiteiras, muitas produtoras de leite tipo B e assim sendo, na maioria, grandes produtores, criados em regime intensivo de manejo, dos quais 12 apresentavam deslocamento do abomaso. Destes, 10 foram casos de deslocamento para a esquerda e 2 para a direita sem torção do órgão.

Casuística

1.Bovino da raça holandesa branca e preta, fêmea, com aproximadamente 4 anos de idade, criado na região de Campinas, em fazenda leiteira-tipo B. O animal, recém parido (2 meses) apresentava-se emagrecido, com queda acentuada da produção, apetite diminuído e seletivo, hipotomia dos reservatórios gástricos e ligeira acetonemia. Comprovou-se o deslocamento do abomaso para a esquerda por percussão-auscultatória com sons metálicos característicos. Tratamento por laparotomia no flanco direito, esvaziamento de conteúdo gasoso do órgão e sua reposição no hipocôndrio direito, não se fazendo a omentopexia à parede lateral. Recuperação após 15 dias, retornos do apetite, produção e da atividade dos reservatórios gástricos(1977).

2.Recidiva do caso anterior após duas gestações, a sintomatologia foi semelhante à já mencionada, sendo o tratamento realizado com fixação do omento na parede do flanco direito, permanecendo o órgão no hipocôndrio direito. Recuperação total(1979).

3.Bovino da raça holandesa branca e preta, fêmea, com 2 anos de idade, pequeno desenvolvimento corpóreo e no 3º mês de gestação. Apresentava emagrecimento acentuado, meteorismos recidivantes, paresia dos reservatórios gástricos e aumento de volume das porções ventrais do abdome(piriforme), fezes pastosas e finamente trituradas. Diagnóstico clínico de dilatação e deslocamento do abomaso para a direita através da prova de sucussão no hipocôndrio e flanco direito, auscultação e percussão auscultatória revelando borbulhar e retinir metálico. Evolução: em 6 dias - decúbito permanente; óbito em 10 dias. À necroscopia confirmou-se o diagnóstico clínico: ectopia abomasal para a direita, com dilatação(aumento de 3 vezes), úlceras e ausência de torção(1978).

4.Bovino da raça holandesa branca e preta, fêmea adulta, recém parida criada em Ampero, diagnóstico de deslocamento do abomaso para a esquerda baseado na sintomatologia apresentada: emagrecimento, hiporexia, diminuição da produção leiteira, apatia, acetonuria de grau leve e ruído metálico característico obtido pela percussão-auscultatória do flanco esquerdo. Tratamento cirúrgico por laparotomia pelo flanco direito com omentopexia. Evolução favorável com recuperação plena(1978).

5.Bovino da raça holandesa branca e preta, fêmea com 24 meses de idade, não gestante, criado em Bragança Paulista. O animal foi internado em estado de pré-choque, em decúbito, hipotérmico, desidratado e com insuficiência respiratória. Após tratamento sintomático, voltando o animal espontaneamente a uma atitude em estação, comprovou-se o deslocamento do abomaso à direita pela prova de sucussão, comprovando-se a dilatação com conteúdo líquido e a ausência de torção pela punção de líquido abomasal de cor amarelada. O tratamento cirúrgico através laparotomia do flanco direito, com o animal em decúbito, resultou apenas no esvaziamento do órgão, retirando-se aproximadamente 40 litros de conteúdo. A evolução foi desfavorável, com morte do animal após 24 horas. Na necroscopia comprovou-se a dilatação do órgão(de 4 a 5 vezes), sua ectopia, associada à torção pós-cirúrgica e inúmeras ulcerações(1981).

6.Bovino da raça Jersey, fêmea, 3,5 anos de idade, criada em Jundiaí, parida há 3 meses, manifestando o seguinte quadro clínico: apetite caprichoso e diminuído, emagrecimento acentuado, quebra da produção leiteira, caindo de 18 litros para 4 litros/ dias; fezes bem digeridas e em pequeno volume. Diagnóstico clínico por demonstração do deslocamento do abomaso para a esquerda através a obtenção de sons metálicos característicos pela percussão-auscultatória do flanco esquerdo. Tratamento cirúrgico por laparotomia do flanco direito, esvaziamento e recolocação do abomaso no hipocôndrio direito com omentopexia. Evolução desfavorável, observando-se recidiva 35 dias após a cirurgia: comprovada por percussão-auscultatória. Tratamento conservador através de relaxamento: sem resultado positivo. O animal foi reoperado, observando-se antiga aderência do abo

maso à parede abdominal esquerda, ao nível das articulações condrocostais. Após o esvaziamento do abomaso e da seção da aderência houve recuperação total do animal, sendo observada a evolução durante 8 meses(1982).

7.Bovino da raça holandesa branca e preta, fêmea, com 10 anos de idade, no 8º mês de gestação, criada em Jundiaí. O quadro sintomático caracterizava-se por inapetência, atonia ruminal com ligeiro timpanismo, gemidos constantes e longa permanência em decúbito. Evidente sensibilidade do retículo às provas específicas. O animal, após realização de laparo-ruminotomia foi enviado à Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. Evolução rápida para a morte, observando-se à necroscopia deslocamento do abomaso para a esquerda de grau médio, reticulo-peritonite traumática e abcesso sub-reticular(1983).

8.Bovino da raça holandesa branca e preta, fêmea, adulta, parida há 1,5 mês, apresentando o seguinte quadro clínico: hiporexia, emagrecimento, desidratação e queda da produção leiteira, fezes escassas e bem digeridas, hipoproteinemia, anemia intensa, acetonemia de grau médio. O diagnóstico de deslocamento do abomaso foi aventado pela percussão-auscultatória do flanco esquerdo, obtendo-se ruídos metálicos e pela punção exploratória do terço médio do 9º espaço inter-costal esquerdo: líquido amarelado, pH 3,0 e ausência de protozoários vivos. Tratamento cirúrgico por laparotomia do flanco direito com omentopexia. Face ao péssimo estado geral do animal, este permaneceu em decúbito esternal persistente, às vezes lateral, apresentando paralisia do nervo radial. Nos 36 dias, de acompanhamento clínico o paciente permanecia em estação, apenas quando suspenso por equipamentos. Na necroscopia comprovou-se espessamento e fibrosamento do píloro e a manutenção por fixação cirúrgica do abomaso no hipocôndrio direito(1984).

9-10. Dois bovinos da raça holandesa branca e preta, fêmeas adultas(6 e 8 anos) recém paridas, enviadas ao Hospital de Bovinos por veterinário, uma com suspeita de deslocamento do abomaso e outra com suspeita de retículo-peritonite traumática. Os quadros clínicos caracterizavam-se por emagrecimento acentuado, hiporexia com apetite seletivo, acetonuria intensa e grande diminuição da produção leiteira(de aproximadamente 25 litros). O diagnóstico de deslocamento do abomaso para a esquerda foi feito baseado no ruído metálico característico obtido pela percussão-auscultatória. Em um dos casos este ruído era generalizado, sendo auscultado tanto no flanco esquerdo como no direito, comprovando-se a afecção através de punção exploradora realizada no terço médio do 9º espaço intercostal esquerdo, orientado pela percussão-auscultatória. Na punção obteve-se líquido amarelo-esverdeado com pH entre 1,5 e 2,5, sem demonstrar-se presença de protozoários vivos. Tratamento cirúrgico através laparotomia do flanco direito com omentopexia. Evolução favorável, dando-se alta após 21 dias, com recuperação total do apetite e da produção leiteira(1984).

11. Bovino da raça holandesa branca e vermelha, fêmea com 5 anos de idade, criada em Sorocaba, diagnóstico de deslocamento para a esquerda pelos sintomas típicos apresentados e presença de ruídos metálicos no flanco esquerdo(por auscultação e percussão - auscultatória). Tratamento cirúrgico por omentopexia após laparotomia no flanco direito. Evolução favorável com recuperação total(1985).

12. Bovino da raça holandesa branca e preta com 4 anos de idade, criada em Itatiba, em fazenda leiteira do tipo B, recém parida, apresentando emagrecimento, apetite diminuído e seletivo, queda da produção, acetonuria moderada, hipotonia ruminal e ruído metálico característico, no flanco esquerdo. Tratamento cirúrgico por laparotomia do flanco direito, ocorrendo extrema dificuldade na redução e recolocação do abomaso no hipocôndrio direito, e realização da omentopexia. Houve recidiva após 10 dias de boa evolução, com regularização do apetite e recuperação da produção. A recidiva do processo foi confirmado pelos ruídos metálicos característicos e pela obtenção de conteúdo líquido

com pH 3,0, por punção do terço médio do 9º espaço intercostal esquerdo. Tratamento cirúrgico realizado com abomasopexia, ao nível da linha média do prolongamento da 9ª costela, após laparotomia no flanco esquerdo. Evolução favorável, com recuperação da produção, observada por acompanhamento clínico durante 5 meses(1986).

RESULTADOS

Os resultados obtidos, configurando o quadro sintomático dos deslocamentos do abomaso para a esquerda e para a direita são apresentados na tabela 1.

DISCUSSÃO

A observação do quadro sintomático dos casos clínicos, acometidos por deslocamento do abomaso, incluídos na presente casuística demonstra concordância com as manifestações destacadas por autores europeus e norte americanos, tanto para o deslocamento à esquerda, como para aqueles à direita(1, 4, 5, 7, 11, 12). Os fatores predisponentes, particularmente os relacionados à gestação, ao parto e ao manejo alimentar intensivo, revelados no presente estudo são coincidentes com os destacados por inúmeros textos clínicos de Veterinária(6, 7), da mesma forma a casuística atual com predomínio do deslocamento do abomaso para a esquerda, sobre aqueles para a direita(5:1) identificam - se com as casuísticas alemãs(6, 11). O diagnóstico clínico dos deslocamentos do abomaso, em particular, aqueles para a esquerda, baseia-se nos seguintes fatos: relacionamento direto com a parturição, ao manejo alimentar intensivo de vacas de alta produção leiteira e ao quadro sintomático característico - emagrecimento, hiporexia, apetite seletivo, hipotomia ruminal, acetonúria secundária, fezes pastosas, untuosas e finamente trituradas - merecendo destaque especial os ruídos metálicos ao nível do flanco (auscultação de borborigmos de grandes bolhas com timbre metálico; sucussão hipocratica resultando' ruído líquido com tonalidades metálicas e percussão-auscultatória, resultando em ruídos metálicos - retinir metálico, patognomônico para o diagnóstico nosológico. Confirmou-se assim acertiva dos vários autores citados(4, 6, 10, 12). Os resultados apresentados confirmam alertas referidos por autores brasileiros(2, 3, 7), concordantes com autores internacionais de renome(6, 12), que afirmaram, ser esta ectopia uma verdadeira "doença da civilização", atingindo animais alimentados intensamente com rações concentradas para alcançar-se produção máxima, e que as razões do tardio reconhecimento desta entidade mórbida, com maior número de diagnósticos, relacionam-se: à deficiência de conhecimento da metodologia semiótica específica; à dificuldade do reconhecimento pos- mortem destas ectopias e à raridade das ocorrências, que gradativamente aumentam suas freqüências nos melhores rebanhos.

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Tabela 1 - Sintomas constatados em bovinos apresentando deslocamento do abomaso para a esquerda ou para a direita. Valores expressos em número relativo(%). São Paulo - Br., 1990.

Características Clínicas	Forma Clínica de deslocamento do abomaso		Total
	Para a esquerda	Para a direita	
1)Bovinos examinados	10/437 - 2,29%	2/437 - 0,46%	12/437 - 2,75%
- Casos clínicos	10/ 12 - 83,3 %	2/ 12 - 16,7 %	5 : 1
- Raças dos bovinos			
Holandesa	9/ 10 - 90,0 %	2/ 2 - 100,0 %	11/ 12 - 91,7 %
Jersey	1/ 10 - 10,0 %	-	1/ 12 - 8,3 %
2)Relação com o parto			
- Recém paridas(até 2 meses)	9/ 10 - 90,0 %	-	9/ 12 - 75,0 %
- Não gestante	9/ 10 - 90,0 %	1/ 2 - 50,0 %	10/ 12 - 83,3 %
- Gestante	1/ 10 - 10,0 %	1/ 2 - 50,0 %	2/ 12 - 16,7 %
3)Quadro sintomático			
- Diminuição da produção de leite	100,0 %	-	
- Emagrecimento	100,0 %	100,0 %	
- Hiporexia	100,0 %	100,0 %	
- Apetite seletivo	100,0 %	-	
- Ruído metálico(percussão e auscultação)	100,0 % (à esquerda)	100,0 % (à direita)	
. Vazio do flanco	29,0 % (à esquerda)	-	
. Sob gradil torácico	71,0 %	100,0 % (à direita)	
- Hipotonia do rume	44,0 %	-	
- Aumento de volume abdominal:	33,0 %	-	
. Vazio do flanco	(à esquerda)		
. Hipocôndrio	-	100,0 % (à direita)	
- Acetonúria secundária	43,0 %	-	
- Aspecto das fezes			
. Bem trituradas	43,0 %	100,0 %	
. Verde escura	29,0 %	-	
. Pastosa-untuosa	14,3 %	100,0 %	
. Muco-sanguinolenta	14,3 %	-	
4)Tratamento			
- Conservativo	20,0 %(frusto)	50,0 %(frusto)	
- Cirúrgico	100,0 %	50,0 %	
- 9 omentopexia e 1 abomaso-pexia	70,0 %(cura)	-	
- Recidiva	20,0%(cura)	-	
- Morte por complicação	10,0 %	100,0 %	

RESUMO

As observações de 12 casos de deslocamento do abomaso para a esquerda ou para a direita servem para descrever a ocorrência destas enfermidades em bovinos criados no Estado de São Paulo, bem como para esclarecer a etiologia, patogenese, quadro sintomático e tratamento das ectopatias do abomaso.

ZUSAMMENFASSUNG

Auf Grund von Beobachtungen an 12 Fällen von links - und rechtsseitigen Labmagen verlagerung werden Vorkommem, Aetiologie, Pathogenese, Symptomatologie und Therapie dieses Leidens beschrieben.

RÉSUMÉ

Au cours de ce travail 10 déplacements à gauche et 2 déplacements à droite sont l'objet d'une étude systematique. Les symptômes cliniques et le cours de la maladie, le diagnostic et les divers procédés de traitement sont décrits.

SUMMARY

This article evaluates systematically 10 left-sided and 2 right-sided cases of abomasal displacement. Clinical findings, course, diagnosis and methods of treatment are discussed.

MARQUEURS DE VIRULENCE CS31A ET COL V DES ESCHERICHIA COLI ISOLES DES FECES DE VEAUX ATTEINTS DU SYNDROME "GASTRO-ENTERITES PARALYSANTES" (GEP).

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INTRODUCTION

Un syndrome diarrhéique associé à des symptômes nerveux généraux et locomoteurs a été décrit chez des veaux Charollais agés de moins de 2 semaines (3). Pour apprécier le role étiologique éventuel de E.coli pathogènes dans cette maladie nous avons recherché si les colibacilles de la flore fécale possédaient certaines caractéristiques associées au pouvoir septicémique. On a étudié principalement la proteine de surface CS31A (1) et la colicine V marqueurs du pourvoir pathogène des colibacilles septicemiques (11). Des travaux réalisés au cours de l'hiver 1987/1988 avaient montré que des colibacilles CS31A+ étaient identifiés très fréquemment chez les veaux à GEP. Afin de préciser la portée de ce résultat, une étude complémentaire a été effectuée au cours de l'hiver 1988/1989 en comparant l'incidence des E.coli CS31A+ et col V+ dans la flore fécale des veaux présentant le syndrome GEP et dans celle de veaux du même âge apparemment en bonne santé dans les mêmes exploitations.

MATERIELS ET METHODES

Collecte des échantillons.

Le syndrome diarrhéique associé à des troubles nerveux (GEP) est décrit chez des veaux d'un âge moyen de 9,38 + 2,5 jours. Les symptômes nerveux sont observés avant ou aprés des épisodes de constipation et (ou) de diarrhée. Les animaux présentent des signes d'abattement avec hypoesthèsie accompagnée de signes ataxiques ou parétiques surtout localisés aux membres postérieurs. Un oedéme des paupières, des signes d'oedème laryngé, des lésions hémorragiques sont observés avec une fréquence décroissante (3).

Des veaux de moins de 15 jours présentant ce syndrome ont été sélectionnés par les Vétérinaires praticiens. Lorsqu'un Veau du même âge, mais apparemment en bonne santé était présent dans l'exploitation, il servait de témoin "veau sain". Des prélévements de matières fécales du veau malade et du veau témoin, ont été envoyés au Laboratoire départemental des services vétérinaires pour analyse. Dix souches E.coli de la flore

colibacillaire dominante ont été isolées et adressées au Laboratoire de Microbiologie de l'INRA Centre de Recherches de Clermont-Ferrand.

Techniques microbiologiques

Pour l'étude électrophorétique de la membrane externe des colibacilles, les bactéries cultivées à 37°C sur milieu gélosé Minca (6) sont récupérées à la surface de la gélose dans 2 ml de tampon PBS stérile. Après chauffage à 60°C pendant 20mn puis centrifugation, le surnageant est collecté puis conservé à -20°C.

Pour l'électrophorése en gel de polyacrylamide à 15%, 7µl du mélange à parties égales du surnageant et du tampon de Laemli (7) sont déposés dans les puits. Après migration puis coloration à l'argent (9) on peut visualiser principalement les bandes correspondant aux molécules de LPS (profil électrophorétique de chaque sérogroupe 0) ainsi que celles correspondant au sous-unités constitutives de certains fimbriae (29 Kd pour CS31A, 18,5 Kd pour K99). Ces électrophorèses permettent de savoir si les 10 souches E.coli isolées des matières fécales d'un veau sont toutes identiques ou se répartissent en plusieurs groupes. L'identification immunologique des protéines de surface CS31A ou K99 est effectuée par une methode immunodot avec un sérum spécifique anti-CS31A ou anti-K99 et un sérum anti IgG de lapin marqué à la peroxydase.

Pour étudier la production de colicine V on utilise la méthode de la double couche (4). Les bactéries cultivées en spot à la surface d'un milieu gélosé de Luria sont lysées par les vapeurs de chloroforme. On coule alors une gélose à 7‰ ensemencée soit avec une souche E.coli sensible à toutes les colicines, soit avec une souche E.coli spécifiquement résistante à la colicine V. Une souche E.coli qui produit une colicine mais qui est sans effet sur un E.coli résistant à la colicine V produit une colicine V.

RESULTATS

Fréquence des facteurs ou marqueurs de virulence parmi les colibacilles de la flore fécale des veaux.

Pour cette étude, 15 veaux à GEP et 9 veaux témoins ont été sélectionnés sur des critères cliniques.

Parmi les 15 veaux malades, 10 étaient colonisés par des colibacilles CS31A+ (chez 3 veaux, E.coli CS31A+ et col V+). Parmi les 5 autres veaux, 2 excrétaient des E.coli K99+ et les 3 derniers, des E.coli col V+ (Tableau 1). Parmi les 9 veaux témoins, 3 étaient colonisés par des E.coli CS31A+ (dont 2 par des E.coli CS31A+ et col V+). Un autre veau hébergeait des E.coli col V+. Parmi les colibacilles isolés des 5 autres veaux témoins, aucun des 3 marqueurs CS31A, col V ou K99 n'a été identifié (Tableau 1).

La différence n'est pas significative lorsqu'on compare, selon un test de Khi-2 corrigé, le nombre de veaux malades ou témoins excrétant des E.coli CS31A+ (tableau 2). On atteint presque la limite de la signification statistique si on prend en compte les E. coli CS31A ou K99 ($p<0,06$). Si on ajoute le marqueur col V, la différence devient nettement significative ($p<0,01$).

Table 1 : Présence ou absence des marqueurs (CS31A+, col V+), (CS31A+, col V-), (CS31A-, col V+) ou K99 parmi dix souches Escherichia coli de la flore colibacillaire dominante de quinze veaux à GEP et neuf veaux témoins.

		Nombre de souches E. coli					
		CS31A+, col V+	CS31A+, col V-	CS31A-, col V+	CS31A-, col V-	K99	Total
Veaux à GEP	1					10	10
	2		10				10
	3	3	7				10
	4			10			10
	5		1	9			10
	6		5 + 2*		3		10
	7		7		1 + 2		10
	8			7 + 3			10
	9	10					10
	10		7 + 3				10
	11		3		6 + 1		10
	12			9 + 1			10
	13		10				10
	14	1	9				10
	15					9 + 1	10
Veaux témoins	1	2		8			10
	2	8			2		10
	3		9		1		10
	4				5 + 5		10
	5				6 + 4		10
	6				10		10
	7				9 + 1		10
	8			10			10
	9				10		10

* Deux chiffres indiquent qu'il s'agit de deux groupes de souches E. coli différentes d'après le profil électrophorétique des extraits aqueux à 60°C.

Tableau 2 : Comparaison des nombre de veaux malades ou sains excrétant des colibacilles possédant ou non des marqueurs de virulence.

marqueurs de virulence des E.coli	Veaux		signification statistique (Khi-2 corrigé)
	malades	sains	
CS31A+	10/15	3/9	non significatif
CS31A+ ou K99+	12/15	3/9	(p<0,06)
CS31A+, ou K99+ ou col V+	15/15	4/9	(p<0,01)

DISCUSSION

Dans la première étude décrivant le syndrome GEP (3), les colibacilles CS31A+ étaient isolés fréquemment chez les veaux malades. Dans une seconde étude au cours de l'hiver 1987/1988, E. coli CS31A+ a été identifié chez 82,1% des veaux à GEP (résultats non publiés).

Afin de préciser une possible relation étiologique entre les colibacilles CS31A et le syndrome GEP, une expérimentation plus fine incluant des veaux témoins a été réalisée au cours de l'hiver 1988/1989. Pour caractériser les colibacilles nous avons tenu compte des marqueurs CS31A et col V mais aussi de K99. En effet, nos études montrent que les gènes gouvernant la synthèse de CS31A sont portés par des plasmides (5). Ces derniers augmentent la capacité des colibacilles à coloniser l'intestin des jeunes animaux (Contrepois et al, publication en cours). De la même façon HW Smith (12) avait montré que le plasmide gouvernant la synthèse de la colicine V augmentait le pouvoir pathogène et la survie des colibacilles dans l'intestin. De même K99 dont le déterminisme génétique est plasmidique est un facteur de colonisation de l'intestin des veaux (10). Si les colibacilles K99+ sont habituellement non invasifs, il est possible que certains soient à la fois entérotoxiques et bactériémiques. Une forte colonisation de l'intestin par des colibacilles septicémiques pourrait être à l'origine d'une bactériémie colibacillaire d'origine digestive. Les colibacilles possédant l'un ou l'autre de ces 3 marqueurs pour l'aptitude des E.coli à coloniser l'intestin du veau, sont présents chez tous les veaux à GEP. La différence est significative comparativement aux veaux témoins. Des colibacilles possédant les marqueurs de virulence chez quelques veaux témoins n'est pas anormale, dans la mesure ou ces animaux sont voisins de malades excrétant des colibacilles CS31A+ ou col V+. Ainsi, dans les enquêtes concernant les diarrhées du veau nouveau-né, des E.coli K99+ sont parfois isolés dans la flore colibacillaire dominante

de veaux apparemment sains (8). Par ailleurs, on sait que la résistance du jeune veau aux infections colibacillaires est largement tributaire de l'immunité colostrale transmise par la mère (11). Chez les veaux à GEP agés d'environ 10 jours, les immunoglobulines sériques sont encore d'origine colostrale. Selon que la gammaglobulinemie est élevée au faible, la résistance des animaux sera plus au moins grande. Il est possible que des veaux ayant une gammaglobulinémie correcte ne soient pas affectés par une forte colonisation intestinale par des E.coli CS31A+ ou col V+ et que les veaux malades soient prédisposés aux infections par un état hypogammaglobulinémique. La mesure des immunoglobulines sériques permettrait sans doute de mieux définir l'étiologie des GEP.

En conclusion, les résultats de cette enquête confortent l' hypothèse selon laquelle certains clones de colibacilles ayant des caractéristiques leur permettant de coloniser fortement l'intestin, pourraient être responsables d'une bactériemie colibacillaire d'origine digestive avec endotoxémie. Une endotoxémie sub aigüe pourrait peut être expliquer les manifestations cliniques des entérites paralysantes. Le dosage des endotoxines dans le sang des veaux malades permettrait de vérifier cette hypothèse.

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RESUME

Un syndrome diarrhéique associé à des troubles nerveux (gastro-entérites paralysantes = GEP) a été décrit antérieurement chez des veaux charollais d'environ 10 jours. L'étude comparative des colibacilles de la flore fécale de 15 veaux malades et de 9 veaux témoins en bonne santé, a montré que le syndrome GEP est significativement associé à la présence d'une flore fécale colibacillaire où dominent des E.coli pouvant coloniser fortement l'intestin (plasmides p CS31A, p col V, p K99). Une hypothèse étiopathogénique est proposée selon laquelle une bact"ériémie colibacillaire d'origine digestive avec endotoxémie pourrait expliquer certaines manifestations cliniques des GEP.

SUMMARY

A diarrheic syndrom associated with nervous troubles (paralysing gastro-enteritis) was previously described in 10 days old Charollais calves. Comparative studies of E.coli from fecal flora of 15 ill calves and 9 control healthy calves indicated that PGE was significantly associated with a dolinating E.coli fecal flora able to hidhly colonize the gut (p CS31A, p col V or p K99 plasmids). An etiopathogenic hypothesis is proposed. E.coli bacteriemia with a digestive origin associated with endotoxemia could explain a part of clinical signs in PGE.

RESUMEN

Un síndrome diarreico asociado a perturbaciones nerviosas (gastroenteritis paralizante = GEP) fue descripto anteriormente en el caso de terneros de raza "Charollais" de 10 días. El estudio comparativo de los colibacilos de la flora fecal de 15 terneros enfermos y 9 terneros testigos en buen estado de salud mostró que el síndrome GEP está asociado de manera significativa a la presencia de una flora fecal colibacilar en la que dominan los E.coli que pueden colonizar fuertemente el intestino (pCS31A, p Col V, p K99 plasmid).

Se propone una hipótesis etiopatogénica según la cual una septicemia con colibacilos de origen digestivo con endotoxemia podría explicar ciertas manifestaciones clínicas de las GEP.

PERIOPERATIVE EVALUATION OF COWS WITH RIGHT ABOMASAL DISPLACEMENT AND ABOMASAL VOLVULUS

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INTRODUCTION

Cows with right abomasal displacement or abomasal volvulus typically have high packed cell volume (PCV) and plasma total solids (TS) concentrations due to dehydration. They have low plasma concentrations of Cl^- and K^+ due to sequestration of gastric contents and anorexia, and metabolic alkalosis with hyperbicarbonatemia and increased base excess (BE) concentrations due to obstruction of abomasal outflow and the resultant accumulation of HCO_3^- in the extracellular fluid (ECF) space (4,6). However, we have observed that some cows presented to our referral hospital with severe abomasal volvulus have a PCV and TS concentration that is not as high as would be anticipated. Plasma concentrations of Cl^- and K^+ have been higher in these cows than anticipated assuming a continued decrease of these electrolytes with increasing severity of volvulus. In addition, blood pH, HCO_3^- and BE were lower than anticipated, indicating the development of metabolic acidosis superimposed upon the existing metabolic alkalosis.

The purposes of this study were to use a large data base of cattle admitted to a referral hospital for treatment of right abomasal displacement or abomasal volvulus to evaluate the association between the outcome and the physical and laboratory parameters at admission, and the findings at surgery.

MATERIALS AND METHODS

Clinical, clinicopathologic and pathologic data were compiled from the medical records of adult cows admitted to the New York State College of Veterinary Medicine in the 8 year period (1980 to 1987) with a final diagnosis of right abomasal displacement or abomasal volvulus. Cows were classified as productive, salvaged or terminal based upon previously published data (4). Tests of association of each study parameter to the status at hospital discharge were performed using one way analysis of variance for continuous variables and the Chi-square test of independence with categorical variables. The Bonferroni test was used for pair-wise comparisons of group means when statistically significant group effects were observed for continuous variables.

RESULTS

The distribution of outcome for the 458 cows comprising the study population was as follows: productive, 346 (75.5%); salvaged, 88 (19.2%); and terminal, 24 (5.2%). Two hundred and eighteen cows (47.6%) had right abomasal displacement, and 240 (52.4%) had abomasal volvulus. The rectal temperature decreased from 38.9 ± 0.6 °C for productive cows to 38.3 ± 1.3 °C for terminal cows. The heart rate increased with increasing severity of disease (80.9 ± 18.6/min for productive cows to 107.5 ± 18.4 for cows classified as terminal). The PCV and TS were

highest in the salvaged group of cows. Cows in the productive and salvaged groups had metabolic alkalosis (BE = 5.6 ± 7.0 mEq/l, and 4.0 ± 6.9 mEq/l, respectively). As the outcome became less favorable there was evidence of superimposed metabolic acidosis, with the BE in terminal cattle, -1.5 ± 9.0 mEq/l. This decrease in BE concentration was associated with an elevated anion gap concentration in the cows classified as salvaged and terminal. Hyponatremia was increasingly severe in the salvaged and terminal cows, the latter having Na^+ concentrations of 131.9 ± 3.8 mEq/l. All groups of cows were hypochloremic and hypokalemic, with the lowest values observed in the salvaged group.

Considering the surgical criteria evaluated, cattle were more likely to survive if they had right abomasal displacement as opposed to abomasal volvulus, surgical correction by abomasopexy as opposed to omentopexy, did not require fluid decompression of the abomasum, and had normal appearing abomasal serosa.

DISCUSSION

The low temperature and high heart rate in the more severely affected cattle were most likely a result of dehydration and decreased peripheral perfusion. The rise in PCV and TS in the productive and salvaged cattle probably resulted from hemoconcentration (4); whereas, the low PCV and TS in the terminal group of cattle most likely resulted from vascular injury to the mucosa of the abomasum, subsequent intraluminal hemorrhage, and accumulation of modified transudate in the peritoneal cavity.

The presence of a superimposed metabolic acidosis in cattle with severe or prolonged abomasal volvulus (2,3) is reflected in the lower values of pH, HCO_3^-, and BE when compared to cattle classified as productive or salvaged. This metabolic acidosis is probably due to hypovolemia, decreased tissue perfusion and septic shock associated with developing abomasal necrosis.

The decrease in plasma Na^+ concentration with increased severity of disease is consistent with recent observations (1,5) as well as earlier reports (4). The terminal increase in plasma Cl^- concentration may be due to increasingly severe hemoconcentration or decrease in abomasal secretion of Cl^-, at the same time as there is strong renal conservation of Cl^-. The hypokalemia present in the salvaged group of cattle probably resulted from anorexia, intracellular movement associated with metabolic alkalosis, and loss in urine and abomasal effluent. Cell necrosis and rhabdomyolysis with endogenous K^+ infusion into the ECF may explain the increase in K^+ in the terminal cattle (5).

The prognosis for cows with right abomasal displacement was better than for those with abomasal volvulus because the former were less likely to have vascular compromise of the abomasum and consequently less severe metabolic derangements. The higher survival rate for cows with abomasopexy probably reflected our surgeons' preference to use the standing approach for cows with abomasal volvulus and the recumbent approach for cows with right abomasal displacement.

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SUMMARY

Clinical, clinicopathologic and surgical data from 458 adult dairy cows with right abomasal displacement or abomasal volvulus were analyzed to determine the association between these variables and outcome, classified as productive, salvaged or terminal. Heart rate was higher in the salvaged and terminal cattle, than the productive cattle. Hyponatremia was increasingly severe in the salvaged and terminal cows. All groups of cows were hypochloremic and hypokalemic, with the lowest values in the salvaged group. Cows in the productive and salvaged groups had metabolic alkalosis. As the outcome became less favorable there was evidence of superimposed metabolic acidosis with elevated anion gap.

RESUMO

Dados clínicos, clinicopatológicos e cirúrgicos de 458 vacas leiteiras adultas com deslocamento de abomaso à direita ou torção do abomaso foram analizados para determinar a associaçção entre estas variações e resultados, classificados em produtivos, descartes ou terminais. Frequência cardíaca revelou-se mais elevada nas vacas descarte e terminais, do que nas vacas produtivas. Hiponatremia apresentou-se progressivamente severa em vacas descarte e terminais. Todos os grupos de vacas apresentaram hipocloremia e hipopotassemia, onde os valores mais baixos foram observados no grupo de descarte. As vacas no grupo de descarte e produtivas apresentaram alcalose metabólica. A medida que o quadro tornou-se menos favorável houve evidência de acidose metabólica superimposta com um elevado gap aniônico.

RÉSUMÉ

Les données cliniques, clinico-pathologiques et chirurgicaux de 458 vaches laitières avec déplacement ou torsion de la caillette ont été analysées pour défenir l'association entre les variables et la condition de l'animal après la chirurgie (productive, devant aller à l'abattoir ou terminale). Le rythme cardiaque était moins élèvé chez les vaches productives. L'hyponatremie était plus marquee chez les vaches en phase terminal aprés la chirurgie ou chez les vaches devant aller a l'abattoir. Toutes les vaches avaient une hypochlorurémie, avec les vaches en phase terminal après la chirurgie ayant l'hypochlorurémie la plus sévère. Les vaches classées comme étant productive ou devant aller à l'abattoir avaient une alkalose métabolique. Quant le pronostic était plus sombre, les vaches avaient une acidose métabolique superposée sur l'alkalose métabolique et une élévation de l'écart anionique.

USING A MULTIPLE LOGISTIC REGRESSION MODEL TO PREDICT PROGNOSIS OF COWS WITH RIGHT ABOMASAL DISPLACEMENT OR ABOMASAL VOLVULUS

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INTRODUCTION

To date, individual or pairs of physical and laboratory parameters have been used to predict the prognosis of cows with right abomasal displacement or volvulus (2,3,5-8). However, the use of individual parameters may be unreliable in prediction, and multivariable statistical modeling techniques can control some of the inherent problems associated with their use (4). Our objective was to develop a multiple logistic regression model for estimating outcome in cows after surgical correction of right abomasal displacement or abomasal volvulus.

MATERIALS AND METHODS

Clinical, clinicopathologic and pathologic data were compiled from the medical records of adult cows admitted to the New York State College of Veterinary Medicine in the 8 year period (1980-1987) with a final diagnosis of right abomasal displacement or volvulus. Cows were classified as productive, salvaged or terminal based upon previously published criteria (8). The model for predicting postsurgical outcome was developed using SAS LOGIST (4) which fits the logistic multiple regression model (10) to a single binary dependent variable or to an ordinal dependent variable using maximum likelihood method. The outcome (0 = productive, 1 = salvaged and 2 = terminal) was handled as an ordinal dependent variable. We used both the forward stepwise approach to consider each variable sequentially in relation to the other potentially significant variables, and the backward stepwise approach to confirm the best candidate models. In the reported models, we retained only those variables that were found to significantly effect outcome.

RESULTS

The distribution of outcome for the 458 cows comprising the study population was: productive 346, (75.5%); salvaged 88, (19.2%); and terminal, 24 (5.2%). The admission model containing complete data from 346 cows was reduced to 3 variables: heart rate, base excess concentration (BE), and plasma Cl^- concentration ($R = 0.368$). The surgical model containing data from 335 cows was reduced to 5 variables: heart rate, BE, diagnosis, decompression used, and appearance of abomasal serosa ($R = 0.495$). Tests of goodness of fit comparing observed and predicted numbers indicated a high degree of fit for both admission and surgical models ($P = 0.876$ and $P = 0.970$, respectively).

DISCUSSION

Logistic regression analysis has proven to be a useful statistical technique allowing simultaneous consideration of multiple explanatory variables when the response variable is dichotomous. Because in this study the response variable had 3 categories (productive, salvaged and terminal) to correspond to the biologic situation, we used a polychotomous extension of the usual logistic model rather than the binary situation. Though the admission model containing heart rate, pH, and Na^+ had a slightly higher R value (0.387 versus 0.368) than the model containing heart rate, BE, and Cl^-, the latter was chosen for the final model because the agreement between predicted and observed values was better. Also, most surgeons are used to evaluating plasma Cl^- concentrations in cows with right abomasal displacement or abomasal volvulus, and both Cl^- and BE have previously been suggested as predictors for outcome (5,7,8). The model containing Na^+ was slightly better than the one with Cl^- because Na^+ decreased in a linear fashion with the severity of the disease while Cl^- increased terminally (1). The surgical model did not include either Na^+ or Cl^-, and the only choice was whether or not to include pH or BE. The model with BE had a slightly higher R value than the model with pH (0.495 vs 0.494). Though agreement between observed and predicted numbers in both the admission and surgical models was good, caution must be used in analyzing the results of the chi-square test because the expected number of observations in several cells was less than five (9).

The multiple logistic regression models developed in this study have several advantages over individual physical and laboratory parameters previously reported. They are useful to surgeons for objective decisions. However, the validation of the models and the economical decision strategy require further research.

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SUMMARY

Data at admission and at surgery were collected on 458 cows with right abomasal displacement or abomasal volvulus, to derive multiple logistic regression models for predicting postsurgical outcome (productive, salvaged or terminal). Three admission variables (heart rate, base excess, and plasma chloride concentration), and five surgical variables (heart rate, base excess, diagnosis, method of decompression used, and appearance of abomasal serosa) were used in the final models. Predicted outcomes using the admission and surgical models were closely related with actual outcomes.

RESUMEN

Datos de 458 vacas con desplazamiento derecho de abomaso o con vólvuvo abomasal obtenidos en el momento de admisión o de cirugía, fueron utilizados en modelos de regresión logística múltiple para predecir resultados post-quirúrgicos (i.e., regreso a la producción, envío a matadero después de un procedimiento quirúrgico o terminal). Tres variables de admisión (i.e., ritmo cardíaco, exceso de bases y concentración de cloruros en el plasma) y cinco variables quirúrgicas (i.e., ritmo cardíaco, exceso de bases, diagnóstico, método de descompresión utilizado y apariencia de la serosa abomasal) fueron incluídas en el modelo final. Se encontró una estrecha relación entre los resultados observados y los predichos por los modelos de admisión de admisión y de cirugía.

RÉSUMÉ

Certains paramètres obtenus durant l'admission et la chirurgie de 458 vaches avec déplacement ou torsion de la caillette furent étudiés. Le but de cette étude était de produire un modèle (déterminé à partir d'une régression logistique multiple) servent à prédire la condition de la vache après la chirurgie (productive, perte à l'abattoir ou terminale). Trois variables (rythme cardiaque, excès de base et le niveau plasmatique de chlorure) obtenus à l'admission de la vache, et cinq variables (rythme cardiaque, excès de base, le diagnosis, la méthode de décompression utilisée, et l'apparence de la séreuse de la caillette) obtenus durant la période chirurgicale furent utilisées dans le modèle. Le resultat prédit était très près du résultat actuel.

TREATMENT OF ACUTE RUMEN DILATION WITH ORAL ADMINISTRATION OF ACTIVATED CHARCOAL

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INTRODUCTION

Even though the cattle can utilize cellulosic feedstaffs, they are fed diets that contain high levels of cereal concentrates. In Japanese dairy cattle husbandry, the controlled feeding is very popular. The feeding ration is profixed to fit the physiological state, to obtain maximum milk yield and to minimise the economical loss. Under this restricted feeding condition, the cattle escaped from the mooring may eat too much concentrates availabie for the cow. Ingestion of large amounts of cereal concentrates provides the substrate for rapid proliferation of rumen bacteria and leads to lactate accumulation, low ruminal pH and decreased fiber digestion. Intake of larger-than-normal qantities of highly fermentable carbohydrates laeds to lactic acidosis, acute dehydration and depression in acute forms. Increased prodution of lactic acid also contributes to metabolic acidosis. Death sometimes happens in serious case. Many investigations of accumulation in the rumen of lacit acid were documented (2,3).

Activated chacoal has been used in the treatment of many intoxications (1). The charcaol is administered orally to absorb the toxins or soae materials and thus prevent them being absorbed and increasing. We have investigated the suitability of activated charcaol as an absorbent material for the treatment of acidosis in cattle.

MATERIALS AND METHODS

Three Holstein steers averaging 250 kg with rumen fistula were given 8kg of crushed barley. Eight hours after feeding, 400g activated charcoal was orally administered to a steer. Two others remained untreated for control. Changes in blood chemistry, rumen fluid and clinical sings were monitored for 24 hours. Steers were sampled after feeding from the rumen fistula at 2-h intervals for 24h. Rumen fluid was stored at -20℃ for later analysis. Rumen fluid pH was determined by a pH electrode immediateiy after sampling. Blood sampling was facilitated by placing a polyvinyl catheter in the jugular vein and collected using a diposable syringe, trasferred to tubes. Rumen fluid samples were analyzed for pH, lactic acid, VFA, osmotic pressure, NH_3-N. Blood samples were analyzed for packed cell volume(PCV), plasma gulcose, blood urea nitrogen(BUN), serum glutamic-ozaloacetate transaminase(GOT), Ca, Na. Rectal temprature and heart rate were checked.

In addtion to the experimental animals, 20 dairy or beef cattle

were clinically examined. Field examination were conducted with cattle suffered from acute rumen dilation by overfeeding of the concentrates from local dairy farm, Chiba, Japan. All of them in the field were orally adminstered charcoal after overfeeding and clinical courses were monitored following treatment.

RESULTS AND CONCLUSION

Table 1,2 shows the changes in the clinical sigas of three strees. The steers given only barley in the experiment developed sings including heart rate, temperature and PCV. In contract, the steer given charcoal did not developed sings and especially packed cell volume did not increase. There was no significant treatment difference in plasma Ca, Na. One untreated cow was significaat higher in plasma glucose and GOT 24h after feeding. Though treated steer ruminal pH droped first below 5.0, it fell slight and rose after the adminstrtion of charcoal. On the other hand, ruminal pH of control steers fell down 4.07, 4.43 respectively 24h after feeding (Table.3). Lactic acid levels and osmotic pressure were significantly different between treated steer and untreated ones. The volatile fatty acid profile and NH_3-N in rumen fluid were shown in Table 3.

Oral adnistration of activated charcoal was applied to 20 cattle suffered from acute rumen dilation by overfeeding of the concentrates. Of these, 19 cattle were cured. The recovery was prominent in severely affected cases, which had not be able to stand 24 hours after overfeeding.

In conclusion, activated charcoal treatment is effective to eliminate excess lactic acid from rumen of the cow with rumen acidosis or rumen dilation caused by overfeeding of the concentrates.

Table 1. Changes in clinical sings of steers fed barley

Time of sampling post feeding	No.	temperature (℃)	heart rate	No.	temperature (℃)	heart rate	No.	temperature (℃)	heart rate
(hr)									
0*		39.1	100		39.0	72		39.4	80
4		39.0	120		39.0	80		39.2	82
8*		39.1	106		39.1	95		38.8	71
12	1	※39.2	98	2	39.0	92	3	39.1	88
16		38.9	82		39.0	92		38.8	86
20		38.4	86		39.7	130		39.0	94
24		38.8	84		39.6	132		38.6	90

No.1 was treated with charcoal. No.2 and No.3 were not treated.
*First feeding of 5kg barley. *Second feeding of 3kg barley.
※ 400g charcoal given.

Table 2. Changes in blood of steers fed barley

No.	Time of sampling post feeding	PCV	glucose	BUN	GOT	Ca	Na
	(hr)	(%)	(mg/dl)	(mg/dl)	(IU/l)	(mg/dl)	(mEq/l)
	0*	28.0	55.5	7.5	68	11.9	141.1
	4	26.0	64.7	6.3	63	10.4	140.6
	8*	24.5	66.6	5.1	55	8.6	138.2
1	※12	26.0	74.1	4.7	71	11.1	137.3
	16	26.0	69.6	4.9	65	11.2	145.2
	20	27.0	63.5	5.3	94	11.1	149.0
	24	28.0	74.5	5.6	93	11.4	145.8
	0*	28.0	89.3	11.9	56	11.2	142.0
	4	29.0	89.9	10.7	47	10.2	144.1
	8*	28.5	87.3	6.4	61	10.2	144.1
2	12	28.5	87.5	8.0	64	12.1	146.9
	16	33.0	76.2	8.0	55	13.0	150.4
	20	36.0	72.9	8.8	103	12.3	150.6
	24	42.0	137.7	11.4	103	13.0	154.8
	0*	31.0	66.4	19.7	47	10.9	138.5
	4	28.0	70.4	17.1	31	10.1	138.6
	8*	28.0	68.9	13.5	40	9.0	141.4
3	12	28.0	70.9	12.1	37	10.9	140.9
	16	29.0	72.3	9.3	41	12.1	141.2
	20	30.0	82.6	9.6	67	11.6	142.2
	24	31.0	77.8	8.6	62	11.2	142.6

Table 3. Changes in rumen characteristics of steers fed barley

No.	Time	pH	Total VFA	V F A ratio			A/P	rumen NH_3-N	lactic acid	osmotic pressure
				Acetate	Propionate	Butyrate				
	(hr)		(m mol/dl)	(%)	(%)	(%)	(%)	(mg/dl)	(m mol/kg)	(m osm/kg)
	0*	6.42	9.8	66	20	11	3.3	4.5	-	236
	4	5.20	10.8	60	23	15	2.6	11.7	0.1	345
	8*	5.22	14.6	56	22	15	2.1	5.5	-	294
1	※12	4.98	13.2	60	26	13	2.3	3.5	-	359
	16	4.81	10.8	66	22	11	3.0	7.6	-	346
	20	4.85	9.6	70	20	9	3.5	13.0	-	353
	24	5.12	6.0	77	7	5	4.5	17.4	-	276
	0*	7.29	7.9	65	21	10	3.1	14.8	-	240
	4	5.86	11.5	64	19	14	3.4	27.2	-	329
	8*	5.54	12.0	63	17	17	3.7	19.1	-	320
2	12	5.04	12.8	56	18	24	3.1	17.5	5.84	432
	16	4.63	8.5	59	15	24	3.9	22.5	12.39	504
	20	4.16	6.0	63	14	21	4.5	22.9	16.86	487
	24	4.20	3.1	68	10	17	6.8	21.8	16.27	490
	0*	7.26	7.0	66	18	12	3.7	15.4	-	248
	4	6.05	14.2	59	19	17	3.1	19.7	-	295
	8*	5.67	13.6	58	19	19	3.1	11.4	-	261
3	12	5.47	16.5	55	18	23	3.0	18.9	-	288
	16	5.36	16.0	58	14	25	4.1	10.7	1.39	369
	20	4.81	18.5	74	8	16	9.3	15.3	8.82	435
	24	4.43	18.4	76	7	15	10.8	20.2	12.99	486

No.1 was treated with charcoal. No.2 and No.3 were not treated.
*First feeding of 5kg barley. ᵇSecond feeding of 3kg barley.
※ 400g charcoal given.

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SUMMARY

Three Holstein steers averaging 250 kg with rumen fistula were given 8 kg of crushed barley. 12 hours after feeding, activated charcoal was orally administered to a steer. Two others remained untreated for control. Changes in blood chemistry, rumen juice and clinical sings were monitored for 24 hours. The treated steer recovered without showing critical signs. There was no increase in osmotic pressure and no acummulation of lactic acid in the rumen juice. Two control steers rumen pH fell to less than 4.5 at the lowest and osmotic pressure in rumen juice suggested lactic acid acidosis. Finally, control steers died.

Oral admistration of activated charcoal was applied to 20 cattle sufferedfrom acute rumen dilation by overfeeding of the concentrates. Of these, 19 cattle were cured. The recovery was prominent in severely affected cases, which had not be able to stand 24 hours after overfeeding.

Zusammenfassung

Drei Holsteiner Stiere mit einem Durchschnittsgewicht von 250 kg und mit Pansenfistel erhielten 8 kg zerkleinerte Gerste. 12 Stunden nach der Fütterung wurde einem Stier Aktivkohle oral zugerführt. Die beiden anderen Stiere blieben zur Kontrolle unbehandelt. Änderungen in der Blutchemie, im Pasensaft und in den klinischen Zeichen wurden 24 S tunden lang überwacht. Der behandelte stier erholte sich ohne kritisch e Zeichen. Es kam zu keiner Zunahme des osmotischen Drucks und zu keiner Ansammlung von Milchsäure im Pansensaft. Bei den beiden Kontrollstieren fiel der pH-Wert im Pansen auf unter 4.5 ab, und der osmotische Druck im Pansensaft deutete auf Milchsäureazidose hin. Die

Kontrollstiere starben schließlich.

Orale Zugabe von Aktivkohle wurde auf 20 Kühe und Stiere angewendet, die durch Überfütterung mit Konzentraten an akuter Pansendilatation litten. 19 der Tiere wurden geheilt. In schwer betroffenen Fällen, die 24 Stunden nach der Überfütterung nicht stehen konnten, war die Erholung prominent.

RESUME

Trois jeunes bœufs de race Holstein d'un poids moyen de 250kg présentant des fistules gastriques ont reçu 8kg d'orge broyée. 12 heures après avoir absorbé cette nourriture, du charbon actifleur a été administré par voie orale. Le groupe de contrôle est constitué de deux autres bœufs non traités. Les changements de la composition chimique du sang, du suc gastrique et les signes cliniques ont été observés pendant 24 heures. Les bœufs traités ont récupérés sans montrer de signes critiques. Il n'y a pas eu d'augmentaion de la pression osmotique et aucune accumulation d'acide lactique dans le suc gastrique. Le pH de la panse des deux bœufs de contrôle est tombé à moins de 4.5 à son niveau le plus bas, et la pression osmotique dans le suc de gastrique suggère une acidose d'acide lactique.

L'administration orale de charbon actif a été pratiquée sur 20 bovins souffrant d'une dilatation gastrique due à une surconsommation de fourrage concentré. Parmi ceux-ci, 19 ont été guéris. La guérison a été remarquable dans les cas les plus gravement atteints alors qu'ils étaiet incapables de se tenir debout 24 heures après s'être suralimentés.

AUSWIRKUNGEN VON ADITOPRIM^{R} AUF DIE FERMENTATIONSVORGÄNGE IM PANSENSAFT DES RINDES (IN VITRO)

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EINLEITUNG

Im Zusammenhang mit der sich ändernden tierärztlichen Tätigkeit (sogenannte Massentierbehandlung) muß über neue, realistische Behandlungsmöglichkeiten auch unter den gewandelten Haltungsformen nachgedacht werden. Die einfachste und schonendste Methode ist die orale Applikation. Für das Rind aber ergeben sich wegen seiner anatomischen und physiologischen Besonderheiten (Vormagensystem) Schwierigkeiten. Nur wenn das per os aufgenommene Medikament weitgehend unverändert den Vormagenbereich passiert und darüber hinaus die ruminale Mikroflora nicht schädigt, kann zu einer solchen Maßnahme geraten werden. Das gilt im besonderen Maße für die Verabreichung von antimikrobiell wirksamen Substanzen. ADITOPRIM^{R} ist ein solches antiinfektiöses Agens, das speziell für den oralen Einsatz am ruminierenden Rind entwickelt wurde. In den nachfolgend beschriebenen in-vitro-Untersuchungen sollte geklärt werden, ob und gegebenenfalls in welchem Ausmaß ADITOPRIM^{R} auf die Fermentationsvorgänge im Pansen einwirkt.

MATERIAL und METHODEN

Versuchstiere, Haltung, Fütterung

Fünf adulte Rinder (2 - 3 Jahre alt, nicht tragend, nicht laktierend, Rasse DSB) mit permanenter Pansenfistel wurden im Anbindestall einzeln auf Gummimatten gehalten, wodurch eine unkontrollierte Futteraufnahme verhindert werden konnte. Die Ration bestand aus Heu und Milchleistungsfutter (5 : 1; auf Trockenmasse bezogen), reichte für Erhaltung und 3 - 5 l Milch/Tag und wurde auf zwei Halbtagsrationen (06.30 und 15.30 Uhr) verteilt. Wasser stand ad libitum aus Selbsttränken zur Verfügung.

Versuchsanordnung

Von jedem der 5 Versuchstiere wurde an 10 Tagen zweieinhalb Stunden nach der Futteraufnahme Pansensaft entnommen und für 5 Stunden im künstlichen Pansen (CZERKAWSKI und BRECKENRIDGE, 1969; SCHOLZ, 1980) inkubiert. Dabei konnten jeweils in dem aus vier Einzelfermentatoren bestehenden System zwei ohne (Kontrollen) und zwei mit ADITOPRIM^{R} (ca. 200 mg/l) beschickt werden. Auf diese Weise entstanden Untersuchungsreihen mit Mittelwertergebnissen aus 50 Einzelmessungen.

Pansensaftgewinnung und -aufbereitung

Der Pansensaft wurde mit Hilfe einer Vakuumpumpe aus der Regio xiphoidea aspiriert, durch ein dreilagiges Gazetuch gegossen und ohne Wärmeverlust (vorgewärmte Thermosflasche) in den künstlichen Pansen verbracht, der zuvor mit Puffer (künstlicher Speichel n. BUNGATE, 1966) und Nährsubstraten (Glukose: 40 mmol/l sowie Harnstoff: 18,5 mmol/l) gefüllt worden war.

In den folgenden 5 Stunden konnten sowohl der Substratabbau als auch die Produktion von mikrobiellen Endprodukten sowie Veränderungen des ruminalen Milieus in Abhängigkeit von einer ADITOPRIM^{R}-Zulage gemessen werden. Letztere orientierte sich an der Hersteller-Empfehlung (20 mg/kg LM), was einer Konzentration von ca. 200 mg ADITORPIM^{R} pro Liter Pansensaft entspricht.

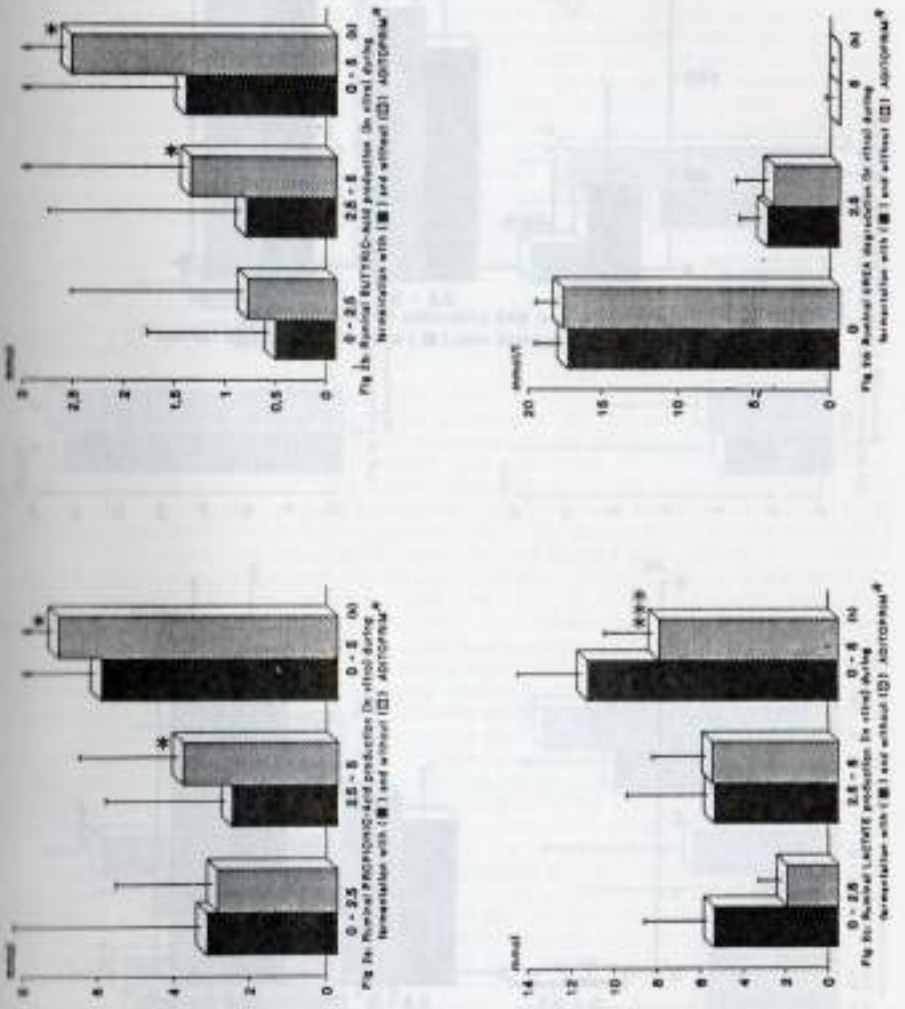
Analytik
Der vor 2.5 und 5 Stunden nach Fermentationsbeginn aus den Inkubatoren entnommene Pansensaft wurde sofort aufbereitet und auf folgende Parameter hin untersucht (Tabelle 1):

Tab. 1: Fermentationskriterien im Pansensaft und deren Meßgenauigkeit (Präzision in der Serie)

Parameter	Meßverfahren	Variationskoeffiz. (%)
ph-Wert	Elektrode	2,0 %
Glukose	enzymatisch	2,4 %
Fl. Fettsäuren	gaschromatographisch	3,5 %
L(+)-Laktat	enzymatisch	2,1 %
Harnstoff	colorimetrisch	3,9 %
Ammoniak	Elektrode	2,1 %
Gasproduktion	volumetrisch	2,0 %
Methan	gaschromatographisch	2,7 %

Statistische Auswertung
Die mitgeteilten Meßergebnisse stellen Mittelwerte aus n = 50 Einzelwerten dar, die im t-Test nach Student auf signifikante Unterschiede hin geprüft wurden.

ERGEBNISSE
Kennzeichnend für das ruminale Milieu ist der ph-Wert-Verlauf (Abb. 1a). Während der Inkubation sank er bei den Kontrollansätzen signifikant stärker ab. Demzufolge bewirkt der ADITOPRIM^{R}-Zusatz eine Milieu-Stabilisierung oder aber er schwächt die Vergärungsintensität. Dieser Eindruck entsteht, wenn man den Glukoseabbau betrachtet (Abb. 1b). Während in den Inkubatoren ohne Medikamentzusatz die Glukoseeinwaage innerhalb der ersten zweieinhalb Stunden zu 2/3 fermentiert wurde, waren in den Versuchsansätzen noch nahezu 50% der zugelegten Glukose vorhanden. Allerdings beschleunigte sich im zweiten Inkubationsintervall die Vergärung erheblich, so daß zu Versuchsende insgesamt nur geringfügig weniger (- 4,3%) Glukose verwertet war. Dieses Fermentationsdefizit ließ zunächst auch eine niedrigere Produktion von flüchtigen Fettsäuren (FFS) im Pansensaft erwarten. Überraschenderweise (Abb. 1c) trat das aber nicht ein. Trotz der deutlich reduzierten Glukosenutzung im ersten Inkubationszeitraum infolge ADITOPRIM^{R}-Zusatzes wurden über 30% FFS mehr gebildet als in den Kontrollansätzen. In erster Linie auf Grund der um über 50% erhöhten Produktion von Essig- und n-Buttersäure; die Steigerung bei Propionsäure betrug knapp 20% (Abb. 1d, 2a, 2b).
Die Überprüfung der in vitro produzierten L(+)-Laktatmengen (Abb. 2c) zeigt, daß die Glukosefermentation in Richtung Milchsäure durch ADITOPRIM^{R} in erheblichem Umfang gedrosselt werden konnte. So fielen in den ersten 2.5 Stunden nur etwa 40% der Vergleichsmenge an. Danach allerdings waren die Kapazitäten weitgehend ausgeglichen, so daß für den Gesamtzeitraum knapp 30% weniger produziert wurden.
Die Dissimilation der Stickstoffverbindungen - hier geprüft am Harnstoffabbau - war durch ADITOPRIM^{R} nicht beeinflußt. So waren weder Differenzen in den Harnstoff- (Abb. 2d) noch den Ammoniakkonzentrationen zu beobachten. Die Gasbildung (Abb. 3a) zeigte eine gewisse Parallelität zu der L(+)- Laktatproduktion: sie betrug zunächst nur etwa 35% der Kontrollen, lag im zweiten



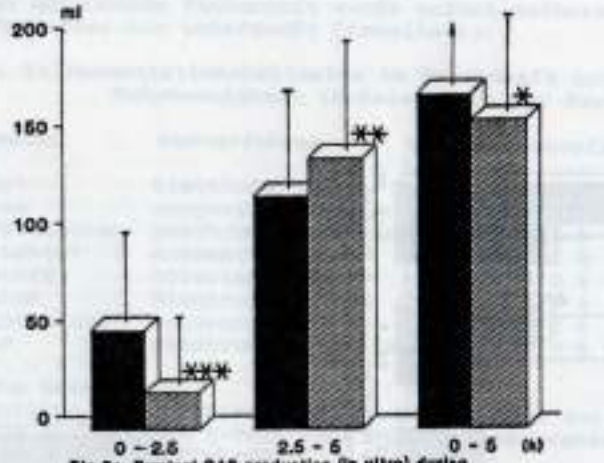


Fig 3a: Ruminal GAS production (in vitro) during fermentation with (■) and without (☒) ADITOPRIM®

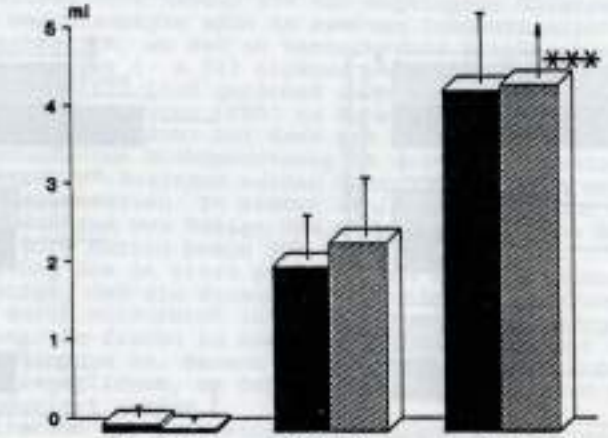
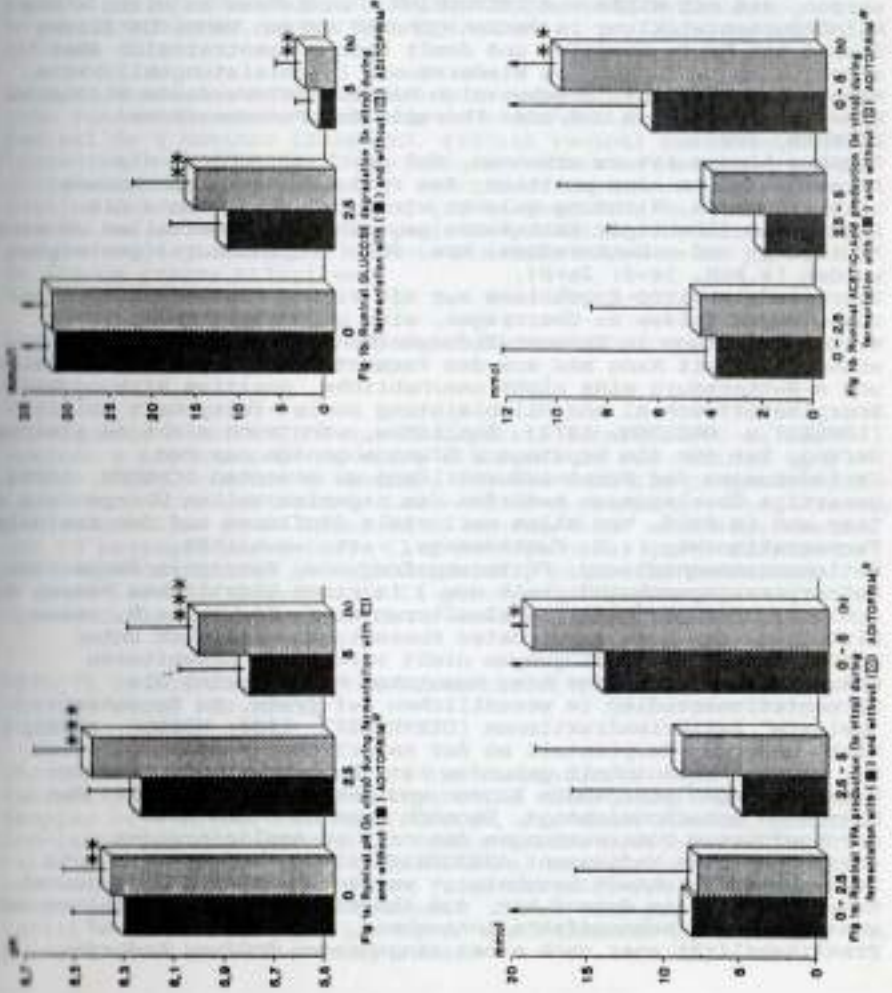


Fig 3b: Ruminal METHANE production (in vitro) during fermentation with (■) and without (☒) ADITOPRIM®

Fermentationsintervall um knapp 20% darüber, ohne aber dadurch das Gesamtvolumen der Kontrollfermentatoren zu erreichen. Der für energetische Überlegungen bedeutsame Gasbestandteil Methan (Abb. 3b) blieb quantitativ bedeutungslos, wenngleich qualitativ sich eine Begünstigung der Methanproduktion abzeichnete (plus 1,6%).



DISKUSSION

Die Untersuchungen zeigen, daß das getestete Medikament den ruminalen Stoffwechsel beeinflußte. Sowohl das Pansenmilieu als auch die mikrobielle Stoffwechselleistung waren eindeutig betroffen, ohne daß mit dieser Technik zu klären war, wo die Primärursache zu suchen ist. Sie kann Folge einer direkten und alleinigen Wirkung auf die Pansenflora aber auch die einer Beeinflussung durch den sich ändernden pH-Wert sein. Letzteres wird jedoch weniger wahrscheinlich, wenn man berücksichtigt, daß mit zunehmender Fermentationsdauer und weiter abnehmendem pH-Wert die anfänglichen Tendenzen sich abschwächten (s.Abb. 1a-d; 2a-c). Bleibt zu fragen, wie diese beobachteten Einflüsse für den praktischen Einsatz zu werten sind. Zunächstmal muß herausgestellt werden, daß mit Hilfe von ADITOPRIM^{R} - zumindest in vitro - die Milchsäureentwicklung im Pansen gehemmt werden kann. Da dieses Risiko bei heute energie- und damit auch konzentratreich aber strukturarm zu fütternden Wiederkäuern (Hochleistungsmilchkühe, Mastbullen) besteht, ergeben sich hieraus interessante klinische Aspekte (Prophylaxe und/oder Therapie der Pansenazidose; DIRKSEN, 1981).

Darüber hinaus ist zu erkennen, daß durch ADITOPRIM^{R} die Fermentation in eine positive, das ruminale Energieaufkommen unterstützende, Richtung gelenkt wird. Immerhin konnte die Produktion flüchtiger Fettsäuren gegenüber den Kontrollen um etwa 50 (Essig- und n-Buttersäure) bzw. 20% (Propionsäure) gesteigert werden (s.Abb. 1c+d; 2a+b).

Zwar sind in-vitro-Ergebnisse nur mit vielen Einschränkungen auf reale Verhältnisse zu übertragen, eine grundsätzliche Wirkungsrichtung im Fermentationsgeschehen ist dennoch zu erkennen. Somit kann man aus dem fermentativen Zugewinn an Essig- und n-Buttersäure eine nicht unerhebliche, positive Wirkung auf Energiestoffwechsel und Milchleistung sowie -fettgehalt ableiten (LENKEIT u. BREIREM, 1972). Ähnliches, wenn auch nicht im gleichen Umfang, ist für die hepatogene Glukoneogenese aus den Veränderungen der Propionsäurebildung zu erwarten (CHURCH, 1988). Derartige Überlegungen bedürfen der experimentellen Überprüfung am Tier und im Feld. Vor allem weil viele Einflüsse auf den ruminalen Fermentationsweg (z.B. Futtermenge, -art, -qualität, Rationszusammensetzung, Fütterungsfrequenz, Resorptionskapazität, Futterpassagegeschwindigkeit usw.) in einem künstlichen Pansen nur schlecht oder gar nicht zu simulieren sind. So wird z.B. reine Glukose in dem hier verwendeten Konzentrationsbereich unter natürlichen Pansenbedingungen nicht vorkommen. Desweiteren beschränken sich in dem hier benutzten Pansensystem die Fermentationsstudien im wesentlichen auf freie, im Pansensaft "gelöste" Bakterienfraktionen (CZERKAWSKI, 1986; HOBSON, 1988), nicht aber den Hauptanteil an der natürlichen Pansenpopulation, der an den Panseninhalt gebunden ist. Auch bleiben in solchen Untersuchungen protozoale Eigen- und Wechselwirkungen mit den Bakterien unberücksichtigt. Dennoch kann nach den hier durchgeführten Untersuchungen dem oral zu applizierenden antiinfektiösen Medikament ADITOPRIM^{R} eine grundsätzlich gute Pansenverträglichkeit bescheinigt werden. Darüber hinaus deutet desweiteren alles darauf hin, daß ihm für Tier und Tierhalter sehr wünschenswerte Nebeneffekte innewohnen, deren Realität und Praktikabilität aber noch einer eingehenden Prüfung bedürfen.

ZUSAMMENFASSUNG

In Pansensaft von 5 Rindern (DSB, permanente Pansenfistel, nicht laktierend, nicht tragend) wurde in vitro der Einfluß von ADITOPRIM^{R} auf das Fermentationsvermögen geprüft. In 10 Versuchsdurchgängen pro Tier ergaben sich Durchschnittswerte mit aus jeweils 50 Einzelmessungen.
Es zeigte sich, daß der ADITOPRIM^{R}-Zusatz das ruminale Milieu stabilisierte, die Milchsäurebildung aus Glukose senkte und die Produktion flüchtiger Fettsäuren erheblich steigerte.

EFECTOS DE ADITOPRIM^{R} SOBRE LOS PROCESOS FERMENTATIVOS EN EL JUGO RUMINAL DE BOVINOS (IN VITRO)
RESUMEN

La influencia de ADITOPRIM^{R} sobre la capacidad fermentativa del jugo ruminal ha sindo examinado in vitro utilisando el jugo ruminal de 5 bovinos (raza DSB, fistula ruminal permanente, non lactantes, non gravidas). Los valores medios se han obtenido por calculacion de un total de 50 mediciones basandose sobre 10 pasajes por animal. Se mostró que la anadidura de ADITOPRIM^{R} estabiliza el medio ruminál, baja la producción de acida lactica partiendo de glucosis, y aumenta considerablemente la producción de ácidos grasos volatiles.

EFFETS DE ADITOPRIM^{R} SUR LES PROCÉDÉS FERMENTATIVES DANS LE SUC RUMINAL DES BOVINS (EN VITRO)
RESUME

L'influence d'ADITOPRIM^{R} sur la capacité fermentative du suc ruminal a été examinée in vitro utilisant le suc ruminal de 5 bovins (race frisonne allemande, fistule ruminale permanente, hors lactation, hors gestation). Les valeurs moyennes ont éte obtenus par calculation de un total de 50 mensurations se basant chacune sur 10 passages par animal. Il a été demontré, que l'additon de ADITOPRIM^{R} stabilise le milieu ruminal, baisse la production d'acide lactique à partir de la glucose, et augmente considérablement la production d'acides grasses volatiles.

EFFECTS OF ADITOPRIM^{R} ON THE FERMENTATIVE PROCESSES IN BOVINE RUMINAL JUICE (IN VITRO)
SUMMARY

The influence of ADITOPRIM^{R} on the fermentative capacity of ruminal juice has been investigated in vitro using ruminal juice samples of 5 cattle (German friesian, permanent ruminal fistula, non-lactating, non-pregnant). Medium values were obtained by calculating a total of 50 measurements, basing upon 10 passages per animal, each. It was shown, that the addition of ADITOPRIM^{R} stabilizes the ruminal environment, lowers the production of laetic acid derived from glucosis, and increases considerably the production of volatile fatty acids.

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THE MICROBIAL FLORA OF THE UPPER AND LOWER RESPIRATORY TRACTS OF FEEDLOT CALVES WITH UNDIFFERENTIATED BOVINE RESPIRATORY DISEASE.

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INTRODUCTION

Undifferentiated bovine respiratory disease (UBRD) is a syndrome frequently affecting recently weaned calves shortly after their arrival in the feedlot (5). The etiology of the disease is complex and can involve multiple viral and bacterial agents. Previous workers have examined either upper airway samples from live animals (1,10), or samples of lung tissue from fatal cases (4), to elucidate the bacterial flora of the respiratory tract in naturally occuring UBRD. While much useful information has been obtained there are limitations to both approaches. Firstly, the reliability with which upper airway isolates can be used to correctly predict the presence of organisms actually in the lung is unknown. Secondly, pulmonary isolates from fatal cases, especially those previously treated with antibiotics, are unlikely to be representative of all calves with UBRD (7). To address these problems a study was undertaken in which nasopharyngeal swabs and bronchoalveolar lavage were used to sample the upper and lower respiratory tracts of both sick and clinically normal feedlot calves. This paper describes the bacteriologic culture results of the samples obtained.

MATERIALS AND METHODS

Study farm and animals

Steer calves (n=136) were observed during a 28 day period following their arrival at a feedlot research facility. All calves were approximately 6 to 8 months old, and had been recently weaned and transported to Ontario from Saskatchewan. The animals were purchased at auction and were from multiple sources none of which advertised any kind of preconditioning or prevaccination program. The calves were housed in pens of 4 in a large slatted floor barn with water and a mixture of corn silage, high moisture grain corn and haylage available ad libitum.

Definition of cases and controls

All calves were observed twice daily for signs of UBRD. Sick cattle were detected based on criteria commonly used by the feedlot industry, such as depression, lack of rumen fill, anorexia, nasal discharge or elevated respiratory rate. Depending on these signs cattle were given a clinical score between 0 and 10. Any animal with a clinical score $\geq$ 2 and with a rectal temperature $\geq$ 40 C was designated as a case. Animals with a score $\geq$ 6 became cases irrespective of body temperature in order that obviously very sick animals would be treated in the event that they had a normal temperature. For each case detected a clinically healthy calf was randomly selected as a control. Inevitably, some control calves later became sick. These were discarded from the control group and were included with the cases. In order to keep the case and control groups approximately equal in number, four controls were selected for every three cases.

Treatment, sampling and microbiological procedures

For the first 2 days following detection, cases were treated with 45,000 i.u./kg body weight of procaine penicillin given subcutaneously. On the third day (48 hours after treatment commenced) the animal's response to therapy was assessed. Those with a rectal temperature less than 40 C were deemed responsive to therapy and recieved two additional days of penicillin therapy. Calves with rectal temperatures $\geq$ 40 C were deemed "non-responders" and a four day course of intramuscular trimethoprim-sulfadoxine (TMS) (Trivetrin, Coopers Agropharm, Ajax, Ontario, Canada) at a dose of 16mg (combined)/kg body weight was initiated. Cases that relapsed following a prior course of treatment were given TMS at the same dose for four days.

Samples for microbiological evaluation were obtained from the upper airway using a guarded nasopharyngeal swab (NS) and from the lung by bronchoalveolar lavage (BAL) (11). The BAL procedure was performed standing, under chute restraint without sedation. A flexible fibreoptic endoscope was passed transnasally and wedged in the right apical lobe bronchus where lavage was performed with two aliquots of 120 mls of phosphate buffered saline. In order to minimise bacterial contamination of the endoscope in the upper airway, it was covered by a sterile plastic sheath which could be removed once it was positioned in the trachea.

Nasopharyngeal swabs and BAL fluid sediment were plated onto heart brain infusion blood agar and incubated at 37 C in 5% CO2 for 48 hours, and onto MacConkey's agar and a selective medium for Haemophilus somnus which were incubated at 37 C in room air. Bacterial colonies were identified using standard bacteriological procedures and quantified as scant, (1-9 colonies) moderate, (10-30) and large (>30 colonies) numbers. Pasteurella spp. isolates underwent antibiotic sensitivity testing by the disk diffusion method. The same samples were cultured for mycoplasmas and colonies were identified by immunofluorescence.

Sampling protocol

On the first day that a calf was determined to be a case (day 1) a NS and BAL were performed. Controls were sampled in the same manner. Cases that responded to penicillin treatment were subjected to the same sampling procedures on days 6 and 12 (ie. 2 and 7 days after finishing the course of antibiotics). Calves deemed "non-responders" on day 3 were resampled at that time and again on days 8 and 13 (ie. 2 and 7 days after finishing TMS therapy). For sampling purposes any animal that relapsed was regarded as a new case and re-entered the sampling protocol.

RESULTS

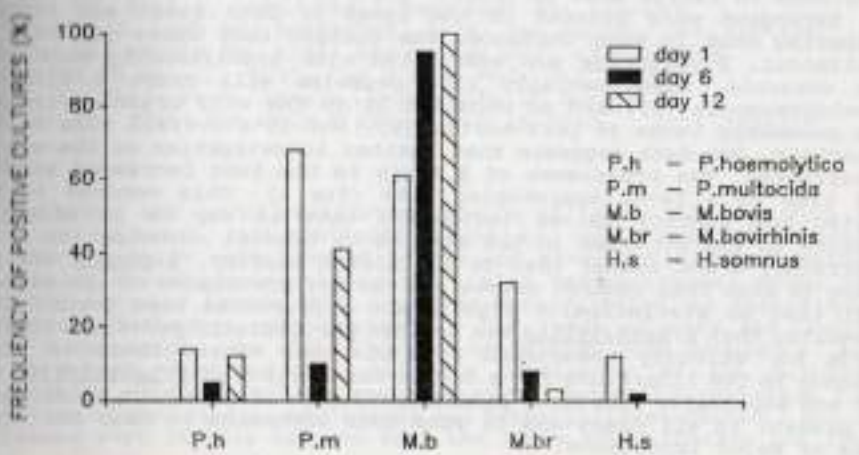
The observed morbidity and mortality rates in this group of calves were 43% and 0% respectively. Samples were obtained from 59 cases and 60 controls. The bacteriologic culture results of all day 1 samples are shown in Table 1. Differences between the isolation rates of various pathogens from cases and controls were evaluated by contingency table chi square analysis. Pasteurella multocida was isolated more frequently from the nasopharynx ($p < 0.05$) and the lung ($p < 0.01$) in cases than controls. Pasteurella haemolytica was more prevalent in nasopharyngeal swabs from controls than cases ($p < 0.05$). Using the Mantel-Haensel procedure, differences in isolation rates from cases and controls were tested for each organism while controlling for the presence of the others. P.multocida was present in BAL fluid from cases more often than controls ($p < 0.05$). No other significant ($p < 0.05$) differences were found. The results of BAL cultures from cases before (day 1), and after

Table 1. Bacteriologic Culture Results in Cases (n=59) and Controls (n=60) on Day 1 (Pre-treatment).

Organism	Number of Positive Isolations			
	Nasopharyngeal Swab		Bronchoalveolar Lavage	
	Control	Case	Control	Case
P. haemolytica	19 (6)*	9 (6)*	7 (6)*	8 (6)*
P. multocida	28 (21)	41 (35)	26 (21)	40 (35)
M. bovis	26 (21)	27 (24)	31 (21)	36 (24)
M. bovirhinis	21 (13)	19 (14)	20 (13)	19 (14)
H. somnus	4 (3)	7 (3)	3 (3)	7 (3)
Streptomyces spp.	2 (2)	0	14 (2)	7
Neisseria spp.	13 (2)	11	2 (2)	0
Bacillus spp.	0	0	2	1

* numbers in parentheses indicate number of calves in which the organism was isolated from both NS and BAL.

FIGURE 1. FREQUENCY OF POSITIVE CULTURES IN CASES BEFORE (day 1) AND AFTER (days 6 AND 12) TREATMENT FROM BAL FLUID.



(days 6 and 12), treatment (Fig 1) show the effects of therapy on the pulmonary flora.

Agreement between NS and BAL culture results for Pasteurella spp., mycoplasmas and H.somnus was determined using the kappa statistic. The summary kappa for all samples on day 1 was 0.53. There was no difference between cases and controls. Kappa values for cases only on days 6 and 12 (after treatment) were 0.43 and 0.46 respectively. Kappa values were also calculated when BAL cultures were only regarded as positive if organisms were isolated in large numbers. In these instances, kappa was 0.38 for all samples on day 1 and 0.27 and 0.18 for cases on days 6 and 12. When isolation rates of different organisms from NS and BAL samples were compared at the group level using McNemar's chi square, no significant differences were identified.

Antimicrobial resistance was encountered only amongst P. haemolytica isolates, and when present was always to a combination of penicillin, ampicillin and tetracycline. Resistant strains were only isolated from cases and were more prevalent after (12/13) than before (2/8) treatment. Antimicrobial sensitivity patterns were the same for NS and BAL isolates within the same animal. Of the 59 cases treated only 5 did not respond to penicillin. Only M.bovis was isolated from BAL fluid in these calves. Eleven cases relapsed (three of which were originally non-responders). M.bovis was isolated from BAL fluid obtained at the time of relapse in all instances. P.multocida and P.haemolytica were isolated from five and two calves respectively.

DISCUSSION

Of the organisms isolated from the upper and lower airways of these calves P.haemolytica, P.multocida, M.bovis and H.somnus are recognised bovine respiratory pathogens (12). Previous attempts to demonstrate an etiologic role for these agents in UBRD have utilised upper airway cultures and serologic evidence (6) as a measure of whether an animal was or had been infected with a particular organism. Using the BAL technique to sample sick and clinically normal calves we were able to evaluate the microbial flora present in the area of lung sampled. The right apical lobe bronchus was chosen as the sampling site as most bacterial pneumonias in calves show an antero-ventral distribution.

Pathogens were present in the lungs of both cases and controls indicating that in many instances the changes they cause (if any) are subclinical. P.multocida was associated with significantly more cases than controls. Experimentally this organism will cause a fibrinous bronchopneumonia (3), and on occasion it is the only organism isolated from pneumonic lungs at post-mortem (8), but it's overall role in UBRD is unclear. Our data suggests that further investigation of the subject is warranted. The prevalence of M.bovis in the lung increased steadily with time to a level approaching 100% (Fig 1). This occured in both treated and control calves indicating that it may be an effect of exposure to the organism in the feedlot or gradual colonisation of the respiratory tract rather than to antibiotic therapy. H.somnus was more common in sick than control calves but the low prevalence of the organism meant that no statistically significant differences were found. It is noteworthy that P.haemolytica was neither particularly prevalent in these calves nor strongly associated with clinical cases. There is strong evidence in the literature for a causal association of P.haemolytica with UBRD and especially fibrinous pneumonia (9,10). However, it is certainly not present in all cases and in some UBRD outbreaks it does not appear to be of major importance.

Streptomyces spp. and Bacillus spp. are common inhabitants of the feedlot environment and are therefore frequently found in the bovine respiratory tract. Neisseria spp. are commensals of the nasopharynx and in our study they were isolated from 24 NS samples but were only recovered twice from BAL fluid indicating that there was minimal contamination of BAL samples by organisms from upper airways.

Comparisons of NS and BAL culture results were perfomed to evaluate the utility of NS cultures in predicting the presence of organisms in the lung. Kappa values were calculated to determine the level of agreement between the two culture techniques at the individual animal level. Kappas of <0.4, 0.4-0.6 and >0.6 reflect poor, moderate and good agreement respectively, thus NS and BAL samples show only moderate agreement at best and it decreases once animals have been treated. When NS and BAL results were compared at the group rather than the individual animal level the results were not significantly different indicating that NS samples can be reliably used for diagnostic or research purposes to predict the pulmonary microbial flora in a group of calves.

Figure 1 shows that bacteria but not mycoplasmas are largely cleared from the lung following treatment (day 6) but later some recolonisation by bacteria occurs. Antimicrobial resistance to P.haemolytica occured frequently following treatment and the resistance pattern found has been reported in previous work (2). In the field, lack of response to treatment and relapses are frequently blamed on the presence of resistant strains of organism and on failure of the antibiotic to clear organisms from the lung. In this study, however, this was not always the case, suggesting that other factors exist to explain the persistance or recurrance of clinical signs.

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SUMMARY

The upper and lower respiratory tracts of feedlot calves with (59 cases) and without (60 controls) signs of undifferentiated bovine respiratory disease were sampled before and after antibiotic treatment (penicillin, trimethoprim-sulfadoxine) using nasopharyngeal swabs (NS) and bronchoalveolar lavage (BAL). Samples were cultured for bacteria and mycoplasmas. Pathogens were present in the lungs of sick and control calves. P.multocida was significantly associated with morbidity. At a group level NS cultures were reliable predictors of BAL cultures. P.haemolytica isolated after treatment were frequently resistant to a combination of penicillin, ampicillin and tetracyclines. Bacteria but not mycoplasmas were largely cleared from the lungs after therapy but later

sone recolonisation occured. Treatment failure was seldomly associated with antimicrobial resistance.

ZUSAMMENFASSUNG

Mikrobiologische Befunde (Bakteriologie; Mycologie) von Probeentnahmen vom oberen Respirationstrakt (Pharynxtupfer [PT]) und von der Lunge (Bronchoalveolar Lavage [BAL]) von Mastkaelbern mit (n=59) und ohne (n=60; Kontrolle) klinischen unspezifischen Luftwegserkrankungen wurden verglichen in beiden Gruppen vor und nach Antibiotikabehandlung der erkrankten Tiere. Pathogene Keime wurden in beiden Gruppen haeufig gefunden. P. multocida waren klar assoziiert mit erhoehter Morbiditaet. Mikrobiologische Befunde der PT-Tiere korrelierten sehr gut mit BAL-Resultaten. In den Faellen wo P.hemolytica nach Antibiotikabehandlung diagnostiziert, wurde eine erhoehte multiple Antibiotikaresistenz festgestellt (Penicillin; Ampicillin; Tetrazykline). Behandlung mit Antibiotika eliminierte die potentiellen pathogenen Keime sehr gut, nicht aber die Mycoplasmen. Gelegentlich wurde Rekolonisation festgestellt. Ein Behandlungsmisserfolg im Einzeltier war nicht auf Antibiotika Resistenz zurueckzufuehren.

SOMMAIRE

Une étude d'échantillonnage bactériologique du système respiratoire supérieure et inférieure fut entreprise chez des bouvillon a l'engraissement avec ou sans des signes cliniques de maladie respiratoire non différenciée. Les spécimens furent obtenu du nasopharynx pour les voies respiratoires supérieure et par lavage bronchoalveolaire pour les voies respiratoires inférieures. Les échantillons ont été soumit pour la culture bactériologique et la culture du mycoplasme. Les agents pathogènes ont été isolés en grand nombres dans les poumons des bouvillons contrôles et malades. Les organismes P.multocida était grandement associe aux tour de morbidité. Si on regarde les résultats des cultures nasopharyngiennes des bouvillons comme groupe, ces derniers semblent avoir des valeurs predictives très comparables aux cultures du lavage bronchoalveolaire. Suivant le traitement, le P.heamolytica isole était très fréquemment résistant au combinaison de pénicilline, ampicilline et tétracycline. De plus, après le traitement, les bactéries étaient en grand partie éliminées du poumon mais non les mycoplasmes. On doit ajouter d'ailleurs qu'il y avait une recolonisation du poumon par les bactéries, dans les jours suivant le traitement. Finalement, une mauvaise réponse du traitement n'était pas associée a une résistance antimicrobienne.

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ETUDE DE L'INTERVENTION DE LA SEROTONINE EN TANT QUE FACTEUR PATHOGENIQUE POTENTIEL DANS LES MALADIES RESPIRATOIRES DES BOVINS

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INTRODUCTION

Les jeunes bovins sont particulièrement enclins aux maladies respiratoires. Les pertes zootechniques qui en découlent ont une importance économique considérable pour la spéculation bovine en général et justifient les efforts réalisés pour élargir notre arsenal thérapeutique dans la lutte contre ce fléau. Si l'antibiothérapie est, et restera, la pierre angulaire de toute thérapeutique dans ce domaine, l'utilisation simultanée des antiinflammatoires est, par contre, discutée. En effet, les effets secondaires des stéroïdes d'une part et l'inconstance des résultats obtenus par les non-stéroïdes d'autre part, suggèrent plus de questions que de réponses. A cet égard, il faut souligner combien le manque de spécificité d'action des antiinflammatoires usuels, stéroïdiens ou non, contraste avec la remarquable diversité des médiateurs présents au niveau des poumons. Une meilleure connaissance du rôle individuel de chacun de ces médiateurs dans les processus physiopathologiques de l'inflammation pulmonaire pourrait permettre d'isoler l'un ou l'autre d'entre eux qui serait plus spécifiquement impliqué dans le développement des altérations fonctionnelles survenant au cours des maladies respiratoires des bovins. Il va de soi que l'aboutissement d'une telle démarche pourrait offrir de nouvelles approches thérapeutiques plus efficaces des pathologies respiratoires des bovidés. Des études récentes de l'activité de la bradykinine (10) et des antagonistes de l'histamine (12) chez les bovins ont révélé que ces deux médiateurs n'ont qu'un impact mineur sur leur fonction pulmonaire. Par contre, la 5-hydroxytryptamine (5-HT, sérotonine) a exercé *in vitro* des propriétés spasmogènes au niveau des vaisseaux pulmonaires et des muscles trachéal et bronchique (9). L'objet de la présente étude était d'étudier *in vivo* l'effet de la sérotonine sur la fonction pulmonaire des bovins.

MATERIEL ET METHODES

Animaux

Six veaux de race Frisonne, âgés de 7 mois et pesant 210±15 kg ont été utilisés dans cette étude. Un examen clinique minutieux, pratiqué le jour de l'expérimentation, a permis d'exclure la possibilité d'une éventuelle pathologie respiratoire ou cardiaque. Les animaux avaient été habitués aux conditions et équipements du laboratoire et avaient subi une période de jeûne de 12 heures avant l'expérimentation. Un cathéter (16G, Vygon, Belgium) avait été placé dans une jugulaire pour permettre l'injection de la solution contenant le médiateur (Imed Ltd 960 volumetric infusion pump, England).

Mesure des *paramètres*

Le débit de l'air (V) était mesuré à la bouche grâce à un pneumotachographe de Fleisch (n°3) adapté à la tête de l'animal via un masque facial testé pour son étanchéité et pour son espace mort (3). L'intégration du débit en fonction du temps fournissait le volume tidal (Vt). Les procédures de calibration étaient répétées avant et après chaque expérience avec un rotamètre pour le débit et en envoyant des volumes connus d'air au travers de la tête de Fleisch pour le volume. La pression oesophagienne (Pes) était mesurée au moyen d'un cathéter dont l'extrémité distale, munie d'un ballonnet, était positionnée dans l'oesophage selon la méthode préconisée antérieurement pour estimer de manière fiable et reproductible la pression pleurale chez les bovins (4). Une ouverture pratiquée dans le masque près des narines permettait, via un deuxième cathéter, la mesure de la pression dans le masque (Pb). Les deux cathéters étaient reliés à deux transducteurs de pression (Bentley Trantec m-600, ACEC, Belgium), les deux systèmes de mesure des pressions étaient calibrés grâce à une colonne d'eau et leur réponse était linéaire entre -50 et + 50 cmH2O. D'autre part, la compatibilité de phase entre le pneumotachographe et les

transducteurs de pression était complète jusqu'à 6 Hz. La pression transpulmonaire (Ptp) était obtenue par soustraction électrique de Pes à partir de Pb. Tous les signaux étaient analysés par un ordinateur qui calculait directement la résistance pulmonaire totale (RL), la compliance pulmonaire dynamique (Cdyn), Vt, la fréquence respiratoire (FR) et le volume minute (VE). Les valeurs "cycle par cycle" de ces paramètres étaient stockées et moyennées au fur et à mesure sur 5 cycles respiratoires réguliers et sans artéfacts.

Epreuves de provocation à la sérotonine

Le chlorhydrate de sérotonine a été utilisé et les doses mentionnées ci-dessous sont exprimées en sérotonine-base. Le ballonnet oesophagien et le masque facial étaient mis en place au moins 10 minutes avant tout test fonctionnel pulmonaire afin que l'animal puisse s'habituer calmement à l'équipement. Au cours de chaque expérience, des valeurs de contrôle de RL, Cdyn, Vt, FR, et VE étaient d'abord mesurées avant et pendant la perfusion d'un soluté physiologique. Ensuite, une perfusion continue de sérotonine (0.050 $mg.kg^{-1}.min^{-1}$) était mise en route et prolongée pendant 5 minutes. Les paramètres mentionnés ci-dessus étaient alors récoltés 1, 2, 3, 4, 5, 10, 15 et 20 minutes après le début de la perfusion. Une semaine plus tard, une épreuve de provocation à la sérotonine identique a été réalisée chez deux animaux 1 heure après administration d'un bloqueur spécifique des récepteurs S2 par voie intramusculaire.

RESULTATS

Paramètres cliniques

Les veaux ont bien toléré le stress expérimental et aucune expérience n'a dû être interrompue suite aux réactions des animaux. L'administration continue de soluté physiologique n'a modifié aucun des paramètres fonctionnels retenus. La réponse à la sérotonine a été similaire chez tous les animaux et a consisté en une congestion des conjonctives, une lacrymation, une toux modérée et une tachypnée sévère. La perfusion de 5-HT a également été suivie d'une émission de selles liquides chez 5 animaux environ 4 minutes après la fin de l'injection.

Paramètres fonctionnels respiratoires

L'évolution des paramètres fonctionnels respiratoires pendant et après la perfusion est illustrée par la figure 1. Aucun des paramètres étudiés n'a été significativement modifié par l'administration de sérum physiologique tandis que la sérotonine a profondément altéré aussi bien la ventilation que la mécanique ventilatoire : (1) VE était fortement augmenté, principalement au niveau de l'augmentation de FR tandis que Vt n'était pas modifié, (2) Cdyn était réduite à environ un cinquième de sa valeur initiale et (3) RL était doublée. Tous les paramètres ont récupéré rapidement leur valeur de base après la fin de la perfusion. Après blocage des récepteurs S2 chez deux animaux, le challenge à la sérotonine n'a modifié aucun des paramètres cliniques et fonctionnels.

DISCUSSION

Les valeurs de contrôle des paramètres de la ventilation et de la mécanique ventilatoire mesurées dans notre étude ne diffèrent pas des valeurs de référence publiées pour des animaux de la même race et de la même taille (5). De plus, et contrairement à ce qui a souvent été rapporté, nous n'avons pas observé de grande variabilité dans les mesures des divers paramètres choisis au cours de l'épreuve de provocation à la sérotonine. Ce résultat a pu être obtenu grâce à une standardisation sévère de l'expérimentation tant au niveau des animaux (état sanitaire, race, âge, apprentissage, jeûne) qu'au niveau des techniques de mesure des paramètres ventilatoires ou des méthodes d'administration du médiateur (dose, vitesse, durée).

Les seules données disponibles au sujet de la sérotonine chez les bovins renseignaient son activité *in vitro* sur la contraction des muscles lisses des vaisseaux pulmonaires et des bronches (9). D'autres expériences avaient montré que

chez le veau éthanol anesthésié, elle modifiait de manière significative le volume minute (1). Par contre, l'effet de l'administration de sérotonine exogène sur la mécanique ventilatoire des bovins n'avait jamais été étudié jusqu'ici.

L'augmentation du volume minute que nous avons observée et qui était principalement due à de la tachypnée confirme les données de la littérature. Cet effet pourrait être lié à la stimulation des corps carotidiens comme cela a été démontré chez le chien et le chat (2). Cependant, l'intervention de la sérotonine dans les mécanismes de la chémoréception est contestée par d'autres études (1, 10). Par conséquent, bien que nous ayons établi que la sérotonine modifie de façon majeure la fréquence respiratoire, le mécanisme exact de ce phénomène reste à préciser.

L'effondrement de Cdyn jusqu'à environ un cinquième de sa valeur de contrôle pourrait être dû à une constriction des petites voies aériennes (1) et/ou à une ventilation inhomogène des lobules pulmonaires. De plus, la présence éventuelle d'oedème pourrait être incriminée. Cependant, la réversibilité rapide et complète des modifications de Cdyn observée ici donne plutôt à penser qu'il n'y avait ni oedème, ni hypersécrétion bronchique importante (6).

Le doublement de RL pendant la perfusion de sérotonine indique qu'une bronchoconstriction était présente aussi au niveau des voies aériennes centrales. En effet, la résistance à l'écoulement de l'air dans les voies aériennes périphériques intervient seulement à raison de 10 à 20% dans les variations de RL (7).

Donc, les modifications simultanées et en sens inverse de RL et Cdyn suggèrent un rétrécissement des voies aériennes centrales et périphériques, probablement causé par une bronchoconstriction diffuse. Ces résultats *in vivo* sont à mettre en parallèle avec les études menées antérieurement *in vitro* qui avaient mis en évidence l'action stimulante de la sérotonine sur la contraction du muscle lisse trachéal, bronchique et bronchiolaire chez les bovidés.

Notre étude préliminaire du blocage des récepteurs S2 suggère que les altérations fonctionnelles observées lors des challenges à la sérotonine sont probablement dues à la liaison de celle-ci aux récepteurs S2. Ce résultat ouvre de nouvelles voies de recherche dans le domaine de la physiologie des médiateurs dans le poumon des bovins.

Les résultats de la présente étude indiquent que la 5-HT modifie de manière importante et spontanément réversible la ventilation et le calibre des voies aériennes des bovidés. Par conséquent, si elle devait être libérée en quantités accrues ou si sa clairance était diminuée au cours des pathologies respiratoires de ces animaux, la sérotonine pourrait être responsable, en partie, du développement des altérations fonctionnelles observées.

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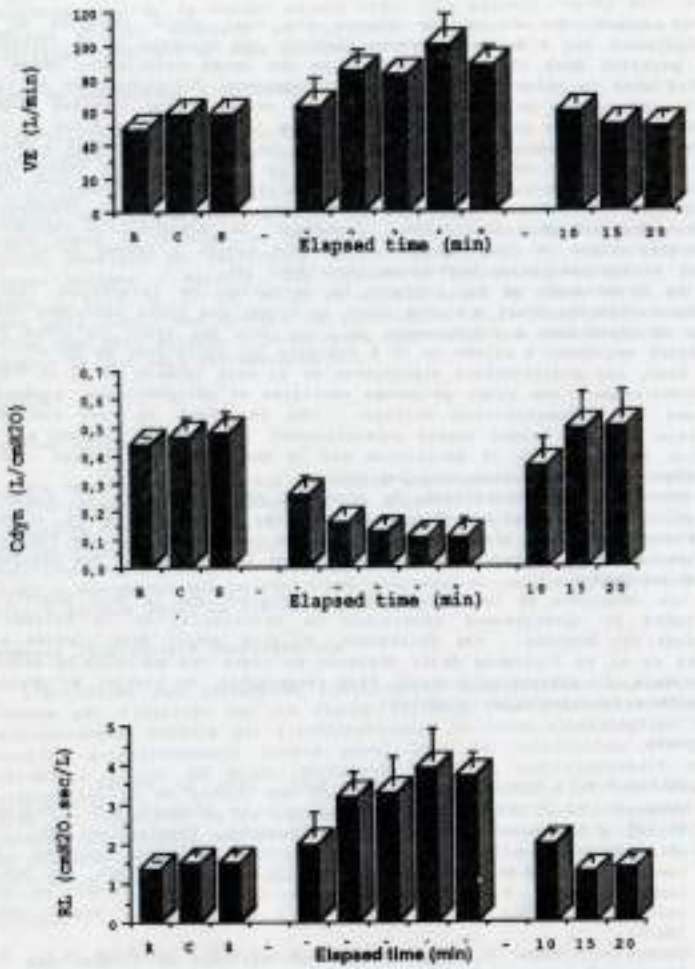

Fig. 1 : *Evolution du volume minute (VE), de la compliance pulmonaire dynamique (Cdyn) et de la résistance pulmonaire totale (RL) pendant et après une perfusion de 5-hydroxytryptamine chez 6 veaux.. (R) : Valeur de référence, (C) : valeur de contrôle et (S) : effet d'un soluté physiologique.*

RESUME

Quoique l'impact considérable de la sérotonine (5-HT) sur l'hémodynamique pulmonaire des bovins ait été décrit depuis longtemps, son influence éventuelle sur la fonction respiratoire n'a jamais, à notre connaissance, été testée *in vivo* dans cette espèce.

Six veaux de race Frisonne, âgés de 7 mois et pesant 210±15 kg, ont été soumis à une perfusion intraveineuse de 5-HT (0,05 mg.kg^{-1}.min^{-1}) pendant 5 minutes (min).

Les animaux étaient équipés d'un masque facial testé pour son étanchéité et son espace mort. Le débit aérien était mesuré grâce à un pneumotachographe tandis qu'un ballonnet installé dans l'oesophage de manière rigoureusement standardisée permettait une mesure fiable de la pression pleurale. Les valeurs de la fréquence respiratoire (FR), du volume tidal (Vt), de la compliance dynamique (Cdyn) et de la résistance pulmonaire totale (RL) mesurées au repos furent respectivement les suivantes : 28±1 min^{-1}, 2,3±0,2 L, 0,55±0,02 L.cmH2O^{-1} et 1,5±0,2 cmH2O.L.sec^{-1}. Ces paramètres n'ont subi aucune modification suite à la perfusion d'un soluté physiologique. Pendant la perfusion de 5-HT, les animaux ont significativement augmenté leur volume minute grâce, surtout, à une augmentation de FR. Les valeurs de Cdyn mesurées 1, 2, 3, 4 et 5 min après le début de la perfusion furent les suivantes: 0,26±0,04, 0,19±0,02, 0,15±0,02, 0,13±0,01 et 0,15±0,05 L.cmH2O^{-1}. Dans le même temps, les valeurs de RL évoluaient en sens inverse : 1,5±0,2, 2±0,3, 3,2±0,6, 3,9±0,5 et 3,7±0,6 cmH2O.sec.L^{-1}respectivement. Tous les paramètres mesurés ont récupéré leur valeur basale 8 min après la fin de la perfusion.

Nous concluons que l'administration de 5-HT chez le veau induit une respiration superficielle et modifie profondément leur mécanique ventilatoire. En admettant que les modifications de Cdyn résultent du changement de calibre des petites voies aériennes alors que les forces résistives sont surtout influencées par le calibre des grosses voies, nous pensons que les évolutions simultanées et opposées de Cdyn et RL rendent compte d'un bronchospasme localisé à la fois aux petites et aux grosses voies aériennes. L'hypothèse de l'intervention de la 5-HT dans le développement des altérations fonctionnelles liées aux maladies pulmonaires chez les bovins nous semble pouvoir être posée.

SUMMARY

Though 5-hydroxytryptamine (5-HT, serotonin) has been reported to be a potent agent in altering the bovine pulmonary hemodynamics, its effects on lung mechanics in the bovine species have not been reported so far. This study deals with the respiratory response of normal calves to 5 minutes perfusion of 5-HT.

Six healthy Friesian calves, 7 months of age and weighing 210±15 kg, were used for this study. All animals were studied in a quiet state when wearing a snugly-fitting face mask. A pneumotachograph (Fleisch n°3) produced a signal proportional to flow which was integrated with respect to time to give tidal volume (Vt). A hole made in the mask allowed the record of mouth pressure (Pm) and a balloon-catheter-transducer system allowed measurement of esophageal pressure (Pes). Airflow and transpulmonary pressure (Pm-Pes) were analysed continuously by a Deres computer (ACEC, Belgium) which calculated total lung resistance (RL), dynamic lung compliance (Cdyn), Vt and respiratory rate (RR). The mean of 5 successive regular respiratory cycles was used for these calculations. Following determination of a base-line value at rest and during intravenous perfusion of saline for all the studied parameters, a solution of serotonin hydrochloride was infused at a rate of 0.05 mg.kg^{-1}.min^{-1} for 5 minutes, using a variable speed infusion pump. RR, Vt, RL and Cdyn values were determined every minute from the beginig of the infusion up to 20 minutes after. Resting values of RR, Vt, Cdyn and RL were 28±1 min^{-1}, 2,3±0,2 L, 0,55±0,02 L.cmH2O^{-1} and 1,5±0,2 cmH2O.L.sec^{-1}. These parameters were not altered by perfusion of saline. There was significant modification of both Cdyn and RL, the Cdyn decreasing and RL increasing, as serotonin was infused. The values recorded for Cdyn at 1, 2, 3, 4 and 5 minutes of perfusion were 0,26±0,04, 0,19±0,02, 0,15±0,02, 0,13±0,01 and 0,15±0,05 L.cmH2O^{-1} respectively, whereas the values of RL varied in the opposite direction : 1,5±0,2, 2±0,3, 3,2±0,6, 3,9±0,5 et 3,7±0,6 cmH2O.sec.L^{-1} respectively. Minute volume was greater during perfusion than in basal conditions, mainly due to an increase in RR. All the parameters returned to pre-injection level 8

minutes after end of infusion. Our data indicate that intravenous serotonin induces mainly a bronchospasm located in both large and small airways. Indeed, the prompt and complete reversibility of pulmonary function values after end of administration suggest that no edema formation has occurred and, therefore, cannot be responsible for the drop in Cdyn. We hypothesize that 5-HT could be an important mediator of lung injury in calves and could be responsible, in part, for the changes in lung function observed in some bovine pulmonary diseases.

ZUSAMMENFASSUNG

Obwohl der gewichtige Einfluß von Serotinin (5-HT) auf die Lungenhaemodynamik der Rinder schon seit langem beschrieben worden ist, wurde - so viel wir wissen - seine mögliche Wirkung auf die Atemfunktion in dieser Rinderart nie untersucht. Sechs 7 monatige Kälber friesischer Herkunft mit einer Körpermasse von durchschnittlich 210±15 kg wurden mit einer 5 minütigen intravenösen Perfusion von 5-HT (0,05 $mg.kg^{-1}.min^{-1}$) behandelt. Die Tiere waren mit einer speziell auf Luftabdichtung und totem Raum geprüften Atemmaske ausgerüstet. Die Atemstromstärke wurde mit einem Pneumotachographen gemessen, während eine auf eine streng vergleichbare Art und Weise in der Oesophagus eingeführte Ballonsonde eine zuverlässige Messung des intrathorakalen Druckes im Mediastinum ermöglichte. Die in Ruhelage ermittelten Werte der Atemfrequenz (Ff), des Atemvolumens (Vt), der Lungendehnbarkeit (dynamisches Compliance = Cdyn) und des Atemwegwiderstandes (RL) lauteten jeweils wie folgt : 28 ± 1 min^{-1} , 2.3 ± 0.2 L, 0.55 ± 0.02 $L.cmH2O^{-1}$ und 1.5 ± 0.2 cm $H2O.sec.L^{-1}$. Die Perfusion von physiologischem Wasser übte keinen Einfluß auf diese Daten aus. Während der Perfusion von 5-HT nahm das Atemminutenvolumen der Tiere bedeutend zu, was vor allem auf eine Erhöhung der Atemfrequenz (Ff) zurückzuführen ist.
Die Werte der Lungendehnbarkeit (Cdyn), die 1, 2, 3, 4 und 5 Minuten nach Anfang der Perfusion ermittelt wurden, lauteten wie folgt : 0.26 ± 0.06, 0.19 ± 0.02, 0.15 ± 0.02, 0.13 ± 0.01 und 0.15 ± 0.05 $L.cmH2O^{-1}$. Gleichzeitig entwickeltes sich die Werte des Atemwegwiderstandes (RL) in entgegengesetzter Richtung : 1.5 ± 0.2, 2 ± 0.3, 3.2 ± 0.6, 3.9 ± 0.5 und 3.7 ± 0.6 cm $H20.sec.L^{-1}$. 8 Minutes nach Beendigung der Perfusion waren alle gemessenen Parameter auf ihren jeweiligen Grundwert zurückgegangen. Aus dieser Untersuchung läßt sich ersehen, daß die Verabreichung von 5-HT zu einer oberflächlichen Atmung bei den Kälbern führt und tiefgreifende Änderungen in ihrer Atemmechanik bewirkt. Angenommen, daß die Änderungen in der Lungendehnbarkeit durch Modifikationen im Durchmesser der kleinen Atemwege eingeleitet werden, während der Atemwegwiderstand vor allem vom Durchmesser der großen Atemwege beeinflußt wird, vermuten wir daß die gleichzeitigen und entgegengesetzten Entwicklungen in den Werten der Lungendehnbarkeit und des Atemwegwiderstandes von einem Bronchospasmus der kleinen und großen Atemwege zugleich zeugt. Unserer Meinung nach kann die Hypothese aufgestellt werden, daß 5-HT einen Einfluß ausübt in der Entwicklung von funktionellen Änderungen wie sie in Rinder-Atemkrankheiten vorkommen.

SEVERE OUTBREAKS OF RESPIRATORY DISEASE IN DAIRY HERDS CAUSED BY BOVINE RESPIRATORY SYNCYTIAL VIRUS

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INTRODUCTION

Bovine respiratory syncytial virus (BRSV) is a nonhemagglutinating pneumovirus of the paramyxovirus family. The virus was named for the characteristic cytopathic effect it produces in tissue culture, that is, the formation of syncytial cells. A syncytium is a multinucleated mass of protoplasm produced by the merging or fusion of cells. In addition to cattle, respiratory syncytial virus have been isolated from sheep and goats (5, 6). The human respiratory syncytial virus has been recognized since 1950s and is currently considered to be the major cause of bronchiolitis in infants and young children (1). Bovine respiratory syncytial virus was first isolated from cattle with respiratory disease in Switzerland in 1970 (7). In the United States it was first demonstrated in 1974 (2). Since then, respiratory tract disease associated with BRSV has been reported from many countries. In Sweden a severe outbreak of respiratory disease occurred in dairy herds, throughout the country, during 1988/89. Incidences of the disease were reported from local veterinarians in many parts of the country during the same period of time (3). The onset of respiratory distress had been sudden and deaths among the cows occurred within a couple of days in a number of herds. A significnt rise in body temperature, cough, subcutaneous emphysema and a marked drop in milk production were also recognized in affected cows. This paper presents serological evidence that these outbreaks of respiratory disease in the dairy herds was caused by BRSV and also shows that it is possible to use an ELISA for detection of BRSV antibodies in bulk milk.

MATERIALS AND METHODS

Sampling procedure

Blood samples were obtained from six herds, four to eight cows per herd in the acute phase of respiratory disease and 3 to 4 weeks later during the convalescense period. Bulk milk samples were, with the assistance of local veterinarians, collected from 21 herds with severe clinical symtoms of respiratory disease and 15 apparently healthy herds within the same area in different parts of the country.
The blood samples were withdrawn from the jugular vein using evacuated tubes (Becton-Dickingson). Bulk milk was collected in 10 ml plastic tubes.
The skim milk was collected from below the fat layer after centrifugation of whole milk for 10 min at 3000 x g (4). Blood samples were centrifugated and the serum was removed. The skim milk and the sera was stored at -20°C until analysed.

Serology

An indirect enzyme-linked immunosorbent assay, ELISA, applying a monoclonal antibody (MAb) to bovine immunoglobulin IgG, was used for detection and titration of IgG antibodies to BRSV in serum and skim milk*. Serum with a mean absorbance value above 0,08 was regarded as positive for antibodies to BRSV. The serum samples were assayed in five-fold dilutions in PBS starting from 1:10.

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The titres were estimated as the highest dilution giving an absorbance value above 0,08. In the table the results are expressed as the reciprocal values of this dilution. Positive and negative control samples of both sera and skim milk were always run in parallel with the test samples.

Eight serum samples with known IgG titres were also analysed for IgM antibodies in both acute and convalescence phases of infection using an IgM ELISA**. The serum was diluted 1:100 and absorbance values >0,4 was according to the instructions regarded as positive.

RESULTS and DISCUSSION

The clinical signs of respiratory disease were very similar in all reported cases though herds in the south of Sweden didn't experience as severe symptoms as herds further north. A sudden onset of respiratory distress was a common feature with high fewer, severe dyspnoea, salivation and a marked subcutaneous emphysema. Most farmers experienced a considerable drop in milk production. A great number of cows were treated with antibiotics and corticosteroids. In spite of treatment occasional deaths occurred in some herds. The financial losses were in many cases considerable due to the decrease in milk production, the cost of medicine and the loss of dead animals.

The clinical symptoms and the pathological findings at post-mortem examination indicated that respiratory syncytial virus was the cause of the disease. The diagnosis of BRSV infection was made by comparison of paired acute and convalescent serum antibody titres.

A signifikant rise in IgG antibody titres were seen in the majority of tested samples (table 1). It is important to collect the first blood sample early in the infection since the antibody levels increases very rapidly, as seen in herd nr 6, table 1, where the first samples were collected a week after the acute phase. Sera from these six herds were also tested for antibodies to bovine virus diarrhoea virus, parainfluenza-3 virus and coronavirus but without significant seroconversions (data not shown).

Furthermore, acute and convalescence sera from eight animals who had shown a significant rise in IgG antibody titres were tested for IgM antibodies as shown in table 2. All eight sera were positive for IgM antibodies in the acute sera whereas no signifcant IgM antibody titres were found in the convalescence sera.

A BRSV outbreak of this magnitude has not been experienced in Sweden before and it was therefore important to chart the prevalence of the infection.

It has been shown that antibodies against a number of viruses eg BVDV, IBR and BLV can be detected in milk by using the indirect ELISA technique applying a monoclonal antibody (Mab) to bovine immunoglobulin IgG (4). The method has been shown suitable both for individual milk samples as well as bulk milk, which can serve as a combined sample from all milking cows in the herd. As seen in table 3 this method also proved to be useful for analyses for antibodies to BRSV in bulk milk. It was a highly significant difference between the absorbance value in bulk milk from diseased and healthy herds. For a country like Sweden which hasn't experienced the infection to any higher extent earlier it's a very suitable method to screen for the prevalence of respiratory syncytial virus infection on a herd basis. This study showed that the numerous outbreaks of respiratory disease in dairy herds in Sweden during 1988-89 were caused by BRSV. The economical consequences of the outbreaks were of the magnitude that it would be of great interest to evaluate the effect of vaccines against BRSV in dairy cows.

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Table 1

Serum IgG antibody titres to BRSV in six herds with a history of severe respiratory disease. Both acute (S1) and convalescence (S2) sera were examined.

Herd 1

Id nr	S1	S2
18	10	250
61	250	250
75	<10	50
76	<10	1250
87	10	250
121	50	250
164	<10	250

Herd 2

Id nr	S1	S2
343	6250	31250
75	1250	1250
574	250	6250
513	<10	6250
477	10	6250
567	1250	6250

Herd 3

Id nr	S1	S2
108	6250	6250
119	10	6250
123	<10	6250
132	<10	31250
144	1250	250

Herd 4

Id nr	S1	S2
112	250	1250
136	50	1250
267	50	50
128	250	1250

Herd 5

Idnr	S1	S2
33	1250	1250
89	>1250	>1250
125	<10	250
175	250	1250
1020	1250	<1250
178	10	250
106	1250	1250
165	<10	1250

Herd 6

Idnr	S1	S2
700	6250	6250
735	250	1250
666	1250	6250
745	1250	1250
721	1250	6250
731	1250	1250
579	31250	6250
551	6250	6250

Table 2

Eight paired serum samples from the acute (S1) and convalescent (S2) phase of BRSV infection were tested for IgM and IgG antibodies to BRSV

	IgM ELISA		IgG ELISA	
Id nr	S1	S2	S1	S2
112	0,53 (+)	0,26 (-)	250	1250
136	0,56 (+)	0,16 (-)	50	1250
423	0,44 (+)	0,36 (-)	10	1250
76	0,60 (+)	0,36 (-)	<10	1250
87	0,46 (+)	0,06 (-)	10	250
128	0,43 (+)	0,19 (-)	250	1250
53	0,49 (+)	0,05 (-)	10	250
179	0,47 (+)	0,03 (-)	10	250

Table 3

Levels of antibodies to BRSV in bulk milk samples from 21 herds with a history of severe respiratory disease and 15 unaffected healthy herds

	Mean bulk milk absorbance ±S.D.	
Dairy herds with respiratory problem n=21	0,94	±0,17
Control dairy herds n=15	0,02	±0,03

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SUMMARY

Bovine respiratory syncytial virus was shown to be the cause of outbreaks of severe respiratory illness in dairy herds throughout Sweden during 1988-89. The diagnosis was made by comparison of paired acute and convalescent serum antibody titres. An indirect enzyme-linked immunosorbent assay, ELISA, applying a monoclonal antibody (MAb) to bovine immunoglobulin IgG was used.

To screen for the prevalence of the infection on herd basis the indirect ELISA-test was also used for bulk milk samples. 21 milk samples from clinically ill and 15 milk samples from apparently healthy herds were tested. The mean absorbance value for positive herds were 0,94 whereas it was 0,02 for negative herds. It proved to be a highly sensitive and rapid method to screen for the prevalence of respiratory syncytial virus infection on a herd basis.

ZUSAMMENFASSUNG

Bovines respiratorisches syncytial - (BRS) - Virus wurde als Ursache für die Ausbreitung ernstlicher respiratorischer Krankheit in Milchbeständen in ganz Schweden während 1988-89 bewiesen.

Die Diagnose wurde durch den Vergleich von Antikörpertitern in Serumpaaren ermittelt. Ein indirekter enzymgebundener Immunabsorptionstest (ELISA) mit einem monoklonalen Antikörper gegen bovines Immunoglobulin IgG wurde benutzt.

Um die Ausbreitung der Infektion in den Beständen zu ermitteln, wurde der ELISA-test bei Tankmilch-proben angewendet. 21 Milch proben von klinisch erkrankten und 15 Milch proben von wahrscheinlich gesunden Beständen wurden geprüft. Der Mittelabsorptionswert von den positiven Beständen war 0.94 und von den negativen Beständen 0.02. Die Methode erwies sich als höchst empfindlich und schnell um die Verbreitung einer respiratorischen syncytial - Virusinfektion auf Beständebasis zu beleuchten.

RESUMEN

Se demostró que el virus respiratorio sincitial bovino fué el causante de brotes severos de enfermedades respiratorias en los establos lecheros de Suecia durante 1988-1989.

El diagnostico fué hecho comparando los titulos de anticuerpos humorales del periodo agudo con los de convalecencia. Se usó el metodo indirecto de ELISA con anticuerpos monoclonales para la inmunoglobulina IgG-bovina. Para determinar la prevalencia de la infection en los establos, se aplico el test de ELISA en pruebas obtenidas de los tanques de leche. Se testaron 21 pruebas de leche de establos con sintomas clinicos y 15 pruebas de leche de establos aparentemente libres de enfermedad. El valor promedio de absorvencia de los establos positivos fué 0.94 mientras que el de los negativos fué 0.02. El método probado es altamente sensitivo y rápido, y puede ser utilizado para determinar a nivel de establo la prevalencia de enfermedad respiratoria ocasionada por el virus respiratorio sincitial bovino.

EFFICACITE DE LA SPIRAMYCINE CHEZ LE VEAU DANS UNE BRONCHO-PNEUMONIE EXPERIMENTALE A PASTEURELLA haemolytica A_1.

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Les bactéries et en particulier Pasteurella haemolytica A_1 (PHA1) sont les agents directement responsables des lésions de bronchopneumonie fibrineuse qui caractérisent les bronchopneumonies infectieuses enzootiques des jeunes bovins (BPIE). Par là-même elles sont à l'origine de la gravité médicale et économique de ces affections considérées comme inéluctables dans la filière viande bovine (2). A ce jour, le contrôle des BPIE est basé à court terme sur l'antibiothérapie, à moyen terme sur l'antibioprévention et la vaccination, à long terme sur l'amélioration globale des conditions d'élevage. Il importe, pour limiter les pertes, de disposer d'antibiotiques actifs sur les pasteurelles et de protocoles thérapeutiques leur permettant d'exprimer pleinement leur potentiel (2). Pour approcher l'efficacité clinique de la spiramycine injectable (SUANOVIL L.A.*), il était intéressant de la confronter à l'une de ses principales cibles PHA1 dans un modèle de pasteurellose expérimentale chez le veau. On sait en effet, que les modèles d'infection expérimentale sont indispensables pour juger de la novation, qu'il s'agisse de molécules ou de stratégies d'activité originales.

MATERIEL ET METHODES (3).

Animaux et environnement : 20 veaux de race française frisonne (13 mâles et 7 femelles), âgés de 2 à 3 semaines et d'un poids compris entre 47 et 61 kg, ont été utilisés dans cet essai. Ces animaux ont bénéficié d'une période d'adaptation de 10 jours avant inoculation. Ils ont été nourris 2 fois par jour, avec un aliment d'allaitement du commerce distribué au seau.

Inoculations : les inoculations ont eu lieu par voie intranasale (cultures agitées de 2 h, titrant entre 3.7×10^7 et 1.6×10^8 UFC/ml) et par voie intratrachéale (cultures agitées de 5 h, titrant entre 8×10^8 et 1.5×10^9 UFC/ml) selon le protocole suivant :

	JOURS		
HORAIRES	J0	J+1	J+2
11 H 15	Intranasale	Intranasale	Intranasale
14 H 15	Intratrachéale	Intratrachéale	-

* SUANOVIL L.A.® : RHONE MERIEUX, FRANCE

Lots expérimentaux : les 20 veaux de l'essai ont été inoculés ; 10 n'ont pas reçu de traitement. 10 ont été traités avec SUANOVIL L.A. à la posologie de 100 000 UI de spiramycine par kg, 2 fois à 48 heures d'intervalle. Les injections ont été réalisées par voie intramusculaire ; la première a été effectuée 1 heure après l'inoculation intratrachéale de J0.

Contrôles cliniques : différents paramètres cliniques étaient enregistrés deux fois par jour, avant chaque repas, à partir du jour de l'inoculation ; ils permettent à chaque contrôle et pour chaque veau de calculer une note moyenne générale de maladie (N.M.G.) (4). Par ailleurs, les animaux ont été pesés avant l'essai, après leur mort ou à leur sacrifice.

Contrôles nécropsiques : les opérations ont été celles décrites en (3) : sacrifice, autopsie, scores pulmonaires, bactériologie.

RESULTATS :

Cliniques : 2 veaux inoculés non traités sont morts, l'un à J+2, l'autre à J+3. Aucune mortalité n'a été enregistrée dans le groupe des veaux traités avec SUANOVIL L.A. 7 veaux traités et 5 veaux non traités ont été sacrifiés à J+4, au matin. 3 veaux de chacun des groupes ont été suivis plus longtemps et sacrifiés à J+10, au matin. Dès le lendemain des premières inoculations (J+1), la note moyenne générale (N.M.G.) des veaux traités avec SUANOVIL L.A. devient très inférieure à celle des veaux inoculés non traités (figures 1 et 2). Des différences, significatives sur le plan statistique (test de MANN et WITHNEY) apparaissent à J+1, J+2, J+3 matin et J+4 matin (figure 1). Ces différences existent vraisemblablement au delà de J+4 mais le nombre trop faible d'animaux ne permet pas d'interprétation statistique. Toutefois, la figure 2 visualise des écarts importants pour ce qui est de la moyenne des N.M.G., en faveur du groupe traité avec la spiramycine. Les animaux ayant reçu SUANOVIL L.A. ont un meilleur comportement général, un appétit plus important, une fréquence respiratoire et une température rectale plus faibles par rapport aux animaux inoculés non traités ; cliniquement, la différence est très nette.

Zootechniques : le gain moyen quotidien (G.M.Q.) des veaux traités à la spiramycine est très supérieur à celui des veaux non traités sur les 4 premiers jours de l'essai : 557 g contre - 45 g. Cette différence est statistiquement significative (degré de signification : 1.8 % - Test de MANN et WITHNEY). Sur les 10 jours de l'essai, le résultat est semblable : 683 g pour les animaux traités versus 283 g pour les animaux non traités.

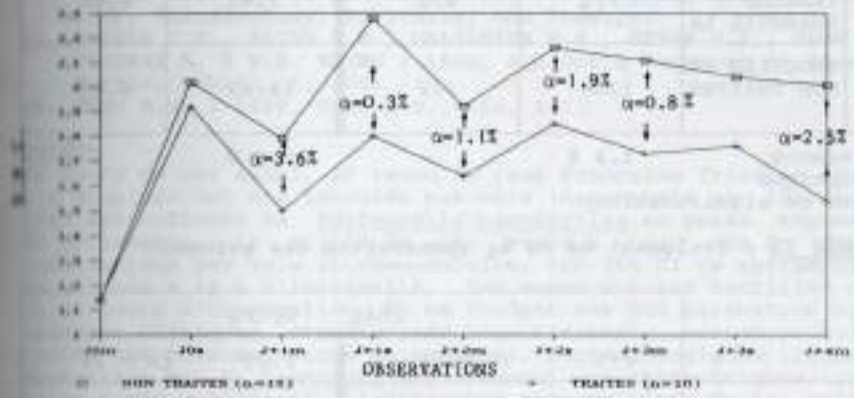
Nécropsiques : les résultats sont présentés dans le tableau I : les animaux traités avec SUANOVIL L.A. présentent nettement moins de lésions du parenchyme pulmonaire par rapport aux animaux non traités, que ce soit 4 jours ou 10 jours après le début des inoculations.

Bactériologiques : les résultats quantitatifs de recherche des PHA_1, sont rassemblés dans le tableau II.
Des PHA_1 ont été isolées en nombre important chez 30 % des animaux traités avec la spiramycine et chez 70 % des animaux non traités. Toutes les souches de PHA_1 isolées avaient une concentration minimale inhibitrice comprise entre 32 et 64 µg/ml comme la souche de l'inoculum.

DISCUSSION

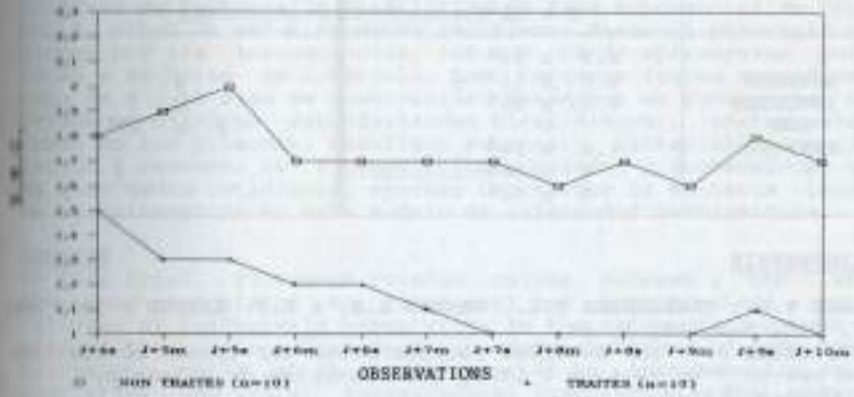
Parmi les modèles proposés pour reproduire un syndrome respiratoire comparable aux BPIE spontanées (14) le modèle utilisé paraît le plus réaliste. L'association en aérosol virus parainfluenza 3-PHA_1 est moins performante. Les associations faisant intervenir PHA_1 précédé du virus de la rhinotrachéite infectieuse bovine ou de Mycoplasma bovis, ne correspondent pas aux situations épidémiologiques les plus courantes dans notre pays (2). Par ailleurs, pour les modèles ne faisant intervenir que PHA_1 l'inoculation par les voies habituelles semble plus légitime que les injections par voie transthoracique.
Le modèle mis au point (3) et appliqué à l'étude de l'efficacité de SUANOVIL L.A. possède les caractéristiques nécessaires et suffisantes pour satisfaire cet objectif (12).
Les animaux utilisés dans cet essai sont des veaux conventionnels, âgés de 1 à 3 semaines au maximum, de race laitière. Les veaux gnotobiotiques n'apparaissant pas comme un matériel indispensable (5). Ce type d'individu a aussi été choisi par AMES et coll (1) pour l'étude de l'efficacité d'une association oxytétracycline-flunixine par rapport à la flunixine seule utilisant des veaux de 10 à 12 semaines. Dans des modèles différents, par exemple injections intra-trachéales d'acide acétique suivies de l'injection intraveineuse d'une culture de Pasteurella haemolytica en suspension dans de l'agar semi liquide ou encore association à 4 jours d'intervalle d'un aérosol de virus de la rhinotrachéite infectieuse bovine et d'un aérosol de PHA_1 (5,8), des animaux plus âgés ont été utilisés (jusqu'à 7 mois). A notre avis, des veaux de 2 à 3 semaines ont plus de chance d'être indemnes de lésions préalablement acquises de l'appareil respiratoire. De plus, comme nous l'avons vérifié en choisissant des animaux au hasard dans un centre de tri, ils ne sont pas porteurs de PHA_1 et ne possèdent pas de trace sérologique pouvant modifier leur sensiblité à l'infection (3).
Dans les études précédentes, le temps écoulé entre l'inoculation et la première administration thérapeutique varie en fonction de la sévérité de l'agression et de l'âge des animaux. L'intervalle de temps est de 1 à 2 heures dans les essais FARRINGTON et coll (8) et SELMAN et coll. (13). Le délai de 1 h adopté dans cet essai est en bonne cohérence avec les précédents et ne paraît pas représenter un facteur de biais. Les résultats de cet essai traduisent la maîtrise de la maladie respiratoire expérimentale par SUANOVIL L.A. :
- les mortalités n'ont été enregistrées que dans le groupe inoculé et non traité.
- la N.M.G. est dès J+1, régulièrement inférieure dans le groupe traité avec SUANOVIL L.A. par rapport au groupe inoculé non traité, de J+1 à J+4 matin ; l'utilisation du test statistique de MANN et WITHNEY démontre cette différence. Le traitement à la spiramycine engendre une nette amélioration des symptômes observés (comportement général des animaux, refus alimentaire, fréquence respiratoire, température rectale).
- les G.M.Q. sont supérieurs chez les animaux traités avec SUANOVIL L.A. ; la différence est significative sur le plan statistique.
- le poids des poumons et les scores pulmonaires sont plus faibles chez les animaux ayant reçu la spiramycine par rapport aux animaux non traités : de plus PHA_1 a été isolée moins souvent dans le poumon des animaux traités avec SUANOVIL L.A. L'efficacité de la spiramycine dans ce modèle de pasteurellose respiratoire expérimentale repose sur sa haute affinité pour les sécrétions bronchiques et le parenchyme pulmonaire des bovins. En effet, plus de 24 h après l'administration par voie intramusculaire de 100 000 UI de spiramycine par kg, le

rapport des concentrations dans les sécrétions bronchiques et le parenchyme est de 20 environ avec un taux dans ces sécrétions de 42.2 ± 27.2 UI/ml (6) ; plus de 24 h après la seconde injection, le rapport des concentrations dans le parenchyme pulmonaire et le plasma est de 113 avec un taux d'activité dans le tissu pulmonaire de 158.6 UI/g de poumon (7). Rappelons d'autre part, que les valeurs extrêmes des concentrations minimales inhibitrices pour PHA_1 varient entre 6.4 et 51.2 UI/ml (9,10,11).



m : matin s : soir α = degré de signification

Figure 1 : Evolution de la moyenne des N.M.G. dans les 2 groupes d'animaux de J0 à J+4 au matin.



m : matin s : soir

Figure 2 : Evolution de la moyenne des N.M.G. dans les 2 groupes d'animaux de J+4 à J+10 au matin.

TABLEAU I : Evaluation des lésions pulmonaires : poids des poumons et score pulmonaire.

		Poids des Poumons (g)		Score pulmonaire	
		J+4 (n=7)	J+10 (n=3)	J+4 (n=7)	J+10 (n=3)
ANIMAUX	INOCULES ET TRAITES SUANOVIL LA	771	692	7.43	0.67
	INOCULES ET NON TRAITES	1348	722	18.43	6.67
Différence statistique (degré de signification)		1.3 %		0.6 %	

TABLEAU II : Isolement de PH A_1 (numération des germes).

	PHA1 UFC/g					
	J+4	(n = 7)		J+10	(n = 3)	
ANIMAUX INOCULES ET TRAITES SUANOVIL L.A	quelques colonies			0		
	0			0		
	quelques colonies			0		
	1×10^6					
	8×10^3					
	3×10^6					
ANIMAUX INOCULES NON TRAITES	2.3×10^8					
	4.5×10^7			0		
	4.01×10^6			0		
	0			9×10^4		
	1.4×10^4					
	4×10^8					
	5×10^4					

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RESUME

Au cours de cet essai, 20 veaux de race française frisonne, âgés de 2 à 3 semaines ont été inoculés par voie intranasale et intratrachéale avec des cultures de Pasteurella haemolytica en phase exponentielle de croissance. La moitié de ces veaux a reçu dès le début des inoculations par voie intramusculaire, 100 000 UI de spiramycine par kg, 2 fois à 48 h d'intervalle. Les veaux ont été sacrifiés après 4 ou 10 jours d'observation. En se fondant sur des paramètres cliniques (examens cliniques standardisés biquotidiens), anatomopathologiques (poids des poumons, score pulmonaire), bactériologiques (recherche et numération de P. haemolytica/g poumon) et zootechniques (gain de poids moyen quotidien), l'efficacité thérapeutique de la spiramycine apparait clairement dans ce modèle de maladie respiratoire.

RESUMEN

En este estudio, se inocularon por vía intranasal e intratraqueal 20 terneros de raza francesa frisona, de 2 a 3 semanas de edad, con cultivos de Pasteurella haemolytica en fase exponencial de crecimiento. La mitad de estos terneros recibieron desde el principio inoculaciones por vía intramuscular, 100 000 UI de spiramycina por kg, 2 veces a 48 horas de intervalo. Los terneros fueron sacrificados al cabo de 4 o 10 dias de observación. Basándose en parametros clínicos (exanenes clínicos estandarizados bicotidianos), anatomopatológicos (peso de los pulmones, resultado pulmonar), bacteriológicos (investigación y recuento de P.haemolytica/g pulmón) y zootécnicos (aumento de peso medio cotidiano), aparece claramente la eficacia terapeútica de la spiramycina en este modelo de enfermedad respiratoria.

SUMMARY

In this trial, 20 French Frisian calves, between 2 and 3 weeks of age, were inoculated by intranasal and intratracheal route with cultures of Pasteurella haemolytica in the exponential growth phase. Half of these calves received two administrations of 100 000 IU of spiramycin per kg at 48 hours interval by intramuscular route one hour after the first intratracheal inoculation. The calves were sacrificed after an observation period lasting 4 or 10 days. On the basis of clinical parameters (twice daily, standard clinical examinations), pathological criteria (lung weight and pulmonary score), bacteriological criteria (isolation and count of P. haemolytica/g lung) and zootechnical parameters (daily mean weight gain), the therapeutic efficacy of spiramycin emerges clearly in this model of respiratory disease.

UTILISATION D'UN INTERFERON $\alpha 1^1$ RECOMBINANT BOVIN DANS LA MAITRISE DES BRONCHOPNEUMONIES INFECTIEUSES ENZOOTIQUES DES JEUNES BOVINS

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INTRODUCTION

Les maladies respiratoires des jeunes bovins essentiellement du type "bronchopneumonies infectieuses enzootiques" (BPIE) restent l'un des problèmes pathologiques majeurs des élevages fournisseurs de viande blanche ou rouge. En Grande Bretagne leur coût annuel est estimé à 50 millions de livres (9). Aux USA on situe les pertes occasionnées par ces affections entre 300 et 500 millions de dollars par an (mortalités, traitements, retards de croissance, mesures préventives). A l'échelon mondial elles atteindraient 3 000 millions de dollars. Pour la France de telles évaluations ne sont pas disponibles, toutefois en considérant que 7 millions de jeunes bovins sont exposés chaque année et en se référant aux valeurs utilisées par les nord américains (4), soit une perte par an de 16 dollars US/tête, le coût annuel des BPIE en France pourrait atteindre 700 millions de francs.

Les BPIE sont des troubles multifactoriels qui se développent lorsque différents évènements du milieu extérieur ont modifié la capacité de résistance des jeunes bovins à une flore pathogène complexe composée de virus, de mycoplasmes et de bactéries. En toutes circonstances les virus apparaissent comme des agents étiologiques majeurs car non seulement ils préparent par les lésions qu'ils déterminent au niveau des voies respiratoires la colonisation des bactéries mais pour la plupart d'entr'eux ils ont un effet immunodépresseur (5).

A ce jour, le contrôle des BPIE est basé à court terme sur l'antibiothérapie, à moyen terme sur l'antibioprévention et la vaccination, à long terme sur l'amélioration globale des conditions d'élevage (6). L'antibiothérapie se heurte souvent à des difficultés d'application des protocoles conseillés suite au coût de la matière première et de la main d'oeuvre. Les phénomènes d'antibiorésistance sont d'autres causes possibles d'échec. L'antibioprévention, selon la technique de la métaphylaxie, offre de nombreux avantages sur le traitement au coup par coup mais rencontre les mêmes difficultés que l'antibiothérapie. La vaccination n'assure la couverture que vis-à-vis de quelques valences virales avec des succès divers, de plus les sytèmes d'approvisionnement et d'élevage ne permettent pas toujours la pleine expression des vaccins disponibles. L'amélioration des conditions d'élevage est certainement le meilleur moyen de prévention des BPIE mais les contraintes et les principes de telles opérations sont souvent difficile à faire accepter aux éleveurs.

De par leur propriétés pharmacologiques (action antivirale et effet immunomodulateur) des molécules comme l'interféron recombinant $\alpha 1^1$ (rBoIFN $\alpha 1^1$)offrent un indéniable intérêt dans la maîtrise des BPIE en intervenant sur des maillons physiopathologiques jusque-là difficilement abordables (2). L'action antivirale peut permettre de désamorcer le processus des surinfections bactériennes responsables des symptômes, des lésions, des mortalités et des pénalités s'exerçant sur les performances zootechniques : l'effet immunomodulateur peut non seulement contribuer à neutraliser la participation virale et à maîtriser les complications bactériennes mais également à minimiser des conséquences des multiples stress auxquels les filières de production de viande bovine soumettent inévitablement les animaux.

Les essais cliniques présentés avaient pour objectif d'évaluer l'efficacité du rBoIFN $\alpha 1^1$ dans la maîtrise d'enzooties de BPIE apparaissant dans des cheptels allaitants de la zone charollaise du centre de la France.

MATERIEL ET METHODES

Elevages et animaux

Huit élevages (A,B,C,D,E,F,G,H), en période d'hivernage ont été le siège des observations. Dans chacun d'eux, composé d'animaux de race charollaise, coexistaient des sujets d'âges différents : femelles reproductrices, parfois veaux nés au cours de la saison de mise-bas, jeunes (mâles et

femelles) issus du précédent hivernage et ayant effectué une saison de pâture. En dehors des veaux nouveau-nés, l'ensemble des animaux vivait à l'attache dans des bâtiments dotés d'une ventilation statique comprenant en général plusieurs salles d'élevage, ne satisfaisant pas toujours aux critères d'hygiène nécessaires à la prévention sanitaire des BPIE (paramètres d'ambiance et paramètres techniques des bâtiments). Pour tous ces élevages à cheptel relativement fixe, les échanges avec l'extérieur étaient très limités mais la plupart avaient des antécédents de maladies respiratoires, soit les années précédentes, soit, pour certains, dès la rentrée à l'étable, pour d'autres, depuis quelques jours ou semaines. Les observations ont concerné les tranches d'âge les plus exposées aux BPIE : les animaux de type "broutards"(mâles et femelles) âgés de 8 à 12 mois et pesant entre 180 et 300 kg de poids vif.

Mise en place de l'essai

Dans chaque élevage le protocole expérimental était mis en place dans des lots de broutards manifestant les premiers signes de BPIE : toux, jetage, anorexie, hyperthermie. Ces lots étaient composés d'animaux malades à différents stades d'évolution mais comportaient surtout des animaux encore indemnes, des sujets en incubation, éventuellement des convalescents. En toutes circonstances, n'étaient retenus que les enzooties de BPIE sans formes cliniques graves, au stade de rhinotrachéobronchite aiguë ou subaiguë.

Dispositif expérimental

Dès que les critères ci-dessus étaient réunis, le lot était divisé en deux groupes de taille identique par tirage au sort, par la suite toutes les opérations étaient pratiquées en double-aveugle. L'un des groupes recevait le rBoIFN $\alpha 1^1$ par voie intramusculaire à la dose de 5 mg par sujet, une seule fois, l'autre un placebo dans les mêmes conditions. A partir de ce jour J0 tous les animaux faisaient l'objet d'un examen clinique quotidien jusqu'à J8. Lors de cet examen les paramètres suivants étaient inventoriés : température rectale, jetage nasal, toux, appétit. Ils faisaient également l'objet d'une notation selon la grille présentée dans le Tableau 1.

A J0 au moins 15% de l'effectif du lot était soumis à un lavage trachéo-bronchique par aspiration transtrachéale dans le but de préciser la flore des premières voies respiratoires essentiellement salmonelles, pasteurelles et Haemophilus somnus.

A J0 et à J21 tous les individus subissaient un prélèvement de sang pour recherche d'une séroconversion vis-à-vis du virus de la rhinotrachéite infectieuse bovine (BHV1) du virus respiratoire syncytial bovin (RSV), du virus parainfluenza 3 (PI3) et du virus du complexe diarrhée à virus-maladie des muqueuses (BVD/MD).

RESULTATS

Le tableau 2 précise l'effectif total de chaque élevage et la taille de chaque groupe de traitement : rBoIFN$\alpha 1^1$et placebo.

Le tableau 3 rassemble les résultats des examens bactériologiques et sérologiques.

La comparaison des moyennes des notes de chaque symptome entre les deux groupes dans chaque élevage et au cours du temps par une méthode statistique (test du chi-deux) fait apparaître les différences significatives rassemblées dans le tableau 4. Le rBoIFN $\alpha 1^1$ assure une bonne maîtrise du symptome toux en particulier dans les élevages G et H.

Une autre approche de l'efficacité comparée des deux traitements selon une méthode non statistique a été réalisée. Pour chaque élevage, chaque traitement, chaque symptome, chaque intervalle de temps (J0-J1, J1-J2, J2-J3 etc...) et chaque animal des deux groupes de chaque lot, est calculée la différence : note (au joue J)- note (au jour J-1). Pour la TR par exemple cette différence peut être nulle, négative (jusqu'à -4) ou positive (jusqu'à +4), idem pour les autres paramètres mais l'écart sera moindre (-2 : +2). Dans chaque classe de variation, pour chaque intervalle de temps et pour chaque traitement est affiché le nombre d'animaux correspondant avec pour les fractions en dénominateur, le nombre d'animaux pouvant évoluer négativement (à gauche) et le nombre d'animaux pouvant évoluer positivement (à droite) (Tableau 5). La figure 1 issue des fractions montre, en pourcentage, la modification dans le temps des évolutions favorables pour chacun des traitements pour le symptome toux dans l'élevage B. La collation de l'ensemble de ces informations dans le tableau 4 permet de confirmer la tendance dégagée précédemment à propos de l'efficacité clinique du rBoIFN $\alpha 1^1$sur l'ensemble des troubles observés.

DISCUSSION

Des résultats d'essais exposés ci-dessus relatifs à 8 élevages de bovins allaitants et concernant 322 jeunes bovins de type broutard, la tendance suivante se dégage :l'utilisation du rBoIFNα 1^1 à la dose de 5 mg par voie intramusculaire dans un effectif où sévit une enzootie de BPIE au stade de rhinotrachéobronchite, permet de maîtriser les troubles sur l'ensemble du lot. L'intervention correspond pour les animaux malades à une thérapie, pour les animaux non cliniquement atteints (non contaminés, en incubation, convalescents) à une prévention rapprochée (métaphylaxie). L'administration de rBoIFNα 1^1 ne s'est accompagné d'aucun effet secondaire systémique ou localisé.

Les résultats favorables obtenus sont très probablement en rapport avec la nature étiologique et le stade d'évolution des différent enzooties. Le tableau 3 montre la participation essentielle de valences virales alors que des bactéries n'ont pas été isolées des exsudats trachéobronchiques de tous les élevages. De plus, la pasteurelle isolée est le plus souvent Pasteurella multocida dont le pouvoir pathogène chez les bovins est discutable (6).

Il est vraissemblable que dans ces enzooties de maladies respiratoires le rBoIFN α 1^1 soit intervenu par son effet antiviral qui a bien été démontré in vivo et in vitro. Sur différents supports cellulaires et en comparaison avec d'autres types d'interféron bovins ou humains, le rBoIFNα1^1 réduit la multiplication des principaux virus bovins impliqués dans les BPIE : BVD/MD, PI3, RSV et BHV1. Ces virus ne sont pas tous également sensibles au rBoIFNα1^1, leur sensibilité semble décroître dans l'ordre de leur citation (8),(7). In vivo l'activité antivirale spécifique du rBoIFNα1^1 a surtout été étudiée dans un modèle expérimental de maladies respiratoires associant le virus BHV1 à Pasteurella haemolytica inoculés en aérosol à 4 jours d'intervalle. La protection conférée par l'interféron est effective tant par voie intramusculaire que par voie intranasale sur la base de critères cliniques et biochimiques. Toutefois, dans ce modèle l'interféron a probablement plus un effet immunomodulateur qu'antiviral étant donné la résistance du virus BHV1 à l'interféron in vitro (1). A noter que ces remarques à propos du virus BHV1 sont applicable également à d'autre virus respiratoires (BVD/ MD, PI3) dont les effets sur l'activité fonctionnelle des principaux effecteurs de l'immunité cellulaire (macrophages, polynucléaires neutrophiles et lymphocytes T) ont bien été démontrés (12).

Malgré les observations de BRYSON et coll.(3) montrant l'absence de protection conférée par un interféron recombinant humain α vis-à-vis d'une infection expérimentale chez le veau à virus PI3, les résultats obtenus sur les broutards charollais sont en bonne cohérence avec ceux de MARTINOD et coll. (12) en Suisse et de LYNN et PHILIP (14) aux USA. En Suisse cinq essais contrôlés ont concerné des veaux de 2 à 3 semaines d'âge, rassemblés en lots en vue de l'engraissement. Les lots expérimentaux de chaque essai comportaient 20 à 22 animaux avec un lot témoin recevant un placebo, et un à deux lots traités avec 0,5, 1 ou 5 mg de rBoIFNα1^1 dès leur arrivée, éventuellement répété 7 jours après. En comparaison avec les témoins placebo, le traitement avec l'interféron a permis une réduction de l'incidence des troubles respiratoires et de la fréquence des rechutes après traitements antibiotiques standarts. Aux USA 7 essais contrôlés ayant interessé 2 200 animaux au total, ont été réalisés avec des veaux de 220 kg en moyenne entrant dans des feedlots. Par rapport à un placebo, l'interféron à la dose de 5 mg était administré soit à l'arrivée dans le feedlot, soit au préalable au centre d'allotissement, soit aux deux époques. Dans un contexte ou la morbidité dans les groupes contrôlés était de l'ordre de 64%, l'utilisation se rBoIFNα1^1 administré 2-3 jours avant l'exposition au risque majeur, c'est à dire au centre d'allotissement, permet de mieux contrôler les troubles respiratoires même si la différence des scores cliniques avec les groupes placebo n'est pas significative dans tous les essais.

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RESUME

L'interféron recombinant bovin α1^1 a été utilisé dans 8 élevages charollais, en période d'hivernage, pour le maîtrise des bronchopneumonies infectieuses enzootiques des jeunes bovins. Chez 162 broutards exposés au risque, l'administration à tous les animaux du lot de 5 mg d'interféron par voie intramusculaire dès l'apparition des premiers signes de rhinotrachéobronchite, permet de réduire l'intensité et la durée des troubles par rapport à un placebo (160 animaux). La mise en place de ce programe de traitement-prévention rapprochée des maladies respiratoires des jeunes bovins ne s'accompagne d'aucun phénomène d'intolérance locale ou générale.

SUMMARY

During the winter of 1989, calves from 8 different farms in the Charolais area (France) were treated with recombinant bovine interferon α1^1 to control bovine respiratory diseases. As soon as some animals in a group exhibited early signs of rhinotracheitis, the remaining calves in that group received, either a 5mg dose of interferon (162 calves) or a placebo (160 calves) by intramuscular injection. Interferon treatment reduced the severity and duration of the disease. No treatment related adverse, clinical reactions were noted at any time during this programme for treatment and prevention of bovine respiratory diseases.

RESUMEN

El interferon recombinante bovino α 1^1 fue utilizado en 8 ganaderias charolesas, en periodo de invernada, para tratar broncopneumonias infecciosas enzooticas de los jovenes bovinos. En 162 terneros expuestos al riesgo, se redujo la intensidad y la duracion de los trastornos, en comparacion con un placebo (160 animales), mediante la administracion a todos los animales del lote de 5 mg de interferon por via intramuscular al aparecer los primeros signos de rinotraqueobronquitis. La realizacion de este programa de tratamiento-prevencion cercana de las enfermedades respiratorias de los jovenes bovinos no va acompanada de ningun fenomeno de intolerancia local o general.

Température Rectale (T.R.)	Notation
< 39°C	1
39°1C < t° < 39°5C	2
39°6C < t° < 40°5C	3
40°1C < t° < 40°5C	4
t° > 40°5C	5
Jetage nasal (J.N.)	
absent	1
séreux	2
muqueux	3
Toux (T)	
absente	1
rare	2
fréquente	3
Appétit (APT)	
normal	1
diminué	2
supprimé	3

Tableau 1 :
Paramètres relevés à chaque examen clinique de J0 à J8 avec leur cotation.

Elevage	Effectif total	rBoIFN alpha1.1	Placebo
A	430	13	12
B	160	18	16
C	270	22 22	22 22
D	250	26	27
E	320	8	8
F	320	23	18
G	260	13	13
H	200	17	20
Total	**2210**	**162**	**160**

Tableau 2 :
Effectifs des élevages et taille des groupes expérimentaux

Elevage	Bactériologie	%	Séroconversion	%
A	Pasteurella multocida	40	RSV BVD	60 56
B	Pasteurella multocida	83	IBR RSV PI3 BVD	6 14 31 22
C	Pasteurella multocida P. haemolytica	50 12,5	PI3 BVD	60 20
D	Pasteurella multocida	40	IBR RSV PI3 BVD	26 68 20 35
E			BVD	25
F	Pasteurella multocida P. Haemolytica	33 33	IBR RSV PI3 BVD	4 52 76 40
G	Pasteurella multocida	33	IBR RSV PI3 BVD	6 23 65 62
H			IBR RSV PI3 BVD	11 49 3 51

Tableau 3 :
Résultat des examens bactériologiques des liquides d'aspiration trachéobronchique (espèces isolées, % d'isolement) et des examens sérologiques (antigènes recherchés et pourcentage de séroconversion).

Elevage	Test du chi-deux Symptome, jour d'observation et signification		Evolution des % des notes Favorables	Défavorables
A	T J2	PBO > IFN	PBO > IFN	PBO > IFN
B			IFN > PBO	PBO > IFN
C	T J, J7 J3, J5 J, J1	PBO > IFN PBO > IFN PBO > IFN	IFN > PBO IFN = PBO	IFN = PBO IFN = PBO
D	T J3, J4, J5	IFN > PBO	IFN > PBO	IFN > PBO
E			IFN = PBO	IFN = PBO
F	T J1&J10	IFN > PBO	IFN > PBO	IFN = PBO
G	T J1&J10	IFN > PBO	IFN > PBO	IFN = PBO
H	T J1, J2	IFN > PBO	PBO > IFN	PBO > IFN

Tableau 4 : Résultats de l'étude statistique de l'évolution des symptomes dans chaque groupe et par élevage, de la comparaison entre les deux groupes du pourcentage d'évolutions favorables ou défavorables de cas (PBO = placebo, IFN = rBoIFN $\alpha 1^1$).

Intervalle de temps	Placebo Nombre d'animaux par classes de variation					rBoIFN alpha 1.1 Nombre d'animaux par classes de variation				
	-2	-1	0	+1	+2	-2	-1	0	+1	+2
J0 - J1			2/12	13	3/18			1/15	17	
J1 - J2			2/13	16				5/15	13	
J2 - J3			5/10	13				10/12	8	
J3 - J4			4/6	14				1/1	15	2/18
J4 - J5			1/2	17				2/2	16	
J5 - J6			0	18				0	18	

Tableau 5 : Nombre d'animaux par classes de variation et pouvant évoluer favorablement ou défavorablement (dénominateurs à gauche ou à droite) pour les deux groupes de traitement pour le symptome Toux dans l'élevage B.

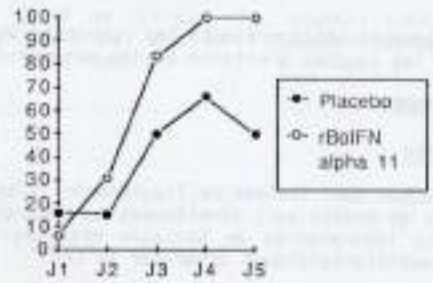

Figure 1 : Pourcentage d'évolutions favorables pour les deux groupes de traitement (placebo et rBoIFN $\alpha 1^1$) pour le symptome Toux dans l'élevage B.

LA RESISTANCE AU TRIMETHOPRIME CHEZ LES PASTEURELLA D'ORIGINE BOVINE

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1- INTRODUCTION

Le caractère multifactoriel des bronchopneumonies infectieuses enzootiques des bovins est désormais bien établi : diverses causes favorisantes sont incriminées dans l'environnement des animaux et relèvent des techniques d'élevage. Les virus respiratoires sont à l'origine d'infections très contagieuses mais souvent inapparentes ou à expression clinique bénigne. La gravité de la maladie, son passage à l'état chronique, sont habituellement attribués à l'intervention de bactéries qui compliquent l'infection virale primaire.

Globalement ce sont les pasteurelles qui arrivent en tête des fréquences d'isolement à partir des liquides de lavage trachéobronchique des bovins malades et des lésions pulmonaires.

Deux espèces de **Pasteurella** sont régulièrement identifiées : **P. haemolytica A1** qui s'avère deux fois plus fréquente dans les cas pathologiques graves que **P. multocida** [8, 9]. Elles justifient l'emploi des antibiotiques pour le traitement des troubles respiratoires des bovins et pour limiter les pertes. Mais le recours à l'antibiothérapie crée une pression de sélection favorable à l'émergence des souches polyrésistantes de **Pasteurella** [1, 2, 3, 6, 7, 11].

Dans le cadre de la surveillance épidémiologique de la résistance aux antibiotiques organisée par le Laboratoire de Pathologie Bovine en France [10] notre attention a été attirée récemment par la résistance nouvelle au triméthoprime, principalement retrouvée chez **P. haemolytica** mais qui existe aussi chez **P. multocida**.

Cette communication présente les résultats de l'étude de ce phénomène de résistance chez les souches d'origine bovine détectées en France.

2- MATERIEL ET METHODES

2.1- Les souches

Les souches sont isolées de liquides de lavage trachéobronchique ou de lésions pulmonaires de bovins soit directement au Laboratoire de Pathologie Bovine (LPB) soit dans des laboratoires de biologie vétérinaire associés au réseau de surveillance de l'antibiorésistance animé par le LPB.

2.2- Les souches résistantes

Les souches résistantes sont détectées par la méthode standard de diffusion de l'antiinfectieux à partir des disques de papier (Diagnostics Pasteur) déposé sur le milieu de Mueller Hinton gélosé additionné de 5 p. 100 de sérum de cheval.

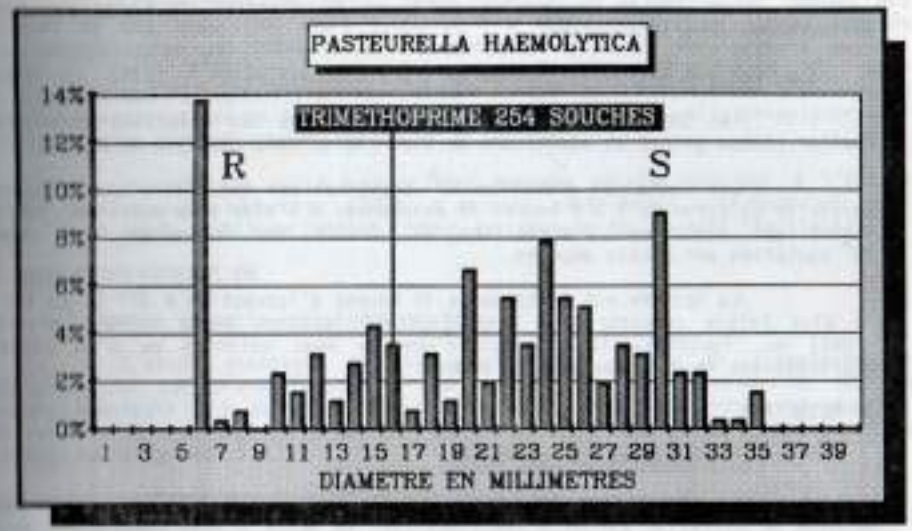

FIGURE 1 : distribution des zones d'inhibition autour des disques de triméthoprime (diamètre 6 mm, charge 5 microgrammes) obtenues par la méthode de diffusion en milieu gélosé (Diagnostics Pasteur) avec un échantillon de 254 souches de **P. haemolytica** d'origine bovine.

Le seuil de 16 mm délimite les souches considérées comme sensibles (diamètre $\geq$16 mm) et les souches considérées comme résistantes (diamètre $<$16 mm).

Les disques de papier de 6 mm de diamètre sont chargés avec 5 microgrammes de triméthoprime.

2.3- Les concentrations minimales inhibitrices (CMI)

Les CMI sont déterminées par la méthode des dilutions en milieu de Mueller Hinton gélosé et additionné de 5 p. 100 de sang hémolysé de cheval.

Les inoculums d'environ 10^7 bactéries par millilitre sont préparés à partir de cultures de 5 à 6 heures et ensemencés à l'aide d'un multiinoculateur de Steers qui distribue 1 microlitre de chacun des inoculums, soit environ 10^4 bactéries par goutte déposée.

La lecture est faite après 18 heures d'incubation à 37° C. La CMI est la plus faible concentration d'antibiotique inhibant toute culture visible à l'oeil nu. Toutefois la présence d'une ou deux colonies ou d'un voile de microcolonies ne sont pas pris en compte.

3- RESULTATS

3.1- Antibiogrammes par diffusion

La répartition des diamètres des zones d'inhibition obtenu par la méthode de diffusion permet d'individualiser au moins 3 sous-populations au sein des souches de P. **haemolytica** (figure 1) :

- une sous-population de souches donnant des diamètres d'inhibition autour des disques de triméthoprime supérieurs ou égaux à 17 mm. Ces souches sont normalement considérées comme sensibles selon les critères thérapeutiques du Commité de l'Antibiogramme de la Société Française de Microbiologie,

- une sous-population de souches ne donnant pas de zone d'inhibition autour du disque ou un anneau limité à 1 ou 2 mm de large. Ces souches sont considérées comme résistantes au triméthoprime selon les critères thérapeutiques,

- une sous-population intermédiaire donnant des zones d'inhibition inférieures à 17 mm.

3.2- Niveaux de sensibilité

Le tableau I présente les limites de distribution des valeurs des CMI.

ANTINFECTIEUX	SOUCHES SENSIBLES	SOUCHES RESISTANTES
Triméthoprime	0,062-0,500	2-64 (P. haemolytica) 1-16 (P. multocida)
0/129	8	16-64

TABLEAU I : limites de distribution des CMI (mg/l) de souches de **Pasteurella** d'origine bovine

Les souches considérées comme sensibles au triméthoprime donnent des valeurs de CMI comprises entre 0,062 mg/l à 0,500 mg/l. Les autres souches considérées comme résistantes ou intermédiaires donnent des valeurs de CMI comprises entre 1 et 32 mg/l (1 à 16 mg pour **P. multocida** et 2 à 64 pour P. **haemolytica**). L'augmentation des valeurs de CMI du triméthoprime s'accompagne d'une augmentation des valeurs de CMI à la 2,4 -diamino- 6,7 -diisopropylptéridine encore appelée composé vibriostatique 0/129 (O/129).

On sait que cette augmentation modérée des CMI est due à l'action d'une dihydrofolate réductase (DHFR) d'origine bactérienne qui reconnait pour substrat le noyau diaminopyrimidine commun aux deux molécules antiinfectieuses.

4- DISCUSSION-CONCLUSION

4.1- Nature de cette résistance croisée

L'étude génétique de ces souches n'a permis de transférer la résistance ni par transformation ni par conjugaison. Par hybridation ADN-ADN aucune homologie n'a pu être détectée entre l'acide nucléique des souches résistantes et les sondes représentatives des 5 DHFR déjà connues chez d'autres espèces bactériennes (5).

4.2- Importance de cette résistance

Bien que cette résistance ne semble pas d'origine plasmidique, l'importante utilisation de l'association triméthoprime-sulfaméthoxazole pour traiter les troubles respiratoires en buiatrie entraine l'émergence de cette résistance qui concerne en France actuellement 15 p. 100 des souches de **P. multocida** et 30 p. 100 des souches de **P. haemolytica** (7).

Par ailleurs la résistance croisée au 0/129 doit être prise en considération au niveau du diagnostic : la sensibilité à ce composé a été retenue comme caractère des pasteurelles pour les différencier des entérobactéries (4). La méconnaissance de cette résistance peut être à l'origine d'erreur d'identification au laboratoire.

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RESUME

La surveillance épidémiologique de la résistance aux antibiotiques a permis de constater récemment en France l'émergence de souches de **Pasteurella** résistantes au Triméthoprime. Le niveau de résistance est moyen et s'accompagne d'une résistance associée au composé vibriostatique O/129.

Cette résistance peut être à l'origine d'échecs thérapeutiques et d'erreur d'identification des **Pasteurella** au laboratoire.

MOTS-CLES : Bronchopneumonies enzootiques des bovins-Antibiorésistance-**Pasteurella** Triméthoprime.

SUMMARY : Resistance to Trimethoprim in bovine **Pasteurella**.

The nation wide monitoring network using antibiograms made in french local veterinary laboratories permits detection of a new coss resistance of bovine **Pasteurella** to trimethoprim and vibriostatic agent O/129.

Emergence of trimethoprim resistance is recent in bovine pathology and may be due to the large use of co-trimoxazol in therapy. Sensitivity must be checked before use in buiatry. Cross resistance to O/129 may result in bacterial misidentification of **Pasteurella** strains.

KEY WORDS : Enzootic bovine bronchopneumonia-Resistance to antibiotics-Pasteurella -Trimethoprim.

LA MECANICA RESPIRATORIA EN TERNEROS SANOS RESPIRANDO HELIO-OXIGENO

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INTRODUCCION

Las enfermedades respiratorias son frecuentes tanto en terneros como en ganado de engorde y resultan en severos disturbios en el proceso de crecimiento e incluso en la muerte. En razas como la Blanc-Bleu-Belge, en la cual por selección genética se desarrollaron caracteres zootécnicos específicos, se encontró que las afecciones respiratorias son la patología más frecuente y figuren entre las causas de mayor mortalidad y morbilidad. Publicaciones hasta la fecha, señalan la existencia de ciertas deficiencias anatómicas y funcionales que pueden estar relacionadas con su gran sensibilidad a las enfermedades respiratorias. Varios trabajos han sido ya realizados referentes a la función pulmonar bovina y han permitido mejorar el conocimiento tanto de la fisiología como de la fisiopatología del sistema respiratorio bovino (6,8,9,12,17). El fraccionamiento de la resistencia pulmonar total así como los ajustes respiratorios al aumento de la resistencia consecutivo a las enfermedades respiratorias obstructivas de las vías aéreas superiores ya han sido estudiadas en terneros (4,5,11). Fue demostrado que en terneros, como en el hombre, perro y caballo, la resistencia de las vías aéreas superiores (Ruaw) representa la mayor parte de la resistencia pulmonar total (RL) y un aumento en la primera induce a un dramático aumento de la segunda, con cambios sustanciales en el modelo respiratorio (4,6).

El propósito de este trabajo fue investigar la hipótesis de que la Ruaw es parcialmente debida a las turbulencias del flujo. Los cambios inducidos en la mecánica respiratoria por disminucion en la densidad del gas respirado fueron estudiados en terneros sanos.

MATERIALES Y METODOS

1) Animales. Seis terneros sanos fueron utilizados para este estudio. Cuatro de ellos (peso: 187 a 208 Kg) eran de la raza Frieslan y los otros dos (peso: 129 y 137 Kg) eran cruza.

Todas las medidas fueron registradas con los terneros en reposo, sin anestesia ni sedantes.

2) Materiales. La presión intrapleural (Ppl) fue medida con un catéter esofágico (9) conectado a un transductor de presión (Gould). La punta del catéter fue colocada en la porción torácica del esófago en el punto donde se cruzan la aorta y los nódulos linfáticos mediastínicos caudales usando la ecuación previamente presentada por Lekeux (9).

Una máscara respiratoria fue adaptada alrededor de la boca y de la nariz de cada ternero, evitando las fugas de aire y minimizando el espacio muerto (10). Un orificio en la máscara permitió registrar los cambios de presión en ella (Pm). La presión transpulmonar (PL) fue obtenida por la diferencia entre la Pm y la Ppl.

El flujo respiratorio (V) fue medido usando un neumotacógrafo Fleish Nº3 conectado a la máscara y acoplado a un transductor diferencial de presión (Gould) con dos catéteres idénticos. El volúmen tidal (Vt) fue derivado electronicamente integrando V con respecto al tiempo.

Una válvula gigante Hans Rudolf fue adaptada al neumotacógrafo Fleish. Un extremo de la válvula fue conectado por intermedio de un tubo a un balón que contenía la mezcla de gases:79%He y 21%O2 a temperatura ambiente. Una válvula manual de tres pasos colocada entre el tubo y el balón permitió el pasaje de

aire o la mezcla cuando fue apropiado.

La calibración fue realizada antes y después de cada experimento con un rotámetro para el flujo e inyectando volúmenes conocidos de aire a través del neumotacógrafo para el volúmen. Ambos transductores de presión fueron calibrados con un manómetro de agua.

3) Protocolo experimental. Un período de 2 a 3 min fue respetado luego de colocar el catéter esofágico, la máscara y el conjunto neumotacógrafo-válvula. Luego el V, Vt, Ppl y Pm fueron registrados simultaneamente en un polígrafo (Gould ES 1000) mientras los terneros respiraban primero aire (Aire 1), luego He-O2 y por último aire nuevamente (Aire 2), durante un minuto cada uno.

4) Cálculos. Los siguientes valores pulmonares fueron medidos en 5 ciclos regulares durante las tres condiciones: frecuencia respiratoria (RF), Vt, pico máximo de flujo inspiratorio y espiratorio (VImax y VEmax), flujo inspiratorio y espiratorio medio (mVI y mVE), presión transpulmonar mínima y máxima (PLmin y PLmax), variaciones máximas de PL (max PL), presión transpulmonar en la capacidad residual funcional (PLFRC), volúmen minuto (Ve), relacion entre el tiempo inspiratorio y el tiempo total del ciclo respiratorio (tI/tTOT) y la compliance dinámica (Cdyn). La RL fue calculada por el método del isovolúmen 50%. Los diagramas de presión-volúmen fueron trazados para las curvas de PL y Vt y el trabajo mecánico respiratorio (Wrm) fue estimado midiendo el area bajo la curva presión-volúmen. El trabajo por litro de ventilación (Wrm/L) también fue calculado.

5) Análisis estadístico. Los resultados son presentados como medias. Los datos tomados mientras los terneros respiraban aire (Aire 1) fueron considerados como valores control. Fueron comparados con los datos obtenidos durante la respiración de He-O2 y Aire 2 usando el test t de Student para datos acoplados.

RESULTADOS

La respiración de He-O2 induce un aumento en el Ve (=0,05) a consecuencia de un aumento en la FR mientras que el Vt permanece incambiado (fig.1). Por otra parte RL, Wrm/L y max PL disminuyen significativamente (Fig.2). El Wrm y tI/tTOT mostraron una disminución significativa durante la respiración de He-O2, mientras que hubo un aumento significativo en VImax, VEmax, mVI y en PLmax. PLmin y PLFRC se vuelven significativamente menos negativas. La Cdyn permaneció incambiada (Cuadro 1).

Todos los datos que cambiaron significativamente retornaron a sus valores iniciales cuando el aire sustituyó al He-O2.

DISCUSION

Este es el primer informe realizado de las medidas de los valores de la función pulmonar en terneros respirando He-O2. En un estudio similar realizado en humanos (15) la respuesta ventilatoria a la disminución de la resistencia fue caracterizada por un aumento en el Ve debido a un aumento en RF mientras que Vt permaneció incambiado. Un aumento en mVE y en VEmax y una disminución en las variaciones de Ppl fueron también observados. El presente trabajo muestra que la respiración de He-O2 induce cambios muy similares en el modelo respiratorio en terneros. En oposición a ésto, fue informado que la respiración de He-O2 en ponies en reposo no estaba asociada con ningún cambio importante en Vt, RF ni Ve (16).

El hecho de que no haya diferencias entre los valores de Aire 1 y Aire 2 sugiere que los cambios respiratorios inducidos por el He-O2 fueron principalmente debidos a cambios en las propiedades físicas del gas más que a otros factores como ser modificaciones en el diámetro de las vías aéreas.

La respiración de He-O2 induce un 23% y un 40% de disminución de la RL en humanos (3) y en ponies (16) respectivamente. En el presente estudio una disminución del 32% en RL fue también observada. En el hombre (14), en el caballo(19,20)

y en el bovino (4), la contribución relativa de Rusw en RL es del 80%. Esta contribución relativa importante es particularmente asignada a la existencia de remolinos turbulentos y fricciones en este parte del tracto respiratorio (2). La reducción de la resistencia de las vías aéreas al flujo de gas está relacionada con la menor densidad de la mezcla He-O2 y consecuentemente con la disminución de las turbulencias. ROHRER en 1915 (18) fue el primero que puntualizó que la densidad del flujo de gas en el árbol traqueobronquial es una de las determinantes de la resistencia de las vías aéreas. En el tracto respiratorio, la relación entre el volúmen del flujo y el gradiente de presión se expresa en la siguiente ecuación:

$P = K_1 V + K_2 V^2$

donde P es el gradiente de presión y V el flujo. La parte lineal de la ecuación representa el componente lineal del flujo y la constante K_1 la viscocidad del gas respirado. La parte cuadrática de la ecuación representa las turbulencias locales cuando ramas de la vía aérea cambian subitamente de diámetro y la constante K_2 incluye la densidad del aire. Sustituyendo el aire por una mezcla de 80%He y 20%O2 la densidad del gas respirado decrece, pero altera sólo ligeramente la viscocidad y por esta razón cambia la constante K_1 sin afectar mayormente K_2. El hecho de que la disminución de RL sea más importante en caballos y en terneros que en el hombre, sugiere que en los grandes animales las turbulencias que ocurren en los turbinetes nasales, encrucijada nasofaringea y laringe son más importantes que en el hombre.

Estudios realizados en vacas (7) y en ponies (1) muestran que a diferencia del hombre, la inertance pulmonar no debe ser despreciada en los grandes animales. La inertance pulmonar es directamente proporcional a la densidad del fluido respirado. Por esta razón, la reducción del trabajo mecánico por litro, aunque ampliamente explicado por la disminución de RL, puede ser también consecuencia de la disminución de la presión necesaria para la aceleración del flujo.

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RESUMEN

La mecánica respiratoria fue investigada en seis terneros sanos respirando aire y una mezcla con 79% de He y 21% de O2 (He-O2). La presión intrapleural (Ppl) fue medida con un catéter esofágico y la presión transpulmonar (PL) fue obtenida como la diferencia entre la presión bucal (Pm) y Ppl. El flujo respiratorio (V) fue medido usando un neumotacógrafo Fleish Nº3 y el volúmen tidal (Vt) fue derivado electronicamente integrando V con respecto al tiempo. El V, Vt, Ppl y Pm fueron simultaneamente registrados mientras los terneros respiraban primero aire (1 min), luego He-O2 (1 min) y por último aire nuevamente (1 min). Los valores de la función pulmonar fueron calculados sobre la base de los registros y los valores "He-O2" fueron comparados con los correspondientes a "aire".

La respuesta ventilatoria a la disminución de la resistencia se caracterizó por un aumento significativo del volúmen minuto exclusivamente debido a un aumento de la frecuencia respiratoria, mientras que Vt permaneció incambiado. Una disminución del 32% en la resistencia pulmonar total y 16% en el trabajo mecánico respiratorio por litro (Wrm/L) fue observada cuando los terneros respiraban He-O2.

La repercución del He-O2 sobre la resistencia pulmonar total y sobre Wrm/L sugiere que en la especie bovina, las turbulencias en la parte superior del tracto respiratorio juegan un rol significativo en la resistencia total de las vías aéreas.

SUMMARY

The mechanics of breathing was investigated in six healthy calves breathing either air or a mixture of 79% helium and 21% oxygen (He-O2). Intrapleural pressure (Ppl) was measured with an esophageal ballon catheter and transpulmonary pressure (PL) was obtained by subtracting the mouth pressure (Pm) from Ppl. Respiratory airflow (V) was measured using a Fleish pneumotachograph Nº3 and tidal volume (Vt) was electronically derived by integrating V with respect to time. Respiratory airflow, Vt, Ppl and Pm were simultaneously recored while the calves breathed first air (1 min), secondly He-O2 (1 min) and finally air again (1 min). The pulmonary function values were calculated on the tracings and the He-O2 values were compared to the corresponding "air" values.

The ventilatory response to resistance unloading was characterized by a significant increase in minute volume exclusively due to an increase in respiratory frequency, while Vt remained unchanged. A decrease of 32% in the total pulmonary resistance (RL) and 16% in the work per litre of ventilation (Wrm/L) were observed when calves breathed He-O2.

The effect of the He-O2 on RL and on Wrm/L suggest that, in the bovine specie airflow turbulences in the upper part of the respiratory tract play a significant role in the total airways resistance.

RESUME

La mécanique ventilatoire a été recherchée dans six veaux sains en respirant de l air et un mélange 79% He et 21% O2. La pression intrapleurale a été mesurée avec un cathéter oesophagien et la pression transpulmonaire comme étant la différence entre la pression bucal (Pm) et l intrapleurale (Ppl). Le débit respiratoire (V) a été mesuré avec un pneumotachograf de Fleish Nº3 et le volume tidal (Vt) par intégration de ce dernier en fonction du temps. Le V, Vt, Ppl et Pm ont été simultanément enregistrés pendent que les veaux respiraient au début de l air (1 min), après He-O2 (1 min) et a la fin de l air de nouveau (1 min). Les valeurs de la fonction pulmonaire ont été calculées sur la base des enregistrements et les valeurs He-O2 ont été comparées avec celles de l air.

La réponse ventilatoire a la diminution de la résistence a été caracterisée par une augmentation significative du volume minute due exclusivement a une augmentation de la fréquence respiratoire, tandis que le Vt est resté inchangé. Une diminution de 32% dans la résistence pulmonaire totale (RL) et 16% dans le travail mécanique respiratoire par litre (Wrm/L) a été observée tant que les veaux respiraient He-

O2.

L impact du He-O2 sur la RL et sur le Wrm/L fait penser que dans l espece bovine, les turbulences dans la partie supérieur du tracte respiratoire jouaient un role important dans la résistence total des voies respiratoires.

Cuadro 1: Valores de la función pulmoner en 6 terneros sanos mientras respiraban aire y He-O2

Valores	Unidades	Aire 1	He-O2	Aire 2
tI/tTOT		0.50 + 0,03	0.48 + 0.04	0.48 + 0.03
VImax	L/seg	2.29 + 0.48	3.06 + 0.88*	2.40 + 0.39
VEmax	L/seg	3.05 + 0.99	3.63 + 0.83*	2.68 + 1.00
mVI	L/seg	1.98 + 0.42	2.34 + 0.34**	1.98 + 0.26
mVE	L/seg	2.00 + 0.40	2.24 + 0.61*	1.89 + 0.37
PLmin	kPa	-2.22 + 0.48	- 2.00 + 0.39*	-2.09 + 0.42
PLmax	kPa	- 0.27 + 0.26	- 0.32 + 0.33	- 0.26 + 0.26
PLFRC	Kpa	-0.73 + 0.21	-0.62 + 0.25*	- 0.68 + 0.24
Cdyn	L/kPa	2.77 + 0.83	2.83 + 0.79	2.85 + 1.10
Wrm	J	1.56 + 0.85	1.34 + 0.87	1.47 + 0.94

*: significativamente diferentes para Aire 1 valores con P = 0.05; **: P = 0.01

Gráfico

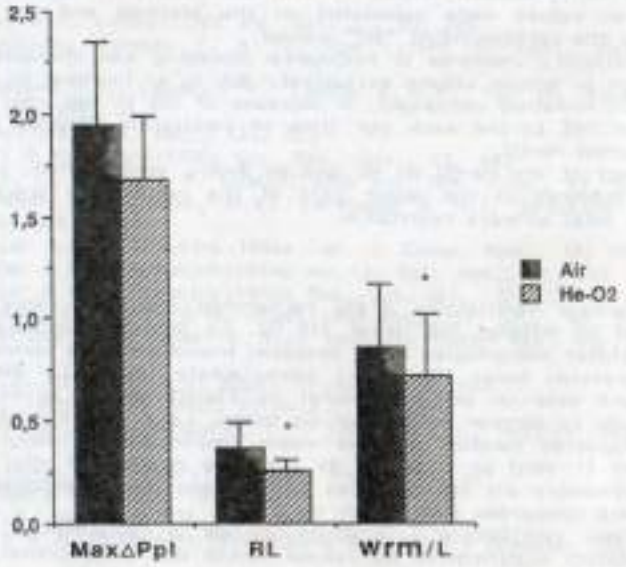
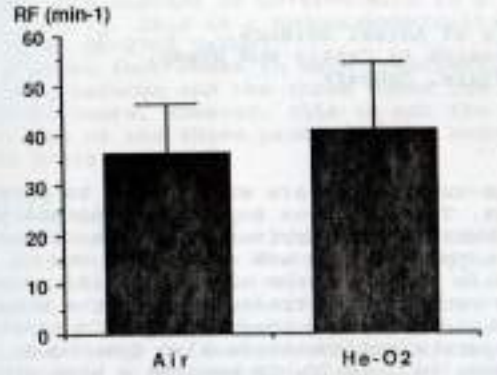
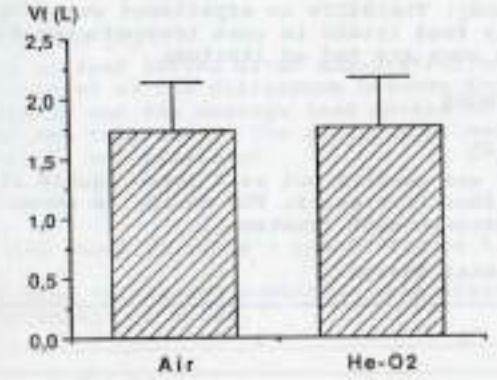
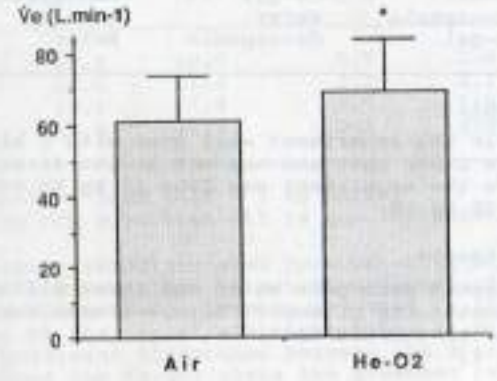


Gráfico 2.







REDUCED FEED INTAKE IN COWS AFTER PERORAL CALCIUM SUPPLEMENTS

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INTRODUCTION

Peroral calcium-supplements are widely used to prevent and/or cure milk fever in cows. These calcium supplements normally contain $CaCl_2$, $2H_2O$ which is a chemical with irritative effect. In an experiment with two different Ca-supplements it was shown that one of these had an irritative/ulcerogenic effect on the rumen wall (3). Other cows had a reduced feed intake rate after a treatment with the same Ca-supplement.

Some practitioners have also observed that Ca-treated cows could have a reduced appetite and sometimes also diarrhoea. A reduced feed intake about calving is undesirable because a high producing cow needs a high feed intake to avoid ketosis caused by a large mobilization of energy from the body. Therefore an experiment was made to show if there is a reduced daily feed intake in cows treated with different Ca-supplements when the cows are fed ad libitum.

MATERIALS AND METHODS

Experimental design

The experiment was carried out as a latin square with four cows, four periods and four treatments. The design is shown in Table 1. There were eight days between each treatment.

Table 1. Experimental design.

Period/Cow	1	2	3	4
1	Water	Ca-oil	Ca-gel	Ca-capsule
2	Ca-oil	Ca-gel	Ca-capsule	Water
3	Ca-capsule	Water	Ca-oil	Ca-gel
4	Ca-gel	Ca-capsule	Water	Ca-oil

Experimental animals

The four cows in the experiment were cows with a high feed intake. Three of them were older cows and one was in its first lactation. The feed intake before the experiment was from 16 kg DM to 24 kg DM per day, average was 20 kg DM.

Experimental treatments

The four treatments were pure water and three different commercially available $CaCl_2$-containing products. All were made for peroral administration. The four treatments were:

1) Pure water, (1 l)

2) Ca-oil, (200 g $CaCl_2$, $2H_2O$ emulgated in 380 g soya bean oil and 200 g water + 20 g aroma-mixture) (Calol, Gunnar Kjems APS, DK-1173 Copenhagen).
3) Ca-capsule, (126 g $CaCl_2$, $2H_2O$, 45 g $CaSO_4$, 7 g $MgCl_2$ and 2 g calciumstearat. The capsule is covered with 10 g animal fat)
4) Ca-gel, (200 g $CaCl_2$, $2H_2O$ in a hydroxyethylcellulose-gel) (H. Lundbeck A/S, DK-2500 Valby).

The cows were given four doses in each treatment. The first dose was given late in the afternoon and the three doses the following day at 0700, 1200 and 1700 hours. However, this is not the recommended time schedule for the use of the three products. The recommendation says four doses in 48 hours.

Feeding procedure

Twice a day the cows were fed a mixed feed containing 52% of dry matter (DM) as grass silage, 37% DM as fodderbeets and 11% DM as concentrates rich on protein. This mixture was fed ad libitum with at least 10% extra. The cows were fed other concentrates according to yield. Two cows had 3.8 kg, one 2.4 kg and one 1.2 kg per day. The feed intake was measured every day and the refusals were analysed for DM to eliminate a difference in DM content of the feed digested and the feed left over.

Calculation and statistics

The reduction in feed intake after administration of Ca-supplements or water is calculated as the difference between the feed intake when the cows are treated and the average feed intake of the 3 days before and 3 days after the treatments. The statistical model contains the effect of cow, period, and treatment.

RESULTS

The results are shown in Table 2 and in Figure 1.

Table 2. Normal feed intake and reduction when treated with different Ca-supplements or water.

Treatment	Normal feed intake		Reduction		% Reduction	
	kg DM	FU*	kg DM	FU	DM	FU
Water	19.2	18.0	0.9	0.8	4.7	4.6
Ca-oil**	18.6	17.4	2.2	2.1	12.2	11.9
Ca-capsule	19.1	17.9	2.1	1.9	11.5	11.0
Ca-gel	18.8	17.7	3.1	2.8	16.7	16.1

* FU = Feed unit. 1 feed unit = 1 kg barley
** The energy in the soya bean oil is not included (about 2.4 FU)

There is a small reduction when pure water is used for peroral administration, but the reduction is not significant. The reduction in DM intake when the three Ca-supplements are used is from 11.5% to 16.7%. This reduction, at the day of administration, is significant ($P<0.05$). There is no significant difference between the 3 treatments but there is a tendency that the Ca-gel gives the greatest reduction. On the

other hand the Ca-oil seems to give an effect for a longer period as shown in Figure 1. This effect on the following days is not significant and it is one cow in particular which is affected.

The energy intake is reduced almost proportionally with the feed intake. The reduction in feed intake is primarily a reduced intake of the mixed ration and not of the concentrates. The soya bean oil in the Ca-oil supplies a lot of energy. How much will depend on the digestibility of the fatty acids. If the digestibility is about 75% the energy supply is 3.2 FU, so even if there is a reduction for a longer period the total energy reduction will be very small.

DISCUSSION

The possible mechanisms involved in the reduction of feed intake are:
1) the handling of the cows,
2) the increase in the osmotic pressure,
3) the effect of unsaturated fatty acids on the rumen function,
4) the effect of etching of the rumen wall.

The reduction in feed intake when the cows were treated with pure water can only be due to the handling of the cows. The effect of that is very small and it is not of any importance.

After administration of the 3 Ca-supplements the osmotic pressure in the rumen will increase. If the rumen contains 80 l the increase after 1 dose of Ca-gel or Ca-oil will be about 50 mOsmol/l. The Ca-capsule will increase the osmotic pressure by about 36 mOsmol/l because of a lower content of $CaCl_2$. The normal osmotic pressure is 240-280 mOsmol/l before feeding increasing to 350-420 mOsmol/l the first 2-3 hours after feeding. After 5-9 hours the osmotic pressure will be at normal level (1).

Above 350 mOsmol/l there is an increasing, negative effect on feed intake, and the effect of the osmotic pressure might explain the decrease in feed intake in this experiment. It is questionable if it is the only reason, because the feed intake would increase if the cows were fed a higher amount of concentrates and so would the osmotic pressure.

When unsaturated fatty acids are administrated to the rumen there will be a great reduction in the cellulolytic activity. This will decrease the rate of passage of digesta and the digestibility of the feedstuff (2), and the result is a decrease in feed intake. In this experiment the Ca-oil has a tendency to decrease feed intake for a longer period than Ca-gel and Ca-capsule, and the inhibition of the bacterial activity might explain that.

Etching of the rumen wall could also be a reason for a decrease in feed intake. The Ca-gel used in this experiment has shown a serious etching effect (3), but it is impossible to decide if that has been the reason for the decrease in feed intake in this experiment.

CONCLUSION

There is no doubt that administration of these three Ca-supplements has reduced the feed intake, but a 10-15% reduction in one day is not of any importance, not even about calving. However, if the reduction increases with decreasing feeding level it might be serious for the cow. On the other hand it must be balanced against a higher incidence of milk fever if the Ca-supplements are not used. An experiment with cows treated at calving will be presented.

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SUMMARY

The influence on daily feed intake of treatment with three different commercially available $CaCl_2$-containing products and pure water was measured. The treatments were four perorally administrated doses of 1) 1 l pure water, 2) Ca-oil containing 200 g $CaCl_2$ emulgated in soya bean oil, 3) Ca-capsule containing 126 g $CaCl_2$ and 45 g $CaSO_4$, 4) Ca-gel containing 200 g $CaCl_2$ in an ethylcellulose-gel. The treatment gave a reduction in DM intake of 4.7%, 12.2%, 11.5% and 16.7% respectively on the day of treatment. The energy intake was reduced about the same except for the Ca-oil treatment where the energy intake was not reduced due to the oil. The mechanisms causing the reduction might be 1) handling of the cows, 2) increase of the osmotic pressure, 3) fat depression in the rumen, 4) etching effect of the rumen wall.

ZUSAMMENFASSUNG

Die Wirkungen von 3 verschiedenen $CaCl_2$ Präparaten nebst reinem Wasser auf die Futteraufnahme der Milchkühe wurden untersucht. Die Behandlungen bestanden von peroraler Zufuhr von 1) 1 l reinem Wasser 2) Ca-Öl mit 200 g $CaCl_2$ emulgiert in Sojaöl 3) Ca-Kapsel die 126 g $CaCl_2$ und 45 g $CaSO_4$ beinhalten 4) Ca-Gel mit 200 g $CaCl_2$ in einem Ethylcellulosegel. Auf dem Behandlungstag haben die Behandlungen eine Ermässigung in der Trockenstoffaufnahme von 4,7% bzw. 12,2%, 11,5% und 16,7% mitgeführt. Die Energieaufnahme war ebensoviel reduziert, aber wegen des Öls hatten die Kühe, die die Ca-Ölbehandlung bekamen, nicht diese Reduktion. Die Vorrichtungen könnten 1) Handhabung von den Kühen 2) Vergrösserung im osmotischen Druck 3) hemmende Wirkung auf Fett in Pansen 4) Ätzung von der Pansenwand sein.

RÉSUMÉ

Les effets de 3 préparations $CaCl_2$ différentes et de l'eau pure sur l'absorption de fourrage chez les vaches laitières ont été examinés. Les traitements ont été un apport peroral de 1) 1 litre d'eau pure 2) Ca-huile, contenant 200 g de $CaCl_2$, émulsionné dans de l'huile soya 3) Ca-capsule, contenant 126 g de $CaCl_2$ et 45 g de $CaSO_4$ 4) Ca-gel, contenant 200 g de $CaCl_2$ dans un gel d'ethylcellulose. Les traitements ont causé une réduction dans l'absorption de la matière sèche au jour de traitement de respectivement 4,7%, 12,2%, 11,5% et 16,7%. L'absorption d'énergie était autant réduite, mais a cause d'huile les vaches q'ont reçu le traitement de Ca-huile n'avaient pas cette réduction. Les raisons peuvent être 1) le maniement de la vache 2) une augmentation dans la pression osmotique 3) un effet frénateur de graisse dans la panse 4) une corrosion de la paroi de la panse.

Figure 1. Feed intake before (day -3, -2 and -1), at (day 0) and after (day 1, 2 and 3) treatment with different Ca-products or water.

RELACION DEL PERFIL ENERGETICO Y CUERPOS CETONICOS EN SANGRE, LECHE Y ORINA A LO LARGO DE LA GESTACION EN BOVIDOS. (*)

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INTRODUCCION

Los problemas dismetabólicos representan en las explotaciones de ganado bovino un elevado porcentaje, destacando entre ellos los concernientes al metabolismo energético, sobre todo como resultado de mayores exigencias, tal como acontece a lo largo de la gestación y puerperio (KAUPPINEN, 1983 y BERGMAN, 1971).

Esta situación puede verse agravada si la alimentación que reciben los animales es pastosa, siendo el ensilado la principal fuente de energía (BALLARINI, 1974 y SIGNORI et al, 1976), además de poder instaurarse un balance negativo al relacionar la alimentación-producción. (HOFECKER, 1973).

Por ello, el perfil energético y la presencia de cuerpos cetónicos en líquidos orgánicos a lo largo de la gestación son, sin lugar a dudas, indicativos del compromiso metabólico, lo que justificará su estudio comparativo en dos razas bovinas, la Frisona y la Rubia Gallega, de aptitud láctea y cárnica respectivamente.

MATERIAL Y METODOS

En el presente trabajo hemos utilizado como material vivo 50 hembras bovinas, 25 de raza Frisona y 25 de raza Rubia Gallega, de diferentes edades, pertenecientes a distintas explotaciones, teniendo en común todas ellas el regimen de semiestabulación y la alimentación uniforme a base de heno y ensilado, suplementando a las de alta producción lechera con un concentrado.

A todos los animales previa anamnesis y exploración general, con el fin de considerarlos clínicamente sanos se les extrajo muestra de sangre, orina y leche con periodicidad mensual, desde el momento de la cubrición hasta el parto, valorando los siguientes parámetros: glucemia, lipidemia, colesterolemia, ASAT, y B-hidroxibutirato y acetoacetato en sangre, leche y orina.

RESULTADOS Y DISCUSION

Para comprobar el estado metabólico general y averiguar si existe una alteración hepática, la glucemia es uno de los principales parámetros a estudiar.

Al valorara la glucemia en las hembras de raza Frisona y Rubia Gallega, hemos comprobado que alcanzan unos valores medios de $53,76 \pm 1_{5,2}$ y $60,48 \pm 1_{5,9}$ mg/dl. respectivamente, siendo superior el valor medio de la Rubia Gallega durante la gestación. (Tabla 1).

La glucemia media de las hembras Frisonas muestran un comportamiento diferente al de las Rubias Gallegas en los dos primeros tercios de la gestación, incluso en el último de se observa una elevación de este parámetro en la raza Frisona, mientras que en la Rubia Gallega desciende. (Gráfica 1).

Esta diferencia significativa se debe a la diferente aptitud entre las razas (BENEITO, 1966), incluso el descenso de la glucemia a lo largo de la gestación, que sufre la Frisona, está de acuerdo con MACOVEI, 1986 y BAGLIONE, 1982.

Al estudiar los valores medios de colesterol y su evolución durante la gestación, en estas dos razas, se observa que el valor medio en la Frisona es de $184,94 \pm 46,85$ mg/dl significativamente superior a la que muestra la Rubia Gallega con $156,07 \pm 46,95$ mg/dl (Tabla 1).

(*) Proyecto Financiado por la Consellería de Educación de la Junta de Galicia. España

TABLA I. Valores medios de los parámetros séricos de los 50 animales muestreados.

		Glucosa (mg/dl)		Lípidos T. (mg/dl)		Colesterol (mg/dl)		ASAT (UI/l)	
		R.G.	Frisona	R.G.	Frisona	R.G.	Frisona	R.G.	Frisona
Cubrición	0	53,61	54,25	592,58	547,40	152,77	188,97	59,60	66,37
Mes de gestación	1	59,60	55,76	611,90	634,81	161,16	190,83	57,60	57,25
	2	56,96	55,13	614,08	535,75	147,14	205,66	60,46	70,25
	3	57,21	51,22	652,48	566,21	178,35	197,38	52,86	63,18
	4	68,12	50,46	620,33	546,98	142,84	198,71	47,20	61,25
	5	66,19	54,12	596,88	576,06	150,57	165,30	47,33	70,81
	6	61,49	53,89	645,17	528,00	151,51	185,56	47,20	65,12
	7	60,63	48,71	599,34	555,00	149,11	183,99	54,33	68,68
	8	62,57	55,50	639,53	501,80	156,01	186,14	56,86	69,87
	9	58,55	58,54	552,52	531,06	171,23	146,68	66,60	72,87
Valor medio		60,49	53,76	612,48	552,31	156,07	184,94	55,00	68,56

ASAT: aspartato aminotransferasa

R.G.: raza Rubia Gallega

Si atendemos a la evolución de la colesterolemia, se aprecia que el comportamiento en el último mes de gestación es inverso a lo que sucedía con la glucemia en ambas razas, igualmente los valores máximos se encuentran para las dos razas en el segundo y tercer mes de gestación, lo que está de acuerdo con lo observado por GHERGARIU, 1986, en este parámetro.

NAGY, 1984 justifica el descenso de colesterolemia al final de la gestación en la raza Frisona a un aumento de los ácidos grasos no esterificados, hecho éste que no ocurre en la raza Rubia Gallega.

La lipidemia tiene valores medios de 612,48 ± 106,95 y de 552,31 ± 115,72 mg/dl. para las hembras Rubia Gallega y Frisona respectivamente, mostrando variaciones poco significativas a lo largo de la gestación. (TABLA I). El descenso en los lípidos totales en suero al acercarse el momento del parto (GOICOA, 1989), se ha vuelto a comprobar a lo largo de este estudio solo para la raza Rubia Gallega.

DUNCAN y GARTON (1963), encontraron una hipolipidemia antes del parto en hembras de alta producción láctea, esta situación la hemos apreciado en la raza Frisona, aunque se observó también valor mínimo de lipidemia en el último mes de gestación, en la raza Rubia Gallega.

La actividad enzimática media en sangre de la aspartato aminotransferasa ha sido de 55,00 ± 14,74 UI/l. para la raza Rubia Gallega y de 68,56 ± 22,57 UI/l. en la raza Frisona (TABLA I). Al estudiar la evolución a lo largo de la gestación se observa un cierto paralelismo durante el primer tercio de la misma apreciándose un incremento a partir del sexto mes, observación ya hecha en 1973 por KAMMERER y FRERKING.

Al igual que BAGLIONI (1987), hemos observado en ambas razas un aumento de la acti-

Tabla II. Valores medios de cuerpos cetónicos en las distintas razas objeto

		BHB(sangre) (microm/l)		BHB(orina) (microm/l)		BHB(leche) (microm/l)		AA(sangre) (microm/l)		AA(orina) (microm/l)		AA(leche) (microm/l)	
		R.G.	Frisona	R.G.	Frisona	R.G.	Frisona	R.G.	Frisona	R.G.	Frisona	R.G.	Frisona
Cubrición	0	180,74	209,72	75,69	164,35	80,74	104,25	38,36	189,36	40,21	47,32	45,20	54,17
Mes de gestación	1	172,21	293,42	80,21	174,20	64,21	132,39	47,92	92,17	42,36	96,32	37,16	62,25
	2	170,14	276,42	72,48	149,80	79,13	136,27	39,64	104,02	41,19	102,14	31,09	56,32
	3	181,32	264,36	84,26	157,20		156,36	40,26	97,29	60,16	100,21		57,49
	4	150,14	311,21	75,45	210,31		167,39	31,27	124,36	52,39	105,17		70,21
	5	168,70	157,17	77,60	154,35		110,41	30,29	70,34	54,16	100,21		64,17
	6	149,21	194,14	74,49	160,49		109,31	40,21	80,72	53,36	97,32		57,96
	7	170,42	342,16	68,16	237,49		91,32	42,16	139,48	57,00	120,23		75,19
	8	192,28	205,17	79,84	175,46			45,94	99,34	62,61	105,15		
	9	175,40	364,38	81,24	199,30			54,32	170,96	60,24	139,48		
Valor medio		171,06	260,81	76,95	178,29	74,69	125,83	40,72	116,73	52,32	106,35	37,81	62,22

BHB: B-betanidroxibutirato

AA : acetoacetato

R.G.: raza Rubia Gallega

GRAFICA 1

GRAFICA 2

GRAFICA 3

GRAFICA 4

idad enzimática en las proximidades del parto, a la vez que los valores mínimos en - los dos últimos tercios de la gestación corresponden con el quinto mes. (Gráfica 2).

La diferencia entre las actividades enzimáticas de estas dos razas puede justificarse por el diferente compromiso hepático, ya que las hembras de raza Frisona tienen un metabolismo energético superior como consecuencia de la alta producción láctea, mientras que en la de raza Rubia Gallega por nosotros estudiadas, es menor este compromiso debido a que a partir del segundo mes de gestación la producción láctea es practica- mente nula.

Respecto a los niveles de B-hidroxibutirato en sangre, orina y leche hemos de indicar que las hembras bovinas de producción láctea tienen siempre unos valores medios superiores a los de las de producción cárnica, esto mismo sucede en el caso del acetoacetato en estos líquidos orgánicos -TABLA II- debido a que en los primeros tienden hacia un balance energético negativo (GARCIA PARTIDA, 1988).

Al estudiar los resultados obtenidos por nosotros se aprecia la diferencia antes citada , siendo la media del B-hidroxibutirato en sangre inferior en las dos razas a las dadas por la mayoría de los investigadores, solamente LYLE en 1984 da valores inferiores a la media obtenida por nosotros en la raza Frisona.

Las oscilaciones a lo largo de la gestación de los cuerpos cetónicos estudiados son mayores en la raza Frisona que en la Rubia Gallega, siendo para esta última raza muy poco significativas. También es cierto que en los máximos valores de acetoacetato y B-hidroxibutirato los encontramos siempre tras el parto. (Gráficas 3 y 4).

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RESUME

On fait une étude de la corrélation du profil energétique et des niveaux de BHB et AA, chez 25 vaches de production laitière (Frisonne) et 25 vaches de production de viande (Rubia Gallega).

Après la frise d'échantillons chez des animaux non gestation, on a procédé à une étude mensuelle de ces paramètres tout au long de la gestation et aussi après le vêlement. Toutes les vaches ont eu une alimentation uniforme (foin et ensilage) en augmentant pour les vaches à forte production laitière un concentré.

Tout au long de ce travail nous avons observé un comportement différent parmi les -

vaches à grande production laitière et celles qui produisent de la viande. La correlation entre glucémie et corps cétoniques a été inversement propotionnelle, tandis qu'il y a eu un parallélisme entre lipidémie avec le BHB et AA, chez ces deux races dans le dernier tiers de la gestation.

On a réalisé une étude enzimograme de la même façon dans ces séra.

SUMMARY

This is de study on the correlation of energetic profile and the levels of betahidroxibutirate and acetoacetate in twentyfive dairy cows (Holstein-Frisian) and twentyfive of the Rubia gallega breed.

After taking the samples on animals wich were not pregnant, we proceded to a monthly control of these parameters through out the gestation period as well as after calven. All cows had a uniform feeding (hay and ensilage) giving a supplement to those with a high milk production.

Through out the experiment we have observed different behaviour between the cows with high milking production and the beet cows. There was an inversive proportion on the correlation beetween de glucemie and cetonic bodies. At the same tine we found betaht there was paralellism of lipidemia with the betahidroxibutirate and the acetoacete te (in both breed during the last gestating period). We also studied the enzimograma on these serums.

ALGUNS PARÂMETROS FISIOLÓGICOS DE BOVINOS NA REPRODUÇÃO EXPERIMENTAL DO HIPERPARATIREOIDISMO SECUNDÁRIO NUTRICIONAL.

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INTRODUÇÃO

Embora o Brasil conte com um razoável rebanho bovino mestiços leiteiros, poucos são os trabalhos de pesquisa que relatam as constantes fisiológicas destes, nas condições climáticas tropicais. A influência de fatores tais como espécie, idade e temperatura ambiente nas freqüências cardíaca e respiratória e na temperatura corporal já foi descrita por vários autores(1,2,3,4).

Os dados que dispomos, relativos a normalidade destas valores, referem-se a observações, que na sua maioria, provem de zonas de clima temperado, com sistema de criação diverso do nosso.

Sendo assim, o presente trabalho visa relatar os valores fisio-seniológicos observados em 15 bovinos jovens mestiços leiteiros, durante um período no qual foram submetidos experimentalmente ao hiperparatireoidismo secundário nutricional.

De acordo com Marek & Mocsy(5) a freqüência respiratória de bovinos, aparentemente sadios, varia entre 10 a 40 movimentos por minuto. Novilhos de até 1 ano apresentam una temperatura corporal que oscila entre 38,5 a 40,0°C.

Segundo Rosenberger(6), bovinos adultos sadios, apresentam um limite máximo para a freqüência cardíaca de 90 por minuto, podendo a respiratória variar entre 15 a 35. Como medida de atividade rumenal normal, admite duas ou três fortes contrações durante dois minutos de auscultação. Com relação a temperatura corporal cónsidera os valores de 38,5 a 39,5°C.

MATERIAL E MÉTODOS

Animais e instalações

Utilizou-se 15 novilhos mestiços leiteiros, não castrados e aparentemente sadios, entre 8 a 12 meses de idade, procedentes da cidade de Nova Serrana, Estado de Minas Gerais.

Durante o período de março a setembro de 1988 os animais permaneceram confinados em baias individuais, num galpão ventilado localizado na Escola de Veterinária da UFMG, para a indução do hiperparatireodismo secundário nutricional. Para isso, dividiu-se os animais em três grupos que receberam uma dieta isoproteica e isocalórica com a mesma composição mineral (segundo NRC, 7) exceto quanto ao cálcio e fósforo, que sofreram modificações que corresponderam aos seguintes grupos: 1(0,45% de cálcio e 0,36% de fósforo); 2 (0,45% de cálcio e 0,72% de fósforo); 3 (0,34% de cálcio e 0,18% de fósforo).

Exame fisio-semiológico

Mensalmente, sempre pela parte da manhã, entre 8 às 10 horas, durante o exame clínico, efetuou-se a verificação das freqüências respiratória e cardíaca e dos movimentos rumenais, mediante auscultação, assim como da temperatura retal através de termômetro clínico veterinário.

Análise estatística

Todos os resultados obtidos submeteram-se a análise de variância correspondente a um delineamento de parcelas subdivididas, testando os três grupos (tratamentos). Para comparação das médias utilizou-se o teste de Tukey ($p < 0,05$).

RESULTADOS

Os valores médios obtidos nos três grupos não apresentaram diferença significativa entre si.

O resultado da análise estatística final dos exames fisio-semiológicos estão relacionados na Tab.1.

Apesar de não ter sido possível o contrôle da temperatura ambiente e da umidade relativa do ar, o desvio padrão elevado encontrado nas freqüências respiratória e cardíaca possivelmente se deveram a influência da temperatura ambiente, conforme ressalta Pugliese(1), Rodrigues(2) e Rosemberger(3), uma vez que o período experimental realizou-se entre o final do verão até o início da primavera.

O presente trabalho conclui que a indução do hiperparatireoidismo secundário nutricional não alterou os valores fisio-semiológicos dos bovinos no presente estudo.

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Variável	M	s
Temperatura (°C)	38,40	0,35
Movimento Rumenal (2 min.)	2,08	0,65
Freqüência Respiratória (1 min.)	28,29	5,19
Freqüência cardíaca (1 min.)	84,84	16,35

N= Número de observações = 90

RESUMO

O presente trabalho teve por objetivo a determinação de alguns parâmetros fisiológicos de bovinos clinicamente sadios durante o período experimental do hiperparatireoidismo secundário nutricional. Entre o período de março à setembro de 1988, foram mantidos confinados 15 novilhos mestiços leiteiros, entre 8 a 12 mêses de idade na Escola de Veterinária da UFMG. Dividiu-se os animais em três grupos para receberem uma dieta isoproteica e isocalórica, com a mesma composição mineral (segundo NRC 1976), exceto quanto ao cálcio e fósforo, que sofreram modificações o que correspondeu aos tratamentos 1, 2 e 3. Quinzenalmente, efetuou-se a medição da temperatura, dos movimentos rumenais e das freqüências cardíaca e respiratória de todos os animais. Não houve diferença significativa entre os resultados obtidos nos três tratamentos e os valores médios finais foram: Temperatura - 38,40 + 0,35°C; Movimentos rumenais 2,08 ± 0,65 durante dois minutos; Freqüência respiratória - 28,29 ± 5,19 movimentos por minuto; Freqüência cardíaca - 84,84 ± 6, 5 batimentos por minutos. Todos os valores encontrados apresentavam-se dentro dos parâmetros fisiológicos estabelecidos pelos autores consultados.

SUMMARY

The objective of this work was to determine some physiological aspects of bovine, clinically healthy during an experimental period of nutritional secondary hiperparathyroidism. From march to september, 1988, 15 yong cross-bred dairy cattle, aging eight to twelve month-old, living under confine condition in the UFMG Veterinary Medicine school. Three groups were composed and were fed with isoproteic and isocaloric food which showed the same mineral composition (NRC 1976), excepting calcium and phosphorus. The composition of these two minerals were modified to form the treatments 1, 2 and 3. In all the animals, the body temperature, ruminal movement and respiratory and cardiac frequency were verified monthly. There was no major difference in values in the three groups and the results found were: Corporal Temperature - 38,40 ± 0,35°C; Rumenal Moviment - 2,08 ± 0,65/two minutes; Respiratory Frequency - 28,29 ± 5,19 /minute; Cardiac frequency - 84,84 ± 16,35/minute. All the results were found to be according to physiological values established by the consulted authors.

RESUMÉ

L'objet du présent travail a été de déterminer quelques paramètres physiologiques de bovins, cliniquement sains, durant la période experimentale de l'hyperparatyroidisme nutritionnel secondaire. Durant la période allant de mars à septembre 1988, quinze bouvillons métis laitiers ages de 8 à 12 mois ont été maintenus confinés dans l'Ecole Vétérinaire de la UFMG. Les animaux ont été divisés en trois group s afin de recevoir une diéte isoprotéique e isocalorique, avec la même composition minérale (selon NRC 1976), excepté en ce qui concerne le calcium et le phosphore qui ont subi des modifications correspondant aux traitements 1, 2 et 3. A chaque mois, les mesures suivantes ont été effetuées pour tous les animaux: température, mouvements respiratoires et rumenaux et fréquence cardiaque. Il n'y a pas eu de différence significative entre les resultats obtenus pour les trois groupes et les valeurs moyennes finales ont été les suivantes: Température - 38,40 ± 0,35°C; Mouvements Rumenaux pendant 2 minutes - 2,08 ± 0,65; Fréquence respiratoire - 28,29 ± 5,19 mouvements par minute; Fréquence cardiaque - 84,84 ± 16,35 batements par minute. Toutes les valeurs déterminées se

sont trouvées dans la zone de paramétres physiologiques établis par les auteurs consultés.

RESUMEN

El presente trabajo tuvo por objetivo la determinación de algunos parâmetros fisiológicos de bovinos clinicamente sanos, durante el período experimental del hiperparatiroidismo secundário nutricional. Entre el período de marzo à seticembre de 1988, fueron mantenidos confinados 15 novilhos mestizos lecheros, entre 8 a 12 meses de edad en la Escuela de Veterinaria de la UFMG. Se dividieron los animales en tres grupos para recibir una dieta isoproteica e isocalórica, con la misma composición mineral (segun NRC, 1976), excepto cuanto al calcio y fósforo; que sufriran modificaciones lo que correspondio a los tratamientos 1, 2 e 3. Quincenalmente efectuose medición de la temperatura, de los movimientos ruminales y de las frecuencias cardiacas y respiratoria de todos los animales. No tuvo diferencia significativa entre los resultados obtenidos en los tres tratamientos y los valores medios finales fueron: Temperatura - 38,40 ± 0,35°C; Movimientos ruminales 2,08 ± 0,65 durante dos minutos; frecuencia respiratoria 28,29 ± 5,19 movimientos por minutos; frecuencia cardiaca 84,84 ± 16,35 batimentos por minuto. Todos los valores encontrados estaban dentro de los parametros fisiológicos estabelecidos por los autores consultados.

METABOLIC PROFILE TESTS IN HIGH YIELDING NORMAL COWS AND IN COWS SUFFERING FROM ABOMASAL DISPLACEMENT*

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INTRODUCTION

High yielding dairy cows very often develop metabolic disturbances as ketonaemia and fatty liver. These abnormalities are frequently accompanied by or complicated with other syndromes, such as hypocalcaemia, abomasal dislocation and infectious processes as mastitis and metritis.

KETONAEMIA AND ITS ETIOLOGY

As far as the etiology of ketonaemia is concerned, initially an energy deficiency was putted forwards. But the pathogenesis of the syndrome seems more complicated and several parameters may play a role in the development of the syndrome. In slide number 1, an attempt is made to combine several factors which may be of importance.

DIAGNOSIS OF KETONAEMIA AND FATTY LIVER : RESULTS AND INTERPRETATION

Determination of ketone bodies in blood and urine is often used as a method for diagnosing metabolic disturbances. However, it has been proved that a postprandial ketonaemia and ketonuria can appear, without any pathological significance, in both non-lactating and high yielding dairy cows. It was the aim of the present investigation to correlate blood parameters to the metabolic profile of the liver as estimated by the quantitative determination of triglycerides (TG) and glycogen in liver biopsies of control cows and cows with metabolic disturbances.

The liver of clinically normal lactating cows contains less than 20 mg TG and more than 20 mg glycogen per gram of wet tissue.

From table 1 it can be seen that in high yielding cows from a farm with metabolic problems, only those animals with both fatty liver and glycogen depletion showed ketonaemia and hypoglycaemia. When the glycogen level was normal, ketonaemia and glycaemia stayed within normal limits, even though these cows had a fatty liver.

TABLE 1. Blood parameters from high yielding cows on a farm with problems of metabolic disturbances

Number of animals n=42	3OHBA mmol/l	glucose mmol/l
Fatty liver : n=20		
- normal glycogen n= 8	0,76 ± 0,36	3,54 ± 0,58
- glycogen depletion n=12	2,13 ± 1,17**	2,59 ± 0,47**
Not fatty liver : n=22		
- normal glycogen n=14	0,71 ± 0,18	3,41 ± 0,23
- glycogen depletion n= 8	0,61 ± 0,26	3,31 ± 0,29

** Significantly different from other groups ($p<0.01$)

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In problem herds, regular control of ketone bodies, eventually associated with liver biopsies, might be indicated to evaluate the energy state of the animals. Besides, determination of blood levels of some ions, especially Ca and Mg, may be a guide to take special care of pre- and post-partum supplementation.

IVGT-TESTS IN COWS SUFFERING FROM ABOMASAL DISLOCATION

In high yielding dairy cows, abomasal dislocation is a frequently occurring syndrome. Going back to slide 1, we can point out 3 factors which certainly play a main role in this syndrome, because they inhibit the emptying of the abomasum : hypocalcaemia, circulating endotoxins and insuline resistance. Using cows with a fistulated duodenum it was shown that insuline, endotoxins and hypocalcaemia induced a definite decrease in abomasal emptying. In cows suffering from LD, it was found that notwithstanding a frequently occurring ketonaemia, these cows showed high blood glucose levels.

TABLE 2. T1/2 of glucose after IVGTT in control animals and animals suffering from LD

T1/2 : min	Control animals (n=40)	LD (n=387)
First hour	52,2 ± 9,3	66 ± 15**
Second hour	77,8 ± 13,1	103 ± 26**

** Significantly different from control animals ($p<0,01$)

TABLE 3. Insuline response after IVGTT in control animals and animals suffering from LD

Time after IVGTT (min)	Control animals non-ketotic(33)	Control animals ketotic(7)	LD (38)
0	47 ± 19[a]	31 ± 5[b]	73 ± 31[c]
15	203 ± 98[a]	42 ± 20[b]	235 ± 62[a]
30	158 ± 108[a]	50 ± 17[b]	230 ± 70[c]
60	117 ± 80[a]	44 ± 10[b]	209 ± 62[c]
120	52 ± 18[a]	36 ± 10[b]	103 ± 52[c]
240	48 ± 22[a]	30 ± 13[b]	79 ± 23[c]

Values pointed by various letters are significantly different ($p<0,01$)

IVGT-tests indicated that LD-cows reacted with high insuline levels, which apparently were not able to decrease the glycaemia. In contrast, in normal non-ketotic cows, the insuline response to the intravenous glucose resulted in a normal glucose clearance. In normal ketotic cows, the glucose clearance was also normal, even though they showed a minimal insuline response. Up till now, there is no explanation available for this.

From these results in LD-cows, the presence of an insuline resistance was put forwards. The causes for this resistance are not very clear yet. Among other things, lipolysis and circulating ketone bodies are supposed to play a role. STH, that reaches a peak at the moment of the parturition, is known to cause insuline resistance in ruminants, as well as

a lipolytic effect.

CONCLUSION

In conclusion, we can say that in dairy herds health management, determination of ketone bodies and some ions is a useful aid to evaluate the energy and ionary state of the herd, so that intervention is possible where needed. As for the causes of insuline resistance which appears in cows suffering from LD, further investigation will be needed.

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SUMMARY

In high yielding dairy cows, the determination of the ketone bodies in blood and urine for evaluation of the energy state, is not always a reliable method, though it can be helpful in dairy herd health management. Determination of Ca and Mg may also be a guide for eventual supplementation in pre- and postpartum diet. Cows suffering from abomasal dislocation were found to be in a state of insuline resistance, of which the causes need further investigation.

RÉSUMÉ

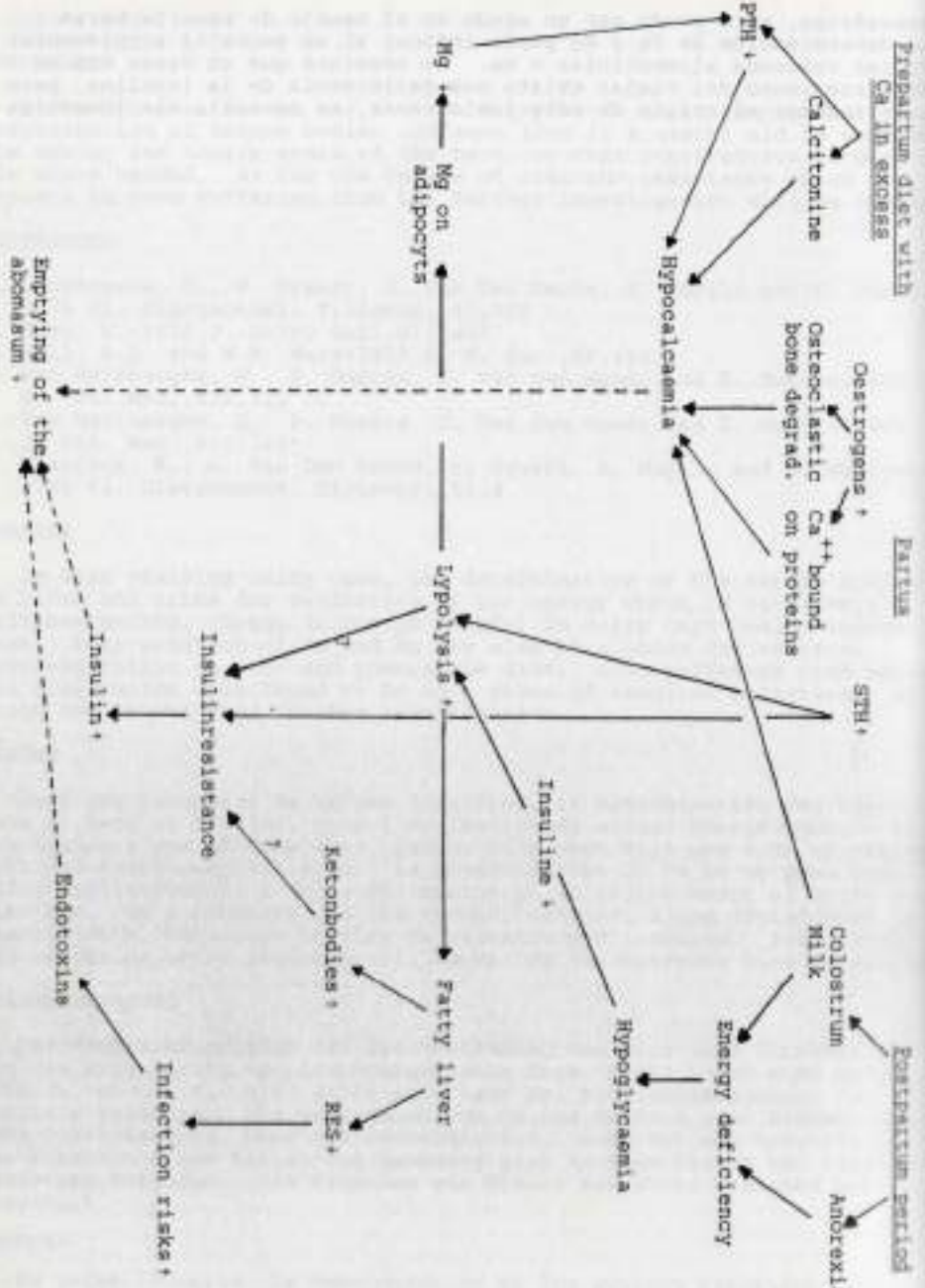
Dans des troupeaux de vaches laitières, la détermination des kétones dans le sang et l'urine, pour l'evaluation du statut énergétique, n'est pas toujours une méthode sûre, quoiqu'elle peut être une aide au management des troupeaux laitières. La détermination du Ca en Mg peut conduire éventuellement à une supplementation au régime avant et après la mise bas. On a constaté que les vaches, atteints d'une déplacement de la caillette, sont dans un état de résistance d'insuline. Pour savoir les causes de cette résistance il faudra de la récherche plus détaillée.

ZUSAMMENFASSUNG

Bei Hochleistungskühe ist die Bestimmung von Blut- und Urineketonen für die Evaluierung von die energetische Lage, nicht immer eine gute Methode, obwohl Sie eine Hilfe sein kann bei Betriebsmanagement von Hochleistungskühe. Die Bestimmung von Ca und Mg kann eine Ergänzung beim Diät anzeigen, wann est notwendig ist. Auch hat man konstatiert das Kühe mit einer Versetzung Labmages sich in eine Status von Insulinresistenz befinden. Die Ursachen von dieser Resistenz brauchen weiter Untersuch.

RESUMEN

En vacas lecheras, la determinación de los cuerpos cetónicos en el sangre y la orina no siempre es una buena manera para evaluar el balance energético, pero puede ser un ayudo en el manejo de vaca lecheras. La determinación de Ca y Mg puede indicar si se necesita supplementar en las raciones alimenticias o no. Se constató que en vacas con un desplazamiento del cuajar existe una resistencia de la insulina, pero para indicar el origin de esta resistencia, se necesita más investigación.



SEQUELAE TO ORAL CALCIUM CHLORIDE GEL DOSING OF COWS

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INTRODUCTION

Oral dosing with solutions containing $CaCl_2$, 2 H_2O is widely used in the Scandinavian countries and elsewhere to prevent hypocalcaemic peripartuient paresis. The standard prophylactic programme consist of 4 dosis given to the calving cow over 48 hours according to Jönsson and Pehrson (1).

Calcium chloride is known to be irritative to tissues and it is well known that cows dislike the taste of such aqueous solutions. Therefore, investigations were designed to examine the possible occurrence of acute mucosal damage (experiment A) and reduced appetite (experiment B).

MATERIALS AND METHODS

Calcium chloride products and their management.

Two commercial products, available in Denmark, were tested.

Product 1: $CaCl_2$, 2 H_2O, 196 g in an aqueous hydroxymetylcellulose gel. Total volume 400 ml (H. Lundbeck A/S, DK-2500 Valby).

Product 2: $CaCl_2$, 2 H_2O, 200 g given in a soja oil emulsion made with 380 g soja oil. Total volume 1 liter (Gunnar Kjems ApS, DK-1171 Copenhagen).

The products were given at the following intervals. First dose in the afternoon. Second, third and fourth dose were given the following day in the morning, at noon and in the afternoon, respectively.

Experimental cows; management and design.

Experiment A: Six in-patients were available. All six had reduced appetite due to mainly infectious diseases such as endocarditis (appetite varied between 1 and 7 kg of concentrate/day). Three of the cows were given product 1 and three cows were dosed with product 2. All six cows were autopsied the day after the fourth dose was given. The oral cavity, pharynx, oesophagus and the forestomacs were examined for mucosal lesions. Standard histopathology was performed.

Experiment B: Six healthy dry cows were used. Three cows were given product 1 and three cows were given product 2, respectively. They were fed hay ad libitum plus 2 kg concentrate in the morning and 2 kg in the afternoon. Before the start of the experiment, all six cows were eating their concentrate rate within 15 minutes.

Clinical methods (experiments B)

Appetite was recorded as normal, reduced or absent by judging the amount of concentrate left one hour after feeding. The glutaraldehyde test was performed as a cow-side test (Glutavac'®'test, J. Kruuse A/S, DK-5290 Marslev) on whole blood in vacutainer tubes. Serum albumin was measured spectrophotometrically by standard bromcresole green method. Fibronogen was determined by refractometer measurement before and after heat treatment (2). Serum protein was done by the Biuret method (3). Glubulin concentrations were calculated as the difference between total protein and albumin.

RESULTS

Experiment A.

All 3 cows given product 1 had lesions in the forestomacs. Two had a 15-20 cm diameter focal lesion in atrium ruminis with central necrosis of the epithelium and periferal hyperaemia. The third cow had more diffuse hyperaemia with minor areas with loosening and necrosis of epithelium in atrium rumenis, reticulum and omasum. Histologically the epithelial lesions were characterized by necrosis, epithelial vesicles filled with plasma/neutrocytes and a sero-fibrinous and neutrocytic inflammation of the underlying propria. The inflammatory processes also involved muscularis and an oedema in subserosa as well as trombosis of lymph and vein/capillary vessels. In the cow with more diffuse lesions, the histological picture was characterised by necrosis and neutrocytes infiltrated epithelium as well as moderate inflammatory oedema in a narrow subepithelial zone of propria.

Cows given product 2 were without macroscopic lesions. Two were without microscopic lesions whereas the third had hyper-dyskeratotic changes of oesophagus and atrium ruminis with localised plasma/neutrocyte exudate superficially and a light neutrocytic infiltrated oedema in subepithelial propria.

Experiment B.

In one of the cows given product 1, lack of appetite was recorded day 2 and 3 and reduced appetite the following two days. Diarrhoea, bradycardia and subnormal rectal temperature and skin temperature on rump and hind legs was recorded at the same time. Among the other two cows given product 1, lack of appetite and reduced appetite was observed for one and two days, respectively. The latter cow had a semi-fluid faecal consistency for four days.

Two of the three cows given product 2 had reduced appetite for 2 days and loose faeces for 3 days. Rectal and skin temperature as well as heart rate was normal.

The glutaraldehyde test became positive and remained so for at least 5 days, among the cows given product 1, whereas cows given product 2 remained negative.

It appears from figure 1 that there was a slight increase in the globulin concentration among the cows given product 1 but that inparticular a marked increase was recorded in fibrinogen of the same group.

DISCUSSION

Although the number of cows was limited, it is clear that side effects occured and that these were pronounced in the cows given product 1. It is likely that the described lesions of that group were responsible for the increase in plasma fibrinogen which again resulted in a positive glutaraldehyd. The latter finding may be usefull to veterinary practitioners trying to differentiate between the described syndrome and a light case of milk fever.

The rather constant albumin concentration indicates that the fibrinogen increase was not due to haemoconcentration caused by diarrhoea.

Although cows were given 4 doses over a 24 hour period in the present experiment rather than over 48 hours as recommended by Jönsson and Pehrson (1) and by most producers of calcium chloride drenches, the described side effects are likely to occur under farm conditions because farmers often repeatedly drench periparturient cows which for one reason or the other do not perform well.

The severity of the described symptoms may not only be seen as a differential diagnostic problem, but also as a noxe to the fragile high yielding calving cow.

ACKNOWLEDGEMENTS

The authors are thankfull to Asger Lundorff, Central Laboratory, Department of Clinical Studies, for fruitfull discussions on relevant blood parameters.

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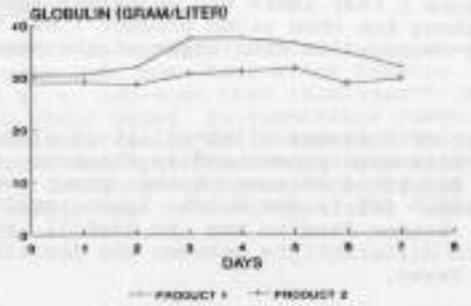
SUMMARY

Focal rumenitis in atrium ruminis with transitory reduction or loss of appetite was found in cows given a calcium chloride gel product intended for the prophylaxis of milk fever. The lesions caused an increase in blood fibrinogen which again resulted in a positive glutaraldehyde test. Clinical examination reveiled temporary diarrhoea and, in one out of three cows, subnormal temperature and bradycardia. Cows given a different product containing the same amount of calcium chloride in an oil emulsion, showed only slight; lesions, clinical symptoms were virtually absent and the glutaraldehyde test remained negative. The desribed symptoms may lead to differential diagnostic problems and they may be seen as a noxe to the potentially high yielding but fragile calving cow.



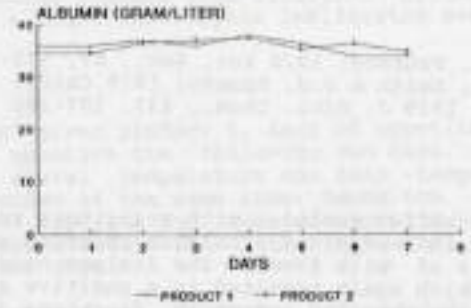


Figure 1. Blood parameters of the cows in experiment B. Day 0 was defined as the day the cows received 3 doses.

RÉSUMÉ

La ruminite focale dans l'atrium ruminis avec réduction transitoire ou perte d'appétite a été trouvée chez les vaches traitées par un produit à base de gel de chlorure de calcium destiné à la prophylaxie de la paralysie puerpérale. Les lésions ont provoqué une élévation du taux du fibrinogène sanguin qui s'est traduit par une réaction positive à l'épreuve au glutaraldéhyde. Des examens cliniques ont révélé une diarrhée temporaire, et, chez une vache sur trois, une température au-dessous de la normale et une bradycardie. Les vaches traitées par une autre produit contenant le même taux de chlorure de calcium mis dans une émulsion d'huile ont présenté seulement de faibles lésions, les symptômes cliniques étaient de fait absent et l'épreuve au glutaraldéhyde est restée négative. Les symptômes décrits peuvent conduire à des problèmes de diagnostic différentiels et peuvent être interprétés comme un facteur de stress chez les vaches à haut rendement potentiel mais à difficulté de vêlage.

ZUSAMMENFASSUNG

Bei Kühen, denen ein Kalziumchloridgel zur Milchfieberprophylaxe verabreicht worden war, trat eine begrenzte Rumenitis im Atrium ruminis mit vorübergehender Appetitminderung oder (temporärer) Futterverweigerung auf. Die Läsionen verursachten einen Anstieg der Blutfibrinogenwerte, was sich in einem positiven Glutaraldehydtest ausdrückte. Bei der klinischen Untersuchung wurden kurzfristig Diarrhoe und in einem Fall subnormale Temperatur und Bradykardie festgestellt. Kühe, denen ein anderes Produkt mit derselben Menge an Kalziumchlorid in einer öligen Suspensionen verabfolgt wurde, wiesen nur leichte Läsionen auf, hatten praktisch keine klinischen Symptome und einen negativen Glutaraldehydtest. Die beschriebenen Symptome könnten differentialdiagnostische Schwierigkeiten bereiten und einen Schaden für leistungsmässig hochveranlagte, aber gesundheitlich labile Kühe darstellen.

PREVENTION OF MILK FEVER BY ORAL ADMINISTRATION OF ENCAPSULATED CA-SALTS

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INTRODUCTION

It has earlier been shown (1) that oral administration of calcium chloride ($CaCl_2$) in a water solution 2-3 times in close relation to calving will have a prophylactic effect on parturient paresis (milk fever). Because of the risk of aspiration the method is, however, unsuitable for use in practice. By mixing the salt with hydroxycellulose into a gel ("Paregel") it was possible to decrease the risk of aspiration, and a good prophylactic effect was achieved after administration of the $CaCl_2$-gel daily from 3-7 days before to 2 days after calving (5). The incidence of milk fever among cows which had contracted the disease at their last calving was reduced by 50 per cent when a modification of this method was performed, implying administration of the gel 3-4 times during the period 24 hours before to 24 hours after calving (4).

The "Paregel" method is commonly used by the farmers in Sweden. It has, however, certain disadvantages. The most evident is the unpleasant taste of the salt, which brings about struggling efforts from the cow at administration. Besides, there are still certain risks of aspiration.

The aim of the present investigation was to test the prophylactic effect on milk fever of orally administered Ca-salts, which were compressed and encapsulated according to a patented method which totally masks the sharp taste of the salts.

MATERIAL AND METHODS

Only cows which according to the Swedish central disease registration system had been treated for milk fever at their last calving were included.

Trial I. The cows in this trial were from herds in which "Paregel" regularly was used for prevention of milk fever to animals which at their earlier calving(s) had contracted the disease. Since it was not considered ethically justifiable to use a placebo group in such herds, every second cow was given Ca-capsules (group IA), whereas every second cow got "Paregel" as should have been done if no trial had been executed (group IB). Altogether 135 cows from 114 herds were included. Except for the administration of Ca-capsules or "Paregel" no preventive measures against milk fever were used.

Trial II. The cows were from herds in which the farmers did not intend to give any prophylactic medicine at all to their milk fever-prone cows. Every second cow got Ca-capsules (group IIA) and every second placebo capsules containing sand instead of Ca-salts (group IIB). Altogether 107 cows from 84 herds were included. This trial was a double blind test.

Administration of treatments. One dose of "Paregel" was recommended to be given 4 times in a conventional way; that is about 24 hours before calving, in close relation to calving, 10-14 hours after calving and about 24 hours after calving. The same time schedule was recommended for administration of the capsules - once at each time. The actual time for the administration of "Paregel" and capsules were recorded by the farmer. The capsules were given with a balling gun (Fig. 1).

Each dose of "Paregel" contained 54 g of Ca as $CaCl_2$ in a hydroxycellulose gel. Each Ca-capsule contained 46 g of Ca as $CaCl_2$ and $CaSO_4$ surrounded by a mixture of fatty acids. This capsule has been shown to be dispersed in the rumen fluid within 10-20 minutes.

Figure 1. Administration of Ca-capsules with a balling gun

The time for first treatment of cows with milk fever was recorded by the veterinarian, who also was asked to take blood samples before treatment for Ca-analyses.

Only the cows in groups IA, IB and IIA which got the first dose earlier than 2 hour after calving were included in the statistical evaluation. Cows in these groups which did not get milk fever were excluded only if they received at least 3 out of the 4 recommended doses at the correct times. Cows in group IIB were excluded if they got milk fever earlier than 2 hours after calving.

A cow was considered to have milk fever if she exhibited paretic symptoms within a week after calving.

The statistical evaluation was made by chi-square analysis.

RESULTS

Of the 242 cows selected for the experiment, 49 were excluded (15 in group IA, 17 in IB, 9 in IIA and 8 in IIB); 46 of them because of not fulfilling the above-mentioned criteria and 3 because of suffering primarily from other diseases than milk fever (1 coliform toxemia in each of group IB and IIB, and 1 extensive muscular injury in group IA).

Among the 193 cows which were included in the final evaluation, 63 got milk fever. Hypocalcemia (<1 mmol Ca/l serum) was confirmed in 43 cases. In the remaining 20 cases the clinical picture clearly indicated a classical milk fever. In 17 of these 20 cases no blood samples were taken, whereas in 3 the serum-Ca levels were close to 2.00 mmol/l (2.01; 2.03 and 2.16, respectively).

No clinical side effects of any of the treatments were reported.

The incidences of milk fever in the different groups are in Table 1. The placebo cows (group IIB) had significantly higher incidence than the cows in the other groups. No significant difference existed between "Paregel"-treated cows and those who got the Ca-capsules in trial I. The disease incidence was higher in group IA than in group IIA, both of which got Ca-capsules. However, the difference was not significant.

There were no significant differences between treatment groups in regard to age or milk production the year before the experiment ($\bar{x}$=7.7 years and 7.265 kg 4% FCM, respectively, in the total material). The breed distribution was equal within each trial but somewhat different between them (Table 1).

TABLE 1. Incidence of milk fever and breed distribution in cows given Ca-capsules (IA and IIA), Paregel (IB) and placebo (IIB). Different letters indicate significant differences ($p<0.05$). SRB = Swedish Red and White Cattle. SLB = Swedish Holsteins.

Group	Number of cows	Incidence of milk fever, per cent	Breed distribution, per cent		
			SRB	SLB	Others
I A	52	28.8[a,b]	65.4	26.9	7.7
I B	51	35.3[a]	62.7	25.5	11.8
II A	48	14.6[b]	52.1	43.8	4.1
II B	42	54.8[c]	54.8	40.5	4.7

DISCUSSION

The prophylactic effect of administration of the Ca-capsules was evident. The disease incidence in the placebo group was of the same magnitude as reported earlier in similar studies (2,3,4). On the other hand the incidence was somewhat higher in the "Paregel" treated group than earlier reported (4). This discrepancy can probably be explained by differences on the herd level, as can the great difference in disease incidence between the two groups that got Ca-capsules (IA and IIA). Since there were no significant differences regarding age or productivity between groups, and since the different breed distribution hardly can explain more than a negligible part of the differences in disease incidence, the explanation must be found in other herd factors. The farmers in trial I had earlier used "Paregel" as prophylactic treatment more or less as a routine. Some cows in that trial had, therefore, probably got milk fever at their last calving despite being "Paregel"-treated. Thus, the cows in trial I might be supposed to be more disposed to milk fever than the cows in trial II and also, in some cases, more refractive to prophylactic treatment with Ca-salts. If this is correct, the incidence of milk fever should have been even higher in a hypothetic placebo group from trial I than that found in group IIB. Therefore, the conclusion from earlier experiments (4) that prophylactic treatment with "Paregel" eliminates every second case of milk fever in cows which had the disease at their last calving, may not be contradictory to the results from the present investigation.

Comparable amounts of Ca were given at the same times with the Ca-capsules and with "Paregel". Even if the difference was not significant, the disease incidence in trial I was somewhat lower in the capsule-treated cows than in the "Paregel"-treated cows (28,8 and 35,3 per cent, respectively). Besides, the capsule-treated cows in trial II had a lower disease incidence than "Paregel"-treated cows in an earlier comparable experiment (14.6 and 22.6 per cent, respectively; ref. 4). This may be interpreted as an advantage from the slow release effect of Ca caused by the gradual dissolution of the capsule within the rumen. Anyhow, it seems evident that the prophylactic effect of the Ca-capsules was at least as good as that of "Paregel". The most evident advantages with the capsule-method are that the risk of aspiration is eliminated and that the sharp taste of the Ca salts is masked.

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SUMMARY

The prophylactic effect on milk fever by repeated oral administration of encapsulated Ca-salts in close correlation to calving was tested in comparison with both a commercially available Ca-gel ("Paregel") and placebo capsules. Two trials involved a total of 193 cows which had contracted milk fever at their last calving. In trial I 28.8 per cent of the cows treated with Ca-capsules got milk fever compared to 35.6 per cent of the "Paregel"-treated cows. In trial II 14.6 per cent of the cows in the capsule-treated group got the disease compared to 54.8 per cent in the placebo group.

RÉSUMÉ

L'effet prophylactique contre la fièvre vitulaire par l'administration orale répétée de sels de calcium capsulés près de la parturition a été étudié en comparant une "gelée-calcium" commerciale (Paregel) aussi bien que des capsules placebo. Les deux études comprenaient un total de 193 vaches, qui avaient souffert de la fièvre vitulaire pendant leur dernière parturition. Dans la première étude 28,8% des vaches traitées avec des capsules-calcium ont souffert de fièvre vitulaire, comparé à 35,6% des vaches traitées avec Paregel. Dans la deuxième étude 14,6% des vaches traitées avec des capsules-calcium ont souffert de la maladie comparé à 54,8% des vaches traitées avec des capsules placebo.

RESUMEN

El efecto profiláctico contra la fiebre de leche por la administración oral repetida de cápsulas de sales de calcio cerca del parto fue estudiado en comparación con un gel de calcio comercial disponible (Paregel) y con cápsulas placebo. Dos estudios involucraron a un total de 193 vacas, las que habían contraído fiebre de leche durante el parto anterior. En el experimento un 28,8% de las vacas tratadas con cápsulas de calcio tuvieron fiebre de leche comparado con un 35,6% de las vacas tratadas con Paregel. En el experimento dos, 14,6% de las vacas en el grupo tratado con cápsulas tuvieron la enfermedad comparado con un 54,8% en el grupo placebo.

ZUSAMMENFASSUNG

Der vorbeugenden Effekt auf Gebärparese von mehrfacher oraler Zufuhr einkapselten Ca-Salzen in engem Anschluss der Geburt war mit dem Effekt eines kommerziell zugänglichen Ca-Gelee (Paregel) und auch mit dem Effekt der Placebo-Kapseln verglichen. Zwei Versuche haben insgesamt 193 Kühen umgefasst, die allen in Milchfieber an der lezten Geburt erkrankt waren. Im Versuch I erkrankten 28,8% der Kühen die mit Ca-Kapseln behandelt waren, was mit 35.6% der "Paregel"-behandelten Kühen verglichen werden soll. In Versuch II erkrankten 14.8% der Ca-Kapsel-behandelten Kühen, weil 54.8% der Kühen in der Placebo-Gruppe in Gebärparese erkrankt waren.

CLINICAL APPRAISAL OF DOWNER SYNDROME IN INDIAN DAIRY ANIMALS

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INTRODUCTION

High Producing Cows and Buffaloes develop downer syndrome generally around calving. In the beginning, it was considered to be an outcome of unresponsive cases of milk fever. Presently, a number of etiological factors have been attributed to cause downer syndrome (1). The clinical management often becomes a challenge for practising veterinarian when a cow assumes recumbent posture. Often the effects of downer posture to the support muscles and nerves are to the extent of no regain even after correction of primary factor(s).

In order to understand the etiopathogenesis and remedy of the disease, studies were made covering symptomatology, blood biochemistry and treatment on 89 milch animals suffering from downer syndrome.

MATERIALS AND METHODS

Animals

Sixty-six cows and twenty-three female buffaloes, 2.5-9 years old, suffering from downer syndrome for varying periods of time, presented either at the college clinics or attended at dairy farms belonging to 446 organised dairies of the Punjab State, were studied.

Clinical Examination

All the animals were subjected to general inspection in respect of posture and general condition. Clinically, body temperature, respiration, rumen movement, rumination, defaecation and urination were recorded.

Blood sampling

Blood samples were collected at different intervals till the recovery or death of the animals. Fresh heparinised blood samples were analysed for Hb, RBC, WBC and its differential patterns, haematocrit, Erythrocytic indices, blood glucose and Blood Urea Nitrogen. Serum was harvested out of plain blood and processed for Ca, P, Mg, Na, K, total protein, cholesterol, AST, ALT, CPK and LDH using the standard procedures. Urine samples were subjected to routine examination.

Tissue sample

Gross pathological examination and collection of samples of muscles, nerves, liver, heart and kidney from unresponsive animals for histomorphological, histochemical and histoenzymic patterns, were carried out using standard techniques.

Treatment

Management

All the patients were provided soft floor covered with thick layer of paddy straw. The sides were changed every 2-3 hrs.

Mechanical slinging was done daily once or twice for 20-30 minutes. The limbs were massaged while standing. Superficial bed sores were applied with antiseptic cream.

Therapeutics

Solution containing Ca, P and Mg was infused intravenously @ 1.5 ml/kg body weight once or twice. Intramuscular injections of Inophosphan (10-15 ml) and Neuroxin-12 (10 ml) on alternate day and Vitacept (5 ml) twice weekly were given. Orally Potassium acetate @ 40 g daily was supplemented. In addition, appetizer and liver tonics were used till their final outcome.

RESULTS AND DISCUSSION

Based on clinical appreciation, the downers were of two categories (i) Alert downers, and (ii) Non-alert downers. The alert downers had peculiar sternal sitting posture with normal appetite, respiration, rumination, defaecation and urination but for inability in getting up. Whereas the non-alerts were anorectic, usually on lateral recumbency and even comatosed. Both the respiratory and pulse rates were increased. Most of the animals had normal temperature but slightly raised as well as low temperature was also recorded.

All the animals were unable to get up particularly with hind legs. Characteristic crawing on ground were observed in alert downers which might be associated with hind leg weakness due to potassium depletion owing to prolonged recumbency (10). Unsuccessful attempts to get up and prolonged unchanged sitting posture caused bed sores. In addition, knuckling and hyperflexon of hind fetlocks were observed, which were the outcome of damage to tibial and peroneal nerves (11) during crawling or uncontrolled efforts by the animals to get up.

Haematology

The haemograms of downer cows were within normal limit, whereas a marginal decrease was noted in downer buffaloes. No marked change in haemotocrit (HCT) and leucocytes was recorded in both species of animals. Erythrocytic indices showed increase in MCV while decrease in MCHC. Downer buffaloes had low RBC count. Relative neutrophilia and lymphopenia were observed in all animals owing to physiological stress of recumbency (12) as well as apparent bed sores. MCH, basophil and monocytic patterns were unchanged.

Blood biochemical and enzyme

Most of the downer animals had hyperglycaemia. As high as 232-250 mg/dl blood glucose was recorded. Hypo-insulinaemia conditioned by low serum calcium was alleged for it in paretic cows (2,11) and in present study most of the animals had hypo-calcaemia. Additionally, increased cortisol concentration has also been opined to cause increased glucose level (2,4). The BUN level was also increased in downer animals and remained high throughout the period of recumbency. Pressure damage of muscle and renal malfunctions might be the reasons for raising BUN level (7). However, the level of cholesterol was unaffected. There was appreciable decrease in serum calcium, phosphorus and potassium level while the level of magnesium and sodium was unchanged. Release of parathormone in response to hypocalcaemia (8) caused enhanced excretion of inorganic phosphorus leading to

hypophosphataemia. Rapid urinary excretion and diminished alimentary absorption of potassium due to decreased feed intake had been attributed to cause hypokalaemia in such animals.

High activities of AST, ALT, CPK and LDH in downer animals were indicative of ischaemic muscular damage owing to prolonged recumbency (3). Sustained raised activities of these enzymes in non-responsive animals were suggestive of persistent muscular damage. Whereas the gradual decline in these haemoenzymes in responsive downers was indicative of arrest of muscle damage giving favourable prognosis of the cases.

The urine analysis showed glycosuria and proteinuria in 30 and 43 per cent downer cows while the respective figures for buffaloes were 75 and 62 per cent. Proteinuria was the result of muscular and renal damage while glycosuria was the result of hyperglycaemia, exceeding renal thresh-hold (110-120 mg/dl) [9].

Histopathological and histoenzymic studies

Grossly the animals were emaciated and sores were on the sides in the animals recumbent for more than four days. Deep sores were on shoulder, tubercoxae, knee and pastern joints. Marked atrophy, hyalinization and loss of cellular content of muscle were observed. These changes were the outcome of pressure ischaemia due to prolonged recumbency (1,6). Both the peroneal and sciatic nerves showed marked degenerative changes. Nerve fibres were detached from its epineural covering suggestive of ischemic effect of pressure in recumbent animals (5). Necrosis of ventral motor neuron in spinal cord and degenerative changes in purkinje cells of cerebellum were apparent. Depletion of hepatic as well as skeletal muscle glycogen was suggestive of exhaustion of stored energy and/or reduced synthesis of it by stressed liver. The muscle fibres, liver and kidneys had increased lipid content.

The histoenzymic studies revealed loss of AKPase in muscle fibres and liver which was suggestive of degenerative changes leading to leakage of this enzyme into blood circulation while ACPase activity was increased in gluteal muscle and Kupfer cells. The atrophied muscle fibre had enhanced ATPase activities and loss in hepatic canaliculi. Marked loss of Acetyl cholinesterase (AchE) in nerve fibre, muscle, purkinje cells was observed. The decreased AchE activity in these neural elements impaired the transmission of nerve impulse leading to muscle atony. The activity of SDH in muscle and kidney was low.

The management as well as therapeutics provided to ailing cows and buffaloes proved successful in 51.5 and 37 per cent cows and buffaloes, respectively. About 30 per cent recovery has been reported in downer cows (10). The response was comparatively poor in downer buffaloes and cows which might be attributed to the heavy weight of the buffaloes. The favourable outcome of downer animals depended on the duration of recumbency. Longer was the duretion of recumbency, poorer was the response. However, 7 weeks old case could respond to the sustained managemental and therapeutic supplementation.

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SUMMARY

High producing cows and buffaloes develop downer syndrome, generally around calving. Clinical management and the duration of illness warrant critical appreciation to deal with this condition in dairy animals. Studies were carried on 89 clinically downer animals (66 cows and 23 buffaloes) belonging to 446 organised dairies of the Punjab State. The animal of 6-7 yr age suffered more. Clinically alert and non-alert downers were recorded. The alert downers were on sternal posture with good appetite but for unable to stand up. On the other hand, the non-alerts were recumbent laterally and had poor appetite. Blood analyses revealed bed sores related neutrophilia, low haemoglobin, erythrocytes, calcium and magnesium - a metabolic outcome and raised blood glucose. Increased activities of serum creatine phosphokinase, aspartate aminotransferase and alanine aminotransferase in downer animals indicated continued damage of dependent muscles and remained elevated in unresponsive cases due to downer posture. Histomorphologically atrophy as well as necrosis of gluteal and biceps femoris muscles, degeneration of myelin sheath of sciatic nerve and necrosis of ventral motor neurons of spinal cord were the outcome of continued recumbent posture. Hepatic and muscle glycogen was low in downer animals. Loss of ATPase in hepatic canaliculi, skeletal muscles and cerebellar capillaries was evident. Marked loss of acetylcholinesterase in motor end plate and sciatic nerve resulted in atony of support muscles contributing downer posture.

Based on clinical and laboratory evaluations, factors like low calcium, magnesium and potassium, dystocia, abortion, hip dislocation, hyperthermia and mastitis-metritis were associated with the development of downers syndrome. A combined therapy comprising infusion of calcium, magnesium, supplementation with Vit. A, D3 and E parenterally and potassium acetate orally along with frequent change of slides and slinging for 20-30 min. daily twice proved successful in 51.5 per cent and 37.50 per cent downer cows and buffaloes, respectively.

NEW TREATMENT OF MILKFEVER.

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Introduction

Peroral calcium chloride has proven its efficiency in treatment of milk fever in many trials both as a supplement to intravenous calcium therapy (1,2) or as a prophylaxis (2,3,7) although the peroral treatment has been troublesome due to the irritative/ ulcerogenic effect of the active substance (4).

A new none-irritating calcium supplement (CALOL/KOVEL) (4), where the calcium chloride is covered with oil, tasty to the cow, has been tested for efficiency and acceptability compared with a calcium chloride containing gel in a prospective, randomized, placebo controlled clinical trial.

Materials and Methods

The material originates from a field investigation carried out in Mols, (Denmark) during 1989.

The present material comprises 31 cows all suffering from milk fever (Parturient Paresis) randomized in three groups.

Table 1

	Race			Yield	Feed		Calvings	Milk fever previous.
	HF	RDC	Y	kilos/year	g Ca	Ca/P	No.	No.
CALOL	2	6	3	325	49	1,19	4,8	2
GEL	5	1	3	326	48	1,32	3,8	2
CONTROLS	3	2	7	313	52	1,21	4,3	4

The cows belong to three different races, Holstein-Friesian (HF), Red Danish Cow (RDC) and Jersey (J). Milk production (yield) is calculated as kilos butterfat per year, calcium intake and calcium/phosphate ratio in the feed given up to the calving were recorded together with information on the number of calvings. Although there is a high number of Jerseys in the control group, the three groups are comparative, as no significant differencies are seen in the above values.

Treatment

All the cows were treated with 8 grams Ca intravenously and 8 grams Ca subcutaneously as calcium boregluconate at time T=0. Two of the groups received a follow up peroral treatment with 54 grams Ca , either CALOL*) or KALCIUM CHLORID GEL **) respectively at time T=4, T=12 and T=24

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**) Lundbeck Pharma A/S, Copenhagen.

hours. The third group acts as controls.

Clinical symptoms were recorded and blood samples taken from the anterior mammary vein at T=0, T=8 and T=24 hours. The blood were collected in vaccutainers, pH measured immediately in the stable with a ATC pH-meter, model Piccolo. The serum was taken apart, frozen and later analysed at Statens veterinaere Serumlaboratorium by atomic absorption spectrometers for total serum calcium and serum magnesium. The farmers were questioned about the reactions of the cow by different treatments and the cows were followed at least 1 week after the treatment.

Results

Clinical symptoms

At the clinical examinations at time T=0, T=8 and T=24 hours position (standing/laying), swaying (yes/no), appetite (none/ poor/normal),rumen activity (none/poor/normal) and faeces (none/diarrhoea/normal) were recorded in the three groups.

Table 2 Clinical Symptoms

	Position No.laying			Swaying No.			Appetite Non/poor			Rumen activity Non/poor			Faeces Non/diarrhoe		
Index value	5			2			1 / ½			1 / ½			1 / ½		
Time hours	0	8	24	0	8	24	0	8	24	0	8	24	0	8	24
CALOL	5	0	0	5	0	0	7/2	0/5	0/1	7/2	0/4	0/0	9/0	0/2	0/0
GEL	5	0	1	4	1	0	10/0	0/5	0/3	9/1	0/4	0/2	10/0	0/0	0/0
CONTROLS	9	0	2	3	2	3	12/0	0/7	1/3	9/2	0/6	2/2	12/0	0/2	3/2

All the clinical symptoms can be summarised in one figure, a "Milkfever Index" calculated by multiplying the number of cows with the individual symptom in each group and at each time respectively by the "Index Value"mentioned in the above table 2.

Table 3 Milk Fever Index

Time hours	0	8	24
CALOL	5,30	0,53	0,06
GEL	5,25	0,65	0,55
CONTROLS	5,58	0,96	1,79

The index indicates that the three groups are comparative as the index at T=0 is almost equal. Further that the cows benefit from the infusions,as the clinical symptoms are markedly reduced at T=8 in all three groups. The effect from the infussion disappears, however, showed as a rise in the index T=24 at the controls.

Recidive

The cows were followed after the treatment and the number of recidive needing further infusion of calcium and the number of cows with difficulties to restore to health are shown in table 4.

Table 4 Recidive

	No.recidives	No.difficulties in restoring	Comments
CALOL	0	0	Non
GEL	1	4	1 cow got collmastitis 36 hours later. Non
CONTROLS	4	0	

The number of recidives at the controls corresponds well to earlier investigations (2,5) and are significant different from the number in the groups receiving follow up peroral treatment (p<0.01). The four cows in the GEL group with difficulties in restoring are in good accordance with earlier results (2,4) and it might be due to damages in the walls of the forestomachs (4).

Acceptability

The farmers took care of the peroral administration of CALOL and GEL. They were interviewed and their opinions on handling the two products at the third dose, where the cows knew the product, summarised in table 5.

Table 5 Acceptability

HOW TO HANDLE ?	easy	moderate	difficult
CALOL	7	1	0
GEL	0	3	6

The cows seem to have a clear preference for the oil based CALOL.

Blood analysis.

The results from the analysis were as follows.

Table 6 Blood analysis

	pH			Se-Calcium			Se-Magnesium		
Time hours	0	8	24	0	8	24	0	8	24
CALOL	7,60	7,57	7,51	1,13	1,95	1,74	1,26	0,90	1,01
GEL	7,62	7,56	7,56	1,18	2,23	2,03	1,24	0,98	0,91
CONTROLS	7,58	7,60	7,59	1,35	2,28	1,84	1,23	1,04	1,03

There is a significant fall in pH in the peroraly treated groups compared with controls (p<0.01) indicating an acidifying effect of peroral calcium chloride as earlier described (6), but no difference between the two treated groups. The decrease in pH probably cause a release of free Ca-ions in the blood (6,9) and this might be the reason why calcium chloride is more effective than other Ca-salts tested (2). The Se-Ca value (totat calcium) varies very much in all three groups, and in average the CALOL treated group are the lowest at all times. When looking at T=0 figures there seems to be no indication of the outcome of the treatment. The difficulties to interpret Se-Ca values at milk fever has been mentioned in many papers (2,6,7,8) The injections of calcium caused a significant rise in Se-Ca in all three groups, but of different magnitude judged by the T=8 values. However from T=8 to T=24 there is a marked difference between the peroraly treated groups and controls with decreases of 0,2 and 0,4 respectively, so the rise in Se-Ca from T=0 to T=24 was significant higher in the peroraly treated groups compared to the controls (p<0.05), indicating an absorption of calcium from the intestine in the CALOL and GEL treated groups.Again there seem to be no difference between the two peroraly treated groups.

The serum-magnesium values are almost all in the normal range from 0,80 mmol/l to 1,40 mmol/l but differs very much al-though there are a clear inverse correlation to the Se-Ca value. It is very difficult to conclude anything from these figures except that none of the cows seems to be in lack of magnesium.

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Summary

A new none-irritating peroral calcium supplement (CALOL/KOVEL) has been tested for efficiency and acceptability compared with a calcium chloride containing gel in a prospective randomized, placebo controlled clinical trial comprising 31 cows suffering from milk fever. The beneficial effect of peroral calcium chloride treatment as suplement to intravenous calcium therapy of milk fever is clearly demonstrated. The differences between the peroraly treated groups and the controls were evident in clinical symptoms 24 hours after the

injections and there was a significant reduction in number of recidive.

In blood analysis a significant reduction in pH was demonstrated in the peroraly treated groups probably causing a release of free Ca-ions in the blood

The Se-Ca values varies very much in all three groups and it is impossible to predict the effect of the treatment from the pre-treatment values. The preoral treatment caused a significant higher rise in Se-Ca from T=0 to T=24 hours.

None of the cows seem to be in lack of magnesium judged from the Se-Mg values, where a clear inverse correlation to the Se-Ca values was seen.

CALOL and calcium chloride containing GEL seem to have equal beneficial effect on milk fever. However, there seems to be differences in side effects Probably due to differences in ulcerogenic effect (4).

Further it was reported, that CALOL were much easier to handle, as the cows accepted this treatment very well.

Zusammenfassung

Eine neue, nichtirritierende, perorale Kalziumergänzung (CALOL/KOVEL) ist in einem prospektiven, randomizieten, placebokontrollierten, klinischen Versuch für Effizienz und Akzeptabilität getestet und mit Kalziumklorid Gel verglei-chend worden. Der Versuch umfasste 31 kühe, die an Gebärpa-rese leiteten.

Der nutzliche Effekt der peroralen Kalziumkloridbehand-lung als Ergänzung von intravenöser Therapie von Gebarparese wurde deutlich demonstriert.

Der Unterschied zwischen den peroral behandleten Gruppen und den Kontroltieren war durch klinische Symptome 24 Stunden nach dem Anfang der Behandlung evident, und es gab eine sig-nifikante Reduktion von tieren mit Rückfall.

In Bluutanalysen fand man eine signifikante Reduktion von pH bie peroral behandelten Tieren, was vermutlich eine abgabe von freien Ca-ionen in dem Blut verursagt. Der Inhalt von Kalzium in Serum variiert in allen drei Gruppen sehr viel, und es ist unmöglisch das Behandlungsresultat vor dem Anfang der Behandlung voraussehen. Die perorale Behandlung hat eine signifikante höhere Steigerung in Se-Ca von T=0 bis T=24.

Es fehlte keinen den kühen Magnesium, wenn man den Inhalt des Serums wertet, man fand aber eine merkbare umgekehrte Korrelation zu den Se-Ca-werten.

Es scheint als ob CALOL und Kalziumklorid-Gel dieselbe nutzlische Wirkung von Gebarparese haben, es gilt aber einen Unterschied der Nebenwirkungen.

Weiter wurde es rapportiert, dass CALOL viel leichter einzugeben ist, weil die Kühe das Praparat gern haben.

Résumé

Un nouveau, non-irritable, supplément perorale de calcium (CALOL/KOVEL) a été contrôlé à l'égard d'efficacité et d'ac-ceptation, et il a été comparé avec le calciumchloride-gel

dans une prospective, randomisé, placebo-controllé expérience clinique comprenant 31 vaches souffrant de fièvre vitulaire.

L'effet avantageux du traitement perorale calciumchloride comme supplément à la thérapie intraveineuse de calcium destinée à guérir la fièvre vitulaire, a été clairement demontré. La différence entre les groupes traités peroralement et les animaux de contrôle etait evident dans les symptômes cliniques 24 heures après le début du traitement, et il y avait une reduction significative dans le nombre de animaux recidivé.

Dans des analyses du sang des groupes traités peroralement on a constaté une reduction significative de pH ce qui suppose un dégagement de ca-ions libres dans le sang.

Le contenu de calcium du serum varie beaucoup dans tous les trois groupes, et ce n'est pas possible à partir des valeurs sérum-calcium de pronostiquer le resultat du traitement avant le début de celui-ci. La cure perorale fait croître le Se-Ca d'une manière significative de T=0 à T=24.

Jugé sur contenu du sérum, aucune des vaches semblaient manquer le magnésium, mais on a trouvé une corrélation renversée evidente de les valeurs Se-CA.

Le CALOL et le calciumchloride-gel semblent avoir les mêmes effets cliniques avantageux sur la fièvre vitulaire. Pourtant, en ce qui concerne les effets secondaires, il semble qu'une différence se manifeste. Cette différence peut être dû à une différence de l'effet irritatif

En plus, on a rapporté que CALOL était plus facile à donner, puisque les vaches ont fait impression de bien aimer ce produit.

EVALUATION OF TOTAL SERUM BILE ACID CONCENTRATIONS FOR THE DIAGNOSIS OF HEPATOBILIARY DISEASE IN CATTLE

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INTRODUCTION

Increased serum bile acid values have been used in the diagnosis of various forms of liver disease in man (19). Limited attention has been given to serum bile acid concentrations in veterinary work because of difficulties encountered with assay methods (1) which have now been overcome (6,7,36,39). The total serum bile acid concentration is influenced primarily by hepatic uptake and intestinal absorption (5,18). The total bile acid concentration is a balance between input of bile acids into the peripheral circulation and the output attributable to hepatic bile clearance (21,33). In hepatobiliary disease the liver loses its ability to extract effectively the serum bile acids (31) and excrete them into hepatic bile. In naturally occurring types of hepatobiliary diseases in horses (39), dogs (6), cats (7) and in a variety of experimental situations in sheep (1, 36) calves and ponies (1) serum bile acid concentrations were a useful diagnostic aid.

The purpose of this investigation was to evaluate the sensitivity and specificity of total serum bile acid concentrations as a test for the diagnosis of hepatic disease and compare these results with those of liver specific enzyme activities and bromosulphalein (BSP) clearance in cattle.

MATERIALS AND METHODS

Animals

The cattle were referred to the University Large Animal Hospital because of suspected hepatic disease. Hepatic disease was considered a possible diagnosis on the basis of history and clinical examination. Histological examination of the liver was used as the ultimate criterion for group segregation.

The cattle were separated into nine groups. The thirty-six cattle in group 1 were suffering from hepatic lipidosis as part of acetonaemia or the 'fat cow syndrome'. Group 2 included adult cattle and calves with hepatic abscessation (15 cattle). Group 3 comprised seven cattle with leptospirosis. The two cows in group 4 had biliary calculi. In group 5 there were eleven cattle with fascioliasis. The six cattle in group 6 had a respiratory syndrome clinically resembling caval thrombosis which was subsequently proven not to involve the liver. Seven cattle with cardiovascular problems, mainly endocarditis, are included in group 7 for comparative purposes. All had a history of weight loss so that liver disease was suspected initially. The six cattle in group 8 were suffering from a variety of infectious conditions, i.e. septicaemia, coccidiosis, mastitis, peritonitis. Initially hepatic involvement was suspected from the clinical history. The eight cattle in group 9 were suffering from other conditions causing weight loss such as abomasal ulceration.

Clinical chemistry

Heparinised EDTA, clotted and oxalate fluoride blood samples were obtained from each animal at diagnosis and before treatment. Where possible the sample was analysed immediately after collection or frozen immediately and stored at -20°C. Plasma total bilirubin concentration was measured (12) and total serum bile acids (SBA) using the Enzabile R enzymatic method (Nyegaard, Oslo)(22). The activities of aspartate aminotransferase (AST) were measured (27), glutamate dehydrogenase (GD) (17), iditol dehydrogenase (ID)(15), γ-glutamyltransferase (γ-GT)(34), 5'nucleotidase (5'NT)(25), leucine aminopeptidase (LAP)(24) and

creatine kinase (CK)(35). Plasma urea concentration was measured (14) using the Boehringer Mannheim test (124788) combination. Serum albumin was measured (13). Plasma ammonia concentration was determined using EDTA plasma (11) and the Boehringer Mannheim test kit 125857. Plasma glucose concentration was determined on oxalate fluoride treated samples by the guaiacum and glucose oxidase method (23) and read from a standard curve. The method of Bakker & White (3) as modified (16) was used to determine total acetone bodies in plasma.

Serial blood samples were taken at regular two or three day intervals during the course of the animal's illness and the measurements repeated.

BSP test procedure

A bromosulphalein (BSP) clearance test was performed on each animal at diagnosis at the time of blood sampling for clinical chemistry investigation (37,38). The BSP concentration in plasma was measured (8,28) and the curves were analysed by computer by appropriate non-linear function fitting methods estimated as best fit to the curve (37,38).

The transfer constants for BSP distribution where 'a' is the rate of transfer from plasma to liver, 'h' from liver to bile and 'b' from liver back to plasma were calculated by computer from the optimised curve parameters as described (28). The BSP halftime ($t_{\frac{1}{2}}$), fractional clearance (k) after allowing 2 minutes for mixing and the 15 and 30 minute retention were also calculated from the plasma clearance graph.

Liver biopsy

Liver biopsy samples were taken (20) in the 11th intercostal space at the time of blood sampling and fixed in 10 per cent formol saline. The sections were stained with haematoxylin and eosin, periodic acid Schiff (with and without diastase treatment), and oil red O. The sections were examined under light microscopy and changes recorded.

RESULTS

Clinical cases

Test efficacy was expressed as sensitivity and specificity (26). Sensitivity represented the proportion of cattle with a particular hepatobiliary disease that had increased test values expressed as a percentage. Specificity represented the proportion of cattle without hepatobiliary disease that have test values within the normal range expressed as a percentage. To be considered significant each test value was compared with ± 2 standard deviations of the mean.

In comparing sensitivities, the total SBA concentrations were more sensitive than the fractional clearance, 'k, and both were equally specific for the different types of liver injury. The total SBA concentrations were the most sensitive of the clinical chemistry estimations evaluated (Table I). The total bilirubin concentration was specific for hepatic dysfunction but not very sensitive. Of the enzymes evaluated GD, ID, γGT, 5'NT and LAP were liver specific. Their sensitivity and that of AST showed variation depending on the type of liver lesions being highest in leptospirosis and fasciollasis. Some of the increase in AST was attributable to elevation in CK activities in plasma. Overall LAP was not very sensitive. GD was more sensitive than ID to a variety of hepatic lesions but was similar in sensitivity to γGT and 5'NT.

Plasma ammonia concentrations were more highly liver specific than glucose, total ketone bodies, urea and albumin concentrations. The sensitivites of these measurements varied in the different types of liver disease and most alteration was observed in glucose and total ketone body concentrations in hepatic abscessation.

Of the measurements of BSP clearance, the fractional clearance, k, was chosen for comparative purposes. In hepatic disease in groups 1, 2, 3 and 5, 'k' was significantly correlated with the transfer constant 'a', the 15 and 30

TABLE I. A comparison of the sensitivity and specificity of the various tests of hepatic function in the different disease categories

Group	Disease category	n	'k'	Total bile acids	Total bilirubin	AST	GD	ID	γGT	5'NT	LAP	Glucose	Total ketone bodies	Urea	Ammonia	Albumin
Sensitivity																
1	Hepatic lipidosis	36	50	86	53.8	94.4	27.6	2.8	36.1	30.6	11.1	100	100	33.3	30.8	19.4
2	Hepatic abscessation	15	33.3	33.3	0	53.3	53.3	33.3	53.3	40	13.3	40	40	6.7	46.7	60
3	Leptospirosis	7	71.4	71.4	42.9	100	71.4	42.9	71.4	71.4	0	57.1	14.3	28.6	14.3	0
4	Biliary calculi	2	100	100	0	0	0	0	0	50	0	0	100	100	100	50
5	Fasciolosis	11	27.3	100	0	45.5	72.7	9.09	72.7	90.9	63.6	9.09	45.5	27.3	72.7	72.7
Specificity																
6	Respiratory conditions	6	100	100	100	100	100	100	100	100	100	100	83.3	83.3	100	50
7	Cardiovascular disease	7	85.7	100	100	85.7	85.7	100	100	100	100	85.7	71.4	100	100	100
8	Infectious conditions	6	100	83.3	83.3	33.3	83.3	100	50	83.3	66.7	50	83.3	33.3	100	33.3
9	Miscellaneous	8	100	100	100	87.5	100	100	100	100	87.5	100	12.5	62.5	100	87.5

minute BSP retention (P<0.001). So 'k' was used for comparative purposes. There was no correlation between 'k' and the transfer constant 'b' in any of these groups and 'k' was correlated with 'h' in groups 2 and 3 only (P<0.001).

Correlation between total serum bile acids and other clinical chemistry tests

There were significant correlations between total SBA values and BSP fractional clearance, k, in groups 1,(P<0.01), 2,(P<0.05), 3 and 5 (P<0.001). In group 4 there were too few cows for a valid comparison to be made. The total SBAs were significantly correlated with total bilirubin in groups 1 (P<0.01) and 2 (P<0.05); with AST in group 1 (P<0.001); with GD in groups 1 (P<0.01) and 3 (P<0.05); with ID in group 1 (P<0.001); with LAP (P<0.01) and total ketone bodies (P<0.05) in group 3 and with urea (P<0.001) in group 1.

DISCUSSION

Measurement of the SBA concentrations improved the diagnostic value of routine hepatic tests in the detection of hepatobiliary disease as bile acid concentrations were increased in all forms of cattle hepatic disease (Table 1). They had high sensitivity and there were no false positive results except in one calf which had some liver dysfunction secondary to an infectious disease (Table 1). The range of SBA values for the various disease groups was wide with considerable overlap between groups as has been reported previously in the dog (6), cat (7) and horse (39).

The SBA concentrations improved the diagnostic capabilities of routine hepatic tests in the detection of hepatobiliary disease (Table 1). Concurrently evaluating combinations of test results improved the overall diagnostic performance of SBAs. (Table 1). The individual interpretation of bile acid values was useful in detecting impaired hepatic function but they were less valuable in the differential diagnosis of hepatobiliary disease (Table 1). Serial measurements of SBA concentrations provide a good guide to the prognosis of different liver lesions in cattle.

The SBAs when used in conjunction with other tests of hepatic disease (Table 1) were useful in establishing a definitive diagnosis owing to certain patterns that develop in specific disorders. Experimental work indicates that GD and ID are released into plasma in hepatic necrosis in cattle (10,15). The present study indicates that GD is more sensitive than ID (Table 1) and more persistent in chronic liver injury. These observations coupled with its higher stability in plasma at room temperature (2) than that of ID are an advantage. The interpretation of elevated values of enzymes in plasma is dependent not only on the tissue and site of origin but also on the half time of clearance of the enzyme (9). Some of the increase of plasma AST may have been due to muscle damage (4) as evidenced by results of CK measurement. The total bilirubin concentrations in cattle were highly specific but only sensitive in diffuse liver conditions (Table 1).

The present study indicates that γGT, 5'NT and LAP may be elevated in intra- and extrahepatic cholestasis in cattle. Certainly experimental evidence exists that γGT is released in biliary tract damage (10); 5'NT leaks into plasma in cholestasis in ruminants (29) and LAP is high in plasma in cholestasis in man (30). 5'NT and γGT had similar specificity but 5'NT may be more persistent in chronic liver damage (Table 1) and may be released into plasma in hepatocellular necrosis as well as biliary obstruction. LAP was insufficiently sensitive to be of value (Table 1).

The fractional clearance, k, was as specific for hepatic injury but not as sensitive as the total bile acids (Table 1). BSP clearance is reliable in diffuse liver lesions or in localised lesions if at least 30 to 40 % of the liver is affected (37). Both measure hepatic uptake and biliary excretion of organic anions but bile acid measurement has the advantage that it does not involve the injection of a foreign dye.

Glucose, total ketone bodies, urea, ammonia and albumin measure the liver's synthetic function. Of these ammonia was the most hepatic specific, but the sensitivities varied depending on the type of liver lesion. Plasma ammonia was most raised in end-stage liver disease and glucose was most sensitive in diffuse hepatic lesions (Table 1). Blood glucose and urea may be raised terminally so results must be interpreted with caution.

The best overall test combination for the detection of hepatobiliary disease was total bile acids and fractional clearance, k. In diffuse liver disease, total bilirubin, GD and ID with bile acids may be useful.

As the animals investigated all came from different commercial herds with varying management regimes and were of different ages, breeds and weights, it was decided that the most meaningful comparison was to classify the degree of illness. To some extent this had to be done retrospectively once the response to therapy was known. Hepatic biopsy was necessary to confirm the diagnosis in diffuse liver lesions. In groups 2 and 4, post-mortem examination was used as the lesions were discrete. In group 5, the fluke eggs were counted in the faeces. In all groups the total SBAs and BSP fractional clearance were the most reliable tests for detecting the degree of clinical illness. The pattern of enzyme release in cattle is altered in chronic advanced liver lesions (32).

On the basis of the present study SBA values >45umol/l in cattle warrant morphological diagnosis of hepatobiliary disease by liver biopsy.

In conclusion, the SBA concentrations provided a convenient rapid and reliable indication of hepatic function and the degree of illness in cattle. They had the highest sensitivity and specificity of all the tests evaluated. Measurement of fasting values is difficult in cattle because of the ad lib feeding system but in practice measurement of SBA concentrations were sufficiently sensitive to make fasting unnecessary. The combination of SBAs and BSP clearance was useful in detecting hepatic disease.

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SUMMARY

The total serum bile acid concentrations, together with other tests of hepatic disease, were evaluated in cattle with various types of hepatobiliary disease (hepatic lipidosis, hepatic abscessation, leptospirosis, biliary calculi, fascioliasis), respiratory, cardiovascular and infectious diseases, and in various other conditions not affecting the liver. Total serum bile acids were the most specific and sensitive indicators of a wide variety of hepatic diseases and were significantly correlated with the degree of clinical illness.

RESUMEN

Los concentreciones sérico totales juuto con otras pruebas pere enfermedades hepéticas fueron evaluades. En bovinos con diversn tipos de enfermedod hepetobilier (lipidosis hepitce, abscesos hepéticos leptospirosis, calculos bilieres, distometosis), enfermedodis respirotorios, cardiovésculosis e infecciosas, como en varios otios condiciones que afectoben al higado. La concentrecion sérice total de acidos biliores fueron los indicodores más especificos y sensiticos de uno amplie voriedod de enfermedades hep éticos y se correlorioneson significativemente con el gredo de enfermedod clinica.

RÉSUMÉ

Le concentration sanguine total acides d'bile sérique avec les autres tests de maladie hépatique sont évaluer en maladie du détail hépatique (stéatose du foie, abcès hépatique, leptospirose, calcul bilaire, parasitose fasciola hepatica), respiratoire, maladie d'coeur et les infections et les autre maladie que ne pas affecter le foie. Les acides d'bile total sont très spécifique et très sensitif indicateur de maladie hépatique et il y a un corrélation significatif avec la sévérité des maladies cliniques.

ESTUDO DO EFEITO VACINAL EM SURTOS DE CERATOCONJUNTIVITE INFECCIOSA BOVINA

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INTRODUÇÃO

Nos últimos anos, têm-se verificado um esforço mundial no sentido de desenvolver-se vacinas eficazes contra Ceratoconjuntivite Infecciosa Bovina. Vacinas produzidas com antígenos somáticos replicantes ou inativados, em ribossomas e outras fundamentalmente de fímbrias (Hughes & Pugh,1972; Pugh et al., 1981; Gil Turnes et al., 1986). Igualmente Lehr et al.(1985), demonstraram que as fímbrias purificadas de M. bovis são excelentes antígenos e permiten significativa proteção contra CIB. Os testes sorológicos entretanto, mostram diferenças antigênicas somáticas e fímbrias entre diferentes amostras, o que sugere dificuldades na produção de uma vacina eficiente (Araújo, 1980; Lehr et al.,1985). Neste particular, vários autores têm observado insucessos quando da utilização de vacinas no controle imunológico desta doença (Freitas, 1964; Pugh et al.,1978; Baptista, 1979 ; Gil Turnes et al., 1986).

Esta heterogeneidade parece ser a principal barreira na profilaxia desta infecção, pois, apesar dos esforços, a doença continua causando enormes perdas econômicas. Só nos Estados Unidos da América do Norte, aproximadamente 10 milhões de bovinos são acometidos anualmente, havendo uma estimativa de perdas próximas aos 150 milhões de dólares (Troutt & Schurig, 1985). No Rio Grande do Sul continuam milhares de bovinos sendo anualmente afetados, causando dificuldades no manejo, comercialização e controle da CIB, principalmente na primavera, verão e outono.

O presente trabalho teve por objetivo avaliar a eficácia de uma vacina contendo isolamentos de M. bovis, aderentes, em surtos já instalado de Ceratoconjuntivite Infecciosa Bovina.

MATERIAL E MÉTODOS

Utilizou-se três propriedades com características semelhantes de exploração pecuária. O número de bovinos foram: 140 novilhas de 1 ano; 145 novilhas de sobre-ano e 148 vaquilhonas de 2 anos de idade por propriedade, respectivamente. Em cada propriedade utilizou-se o mesmo esquema, separando-se em: Lote 1 - bovinos testemunhas; Lote 2 - que receberam 2 doses de vacina*; Lote 3 - receberam somente 1 dose de vacina.

A vacinação foi realizada quando o surto já estava instalado e o experimento durou de otembro a janeiro.

A avaliação da proteção vacinal foi feita observando-se a evolução clínica nos casos já instalados, classificando-se em cinco grupos, sem lesão,

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com lágrima e/ou opacidade, Grau I onde a córnea mostrava uma lesão de 1 a 2 mm de diâmetro, Grau II lesão de 3 a 4 mm e Grau III lesão de córnea superior a 4 mm de diâmetro.

A análise estatística foi feita através do teste do qui-quadrado, classificação dupla onde a hipótese testada éa de de independência entre os atributos das linhas e das colunas.

RESULTADOS

A intensidade média de CIB na 1ª visita foi 0,65; 0,72 e 0,81 respectivamente aos lotes 1, 2 e 3. Na 2ª visita as médias foram 0,75; 0,60 e 0,76; na 3ª visita 1,22 ; 0,32 e 0,47; as diferenças de médias só foram significativas (= 0,001), quando se compararam as médias da 3ª visita entre os lotes 1 e 2; 1 e 3 e 2 e 3. A prevalência da CIB foi na primeira visita de 28,6; 34,8 e 36,2% para os lotes 1, 2 e 3 respectivamente, na 2ª visita 30%; 25,6 e 31,2% e na 3ª visita 41,5; 9,8 e 14,4%. A hipótese de independência entre número de animais doentes e utilização da vacina foi rejeitada (= 0,01). A diferença entre os lotes que receberam 1 ou 2 doses, porém, não é significativa.

DISCUSSÃO

Vários autores vêm testando vacinas na tentativa de controle da CIB, demonstrando quase sempre que as mesmas se apresentam pouco eficazes, principalmente quando os testes são realizados contra amostras heterólogas da vacinal (Pugh et al, 1978; Baptista, 1979; Gil Turnes et al, 1986). Ultimamente no Rio Grande do Sul (Brasil), Uruguai e Argentina, vem sendo usada preventivamente vacinas contendo Moraxella bovis aderentes, com resultados satisfatórios (Gil Turnes et al, 1986). Mesmo assim, milhares de bovinos são acometidos em períodos de primavera, verão e outono.

No presente trabalho, testou-se uma vacina contendo amostras de M. bovis aderentes, inoculando-se bovinos de raças européias e com a doença já instalada, apresentando um quadro clínico em diferentes estágios; a avaliação foi inédita, pois na literatura consultada não encontrou-se menção a testes de vacina em surtos já começados de CIB (Hughes et al, 1971;1972 e 1979; Baptista, 1975; Pugh et al, 1981). Os resultados indicam que o uso da vacina foi benéfico, porque diminuiuo número de casos e a intensidade da doença nos lotes vacinados. Demonstrou-se igualmente, que mesmo em rebanhos apresentando clinicamente a enfermidade, a aplicação de 1 ou 2 doses de vacina permite diminuir as perdas econômicas acarretadas pela CIB.

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RESUMO

Avaliou-se a influência da aplicação de uma vacina contendo antígenos de adesinas de M. bovis em três rebanhos bovinos, onde surtos de Ceratoconjuntivite Infecciosa Bovina (CIB) estavam começando. Comprovou-se que a administração de um ou duas doses de vacina fez diminuir a prevalência e a intensidade da doença.

SUMMARY

The influence of a vaccine containing adhesins of M.bovis in the development of outbreaks of Infectious Bovine Keratoconjunctivitis was evaluated in three herds. The groups vaccinated with one or two doses had lower prevalences and milder clinical signs than those unvaccinated.

RESUMEN

Se evaluó la influencia de la aplicación de una vacina con antígenos de adhesinas de M. bovis en tres rebaños bovinos en los que brotes de CIB estaban iniciando. Se comprobó que la administración de una o dos dosis de vacina hicieran disminuir la prevalencia y la intensidad de la enfermedad.

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BOTULISMO EPIZOÓTICO DOS BOVINOS NO BRASIL

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INTRODUÇÃO

O botulismo, a intoxicação alimentar causada por toxina neurotrópica de Clostridium botulinum, manifesta-se por paralisia flácida da musculatura esquelética, seguido de alto índice de letalidade. A doença no bovino é causada, na grande maioria dos casos, pelas toxinas Cβ e/ou D e, em pequena escala, pelo tipo B. As toxinas Cβ e D são formadas em cadáveres de animais; a toxina Cβ , adicionalmente, foi encontrada em águas empoçadas e em "cama de frangos", e a presença da toxina B foi demonstrada em silagens. Os casos esporádicos de botulismo tem origem acidental, geralmente quando o homem fornece aos animais alimentos ou água contaminados com a toxina botulínica. Muito mais prevalente e de maior importância econômica é a ocorrência do botulismo epizoótico dos bovinos em regiões carentes de fósforo no solo e na pastagem. Nestas regiões, o gado, sendo criado extensivamente sem ração suplementar, leva os bovinos ao curioso hábito de roerem ossos para suprir a deficiência de fósforo das forrageiras e predispondo-os assim ao botulismo. Nessa forma do botulismo cada cadáver de bovino, vítima da intoxicação botulínica, se torna um novo elo da cadeia epizootiológica, pois se ingerir a toxina letífera também ingere esporos e estes, por ocasião da decomposição do cadáver, produzirão toxinas que se embebem nos ossos, ligamentos e aponeuroses, permanecendo ativas por longos períodos. Novo elo se forma quando outro bovino roer os ossos e anexos deste cadáver.

Antes de ter sido esclarecida a etiologia do botulismo epizoótico em bovinos, a síndrome da doença era conhecida por "loin disease" no Texas (17), por "lamsiekte" na África do Sul(21), por "paralise bulbar" ou "Tasmanian Midland disease" na Austrália (18,19), por "gniedo" no Senegal (3) e por "doença da mão dura" no Piauí, Brasil (23).

Em regiões, mesmo sem carência de fósforo, nas sujeitas a longas estiagens, as criações extensivas de bovinos podem estar expostas à intoxicação botulínica de origem hídrica, à semelhança do que acontece com o botulismo dos palmípedes silvestres (16,7,22). Desde modo a perpetuação ubiquitária e o aumento do número de esporos de Cl.botulinum no solo, como também Souza e Langenegger (20) demonstraram, ocorre por bio-ecosistemas naturais em que se estabelece o indispensável ambiente de anaerobiose no qual os clostrídios se multiplicam, dando origem a surtos de botulismo epizoótico entre os bovinos. Concomitantemente, novas práticas agro-pastoris permitiram criar condições, bio-ecosistemas artificiais de Cl. botulinum, nas quais o germe, presente na forma de esporos, passe à fase vegetativa, multiplicando-se e produzindo a endotoxina. Dentre estas práticas destaca-se a do uso de "cama de frango" como alimento para bovinos ou então aproveitada como adubo nas plantações e pastagens. Com a ampliação da prática da ensilagem estão sendo utilizadas outras espécies forrageiras além do milho, as quais sofrendo um processo menos rápido de acidificação, podem favorecer a eclosão dos esporos de Cl. botulinum e a produção da toxina B (9,2). Outras condições semelhantes foram relatadas na literatura recente.

"Doença da mão dura" no Piauí

Desde 1960 foi observada no Estado do Piauí, no nordeste do Brasil, uma doença conhecida como "doença da mão dura". Ocorria no "agreste" onde predomina vegetação pobre, de arbustos e árvores baixas; mas a maior incidência tem ocorrido nos "campos do capim mimoso penasco" do município de Campo Maior, área plana coberta de pastagem natural com vários capins e plantas baixas. (Fig.1) Em ambas regiões há acentuada deficiência de fósforo no solo e a osteofagia era comum nos bovinos, mantidos soltos em rebanhos comuns. Anualmente a doença atingia até 10% dos rebanhos. Deste foco inicial, a doença se alastrou lentamente em direção ao sul do Estado. Ela afetava principalmente bovinos adultos, sendo mais freqüente em vacas paridas ou em adiantado estado de gestação, raramente novilhos de 2 a 3 anos. Tokarnia et al.(24) descreveram o quadro clínico do botulismo, de evolução superaguda (horas) a subaguda (4-8 dias) e até crônica (18 dias). Observaram que o animal afetado isolava-se do rebanho, mostrava andar trôpego com fraqueza dos membros, sobretudo dos anteriores ("mãos duras"), decúbito esterno-abdominal, estrabismo normal, sobrevindo a morte geralmente dentro de 2 a 10 dias, em posição esternal ou caído de lado. Havia poucos casos de recuperação espontânea. A necrópsia dos 14 bovinos com diagnóstico clínico de botulismo não revelou outros achados dignos de nota senão a presença de fragmentos de ossos e/ou restos de tendões, aponeuroses no rúmen e no retículo em 7 bovinos. Foi evidenciada a presença da toxina botulínica indiretamente, nas culturas de materiais (conteúdo do rúmen e dos intestinos, fragmentos de fígado e de baço) semeados em meios apropriados e cujos filtrados bacterianos eram submetidos a testes de soro-proteção em camundongos, frente aos soros antibotulínicos tipos C e D. Também foi demonstrada a presença de esporos de Cl. botlinun em 8 das 11 amostras de solo colhidas no local onde se decompuseram cadáveres de bovinos que, segundo o histórico clínico, tinham morrido de botulismo. A extração da toxina diretamente do material colhido dos bovinos foi possível em um caso.

O diagnóstico clínico do botulismo no bovino assume importância especial porque, sendo esta espécie animal altamente sensível às toxinas C, C e D, torna-se difícil, em muitos casos, comprovar a presença de pequenas quantidades de toxina no material fonte de contágio, no conteúdo do rúmen, nas fezes, no fígado ou no sôro sangüíneo, através do teste biológico em camundongos ou de outras técnicas (1,8).

A ocorrência do botulismo epizoótico em outros Estados

Posteriormente à constatação do botulismo nos rebanhos bovinos no Piauí, a doença foi diagnosticada no sul do Estado do Maranhão e norte do Estado de Goiás, sempre em regiões de cerrado com solos deficientes em fósforo (23). (Fig. 1)

Na mesma época observou-se no sul de Goiás, em propriedades com solos férteis, de cultura, uma doença em bovinos, a qual se deu o nome de "paraplegia do palhada". Pois durante a seca, quando os animais foram mantidos em cercados para o aproveitamento das palhadas, após a colheita do milho, muitos deles adoeceram e morreram com sintomas de paralisia, de evolução clínica subaguda a crônica. Suspeitou-se de botulismo devido à presença de carcaças de pequenos animais silvestres na palhada, mortos por defensivos agrícolas usados nas culturas. Pela administração de vacina antibotulínica, em algumas propriedades, conseguiu-se evitar a doença. (6)

Nos anos subsequentes ocorreram grandes mortandades pelo botulismo em bovinos, criados em pastagens formadas em áreas de cerrado, deficientes em fósforo na Região Centro-Oeste, no sul de Goiás (5) e no Mato Grosso

do Sul, no município de Cassilândia (5). Independentemente disso, botulismo foi diagnosticado como causa de mortes em bovinos na Ilha de Marajó, Pará (15) e em áreas deficientes em fósforo perto de Alegrete, Rio Grande do Sul (11). (Fig. 1)

Figura 1 Regiões do Brasil onde foi diagnosticado botulismo epizoótico em bovinos (B) e a distribuição dos cerrados e campos nativos, geralmente deficientes em fósforo.

Mais recentemente, o botulismo foi reconhecido como causa de mortandades de bovinos no norte do Estado da Bahia (23), na região de Campo Grande, Mato Grosso do Sul (5), próximo a Brasília (5) e no Estado de São Paulo, sobretudo ao sul e oeste de Marília (5). Também foram diagnosticados surtos de botulismo em bovinos no Triângulo Mineiro, como na região de Uberlândia (4). Todas essas regiões eram anteriormente cobertas por vegetação de cerrado e onde foram então formados pastos, principalmente de Brachiaria decumbens. (Fig. 1)

Os diagnósticos de botulismo sempre se basearam no conjunto dos dados: 1) no histórico da doença, 2) no seu quadro clínico-patológico, incluindo diagnóstico diferencial, sobretudo com encefalites e encefalomácia, 3) no resultado da adoção de medidas de profilaxia contra o botulismo, a serem suplementação adequada de fósforo, vacinação anti-botulínica e eliminação de cadáveres e outras fontes de toxina de Cl. botulinum dos pastos, e 4) nos exames bacteriológicos diretos e indiretos, i.e. na verificação da presença de toxina botulínica, possível somente em poucos casos, e/ou na obtenção de culturas toxígenas de Cl. botulinum, com identificação, em alguns casos, dos tipos envolvidos através de testes de soro-proteção em camundongos ou cobaios.

Discussão

O histórico do botulismo epizoótico dos bovinos no Brasil evidencia que a ocupação e transformação do cerrado, com o objetivo de melhor aproveitamento dessas imensas regiões para a produção pecuária, levaram aos grandes surtos de botulismo e à perda de centenas de milhares de cabeças de bovinos no decorrer dos anos, desde 1960. O Brasil possue uma área total de 2.500.000 km² de campos, cerrados e outras vastas regiões originalmente cobertas por vegetação de cerrado, arbutos e árvores pequenas, onde há no solo fatores químicos limitantes ao desenvolvimento da vegetação, entre os quais se destaca a deficiência de fósforo (12). Os cerrados estendem-se desde os Estados do Maranhão e Piauí ao de Mato Grosso do Sul, englobando grandes partes de Goiás, Minas Gerais e São Paulo. Durante séculos os cerrados serviram, como pastos naturais, à criação de bovinos. Tratavam-se geralmente de animais de pequeno porte, adaptados às condições do meio, o chamado "pé duro". Com o aumento da população bovina e a introdução de raças zebuínas melhoradas, inicialmente de touros para cruzamento, criaram-se condições que acentuavam o efeito da deficiência de fósforo no solo sobre os animais. A osteofagia acentuada e a elevação do número de fontes da toxina botulínica resultaram então nos primeiros surtos de botulismo no norte do Piauí na década de 1960/70. Logo após, iniciou-se a introdução de gramíneas que podiam servir à formação de pastagens nas regiões de cerrado, a fim de permitir maior produção de forragem nos solos de baixa fertilidade (13).

Foi selecionada Brachiaria decumbens que cresce bem apesar do baixo teor de fósforo no solo e evidencia boa competitividade com outras forrageiras e invasoras. Em vez da lotação de 1 bovino por 1 ou mais alqueires de cerrado natural, colocaram-se nos pastos formados de B. decumbens até 4 bovinos. Na ausência de suplementação adequada de fósforo para os bovinos criaram-se condições propícias à ocorrência do botulismo na sua forma epizoótica. (10)

Como o bovino é muito sensível à toxina botulínica e estas pequenas quantidades da toxina freqüentemente não são detectáveis pelos métodos de laboratório conhecidos, impõe-se um diagnóstico essencialmente clínico, levando-se em conta o conjunto dos dados e evidências existentes. O sucesso da adoção das medidas de profilaxia confirmará esse diagnóstico. Já pela suplementação correta de fósforo consegue-se evitar a doença, porém o uso de vacina antibotulínica pode compensar eventuais falhas na suplementação mineral. A vacina contra o botulismo,

bem elaborada, é de boa eficiência. As vacinações devem ser feitas anualmente no fim da época de seca/início das águas, isto é no Brasil nos meses de agosto/setembro. Os animais vacinados pela primeira vez necessitam ser revacinados 45 a 60 dias após. Na eliminação de cadáveres dos pastos deve-se ter em mente que carnívoros silvestres podem espalhar as ossadas, mesmo quando são enterrados no pasto. A retirada completa de animais mortos da área dos pastos é medida mais indicada para a eliminação de fontes de toxina botulínica. Os conhecimentos sobre a existência do botulismo de origem hídrica permitem tomar medidas que evitem a presença de aguadas contaminadas.

Lobato (14) avaliou as vacinas antibotulínicas bivalentes C e D de quatro laboratórios no país e concluiu que nenhuma delas atendia satisfatoriamente as exigências mínimas para a imunização dos bovinos. Por isso, um controle da eficácia das vacinas faz parte imprescindível das medidas de profilaxia contra o botulismo.

Onde é viável, a correção e a adubação do solo através da rotação de culturas e reforma de pastagens constituem-se no método ideal para se evitar a deficiência de fósforo e conseqüentemente a osteofagia nos bovinos, impedindo-se assim o surgimento do botulismo e assegurando-se um bom desenvolvimento dos animais.

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RESUMO

Desde o surto do botulismo pelos tipos de toxinas C e D em bovinos do Estado do Piauí, no nordeste do Brasil, publicado em 1970, a doença tem sido diagnosticada na região Centro-Oeste do País. A transformação de vastas áreas de cerrado em pastagens cultivadas de Brachiaria decumbens permitiu uma lotação maior de bovinos. A gramínea é pouco exigente ao teor de fósforo no solo. A substituição de raças nativas por gado zebu mais precoce resultou em exigências nutricionais mais elevadas não satisfeitas pela pastagem com baixo teor de fósforo. Osteofagia é comum, principalmente em vacas no final da gestação e com bezerro ao pé. Muitos desses animais morreram pelo botulismo epizoótico nos Estados de Maranhão, Goiás, Mato Grosso do Sul, Bahia, Minas Gerais e São Paulo durante os últimos 25 anos. A grande sensibilidade do bovino à toxina botulínica dificultou o diagnóstico laboratorial. Freqüentemente, a suplementação de fósforo não adequada e a muitas vezes baixa eficiência das vacinas antibotulínicas disponíveis no comércio, deram origem a diagnósticos falsos. Medidas de profilaxia contra o botulismo, consistindo na suplementação correta de fósforo, na vacinação com toxóides eficientes e na eliminação de cadáveres dos pastos, deram bons resultados no controle da doença.

SUMMARY

Epizootic Botulism of Cattle in Brazil.- Since the outbreak of botulism of cattle by type C and D toxins in the State of Piauí, Northeastern Brazil, published in 1970, the disease has been diagnosed in the Central-Western Region of the country. The transformation of large "cerrado" (tree savanna) areas into cultivated pasture, by clearing the land and seeding of Brachiaria decumbens grass, allowed a higher stocking rate. The relatively low demanding grass grew well on low phosphorus deficient soils. The substitution of native breeds by more precocious zebu cattle resulted in higher nutritional requirements to be met by the pasture low in phosphorus. Bone chewing is common, mainly in cows before and after calving. Many of these animals succumbed due to epizootic botulism in the States of Maranhão, Goiás, Mato Grosso do Sul, Bahia, Minas Gerais and São Paulo during the last 25 years. The high susceptibility of cattle to botulism toxins made the laboratory diagnosis difficult. The frequently insufficient phosphorus supplementation and a many times low efficiency of toxoid vaccines available on

the market gave origin to often erroneous diagnoses. Prophylactic measures consisting in adequate phosphorus supplementation, vaccination with efficient toxoides and elimination of carcasses from the pasture gave good results in avoiding the disease.

ZUSAMMENFASSUNG

Epizootischer Botulismus der Rinder in Brasilien.- Seit der im Jahre 1970 veröffentlichten Diagnose des durch Toxin C und D verursachten Botulismus bei Rindern im Staate Piauí, im Nordwesten Brasiliens, traten schwere Verluste durch die Krankheit auch im Mittleren Westen des Landes auf. Die Kultivierung weiter "Cerrado" (Baumsavannen)-Gebiete mit Brachiaria decumbens-Gras erlaubte eine grössere Bestockung des intensivierten Weidelandes. Das verhältnismässig anspruchslose Gras wuchs gut auf den phosphorarmen Böden. Die Ersetzung des bodenständigen Viehs durch frühreifere Rassen von Zeburindern erhöhte die Anforderungen an die Ernährung dieser Tiere. Osteophagie ist weit verbreitet, besonders bei Kühen vor und nach dem Kalben. Viele dieser Tiere starben an Epizootischen Botulismus in den Staaten von Maranhão, Goiás, Mato Grosso do Sul, Bahia, Minas Gerais und São Paulo während der letzten 25 Jahre. Die hohe Empfindlichkeit des Rindes gegenüber den Botulinustoxinen erschwerte die Laboratoriumsdiagnose. Die häufig ungenügende Zufütterung von Phosphor und die oft ungenügend wirksamen, im Handel erhältlichen Toxoidvakzinen führten vielmals zu falschen Diagnosen. Prophylaktische Massnahmen, die in angemessener Phosphorversorgung, Vakzinierung mit wirksamen Toxoiden und Beseitigung von verendeten Tieren auf der Weide bestanden, ergaben gute Ergebnisse bei der Bekämpfung der Krankheit.

BOTULISMO DE ORIGEM HÍDRICA EM BOVINOS NO BRASIL

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INTRODUÇÃO

O botulismo constitue atualmente uma das principais causas da mortalidade de bovinos adultos no Brasil. Os surtos da doença estão relacionados, na maioria das vezes, com o hábito da osteofagia que os animais adquiren quando mantidos em áreas deficientes em fósforo (5,2,6), sem a adequada suplementação mineral, e com a presença nas pastagens de restos de cadáveres contaminados por *Clostridium botulinum*. Vários surtos têm sido registrados nos últimos anos, com a estimativa de milhares de mortes sendo atribuidas à doença (6). Contribui ainda para o aumento da importância econômico-sanitária do botulismo no nosso meio a dificuldade na eliminação de cadáveres das pastagens ou ainda o desconhecimento da prática de se fazer corretamente a eliminação de fontes de intoxicação. Em algumas áreas tem-se observado que considerável número de animais pertencentes a outras categorias, além de novilhas e vacas paridas, são acometidas pela enfermidade. Neste sentido, são descritos três surtos de botulismo em propriedades situadas nos Estados de São Paulo e Mato Grosso do Sul, onde categorias animais constituidas por bezerros, novilhos, touros, além de vacas estiveram envolvidas. Nestas circunstâncias, as evidências epizootiológicas indicam que a intoxicação tenha ocorrido através da ingestão de águas estagnadas.

MATERIAL E MÉTODOS

Observação dos surtos e colheita de material

Surto nº 01: Fazenda C., Município de José Bonifácio-SP. Num período de 3 dias morreram 6 animais, de um total de 130 garrotes, introduzidos recentemente numa área de 10 alqueires que estava vedada há 3 meses. O exame clínico de 1 animal com intoxicação aguda permitiu a observação de paresia dos membros posteriores, decubito esternal quando submetido a esforço físico, não se levantando mais, diminuição do tonus da musculatura da língua, respiração dificultada, sendo bifásica na inspiração e psiquismo normal. Em exame minucioso da pastagem não foram encontrados restos de cadáveres. Havia apenas um cocho de sal, ao redor do qual encontramos uma pequena coleção de água estagnada, com bastante limo, fezes e sinais da presença dos animais. Nesta propriedade foram coletadas amostras da poça d'água ao redor do cocho para o diagnóstico laboratorial direto e indireto do botulismo.

Surto nº 02: Fazenda S.J., Município de Campo Grande-MS. Pelo histórico levantado, nesta propriedade morreram cerca de 2.500 animais de um total de 7.000, num período de 2 anos. Inicialmente as categorias animais mais acometidas eram constituidas por novilhas e vacas prenhes e vacas com bezerro ao pé, observando-se na maioria das vezes, quadro clínico caracterizado por dificuldade na locomoção e morte num período variando de horas a dias. Mais recentemente, no entanto, considerável número de bezerros, novilhos e touros, além de novilhas e vacas havian sido acometidos pela doença. Devido às dificuldades na eliminação de cadáveres, os mesmos eram deixados no pasto; nesta situação pode-se verificar dezenas de cadáveres, inclusive animais em decomposição dentro de valas de retenção, águas de chuva estagnadas e bebedouros artificiais. Foram coletados soro sangüíneo e conteúdo rumenal de 14 animais com diferentes evoluções clínicas, além de amostras d'água de diversos locais.

Surto Nº 03: Fazenda B., Município de Piratininga-SP. Nesta propriedade morreram, num período de 7 dias, 60 garrotes de um total de 130. Examinamos 1 animal, que segundo o proprietário, apresentava a mesma sintomatologia que os demais; dificuldade na locomoção, respiração bifásica na inspiração, psiquismo normal e diminuição do tonus da língua. Devido à problemas com erosão no pasto, o proprietário havia construído uma vala de retenção para água de chuva, sendo esta a única fonte da qual os animais ingeriam água. A mortalidade iniciou-se cerca de duas semanas após a introdução dos animais neste pasto, que estava vedado há alguns meses. Foram coletadas amostras de água e limo da vala de retenção.

Exame laboratorial

Para o diagnóstico laboratorial direto os materiais coletados foram centrifugados, filtrados e o sobrenadante inoculado em camundongos para verificação da presença de toxina botulínica. Os soros sanguíneos foram apenas centrifugados e inoculados em camundongos. A verificação da presença de esporos de *Clostridium botulinum* no sedimento do material centrifugado foi realizada de acordo com Tokarnia *et al* (5). Neste sentido, foram inoculados em meio de cultura Wright, mantidos a 30°C durante 5 dias, centrifugados, filtrados e inoculados em camundongo. Em todos os casos foram inoculados amostras pareadas, sendo uma constituida pelo material inativado por 10 minutos a 100°C. A soroneutralização "in vitro" com antitoxina botulínica tipos A, B, C, D e F (Seruminstitut, Copenhagen e CDC, Atlanta), seguida da inoculação em camundongos foi realizada nos materiais positivos.

RESULTADOS E DISCUSSÃO

Desde o seu diagnóstico há vinte anos atrás por Tokarnia e colaboradores (5), o botulismo epizoótico dos bovinos vem assumindo, cada vez mais, grande importância econômico-sanitária no Brasil. Extensas áreas foram incorporadas ao criatório nacional, sem a correspondente e necessária suplementação mineral dos animais. Aliado a tal fato devemos acrescentar a inexistência de campanha sistemática em âmbito nacional no sentido de esclarecer o criador quanto à doença e ainda do seu desconhecimento quanto à prática correta de se eliminar cadáveres. Assim, a contaminação ambiental por esporos de *C. botulinum* a partir de cadáveres de bovinos nas pastagens ocorre intensamente (3). Estabelece-se, pois, toda condição epizootiológica para a ocorrência em larga escala de surtos da doença. Paralelamente à ocorrência do botulismo epizoótico dos bovinos, tem-se observado que em algumas situações considerável número de animais não pertencentes às categorias mais susceptíveis são acometidos.

Através do acompanhamento clínico foram observados, nos surtos descritos, quadros de intoxicação hiper-aguda, sub-aguda e crônica. Enquanto as análises para detecção de toxina botulínica no soro sangüíneo e conteúdo rumenal de 12 animais com evolução clínica aguda, sub-aguda e crônica revelaram-se negativas no bioensaio em camundongos, a presença de esporos de *C. botulinum* foi evidenciada em todas amostras do conteúdo rumenal. Soros sangüíneos de duas novilhas, com intoxicação hiper-aguda, revelaram-se positivos no exame direto, sendo identificado o tipo C de toxina através da soroneutralização. Amostras do limo das coleções d'água foram negativas para o exame direto de toxina, sendo no entanto todas positivas no teste indireto, predominando nestes casos *C. botulinum* tipos C e D.

O histórico da doença, a sintomatologia clínica dos animais examinados, as categorias envolvidas, os achados laboratoriais e as condições epizootiológicas dos surtos indicam a água como fonte de intoxicação. A detecção de toxina botulínica em fontes naturais de intoxicação é um processo normalmente dificultado pelas limitações técnicas, pelas características biológicas da toxina e pela amostragem obtida na colheita de material. Desta maneira, diante de tais evidências, resultados negativos não invalidam o diagnóstico. Surtos de botulismo em bovinos atribuidos à ingestão hídrica têm sido registrados no Senegal (1,4), sendo os *C. botulinum* tipos C e D frequentemente isolados das fontes de intoxicação suspeitas. Nos surtos 01 e 03 considerável número de machos foram acometidos num curto espaço de tempo. A inexistência de outra fonte de intoxicação e a ocorrência de *C. botulinum* no limo das coleções d'água corroboram tal alternativa. No surto 02, além do botulismo epizoótico, foram criadas condições para o surgimento do botulismo de origem hídrica, através da presença de numerosos cadáveres de bovinos em poças de onde os animais ingeriam água, ocorrendo aí uma modificação no quadro epizootiológico da doença.

A existência de coleções de águas estagnadas, sejam elas oriundas das práticas de conservação do solo ou formadas naturalmente após intensas chuvas, com a presença de matéria orgânica em decomposição, em áreas com alta contaminação ambiental por esporos de *C. botulinum*, pode, portanto, constituir fonte de intoxicação botulínica.

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RESUMO

O botulismo assumiu nos últimos 20 anos grande importância econômico-sanitária no Brasil, estando os surtos, na maioria das vezes, relacionados com a osteofagia que os bovinos adquirem quando mantidos em áreas deficientes de fósforo. Devido às dificuldades e o desconhecimento por produtores da prática de se eliminar corretamente cadáveres, forte contaminação ambiental tem ocorrido. Neste sentido, são descritos 3 surtos de botulismo onde considerável número de bezerros, novilhos e touros, além de vacas, estiveram envolvidas. As evidênciaa epizootiológicas indicaram a ingestão de águas estagnadas nas pastagens como sendo a fonte de intoxicação. Duas amostras de soros sangüíneos de animais que apresentaram evolução clínica hiper-aguda revelaram-se positivo no bioensaio, sendo identificado o tipo C de toxina botulínica através da soroneutralização em camundongos. Análises para detecção de toxina no limo de poças d'água e conteúdo rumenal de animais foram negativos, isolando-se, no entanto, em todas amostras *Clostridium botulinum* tipos C e D.

SUMMARY

Botulism of Hydrous Origin in Cattle in Brazil. Three outbreaks of bovine botulism involving calves, heifers, bulls and cows are described. Two outbreaks were characterized by lack of carcass debris on the pasture, and the epizootiological evidence revealed the presence of organic matter in water collections as the source of intoxication. In the third outbreak besides botulism due to osteophagia also botulism of hydrous origin was diagnosed. The latter was due to the presence of carcass debris in natural and artificial water collections used as

drinking water. Serumneutralization in mice revealed serotype C botulinus toxin in sera from two heifers with a peracute course of the disease. The examination of ruminal contents and water samples gave negative results for botulinus toxin, but was positive for *Clostridium botulinum* type C and D.

ZUSAMMENFASSUNG

Verschmutzte Tränken als Ursache von Botulismus bei Rindern in Brasilien. -Botulismus hat sich im Laufe der letzten 20 Jahre in Brasilien zu einer wichtigen Rinderkrankheit entwickelt. Es wird über drei Ausbrüche berichtet, bei denen Kälber, Färsen, Bullen neben Kühen verendeten. In zwei Fällen deutete das Fehlen von Kadaverresten auf der Weide sowie die epizootiologischen Daten auf das Wasser als Intoxikationsquelle hin. Auf einer der Farmen wurden in den Tränken Reste von verendeten Rindern angetroffen. In Panseninhalt und Schlammproben wurde kein toxin, jedoch eine grosse Zahl Sporen von *Clostridium botulinum* Type C und D festgestellt. Der Neutralisationstest in Mäusen ermöglichte, Type C-Toxin im Blutserum zweier perakut verlaufender Fälle bei Färsen nachzuweisen.

ELECTRORETINOGRAM (ERG) IN CATTLE AND ITS CLINICAL APPLICATION IN CONGENITALLY BLIND COWS.

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INTRODUCTION

Recent technical advances enable us to record the electroretinogram(ERG), and normal electroretinograms have been characterized for several small animals(1,5,6,8,9,12,14,15,16,17,18,20), but cow's electroretinograms are still rare(18). An attempt has been made here to establish a normal or characteristic wave pattern for cattle's bright flash ERGs, so that it too may be used to aid in the diagnosis of retinal dysfunction(1,13).
The first experiment was made to establish the methods of measurement, recording and evaluation of the a-,b-wave and the oscillatory potentials of ERG in cattle. The a-wave is produced by the photoreceptor cells. The main b-wave results from the activation of Muller cells and to a smaller extent of bipolar cells, and the oscillatory potentials of the b-wave from amacrine cells and bipolar cells.
The second experiment of this paper is the clinical application in congenitally blind cows. We are studying the feasiability of including ERG in certain retinal dysplasia in cattle.

MATERIALS AND METHODS

Experimental animals

Experiment 1: ERGs were recorded from 24 healthy Holstein Friesian adults(H-1) and 11 calves(H-2) and 5Japanese Black beef cattle adults(B-1) and 12 calves(B-2). H-1,H-2,B-1,B-2 were in healthy bodily condition and had normal eyes.

Experiment 2: ERGs was carried out on 11 cases of retinal abnormalities of Holstein Friesian calves(H-3) and 5 cases of retinal abnormalities of Japanese Black beef cattle(B-3). Symptoms of H-3 and B-3 were evident blindness congenitally .In each case the condition of H-3 and B-3 was bilateral with both eyes equally affected.
The H-3 affected cows had signs of visual defects, and ranged from barely perceptible ataxia to complete absence of coordination and ability to maintain normal posture. The palpebral reflex was absent and the pupillary reflex to light was absent or diminished. But sometimes dilated pupil reflex to light could be elicited by several photoflashes repeated at short intervals. The B-3 affected cows were in normal bodily condition except for the eyes. They were very nervous, and bumping into objects was sometimes observed. The pupils of both eyes of all cows remained dilated and the pupillary reflex to light was absent or diminished.

Stimulating and recording system

The cows were treated with 0.5% tropicamide and 0.5%

phenylephrine HCl to dilate the pupils. A local aneesthetic was used in the eye prior to inserting the o 20mm corneal contact lense. 1.5% hydroxyethyl cellulose-0.55% NaCl solution was used in the contact lens to provide a conducting bridge between the corneal surface and the contactelectrode.
A reference electrode was positioned in the midline over the frontalis, and a ground electrode at the tip of the left ear.
The ERG were recorded following 15 minutes of adaptation to the dark room. General anesthesia was not used at all in the experiments. The light source was placed 30 cm. in front of the cornea of the cows. The photic stimulator with a xenon flash lamp, and power settings 40J. was used for single-flash stimulation in 1 minute intervals. The mean of 5 to 10 single flashes from the single-flash stimulator was calculated.
ERG were measured using a MES-2102 Electororetinograph(NIHON KOHDEN). The amplification sensitivity of a- and b-wave was used 200uV/DIV for experiment 1, 50-200uV/DIV for experiment 2, and amplification sensitivity of the oscillatory potentials 50-100uV/DIV. The sweep speed was 10msec/DIV. The band width of the a- and b-wave was limited by a lowcut filter to 0.5Hz and by a highcut filter to 1KHz. The band width of the oscillatory potentials was limited by a lowcut filter to 50Hz and by a highcut filter to 1KHz.
Before the routine investigation of the ERG, the cows underwent regular ophthalmoscopic examinations. The fundus was regularly photographed using a R.C.Kows fundus camera. All H-1,H-2,B-1,B-2 had ophthalmoscopically normal retinae. Abnormalities of the fundus of H-3,B-3 were present in both eyes and were similar in nature. The retinal findings of H-3 was characterized by greyness of the optic disk with vascular attenuation ,bluish discoloration of the tapetal fundus and slight hyperreflectivity or almost normal blood vessels, but with retinal hemorrhage. The retinal findings of B-3 were a reduced size and number of retinal bloodvessels, smaller optic disks and the tapetal retina was hyperreflective.
Pathological examinations were done on 9 cows of H-3 and 3 cows of B-3.

RESULTS

In the 15 minutes dark adapted conscious cow, photic stimulation with white light flashes at 40J caused an ERG which consisted of two major components, including an early negative deflection (a wave) followed by a positive deflection (b wave). The rising slope of the b wave includes four successive positive, oscillatory potentials (O1,O2, O3 and O4). The ERG for experiment 1 were recorded in Table 1 and 2. The waveforms of the ERG were found to be similar in form and latency (Fig.1), and the ratios of b/a wave amplitude were 1.91(H-1), 1.88(H-2), 1.88(B-1), 1.91(B-2).
Two cows of H-3 resulted in non-recordable ERG.
a-wave amplitude of H-3 ranged from 35 to 488uV (average 196.67). Latency of the a-wave ranged from 6.4 to 15.4 msec(average 10.94). b-wave amplitude ranged from 65 to 929uV(average 332.84). Latency of the b-wave ranged from 29.2 to 44.3 msec(average 36.78). Ratio of b/a wave amplitude was in 1.70.
a- and b-waves of all B-3 cows resulted in non-recordable ERG. Microscopic observation showed diffused hydropic degeneration of

Table 1:Means and SD of the a-and b-wave amplitudes and latencies in healthy cows

	a wave				b wave			
	Amplitude(μV)		Latency (ms)		Amplitude(μV)		Latency (ms)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
H-1	667.88	106.67	10.5	1.61	1270.38	208.96	39.86	5.38
H-2	572.70	160.56	11.69	1.83	1074.75	260.38	36.16	5.52
B-1	695.39	174.81	9.13	1.33	1305.15	302.83	40.82	3.32
B-2	667.87	189.66	10.58	1.93	1274.58	248.95	38.87	5.50

Table 2:Means of the oscillatory potential amplitudes and latencies in healthy cows

	amplitudes		latencies							
	Σ-O	Σ-N	O-1		O-2		O-3	O-4	N-1	N-2	N-3
	Mean	Mean	Mean		Mean		Mean	Mean	Mean	Mean	Mean
H-1	404.95	275.56	17.85		22.23		28.17	35.08	19.87	25.43	32.13
H-2	383.17	272.48	18.83		23.28		28.94	35.21	20.88	26.55	32.67
B-1	435.02	241.52	14.03		18.79		23.15	28.53	16.38	20.85	25.84
B-2	376.82	248.96	18.34		22.88		27.73	34.37	20.35	25.84	32.16

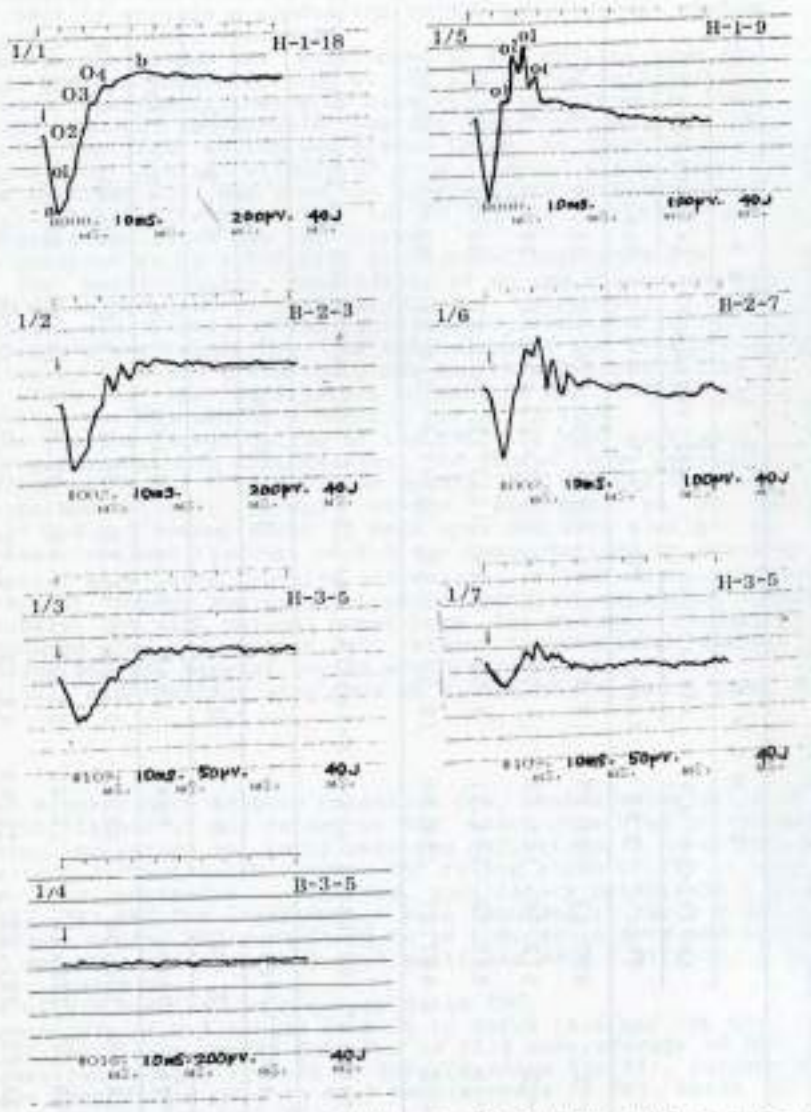

Fig.1 The bright flash cattle ERG resulting from photic stimulation with xenon flash at 40J. Individual components include a- and b-wave, with four oscillatory potentials(O1,O2,O3,O4) on the rising slope of the b-wave.
Fig.1/1 - 1/4 using lowcut filter of 0.5Hz and highcut filter of 1KHz.
Fig.1/5 - 1/7 using lowcut filter of 50Hz and highcut filter of 1KHz.

ganglion cell layers, hypoplasia of ganglion cells, loss of and scarring with granular layers in H-3, and extremely thin granular layers, vascuolar degeneration of the Muller cells and hydropic degeneration of outer granular cells in B-3.
62% of H-3 cows showed cerebellar hypoplasia, 91% hydrocephalia and 73% both symptoms.

DISCUSSION

The cattle ERG is basically similar to the ERGs recorded from other mammals with mixed receptor retinae(5,9,14,15,18,20) The results from the present study indicate that the components of the cattle ERG are almost the same regardless breed and age. The same procedures were used to determine the data of congenitally blind cows of H-3 and B-3. The ERG value depends on the degree of the retinal degeneration. The greatly reduced depth of the retina in all B-3 cows resulted principally from the loss of photoreceptor cells. It was probably the cause of the non recordable ERGs. The cause of ophthalmic degeneration remained unknown(3,4,11). H-3 was probably caused by the virus of bovine viral diarrhea-mucosal disease (BVD-MD), because etiological findings and clinical symptoms of cerebellar and ocular lesions were seen (2,7,10,11). From the result described above, it is concluded that stable ERG can be recorded repeatedly from conscious and comparatively nonstressed cows, this recording procedure is applicable for clinical use in blind cows.

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SUMMARY

This study was made to establish the method of measurement, recording and evaluation of the a,b-wave and oscillatory potentials of electroretinogram (ERG) in healthy cattle and its clinical application in congenitally blind cows.
The usage of different electrodes, photostimulators, amplifiers and fixation of cows to record the ERG were researched. The cattle ERG was basically similar to that recorded from other mammals.
The congenitally blind cows ERGs were showing decreased amplitudes or were nonrecordable.

ZUSAMMENFASSUNG

Diese Studie wurde gemacht um eine Methode der Messung, Aufzeichung und Auswertung der a-Wellen, b-Wellen und des oscillatorischen Potentials der Elektroretinogramme (ERG) in gesunden Rindern, und ihre klinische Anwendung in kongenital blinden Rinden zu finden. Die Verwendung von verschidenn Elektroden, Photostimulatoren, Verstarkern und Fixierungen der Rinder um ERGs aufzuzeichnen wurden untersucht. DieERG der Rinder waren grundsatzlich ahnlich der ERGs anderer Saugetiere. Die ERGs der kongenital blinden Rinder zeigten verringerte Amplituden oder waren nicht messbar.

FACTOR DE RIESGO EN LA GENESIS DEL TUMOR DE GLOBO OCULAR EN BOVINOS : PIGMENTACION CORNEO-ESCLEROTICA.NUEVOS APORTES DE SU IMPORTANCIA EN LA PREVENCION PRECOZ DEL TUMOR OCULAR.

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INTRODUCCION

El motivo de realización de este trabajo es aportar datos complementarios de factores que inciden positiva o negativamente en la presencia de tumores oculares bovinos.Se conoce la importancia que una buena funcionalidad ocular tiene en el bovino(1,2).El animal con visión francamente disminuida presentará,en general,un "estado de carnes"desmejorado con respecto al resto de sus compañeros así como comportamientos sociales y sexuales alterados.Consideremos al toro sexualmente apto,la visión y la correcta funcionalidad articular son elementos básicos para lograr el primero de los tres objetivos que debe de cumplir en el rodeo:localizar la hembra en celo.Las patologías oculares no solo representan problemas del vacuno sino tambien del productor quien deberá destinar recursos humanos y económicos por las medidas preventivo/terapéuticas que se aplican en estas situaciones.El destino final al que se puede llegar en casos avanzados es generalmente el mismo:miasis local y/o metastasis ganglionar o pulmonar,perdida de peso y muerte.En definitiva un vacuno con afeccion ocular altera el esquema productivo del establecimiento.Trabajos realizados indican la importante prevalencia que el proceso tumoral tiene en la raza Hereford y la influencia de la pigmentación palpebral en la presencia/ausencia de tumor.

MATERIAL Y METODO

Se realizan estudios macroscópicos,histopatológicos y estadísticos de 3794 ojos bovinos,Raza Hereford,de distintos puntos del Uruguay(30º a 35º latitud Sur y 53º a 58º longitud Occidental).Las muestras para histopatología fueron fijadas en formol(10%) y coloreadas en Hematoxilina-Eosina.El objetivo básico del trabajo no es el estudio histopatológico,el mismo se realiza solo como referencia ya que en la mayoría de los ojos las patologías eran incipientes y muy pequeñas(2mm x 5mm) Para lograr el estudio detallado del globo ocular,se procede a dividir el mismo,arbitrariamente,en 4 cuadrantes, tales que el eje O-X sea perpendicular a la direccion del maxilar inferior(Figura 1).Dejando de lado la pigmentación palpebral nos ocuparemos solamente de la esclerocorneal.A los efectos de puntuar esta pigmentación la dividiremos arbitrariamente en 20 grados,siendo 1 el menor y 20 el mayor.En caso de que el ojo tenga pigmentación entrecortada se puntua cada zone en forma individual, para luego hacer la sumatoria de partes.La edad se determina por el nivel de desarrollo de incisivos y molares.

Figura 1. Grados de pigmentación corneo-esclerotica, del 1 al 20. Cuadrantes I, II, III y IV. Ojo izquierdo.

RESULTADOS

Observaciones Macroscopicas e Histopatológicas: las tumoraciones oculares observadas fueron clasificadas clínica e histopatológicamente como placas y t mores, mayoritariamente carcinoma-espinocelular. En todos los casos se trata de patologías incipientes (2mm x 4 mm). Esto se ha hecho de exprofeso pues lesiones de mayor tamaño desvirtuan la anatomía del globo ocular.

Observaciones Estadísticas:

Tabla 1. Grados de pigmentación entre ojos derechos e izquierdos.

Similitud de grado pigmentario entre ojo derecho e izquierdo. Total: 2992 ojos.	Tolerancia ±2 grados 41,1 % desiguales.	Tolerancia ±2 grado 58,9 % iguales.
Grados extremos y opuestos de pigmentación entre ojos de un mismo animal.	Solo en 0,4 % de los animales muestreados.	
	En el ojo opuesto:	
Ojo con grado 0 de pigmentación. Total: 960 ojos.	56,2 % poseen pigmento.	43,8 % no poseen pigmento.

Tabla 2. Porcentaje de ojos pigmentados en cada cuadrante segun edad.

edad (meses)	ojos derechos				ojos izquierdos			
	I	II	III	IV	I	II	III	IV
40	14,9	27,1	22,2	35,8	13,9	25,5	25,5	35,1
39	14,3	27,5	20,3	37,9	15,8	15,9	22,8	45,5
38	11,7	26,7	21,6	40,0	12,4	25,8	22,0	39,8
34	12,6	31,3	21,9	34,2	16,8	31,7	19,2	32,3
30	12,9	22,5	25,8	38,8	16,2	25,8	19,3	38,7
29	18,5	31,5	23,6	26,4	12,0	28,0	24,0	36,0

Tabla 3. Imagen especular entre ojos derechos e izquierdos.

Incluidos ojos con grado 0 de pigmento. Total 2900 ojos.	Excluidos ojos con grado 0 de pigmento. Total 2690 ojos.
44,6 % con imagen	40,3 % con imagen
55,4 % sin imagen	59,7 % sin imagen

Tabla 4. Edad, porcentaje de ojos y grado pigmentario (por ojo).

Edad (meses)	Grado de pigmentación por ojo			
	0 a 5	6 a 10	11 a 15	16 a 20
40	52,3%	12,4 %	18,8 %	16,5 %
39	52,5%	15,3 %	17,1 %	15,1 %
23 a 34	52,7%	19,9 %	12,3 %	15,1 %

Tabla 5. Origen de la pigmentación corneo-esclerótica. Ubicación de pigmento según cuadrante. Solamente ojos con grado 2,5 o inferior.

Total de ojos	I	II	III	IV	Cuadrantes
350	2,3%	10,8%	10,8%	76,1%	

Tabla 6. Simultaneidad de pigmentación en los cuadrantes II y III con el IV. Total de ojos 76 (con grado 2 o inferior).

	II. SI	III. SI
IV. SI	15,7 %	0 %
IV. NO	84,3 %	100 %

Tabla 7. Distribución de tumores segun cuadrante.

Cuadrantes ojo derecho.				Cuadrante ojo izquierdo.			
I	II	III	IV	I	II	III	IV
3,1%	5,9%	7,9%	83,1%	1,2%	5,6%	11,2%	82,0%

Tabla 8. Animales con tumores en uno o ambos ojos simultaneamente

Total 314 vacunos.	12,1% de vacunos con tumores en ambos ojos.
	87,9% de vacunos con tumores en uno de los dos ojos.

Tabla 9. Ubicación del tumor y ubicación de la pigmentación.

1,6% de los tumores coinciden con la pigmentación corneo-esclerótica
1,4% de los tumores no coinciden con la pigmentación.

Tabla 10. Cuadrante IV. Contacto tumor/pigmento corneo-esclerotico.

17,8% de ojos pigmentados en IV y con tumor en dicho cuadrante.
72,1% de ojos no pigmentados en IV y con tumor en dicho cuadrante.

Tabla 11. Relaciones de grupos etarios con tumores oculares y grado de pigmentación.

Edad (meses)	Integre población cancerosa	Grado de pigmento en ojo problema y porcentaje de animales.	Promedio grado pigmento entre ambos ojos y % de animales.
23 a 34	Si	≤ grado 5 en 83,8 %	≤ grado 5 en 79,1 %
39	Si	≤ grado 5 en 85,7 %	≤ grado 5 en 85 %
40	Si	≤ grado 5 en 78,9 %	≤ grado 5 en 71,4 %

Tabla 12. Tabla de Contingencia.Nivel de significación del 5 %
P.C.E = pigmentación corneo-esclerótica.
O.D= ojo derecho. OI= ojo izquierdo.

	J	J crítico	Total de ojos
P.C.E/O.D-O.I	1,45	7,81	3260
Tumor/O.D-O.I	0,52	3,84	3294
Edad/Tumoración	42,7	9,49	1658
P.C.E/Tumor O.D	73,6	7,81	1732
P.C.E/Tumor O.I	84,5	7,81	1572
Edad/P.C.E O.D	25,5	21,0	1592
Edad/P.C.E O.I	21,7	21,0	1620

CONCLUSIONES

Si bien la mayoría de animales posee igual grado de pigmentación entre ojos derechos e izquierdos,Tabla 1, la misma no se distribuye de igual forma en ellos,Tabla 3, Afortunadamente los grados extremos de pigmentación solo se dan en una minoría de vacunos,Tabla 1.Consideremos que frente a cualquiera de las situaciones mencionadas debemos siempre buscar el promedio pigmentario de ambos ojos.No olvidemos juzgar la ubicación de la pigmentación en el globo ocular,dando mayores créditos a aquel que tenga el cuadrante IV pigmentado,Tabla 7.Al considerar la Tabla 4 encontramos que,por ejemplo, para los 39 meses la mayoría de los vacunos posee entre grado 0 y 5 de pigmentación.La Tabla 11 indica que de los ojos con tumores la mayoría tiene grado de pigmentación individual o promedial por debajo de 5.Todo esto se mantiene para las edades de 23 a 40 meses.En tal sentido la selección a favor de esta características ayudará a disminuir los riesgos(2).Respecto al origen del pigmento corneo-esclerótico,según la Tabla 5,y considerando valores puntuales de pigmento,2,5, notamos que existen valores bajos en todos los cuadrantes. O sea que todos, con variaciones individuales, se encuentran capacitados como para pigmentar.Sin embargo la mayoría de ojos posee pigmento en el IV cuando aún no ha aparecido en los otros,Esto indica una tendencia clara a iniciar el proceso ahí contra la escasa posibilidad que se inicie en el I,por ejemplo.Consideramos que vacunos con los cuadrantes I,II,y III pigmentados y sin pigmento en el IV, seguramente ya no desarrollaran pigmentación en este último, pues las posibilidades normales estén invertidas.Estos son ojos potencialmente peligrosos.La interrelación de presencia de pigmentación aislada entre los cuadrantes,Tabla 6, nos muestra que cuando hay pigmento en el IV no la hay en el III y en bajo porcentaje en el II.Destacamos que cuando hay pigmentacion en el III nunca la hay en el IV.Todo esto afirma lo dicho respecto a la génesis pigmentaria y nos aporta además ,el concepto del peligro que el ojo con pigmentación aislada en el III representa, pues no habrá desarrollo pigmentario en el IV.Algo similar sucede con el cuadrente II pero en menor medida.Por lo visto en la Tabla 2 el cuadrente que la mayoría de las veces no posee pigmento es el I, lo contrario sucede con el IV.Esto indica que el depósito de

pigmento a distintas edades nunca llega a ser tan importante como para que un cuadrante supere a otro,estadísticamente, entre los 29 a 40 meses.De igual manera decimos que la distribución pigmentaria hasta los 40 meses no se modifica.Afortunadamente la Tabla 8 nos muestra que la minoría de animales poseen tumores en ambos ojos, hecho este importante para el futuro del vacuno afectado.La mayoría de los tumores,71,4 % ,según la Tabla 9, se ubican en regiones del ojo que no poseen pigmentación esclero-corneal.Resultados similares encontramos en el cuadrante IV, Tabla 10.Busquemos pues, ojos pigmentados en este borde y fundamentalmente en el cuadrante IV.En colaboración con el Ing. Jorge Martinez Pena se procede a estudiar la dependencia estadística entre distintos parámetros del globo ocular,Tabla 12. No existe dependencia estadística entre ojos derechos o izquierdos con una mayor o menor pigmentación.Igualmente no encontramos dependencia que asegure que los tumores se desarrollan más en el ojo derecho que en el izquierdo o viceversa.Sin embargo existe si clara dependencia entre grupo etario y presencia de tumor, y en términos generales entre edad y tumor, es decir que al aumentar la edad aumenta la presencia tumoral.También existe dependencia entre grupo etario y pigmentación,en términos generales entre edad y pigmentación, de tal manera que,al aumentar la edad aumenta el grado de pigmento.Dependencia estadística se encontró tambien entre el grado de pigmentación y presencia tumoral,para ambos ojos.A mayor grado pigmentario menor presencia tumoral.Es evidente que todo mecanismo de oncogénesis no puede ser considerado como monocausal, por el contrario son numerosos los factores que participan en su génesis.En este caso : tipo de raza,ubicación con respecto al Ecuador,exposición a horas luz,individualidad génetica,edad y pigmentación corneo esclerótica.Es necesario considerar al tumor ocular bovino como de etiología múltiple y a la pigmentación corneo-esclerótica y al cuadrante IV como dos elementos a considerar en su prevención.

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RESUMEN

La pigmentación corneo-esclerótica(P.C.E) de 3294 ojos se estudia como factor de riesgo en la génesis tumoral bovina.Se indican aportes para prevenir precozmente dichas tumoraciones.Los ojos estudiados son de raza Hereford.Cada ojo se dividió en 4 cuadrantes y 20 grados.Estudios anatómicos/histopatológicos y estadísticos se realizan.No se encontró dependencia estadística entre tumor/ojo derecho o izquierdo,grado de P.C.E/ojo derecho o izquierdo.Sí se encontró dependencia entre

edad y presencia tumoral así como con el grado de P.C.E.La P.C.E tiene dependencia con la presencia tumoral y su valor crítico de riesgo es 5 El inicio de la pigmentación se da principalmente en el cuadrante IV y es aquí donde hay mayor presencia tumoral.La coexistencia de pigmento aislado entre los distintos cuadrantes es estudiada.Concluimos: evalue mos ambos ojos simultaneamente, busquemos cuadrantes IV pigmentados y P.C.E de grado 5 o más. La P.C.E disminuye riesgo tumoral.La P.C.E aislada en cuadrante III anula P.C.E en el IV.

SUMMARY

Cornee-sclerotic pigmentation(C.S.P) is studied as risk factor in bovine tumoral genesis.3294 Herefords eyes were studied,Each eye is divided in 4 quadrants, and 20 degrees.No dependence was found between tumor/right or left eye and C.S.P/right or left eye, but dependence between age/tumor and age/C.S.P was found.Starting of pigmentation is principally found in quadrant IV,which has the greater tumoral presence.Pigmentation coexistence in several quadrants is also studied. C.S.P decreases tumor risk. C.S.P in quadrant III annihilates C.S.P in quadrant IV.

RÉSUMÉ

Nous étudierons le pigment cornee-sclerotique(P.C.E) et les tumeurs des 3294 globes oculaires des bovines Hereford, âgés 23 à 40 mois.Nous avons observé les caractères anatomiques,macroscopiques et microscopiques du tumeur et le pigment des globes oculaires.L'oeil est divisé par deux axes en 20 dégrés et quatre parties,cadrans.L'examen histologique a montré des lésions carcinomateuses ou pré-carcinomateuses.Nous étudierons,statisquement,les suivantes rélations.Sans dépendence: tumeur/oeil droit ou gauche; dégré du P.C.E/oeil droit ou gauche.Avec dépendence: âge/tumeur; âge/dégré du P.C.E; tumeur/dégré P.C.E.La valeur critique du P.C.E est 5 dégrés.Le debout du P.C.E est localisé au cadran IV.La présence du P.C.E dans le cadran III annule la présence du P.C.E dans le cadran IV.Le P.C.E est très abondant dans le cadran IV et en est très faible dans le cadran I. La présence du tumeur dans le cadran IV est très abondant.Si l'on ajoute à ces dispositions anatomiques une sélection qui recherche une pigmentation très abondante spécialement aux cadrans IV on supprime ainsi la tumeur.

BOVINE SPONGIFORM ENCEPHALOPATHY DIAGNOSTIC PROCEDURES

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INTRODUCTION

The initial report of a novel progressive neurological disease of adult bovines (1) provided sufficient histopathological evidence, which included the demonstration of scrapie associated fibrils (SAF), to link this disease, termed bovine spongiform encephalopathy (BSE), to the group of conditions referred to as slow viral encephalopathies.

Physical properties characteristic to this group which includes scrapie in sheep and goats, Creutzfeldt-Jakob disease (CJD) and Kuru in humans, chronic wasting disease (CWD) of captive mule deer and transmissible mink encephalopathy (TME) in farmed mink are a prolonged incubation period with a short invariably fatal clinical course, a predilection for the central nervous system (CNS) with typical histopathology of neuronal degeneration and vacuolation, the absence of a host immune response (2) and transmission to a variety of laboratory animals (3).

This paper describes the clinical findings of BSE cases and presents the results of neurological examination, cerebrospinal fluid (CSF) analyses and electroencephalography (EEG) studies.

MATERIALS AND METHODS

Sampling Procedures

Cerebrospinal fluid samples were collected from the lumbosacral site under local anaesthesia with the animal restrained in cattle stocks. Samples were examined for total and differential white cell counts using a standard haemocytometer and Leishman stained smears. Protein fractionation was performed by agarose gel electrophoresis.

Electroencephalographic Procedure

Recordings were carried out on the farm with the cow restrained in metal cattle stocks. The electrode montage is presented in Figure 1. The skin was thoroughly cleaned with acetone and the surface 6mm pediatric electrodes glued in position with collodion. Contact gel was infused between the skin and the recording electrode to obtain a good contact at less than 2KΩ impedance. Recordings were made on an 8-2 channel SLE electroencephalograph neuroscribe (SLE Ltd., Croydon, England).

RESULTS

The incidence of the probable brain syndrome involved in the observed clinical abnormalities of twenty cases of BSE is presented in Table 1.

Myoclonus, defined as an irregular sporadic contraction of a single muscle often resulting in the movement of a joint or the overlying skin, was observed in 60% of cases, two of which showed a marked startle response.

There was no haematological evidence of an inflammatory response in BSE cases. Cows with BSE had a non significant elevation in the mean serum globulin concentration which was shown to be largely caused by an increase in the IgG fraction.

The results of CSF analysis including protein electrophoresis of normal, BSE-affected and listeriosis cases are presented in Table 2. There was no significant difference between BSE-affected and normal cows ($P>0.05$) but both Listeriosis cases showed a marked inflammatory response and disruption of the blood brain barrier.

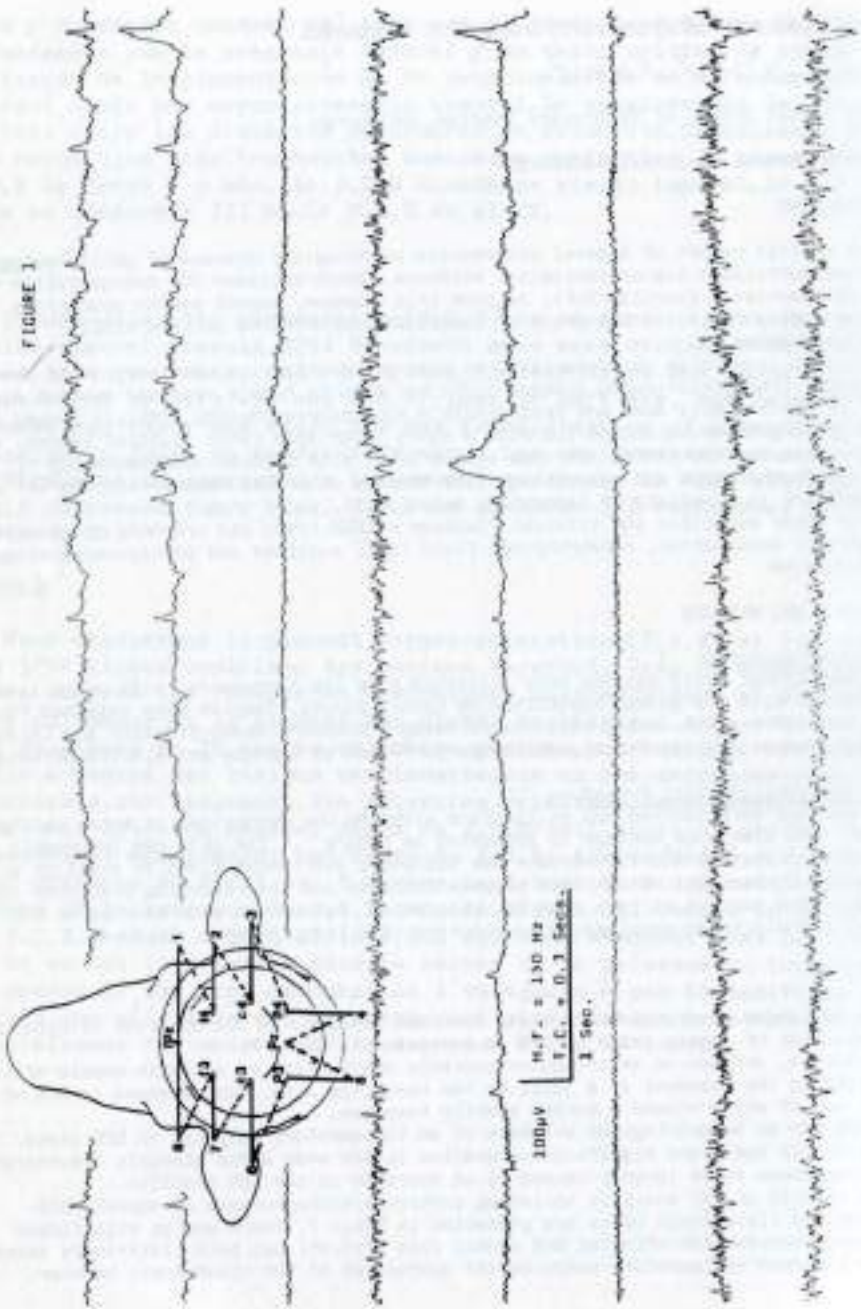

FIGURE 1

TABLE 1

Incidence of the probable brain syndrome involved
in the observed clinical abnormalities in 20 cases of BSE

CNS syndrome	Incidence	Clinical signs
Cerebral	100%	Somnolence, head pressing. Hyperaesthesia, aggression, head kicking.
Hypothalamus	60%	Apprehension/aggression. Abnormal temperature, appetite regulation. Polyuria.
Midbrain	0%	Cranial nerve III deficits. Ptosis. Depression, coma.
Cerebellar	100%	Ataxia without paresis. Dysmetria (hypermetria). Wide-based stance.
Vestibular	0%	Loss of balance, head tilt, nystagmus.
Pontomedullary	5%	Cranial nerve deficits (usually V, VII, VIII). Paresis (hemiparesis, tetraparesis).

Electroencephalographic studies were carried out on ten cows with BSE and six similarly-aged normal cows which acted as controls. The EEG traces from the six normal cows displayed a consistent pattern with a reasonably organised background activity predominantly in the alpha range in the occipital regions but with some beta range activity. The overall amplitude of the trace varied from 50-150μV.

The first changes in the EEG trace considered to be the result of BSE were the appearance of brief bursts of complex activity, periods of increased amplitude background activity (up to 300μV) most marked posteriorly and the presence of sharply-contoured waveforms which were similar in configuration to each other and most prominent in the posterior regions. These changes were observed in all BSE affected cows but in none of the similarly aged control animals.

Serial EEG studies in four cows revealed that there was a loss of normal EEG activity over the period studied, there was little distinction between electrical activity in anterior and posterior leads, the posterior complex activity had gradually disappeared and the periodic complexes became more clearly defined. There was a loss of organised background activity with a generalised slowing of the waveform from predominantly alpha range to theta range activity. In two cases periodic discharges were clearly visible occurring at a rate of approximately one per second and were enhanced within ten seconds following the intravenous administration of diazepam at a dosage rate of 0.05 mg/kg bodyweight. An example of one of these EEG traces recorded six months before the humane destruction of the cow is presented in Figure 1.

Discussion

In this series BSE presented as a slowly progressive, invariably fatal central nervous system disease with clinical features of the cerebral and cerebellar neurologic syndromes. This report is similar to previous case reports (4) (5) and herd studies (6) of BSE.

A hitherto unreported clinical feature of some BSE cases observed in this study were sudden changes in behaviour from an aggressive to a passive attitude and vice versa without obvious external stimulation. Similar changes in mental state have been reported in chronic wasting disease (CWD) of captive mule deer (7).

TABLE 2

Cerebrospinal fluid analysis in 20 cows with BSE, 10 normal similarly aged cows and two Listeriosis cases

Variable	BSE		Normal		Listeriosis
	Mean	SD	Mean	SD	Mean
Specific gravity	1.006	0.0008	< 1.010		1.009
Total WBCs ($x10^6$/l)	<12.5		< 5.0		700
Lymphocytes (%)	70		>95		2
Neutrophils (%)	30		< 5		98
Total protein (g/l)	0.37	0.13	0.43	0.19	2.2
Total albumin (g/l)	0.16	0.05	0.18	0.09	1.22
Albumin quota	0.0045	0.0022	0.0033	0.0016	0.038
Albumin %	44.2	5.7	37.8	6.0	56.4
Alpha-1	13.3	3.2	16.7	4.1	11.2
Alpha-2	11.2	2.8	15.1	5.0	3.6
Beta 1 + 2	21.0	2.2	20.2	2.2	16.4
Gamma	9.6	2.8	9.8	4.3	12.2

The absence of cranial nerve involvement in this study indicates a poor correlation between the neurologic syndromes observed in BSE and the site of spongiform change in brainstem locations (1).

In scrapie studies (8) (9) replication of the scrapie agent occurs in the spleen and visceral lymph nodes with neuroinvasion along the autonomic nerve fibres to the mid-thoracic spinal cord, infection then spreads to the rest of the spinal cord and major regions of the brain in a caudal-rostral sequence (9) (10). In this series early behavioural changes were suggestive of cortical involvement. However, it is possible that mild postural and gait changes indicating a cerebellar lesion could have been overlooked by the farmer. The development of fatal scrapie has been postulated to be the result of the scrapie agent replicating and causing cell dysfunction in a small number of clinical target areas (11) with the rate of transportation to these sites governed by factors other than distance (12).

The absence of conventional immunological response to the causative agent of BSE is a property unique to the slow viral encephalopathic diseases which is surprising in view of an initial, probably obligatory, replicative phase in the peripheral lymphoreticular system and non neuronal tissues (13) before gaining access to the CNS. Analysis of CSF samples either during the development of clinical disease or in the terminal stages of BSE did not reveal any evidence of blood brain barrier disturbance or intrathecal immunoglobulin production. Meningo-encephalitis caused by Listeria monocytogenes is the most important differential diagnosis in BSE cases. The immune response in the CSF in Listeriosis cases observed in this series is similar to previous reports (14) and permits the exclusion of Listeriosis from the BSE differential diagnosis list. No significant changes in CSF parameters have been observed in either scrapie (15), CWD (7) or CJD (16).

An important application of EEG studies in BSE could be the accurate identification of affected animals in the early stages of the disease before the development of advanced clinical features. The role of EEG examination in the early diagnosis of experimentally induced TME is illustrated in the detailed studies (17) where EEG tracings in mink were made weekly after intracerebral inoculation of the TME agent.

Consistent EEG changes appeared 102 days later, 39 days before clinical signs of disease which lasted only 12 days (mean figures quoted). By extrapolating this sequence of events to BSE with an apparent incubation period of 3-6 years (18) and a mean clinical course of 3 months (19), EEG changes may be present 12-18 months before the first clinical signs of disease are recognised. This hypothesis should now be tested following the successful transmission of BSE to cattle (20).

The EEG trace was characteristic in over 75% of subacute CJD cases in spite of the fact that the majority of patients had only one recording early in the course of the illness (16). The appearance of well-defined complexes that were truly periodic in two BSE cases in this study show some similarity to the highly periodic and symmetrical discharges observed in CJD patients (21). The EEG similarity may be the result of the similar neuropathology of neuronal fusion which has been postulated (22) to cause pathological electrical coupling and hence synchronous EEG spikes. Reports of other bovine EEG studies in the literature (23) (24) have produced inconsistent results due to poor case selection.

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SUMMARY

Bovine spongiform encephalopathy (BSE), a slow viral encephalopathic disease, necessitates the destruction of approximately 700 adult cows per month in the United Kingdom.

Clinical signs were of the cerebral neurological syndrome; apprehension, aggression and hyperaesthesia, and the cerebellar syndrome; posterior ataxia without paresis, dysmetria and wide-based stance. There was no evidence of the pontomedullary neurological syndrome.

Serial serum and cerebrospinal fluid samples were collected from BSE cases and assayed. There was no evidence of an immune response to the BSE agent.

Normal bovine EEG traces revealed a relatively organised background activity of between 80-170 μV. In BSE cases there was a loss of organised background activity and a generalised slowing of the wave pattern from alpha range activity to theta range activity. In addition, periodic discharges were clearly visible in the majority of cases occurring at a rate of approximately one per second. These periodic discharges were highlighted within ten seconds of the intravenous administration of diazepam.

RESUMÉ

L'encéphalite spongiforme bovine (BSE en anglais) est une maladie encéphalique à virus lent qui rend nécessaire la destruction de quelque 700 vaches par mois au Royaume-Uni.

Les signes cliniques sont ceux du syndrome neurologique cérébral - appréhension, agression et hyperesthésie - ainsi que ceux du syndrome cérébelleux - ataxie des membres postérieurs sans parésie, dysmétrie, écartement caractéristique des pattes. Aucuns signes du syndrome neurologique ponto-médullaire n'ont été détectés.

Des séries de prelévements de fluides cérébro-spinal et de sérum ont été effectuées sur des cas d'encephalite spongiforme bovine puis soumises à des tests. Ces tests n'ont révélé aucune réaction immunitaire a l'agent infectieux responsable de l'encéphalite spongiforme bovine.

Les électro-encéphalogrammes des vaches saines révèlent une activité de base relativement organisée qui se situe entre 80 et 170μV. Ceux des cas d'encéphalite spongiforme bovine attestent d'une perte d'activité de base organisée et d'un ralentissement généralisé du tracé des potentiels électriques, d'une activité d'amplitude alpha à une activité d'amplitude théta. De plus, des décharges périodiques, clairement visible dans la majorité des cas, se produisent au rythme d'une par seconde environ. Ces décharges périodiques apparaissent dans les dix secondes qui suivent l'injection intraveineuse de diazepam.

ZUSAMMENFASSUNG

Schwammförmige Rinder-Enzephalopathie (BSE) ist eine langsam wirkende, durch Virus verursachte Erkrankung, der Grossbritannien pro Monat etwa 700 erwachsene Kühe zum Opfer fallen. Die klinischen Anzeichen bestehen aus dem Zerebralsyndrom mit Furchtsamkeit, Aggression und Hyperaesthesie und dem Kleinhirnsyndrom: Ataxie der Hinterbeine und Lähmung, Dysmetrie und breitbeinigem Stand. Anzeichen für das Pontomedulla-Nervensyndrom lagen nicht vor.

Von BSE Fällen wurden seriemäbige Proben von Serum und Zerebrospinalflüssigkeit entnommen. Es lagen keine Zeichen für Immunreaktion auf BSE-Wirkstoff vor.

Die normalen EEG-Kurven der Rinder zeigten eine relativ organisierte Hintergrundtätigkeit von 80-170μV. Bei BSE Fällen lag ein Verlust der organisierten Hintergrundtätigkeit vor und eine allgemeine Verlangsamung der Wellen von Aktivität im Alphabereich bis zum Thetabereich. Ferner waren gelegentliche Entladungen bei den meisten Fällen deutlich sichtbar, die mit einer Geschwindigkeit von etwa 1 pro Sekunde auftraten. Diese periodischen Entladungen waren innerhalb von zehn Sekunden nach intravenöser Verabreichung von Diazepam verstärkt.

CREATION OF PSEUDOARTHROSES IN YOUNG CALVES: A CLINICAL AND EXPERIMENTAL STUDY

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INTRODUCTION

Infectious arthritis is of appreciable economic importance to the cattle industry. In the past, calves with infectious arthritis were treated medically using antibiotics; occasionally joint lavage was performed. The success rate in such procedures was sub-optimal and the limited literature available suggests that surgical intervention may be warranted (1,2,3,4).

Preliminary procedures on a limited number of calves with severe clinical infection of the metacarpophalangeal joint has shown that a simple, "field quality" surgical procedure can be effective clinically and still be economical. Surgical curretage of septic bovine metacarpo-phalangeal joints yields good results with return to milk production, weight gain and efficient breeding function (5). The curretage results in a clinically movable joint or pseudo-arthrosis of the joint (6).

The biological basis of wound healing subsequent to joint curettage has not been explained adequately. This project was designed to evaluate the healing of curreted bovine metacarpophalangeal joints by clinical and radiographic assessment at various times after surgery.

The term "pseudoarthrosis" has been described in different ways and needs to be clarified for purposes of this paper. The authors consider pseudoarthrosis to occur in stages as described in Jubb, Kennedy, and Palmer (1980). In this classification, the simplest form of pseudoarthrosis is a fibrous union between two bones ends An extension of this is the stage at which either fibrous or hyaline cartilage develops within the uniting fibrous tissue. In the highest stage of refinement of a pseudoarthrosis, clefts form in the cartilaginous tissue and a synovial-like tissue lines the "new" joint, this final form of pseudoarthrosis may then be termed a neoarthrosis. The above sequence of events, if surgically designed to occur, does not have the same pathological connotation which has frequently been associated with a pseudoarthrosis.

MATERIALS AND METHODS

Experimental animals:

Eight clinically healthy, Holstein-Friesian bull calves aged one to two months were utilized for this study. The calves were starved overnight. On the day of surgery, calves were premedicated with Xylazine hydrochloride , placed in right lateral recumbency, and then induced and maintained under general anesthesia with a Halothane-oxygen mixture in a semi-closed system. A left lateral metacarpophalangeal arthrotomy was performed on all the calves by making a 50 mm incision through the skin, subcutaneous tissue and the joint capsule at right angles to the axis of the limb.

The articular cartilage and some subchondral bone on the distal third metacarpal and proximal first phalangeal condyles was removed using a number four curette (approximately 3 cm wide) and the joint was then lavaged with normal saline to remove all cartilage and bone

fragments. The joint capsule and the subcutaneous tissue was sutured in one layer in a simple continuous pattern followed by skin closure in a cruciate pattern. A non-adhesive pad was then placed over the suture line and a light bandage applied from the carpus down to the hoof.

On the second post-operative day, the bandages were removed, lateral and antero-posterior radiographs of the left metacarpophalangeal joints taken, followed immediately by casting of the left forelimbs from the level of mid-radius distally to enclose the hoof. Standard cast management protocol was followed for the next 21 days and a description of clinical assessment recorded daily. Test animals were sacrificed 60 days following surgery.

Clinical animals

The non-experimental cases used in this study were obtained from clinical cases of septic arthritis presented to the Large Animal Clinic of the Western College of Veterinary Medicine. The majority were presented within the first few months of life and were of both beef and dairy breeds.

The clinical calves were managed using the same general methods as described for experimental animals. Surgical techniques were identical, the only variation being the degree of curettage which was determined by the extent of bone involvement present in the joint. The animals were placed on pre and post-operative antibiotics, post-operatively the curettaged joint was lavaged with saline or Ringers solution using an indwelling rigid walled catheter. When the affected joint was considered to be free of infection, the support bandage and catheter were removed and a plaster or fiberglass cast applied using the same schedule as the experimental calves.

Long term follow-up information and radiographs were documented at varying dates depending upon location of the animal.

RESULTS

General Clinical Observations:

Every calf walked on the operated limb subsequent to cast application. During the 21 days in which their left forelimbs were cast, the calves gained weight and were very active. All had only a slight limp, and there was no noticeable difference in gait between the test and control calves.

On manipulation, the left metacarpophalangeal joints of test calves were stiff when compared to the non-operated limb. In two calves, the angles of flexion of test joints were approximately 45 degrees while the angles of the other three were estimated to be 20 degrees. Pain was elicited when the joints were flexed beyond these angles.

Clinically affected calves were presented to the hospital one to three weeks after onset of signs with obvious swelling, lameness, and evidence of infection of the fetlock joint. In some instances the joint was aspirated and contained purulent material. Following surgery, and routine postoperative care including lavage, casting, bandaging etc, the animals were sent home and monitored by subsequent hospital visits, and/or telephone contact with the owner. The degree of lameness diminished greatly after initial surgery and lavage, and continued to improve over the next few weeks. By the fourth week most calves were walking with minimal gait abnormalities. The owners reported a gradual increase in weight gain approaching normal within one month following surgery. Although there was some variation in the

final conformation of the joint, one animal recovered enough to enable him to enter the show ring and do well within six months of surgery.

Radiographic Observations:

Clinically affected animals were radiographed before surgery to confirm and quantitate the degree of damage to the joint. Frequently there was gas production seen in the periarticular region accompanied with considerable soft tissue swelling. Varying degrees of bone lysis was evident depending upon the duration of the condition. Early cases of septic arthritis (confirmed cytologically) frequently showed no radiographic change in boney contour of the joint components. Long term radiographic follow-up showed a typical periosteal reaction and "filling in" of the defect which coincided with clinical soundness.

Gross Appearance of Test and Control Joints:

When the joints were opened, there was a fibrous tissue reaction involving the subcutaneous tissue and the joint capsule at the surgical site of each of the test and control calves.

The articular cartilage of each control joint was pink, entire and glistening and the joints contained straw-colored, viscous and sticky synovial fluid. In contrast, there was no articular cartilage in any test joint except near the proximal sesamoid bones, laterally and medially. The distal end of the third metacarpal bone and the proximal end of the first phalanx were covered by a dark brown tissue with thick fibrous bands joining the central parts of the distal end of the third metacarpal bone and the opposing surface on the proximal end of the first phalanx. A synovial membrane-like tissue joined these fibrous bands to the joint capsule. The synovial-like fluid of test joints was similar to that of the control joints but blood-tinged.

The interosseous ligament between the left medial and the left lateral metacarpophalangeal joints of test calves was very tough and thicker than that of the opposite limb of the same calves and that of control joints.

In two test joints, the lateral proximal sesamoid bone was united to the opposing side of the third metacarpal bone with bony tissue.

DISCUSSION

The general clinical observations demonstrate that the removal of the articular cartilage and subchondral bone of the left lateral metacarpophalangeal joint of calves is well tolerated and does not impair the general well being of the animals.

Although the arthrotomy incisions in both groups of calves were healed, the test calves became lame when the casts were removed. The lameness could have resulted from incomplete healing of the articular cartilage and subchondral bone defects, the uneveness of the articulating surfaces, the decreased mobility of the joints due to formation of intracapsular adhesions and deposition of the reparative tissue in the joints. Studies on the healing of deep articular cartilage and subchondral bone defects show that the defects are filled with a hematoma and inflammatory exudate soon after they are created (8). The progressive osteolysis of the distal third metacarpal bone and the proximal first phalanx, in response to the trauma of surgery and subsequent joint instability caused greater widening of the area occupied by reparative tissue. The organization of the initial hematoma and the deposition of reparative tissue gave the area a detectable radiodensity. The cause of osteolysis of the distal end of the third metacarpal bone and the proximal first phalanx

is not entirely clear. Infection, decreased or increased pressure on the bone end could cause similar changes.

The gross examination of opened test joints showed that the healing of cartilage and subchondral bone defects was incomplete. The examination also revealed that not all articular cartilage had been removed from the test joints indicating that joint curettage as applied in treating septic joints may not remove all the articular cartilage. In this study, the areas of articular cartilage adjacent to the sesamoid bones were inaccessible by a number four curette through the 55 mm long arthrotomy incisions. Better accessibility to all the articular cartilage can be achieved by using a smaller curette and making larger arthrotomy incisions.

The reason for the thickening of the interosseous ligament in the test joints is not entirely clear. It is, however, possible that the tissue hypertrophied in response to unequal weight bearing between the medial and the lateral claw.

From the observations made in this study the following conclusions can be made:

1) The removal of articular cartilage and subchondral bone of the bovine metacarpophalangeal joint of control calves with subsequent cast immobilization of the operated limbs for three weeks followed by bandaging of the limbs is well tolerated and does not impair the general well-being of the animal.

2) Surgical curettage of the infected metacarpo-phalangeal joint in calves when used in conjunction with local and systemic antibiotic therapy plus lavage, results in control of septic arthritis and osteomyelitis.

3) The surgical creation of a pseudoarthrosis in one half of the metacarpophalangeal joint in control calves and those affected by septic arthritis results in a clinically sound animal.

4) Further studies are warranted to investigate whether or not the repair process, given more time, would have produced cartilaginous pseudoarthrosis and thus a functional new joint.

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SUMMARY

Surgical intervention is an important modality in the treatment of septic arthritis in cattle. The surgical creation of a pseudoarthrosis can result in clinical control of the disease process and produce a functional joint as an end result. Clinical, radiographic, and histologic data suggest the repair process leading to a functional "false joint" has been initiated but not completed by 60 days following surgery.

RESUMEN

La intervención quirúrgica es una modalidad importante en el tratamiento de artritis septica en el ganado. La creación quirúrgica de una pseudoartrosis puede dar como resultado el control clínico del proceso infeccioso y producir como resultado final una articulacion funcional. Los datos clínicos, radiograficos e histologicos sugieren que el proceso de reparación esta encaminado a una "falsa articulacion" funcional que se ha iniciado pero no completado a los 60 dias después de la operación.

RÉSUMÉ

La chirurgie est une importante option pour le traitement de l arthrite septique chez les bovins. La création chirurgicale d'une pseudarthrose peut permettre le contrôle des signes cliniques de la maladie en cours et aboutir à la création d'une articulation fonctionnelle. Les données cliniques radiographiques et histologiques suggèrent que le processus de réparation menant à une "fausse articulation" fonctionnelle est en cours mais n'est pas complété 60 jours après l'intervention chirurgicale.

ZUSAMMENFASSUNG

Eine wichtige Alternative für die Behandlung der septischen Arthritis des Rindes ist der chirurgische Eingriff. Die Schaffung einer Pseudoarthrose kann den klinischen Stillstand des Krankheitsprozesses mit einem funktionsfähigen Gelenk bewirken. Klinische, röntgenologische und histologische Ergebnisse weisen darauf hin, dass der Reparaturvorgang, der zu einem funktionsfahigen "falschen Gelenk" führt, 60 Tage nach der Operation eingeleitet aber noch nicht abgeschlossen ist.

LATHYRISM AND THE VITAMIN D REQUIREMENT IN INTENSIVELY FED CATTLE

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INTRODUCTION

Previous studies have shown that in herds of cattle in which skeletal disorders occur together with ruptures of the Achilles tendon, vitamin D deficiency is an important aetiological factor (8). The herds were all housed, fed concentrates ad libitum together with a small amount of barn-dried hay, and received no additional vitamin D. However, there was also the possibility that the substitution of meal from home-grown peas (Pisum arvense) for imported soya bean meal might have been a factor. This study was designed to investigate this possibility.

MATERIAL AND METHODS

Animals and experimental design. - Eight Swedish Red and White bull calves were used. Immediately after weaning at about 9 weeks of age they were divided into two equal groups, matched for bodyweight, and fed 0.4 kg of barn-dried hay daily and concentrates (67% oats, 24% peas, 6.5% dried beet pulp and 2.5% of a mineral supplement containing 20% Ca and 10% P) ad libitum. One group received no additional vitamin D, while the other group each received 8000 IU D_3/day for the first 13 weeks of the experiment and 15,000 IU D_3/day during the subsequent 13 weeks. The calves were loose housed on slatted floors and weighed every second week. Blood samples were taken at the beginning, and then once a month during the trial, and the separated serum was stored at -20°C until analysis. One calf from each group was slaughtered 10 weeks after the beginning of the experiment, and the other calves were slaughtered 16 weeks later, i.e. at an age of 35 weeks. The serum concentrations of 25-OH-D_2 and 25-OH-D_3 were measured by high performance liquid chromatography, with a detection limit of 2 ng/ml (5), and samples below this limit were assigned the arbitrary value of 1.8 ng/ml. Calcium and magnesium concentrations were measured by atomic absorption spectrophotometry, and inorganic phosphorus by the method of Fiske and Subbarow (2).

At slaughter the tarsus and distal metatarsus were removed from the hind limbs, and radiographed with a standard diagnostic X-ray machine. The tarsal joints were then opened and examined. Transverse slabs, about 5 mm thick, of the distal metatarsal bones, including the metaphyseal growth zones, were cut with a band saw.

Sagittal slabs, about 5 mm thick, of the left calcaneus and Achilles tendon were also cut.

The slabs were radiographed on envelope-wrapped Kodalith Ortho type 3 film, using a X-ray machine with a Machlett AEG 50-A tube, at 25 kV and 18 mA for five to nine minutes, with a focus-film distance of 55 cm. Bone specimens for microradiography were embedded in methyl methacrylate and ground to a thickness of 100 µm. They were radiographed on Kodak electron image film 4489 at 15 kV and 4 mA for three minutes, with a focus-film distance of 42 cm.

Sections from the distal metatarsus and the calcaneal tuber, including the Achilles tendon, were fixed and decalcified. After embedding in paraffin, 6 µm sections were cut and stained with Ehrlich's haematoxylin and eosin.

RESULTS

Clinical findings. - Ten weeks after the start of the experiment two of the calves not receiving extra vitamin D had acute tetanic convulsions. They were both severely hypocalcaemic (1.23 and 1.32 mmol/l) and slightly hypomagnesaemic (0.65 and 0.68 mmol/l). The convulsions were relieved by an intravenous infusion of Mg-containing Ca-borogluconate solution, but one of the calves was unable to rise. It was slaughtered and found to have comminuted fractures of the neck and diaphysis of both femurs. The other two calves in the group were also moderately hypocalcaemic, and the group had a significantly lower mean serum Ca concentration than the group

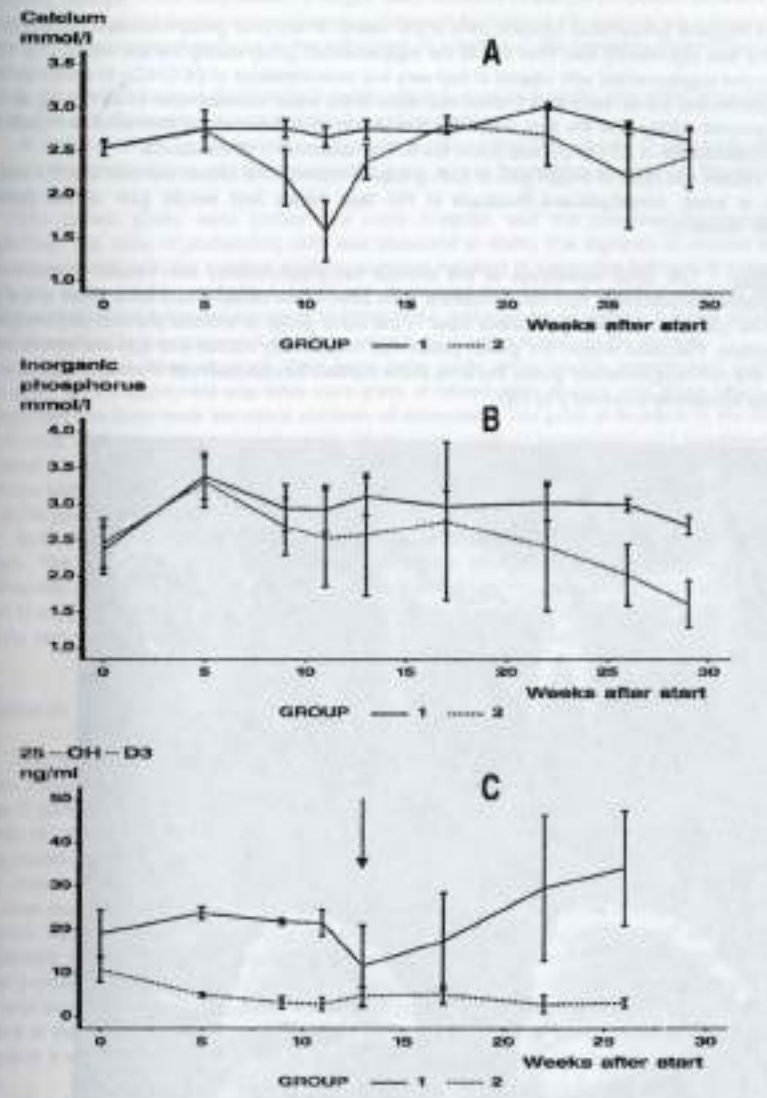

Figure 1. Mean (±s.e.m) serum calcium (A), serum inorganic phosphorus (B) and serum concentrations of 25-OH-D_3 (C) in two groups of calves fed a diet of barn-dried hay and concentrates containing peas, either supplemented with vitamin D (group 1) or unsupplemented with vitamin D (group 2). The arrow (C) indicates when the vitamin D supplement was increased from 8000 IU/day to 15,000 IU/day.

supplemented with vitamin D (Fig 1A). However, during the next two months the mean serum Ca level returned to normal.

The mean inorganic phosphorus concentration of the vitamin D-deprived group decreased gradually during the experiment and was significantly less than that of the supplemented group during the last month (Fig 1B). The calves which were not supplemented with vitamin D had very low concentrations of 25-OH-D_3 in serum throughout the trial. The supplemented calves showed a marked decrease in the mean concentration of 25-OH-D_3 after three months, but it recovered quickly after the daily supplement of vitamin D was increased from 8000 to 15,000 IU (Fig 1C). The serum concentration of 25-OH-D_2 was below the limit of detection in all the calves.

The feed intakes and rates of weight gain of both groups of calves were almost identical until the last month when there was a small, non-significant decrease in the feed intake and weight gain of the calves not supplemented with vitamin D.

Radiological findings. - The distal metatarsus of the animals not supplemented with vitamin D showed more irregular growth plates. Occasionally, cyst-like formations were seen in the metaphyseal bone close to the growth plates (Fig 2A). The specimens from the calcaneal tuber of the same group of animals showed very irregular and widened growth plates. The bone around the growth plates was occasionally rarified and cyst-like formations were seen (Fig 2B). In the non-supplemented group, the area of the Achilles tendon insertion showed a thinner irregular cortex and cyst-like formations occurred (Fig 2B).

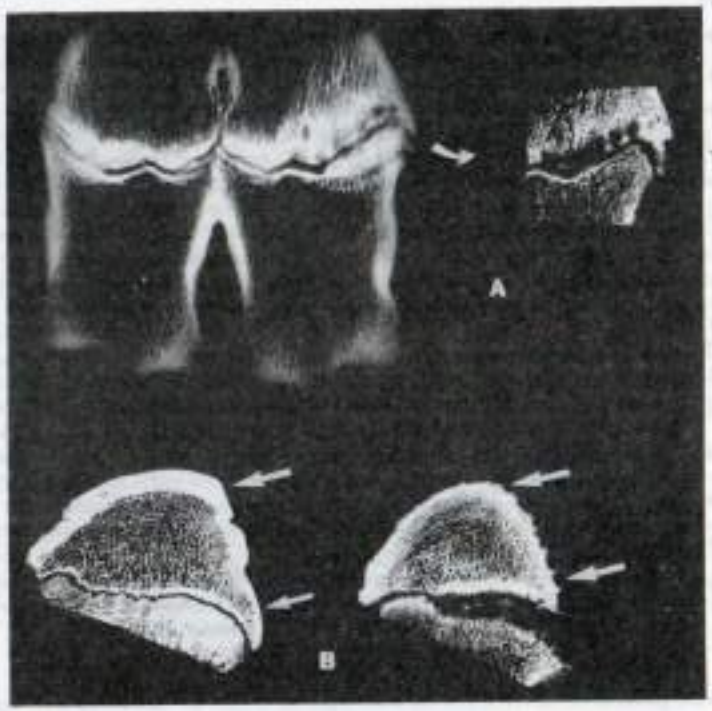

Figure 2. A. Radiographs of a transverse slab of distal metatarsus (left) and of a 100 µm section of the growth plate (right) from a non-supplemented bull. B. Radiographs of sagittal 100 µm sections of calcaneus from a vitamin D-supplemented (left) and a non-supplemented (right) bull. Achilles tendon insertion is between the arrows.

Pathological findings. - The macroscopic changes were localized to the growth plates, adjacent metaphyseal and epiphyseal bone and the Achilles tendon insertion. The growth plates of the animals not supplemented with vitamin D were more irregular, especially so in the calcaneus. Furthermore, the transition tendon/fibrocartilage/bone was very irregular.

In the specimens from the vitamin D supplemented group of animals, only slight irregularities of the growth plates were found. The tendon insertion showed no abnormalities.

At the end of the experiment the calves not supplemented with vitamin D invariably showed histological changes in the growth plates and the surrounding bone, and at the point of insertion of the Achilles tendon in the calcaneus.

The growth plates were thicker and more irregular, and the columnar arrangement of cells was disorganised. The zone of proliferating cells was increased in width. The ingrowth of vessels was disturbed and areas of fragmentation of the calcified cartilage occurred resulting in separation between the growth plate and the metaphysis. In the primary spongiosa, areas with decreased resorption of calcified cartilage were visible. There was also a marked increase in the number of osteoblasts, and a tendency towards a larger amount of osteoid and thicker trabeculae. On the epiphyseal side there were focal areas of fibrosis in the marrow.

In addition to these changes, there were large cystic areas on the metaphyseal side of the calcaneal growth plate; on the epiphyseal side there were areas of inflammatory change, and areas where bone remodelling was disturbed and there were increased numbers of osteoclasts. The point of insertion of the Achilles tendon into the calcaneal tuber showed marked changes. There were areas of hyperaemia and increased cellularity. There were focal disruptions and haemorrhages at the transition between tendon and bone, and fragmented bone trabeculae surrounded by osteoclasts were observed. Degenerative changes were visible in the tendinous tissue close to the point of insertion.

In the calf with femoral fractures, which was slaughtered after 10 weeks, the findings were different in some respects. The metatarsal growth plates were also thicker and disorganised, but there was a wider zone of hypertrophied cells; focal separations between the cartilage and the bone had occurred. In comparison with the vitamin D supplemented calf slaughtered at the same time the number and thickness of the bone trabeculae was markedly reduced. In the calcaneus there were necrotic areas in the bone on the epiphyseal side of the growth plate.

DISCUSSION

The two cases of hypocalcaemic tetany, and the hypophosphataemia, compensated hypocalcaemia and very low concentrations of 25-OH-D_2 and 25-OH-D_3 in the unsupplemented calves clearly show that they became vitamin D deficient. Hypocalcaemic tetany as a result of vitamin D deficiency has been described in lambs (1) and in calves (6). However, no clinical signs attributable to skeletal changes were observed, and the vitamin D deficient calves gained weight satisfactorily until the last few weeks.

There were no pronounced macroscopic or gross radiological changes in the vitamin D deficient calves, but there were marked histological changes which only partially resembled those characteristic of classical vitamin D deficiency rickets. There was an irregular widening of the growth plate, and the arrangement of cells was disorganised. In rickets the widening affects primarily the hypertrophic zone. In the vitamin D deficient calves the zone of proliferation was thickened, and there were areas where the growth plate was separated from the bone; but there was more bone and less osteoid than would have been expected in rickets. The changes at the point of insertion of the Achilles tendon could be explained partly in terms of the rachitic-type skeletal changes, but the changes in the tendons themselves and at the transition between tendons and the fibrocartilage were of uncertain origin.

The skeletal changes also resembled those described in rats fed the seeds of the pea Lathyrus odoratus. Several species of pea contain lathyrogens, some of which induce skeletal changes. These osteolathyritic changes affect the growth plates and include: a proliferation of cartilage cells, with an irregular zone of calcified cartilage and a retardation of endochondral ossification; a loss of orientation of cells, with clumps of chondrocytes scattered through a voluminous matrix which shows degeneration of the ground substance and fragmentation; and a loss of cohesion of the cartilaginous matrix and a loosening and detachment of the tendinous and ligamentous insertions (3, 4). The morphological changes observed in the skeleton of the vitamin D deficient calves were not as severe as those in experimental osteolathyrism in rats.

Furthermore, unlike the lesions of osteolathyrism in rats (7), the lesions in the calves were prevented by supplementing their diet with between 110 and 50 IU vitamin D/kg bodyweight daily. However, in spite of being well above the recommended level, this dose of vitamin D failed to maintain normal concentrations of 25-OH-D_3 in serum for longer than 12 to 13 weeks, when the level of supplementation had to be increased to 15,000 IU/day, providing from 80 to 60 IU vitamin D/kg bodyweight during the second half of the experiment. This failure to maintain normal concentrations of 25-OH-D_3 probably indicates that some component of the diet was interfering either with the availability of the dietary vitamin D, or with the metabolism of the vitamin. It is possible that the addition of the peas to the concentrate could have had such an effect, and thus have exerted an osteolathyritic effect on the skeleton. However, no definitive conclusions can be drawn about the aetiology of the syndrome, and several dietary factors may have been involved.

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SUMMARY

Of four calves fed a diet of barn-dried hay and concentrates containing peas, which was unsupplemented with vitamin D, two developed hypocalcaemic tetany after 10 weeks, and one of them suffered bilateral comminuted fractures of the femur and was slaughtered. The others were slaughtered 16 weeks later. The calves' skeletons had lesions which in some respects resembled the lesions of rickets, and in some respects resembled the lesions of osteolathyrism of rats. The possible influence of feeding peas on the metabolism of vitamin D and on the development of the skeletal lesions is discussed.

RÉSUMÉ

Dans un groupe de veaux alimentés de pois et déficitaires en vitamine D, deux sur quatre ont eu des tétanies hypocalcémiques dix semaines après le commencement de l'étude. Un des veaux, qui a eu des fractures multiples et bilatérales du fémur, a été immédiatement abattu. Les autres veaux ont été étudiés encore seize semaines avant d'être abattus. L'examen histologique du squelette a révélé des lésions qui ressemblaient en partie le rachitis mais aussi en partie l'ostéolathyrisme du rat. On discute aussi l'influence de l'alimentation de pois sur le métabolisme de la vitamine D et le développement des lésions du squelette.

RESUMEN

En un grupo de terneros deficientes en vitamina D, alimentados con guisantes, dos de cuatro adquirieron hipocalcemia tetanizante diez semanas después del inicio del experimento. Uno de los terneros adquirió varias pequeñas fracturas femorales bilaterales y fueron inmediatamente sacrificadas. El resto de los terneros fue sacrificado dieciseis semanas más tarde. La investigación histológica del esqueleto, reveló lesiones parcialmente consistentes con las encontradas en raquitismo, similares a las descritas en osteolatirismo en ratas. Se discute la influencia de la alimentación de guisantes sobre el metabolismo de la vitamina D y el desarrollo de las lesiones en el esqueleto.

ZUSAMMENFASSUNG

In einer Gruppe von erbsengefütterten Kälbern, welchen auch ein Defizit des vitamin D hatten, erkrankten 2 Kälbern in hypokalzämischen Tetanien 10 Wochen nach dem Anfang des Versuches. Ein von diesen Kälbern erhielt bilateralen komplizierten Femurfrakturen und war unmittelbar geschlachtet. Die übrigen Kälbern waren 16 Wochen später geschlachtet. Die histologische Untersuchung der Skelettet zeigte Veränderungen, die nur teilweis von Rachitis erinnerten, aber waren teilweis den Veränderungen beim Osteolathyrismus der Ratten ähnlich. Der Einfluss der Erbsenfütterung auf dem D-vitaminstoffwechsel und auf der Entwicklung der Skelettläsionen ist diskutiert.

SYNDROME ARTHROSIQUE D'ORIGINE METABOLIQUE CHEZ LE TAURILLON

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INTRODUCTION

En France 22 p.cent de la production de Viande Bovine est assurée par l'élevage des taurillons dans des conditions intensives : les animaux sont mis en lots en claustration, sur sol paillé ou sur caillebotis, ils reçoivent une alimentation à base d'ensilage de maïs complétée par un apport de céréales et de concentré azoté (tourteau de soja le plus souvent). Les taurillons placés dans ces conditions intensives ont deux origines différentes :

- des mâles issus de souches productrices de viande ou de croisements à base, de Charolais, Limousin, Salers, etc...Ces animaux appelés broutards, élevés avec leur mère, sont sevrés vers 8 mois et transférés à cet âge dans les ateliers d'engraissement.
- des mâles issus de souches laitières ou mixtes (Normand, Montbéliard), ces veaux dits "laitiers" sont séparés de leur mère très précocement (entre 1 et 3 semaines) et regroupés à cet âge dans les exploitations d'engraissement.

Dans le cadre d'une étude préliminaire regroupant 1500 sujets des deux types, il a été montré qu'en moyenne 50 p.cent des lots présentaient des sujets boiteux dans une proportion de l'ordre de 10 p.cent (soit 5 p.cent du nombre total de sujets mis en place). Le résultat le plus important de cette première étude a été de montrer que le siége des boiteries dépendait du type d'animaux étudiés :

- lésions du pied de type "pododermatite aseptique diffuse" (fourbure) chez 9 p.cent des broutards (1-3-9)
- lésions articulaires chez 17 p.cent des sujets d'origine laitière (5-6-10).

La fréquence et la gravité des lésions articulaires chez les sujets "laitiers" ont justifié la présente étude.

MATERIEL ET METHODE

Etude clinique

Chez 108 sujets ayant présenté, dans le cadre de l'étude préliminaire (5), des boiteries de type articulaire, les articulations lésées ont été prélevées à l'abattoir et disséquées après prélèvement stérile de liquide synovial pour examen bactériologique. Des examens radiologiques ont été réalisés sur différentes pièces osseuses (carpes,métacarpes,radius, humerus) et des examens histologiques ont été réalisés sur des fragments de cartilage prélevés sur des articulations saines et lésées. Afin d'évaluer la fréquence des lésions sur les différentes articulations, l'étude a été complétée par l'examen de plusieurs centaines d'articulations prises **au hasard** dans l'atelier de désossage d'un abattoir industriel.

Etude biochimique

Sur 126 sujets âgés de 8,5 mois (66 laitiers et 60 broutards), les contrôles biochimiques plasmatiques suivants ont été réalisés

- Calcium-Phosphore
- Lactates - Urée
- Phosphatase alcaline
- Aspartate Amino transferase (ASAT)
- Hydroxyproline (sur 20 sujets)

Par ailleurs, 350 sujets (180 laitiers et 170 broutards) ont fait l'objet de semblables contrôles tous les 3 mois en moyenne pendant la totalité de leur présence dans les élevages.

Etude zootechnique

Les croissances individuelles et les caractéristiques alimentaires par lot ont été étudiées chez les 350 sujets regroupés en 13 lots (1).

Etude statistique

Les résultats ont été analysés par la méthode du CHi carré, par le test "t" de Student et par Analyse Factorielle des Correspondances.

RESULTATS ET DISCUSSION

Etude clinique

L'affection débute le plus souvent vers l'âge de 12-13 mois, elle intéresse d'abord les membres antérieurs ; l'articulation radio-carpienne est plus ou moins ployée et les animaux répugnent à se déplacer alors que l'examen attentif des pieds démontre l'absence totale de lésion au niveau des onglons.

Les lésions siégent essentiellement au niveau des cartilages articulaires et elles vont de la simple érosion jusqu'à l'ulcération avec attaque de l'os sous chondral et remaniements osseux plus ou moins importants.

Les examens radiologiques n'ont mis en évidence aucune anomalie de la trame osseuse et les exemens bactériologiques réalisés sur plus de 100 prélèvements de liquide intra articulaire et de membrane synoviale n'ont mis en évidence aucune bactérie ou mycoplasme.

L'examen histologique des cartilages articulaires a montré la nature dégénérative des lésions ; l'amincissement ou la disparition totale du cartilage avec attaque de l'os sous jacent est typique du processus arthrosique (4-8).

La localisation et la fréquence des lésions observées sur plusieurs centaines d'articulations prélevées au hasard dans un atelier de désossage de taurillons est résumée dans le tableau 1. Seules les lésions siégeant sur les membres antérieurs ont été décrites en raison des opérations de désossage portant essentiellement sur les avants de taurillons, mais les mêmes lésions ont été constatées au niveau des différentes articulations des membres postérieurs. Les lésions sont souvent asymptomatiques, mais elles peuvent également revêtir une gravité extrême, notamment au niveau des articulations scapulo humérale et coxo fémorale allant jusqu'à l'impossibilité totale pour l'animal de se déplacer.

Etude biochimique

Les résultats des contrôles biochimiques réalisés au même âge (8,5 mois) sur taurillons laitiers et broutards et résumés dans le tableau 2 montrent qu'au même âge les animaux ont des profils totalement différents en rapport avec leur alimentation (7).

Par rapport aux broutards élevés au lait maternel et sevrés progressivement avec un régime à base de fourrage et d'herbe, les sujets laitiers soumis depuis l'âge de 2-3 semaines à un régime hyperénergétique se distinguent par une phosphorémie très élevée (90 mg/l versus 55 mg. P 0,001) et surtout par une activité phosphatasique trois fois plus élevée (218 U/l versus 65 U/l. P<0,001). Ces différences peuvent être considérées comme le reflet d'un apport excessif de phosphore par les céréales et comme le signe d'une hyperactivité osseuse en relation avec une croissance très rapide du squelette. L'accroissement de la lacticémie (5,5 mmol/l versus 2,9 mmol/l) correspond chez les laitiers à l'ingestion d'une alimentation hyperglucidique et la différence observée au niveau de l'hydroxyproline plasmatique peut être interprétée comme le signe d'une résorption ou d'une dégradation du collagène des cartilages, intervenant dès le 8ème mois.

L'étude cinétique des différents paramètres biochimiques montre que chez les laitiers, les niveaux plasmatiques de Phosphore, de Lactates et de Phosphatase Alcaline sont maximum entre 4 et 8 mois, ils décroissent ensuite régulièrement jusqu'à l'âge d'abattage (16-17 mois).

Etude zootechnique

L'analyse des courbes de croissance obtenues par contrôle individuel des performances tous les 3 mois, montrent des profils tout à fait différents entre laitiers et broutards.

Chez les broutards sevrés à 8 mois le gain moyen quotidien (GMQ) atteint son maximum vers 13-14 mois (de l'ordre de 1400 g/j dans le cadre de l'étude), il diminue ensuite régulièrement jusqu'à l'âge d'abattage.

Chez le taurillon de type laitier, la courbe de croissance culmine entre 4 et 8 mois et les niveaux atteints à cet âge atteignent souvent 1700 g/j ; on assiste ensuite à partir de 8-9 mois à une diminution rapide et régulière du GMQ.

D'une façon générale, la chute de croissance est d'autant plus rapide et prononcée que le pic de croissance aura été précoce et élevé.

L'étude des caractéristiques alimentaires des taurillons élevés de façon intensive fait ressortir une anomalie flagrante : la teneur en Matière Sèche (MS) est encore considérée comme un critère d'appréciation de la qualité d'un ensilage alors que ce paramètre est totalement indépendant de la teneur de l'ensilage de maïs en amidon et en cellulose. En raison de l'importance de l'ensilage dans la ration (jusqu'à 70 p.cent de la matière sèche) une mauvaise appréciation de ses qualités nutritives peuvent conduire à un déséquilibre par excès d'apport d'amidon (jusqu'à 35 p.cent de la MS de certaines rations) et par défaut de cellulose (taux inférieur à 12 p.cent de la MS). A cela s'ajoute, lorsque le maïs est récolté sur terrains granitiques, une teneur en minéraux et en certains oligo-éléments (Mn, Cu, Zn) très inférieure à la normale.

Relations croissance-Age-Biochimie

La mise en classes des données concernant les croissances, les âges et les valeurs biochimiques et leur exploitation par analyse factorielle des correspondances, montre les relations suivantes :

- Les croissances les plus élevées chez les taurillons laitiers sont observées entre 5 et 10 mois.

- Plus les croissances sont élevées entre 5 et 10 mois plus elles chutent rapidement ensuite.
- Le niveau plasmatique des paramètres suivants : Phosphore, Lactates, Phosphatase Alcaline, est synchrone du niveau de croissance, les valeurs maximum sont atteintes entre 5 et 8 mois chez les laitiers, elles chutent ensuite très rapidement contrairement aux observations faites chez les broutards.

CONCLUSION

L'étude comparative des troubles locomoteurs observés chez les taurillons de deux origines différentes (broutards âgés de 8 mois ou veaux laitiers âgés de 3 semaines, montre que chez ces derniers prédominent les troubles articulaires de nature arthrosique alors que chez les broutards les lésions podales (fourbure) sont les plus fréquentes.

Les raisons de cette sensibilité aux problèmes d'arthroses tiennent aux objectifs de croissances imposées aux très jeunes animaux et à l'alimentation déséquilibrée qu'ils reçoivent. L'alimentation hyperglucidique et hypocellulosique provoque chez le jeune taurillon une série de modifications biochimiques importantes dominées par une hyperactivité phosphotasique. Dès l'âge de 6-8 mois, l'intégrité des cartilages articulaires semble compromise et l'augmentation très rapide des pressions exercées sur des articulations mal protégées aboutit à la formation de lésions arthrosiques d'intensité variable dont la fréquence peut atteindre au niveau du carpe 78 p.cent des sujets. Bien que soumis à partir de 8 mois au même régime alimentaire que les veaux laitiers, les conditions de croissance et d'alimentation du jeune broutard sont différentes et son squelette édifié de façon plus rationnelle résiste beaucoup mieux aux contraintes de l'élevage intensif. Chez le broutard l'acidose lactique générée par une alimentation hyperglucidique et hypo cellulosique, se traduira par des problèmes podaux de type podermatite aseptique diffuse (fourbure). Compte-tenu de l'effondrement très précoce de la croissance chez le taurillon de type laitier dans les conditions de l'étude, on peut affirmer que les arthroses ne représentent en fait que les conséquences plus ou moins visibles de perturbations métaboliques profondes entraînées par une alimentation hyper énergétique et hypo cellulosique en jeune âge.

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RESUME

L'étude systématique des principales causes de boiteries des taurillons élevés en ateliers spécialisés, réalisée sur 1500 sujets, a permis de mettre en évidence la prédominance des boiteries d'origine articulaire chez les animaux d'origine laitière. Des lésions ulcératives des cartilages ont été observées chez un très grand pourcentage d'animaux ; l'étude bactériologique et histologique montre que les lésions ne sont pas de nature infectieuse mais dégénérative (arthrose) et vraisemblablement d'origine trophique. L'origine de ces arthroses a été recherchée en comparant les rythmes de croissance, l'alimentation et les caractéristiques biochimiques des taurillons d'origine laitière et des mâles broutards sevrés à 8 mois.

SUMMARY

A survey of main causes of .lameness in 1500 young bulls intensively reared gives evidence that joints troubles are prevalent in animals of dairy origin (D-O) and that bulls of meat origin (M-O) are mainly affected by laminitis. Ulcerative lesions of cartilages are observed on a high percentage of animals slaughtered a 16-18 months (up to 78 p.cent for carpal joints). Bacteriological and histological investigations exhibit the degenerative and not infectious nature of lesions (arthrosis). Causes of arthrosis have been investigated by camparing growth rates, feeding and biochemical parameters in both D-O and MO bulls.

RESUMEN

El estudio sistematico de las principales causas de cojera en toretes criados en establos especializados, realizado sobre 1500 individuos hapermitido poner en evidencia la predominancia de cojeras de origen articular en los animales de tipo lechero. Lesiones ulcerativas de los cartilagos han sido observadas en un porcentage muy elevado de animales, el estudio bacteriologico e histologico revela que las lesiones no son de naturaleza infecciona sino degenerativa (artrosis) y aparentemente de origen trofico. El origen de estas artrosis ha sido investigado comparando el ritmo de crecimiento, la alimentacion y los niveles bioquimicos en los animales de tipo lechero y en los toretos en pastoreo.

TABLEAU 1. Localisations, intensité et fréquence des lésions d'arthrose sur les articulations du membre antérieur

Localisation	Nombre d'observations	Erosions p.cent	Ulcérations p.cent
Carpes	324	78	13
Radius (proximal)	113	53	37
Humerus (distal)	79	18	-
(proximal)	88	5	-
Scapula	126	30	3

TABLEAU 2. Comparaison de différentes valeurs plasmatiques entre taurillons laitiers et broutards à 8,5 mois

	Effectifs	Ca mg/l	P mg/l	Ca/P	Lactates mmol/l	Urée mg/l	Phosphatase Alcaline U/l	ASAT U/l	Hydroxy Proline* mg/l
Laitiers	66	93 ±11	90,5 ±12	1,05 ±0,14	5,5 ±2,3	240 ±72	218 ±52	75 ±12	19,2
Broutards	60	86 ± 5	55 ±11	1,7 ± 0,49	2,9 ± 2,4	197 ± 82	65 ± 37	67 ± 22	13,8
P<		0,001	0,001	0,001	0,001	0,01	0,001	0,05	0,001

* Sur 10 sujets laitiers et 10 sujets broutards

RELATIONSHIP BETWEEN THE OCCARRENCE OF HYENA DISEASE IN CATTLE AND HYPERVITAMINOSIS

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INTRODUCTION

Hyena disease was first reported by Parodi and Espinasse in 1975 in France (4). Since the outline of Hyena disease were reported by them. Thisdisease has been recognized in many contries, also in Japan (3,6,8). The clinical and pathological definition has been established, but the etiological agents have not been fully understood.

At this moment, there are two main hypothesis which are metabolic disease by trace minerals (1) and hypervitaminosis D (5), and an autoimmune disease caused by BVD-MD virus (2). We investigated causative agents of naturally affected cases, and tried to reproduce it by excessive doses of vitamin A, D and ADE.

MATERIALS AND METHODS

Natural cases

Observation of clinical signs and hematorogical, pathological and virological examination in 6 cases of 5 farms were carried out by the routine methods (Table 1).

A relationship between incidence of skeletal abnormality and dose level of vitamin A and D was investigated, excepting farm D of case NO.5 which was bought from a market.

Experimental cases

Eight calves were used to reproduce hyena disease. These calves were divided four groups, ADE, A, D and control, and treated as shown Table 2. Clinical signs, hematological and pathological changes by routine methods were oberved during 6 to 8 months after beginning the treatment.

RESULTS

Natural cases

Epidemiological findings: Farm B was a feed lot , and the other were dairy farms. Twenty one of 50 calves at farm A, all of 28 calves at farm B, 6 of 10 calves at farm C, and 1 of 16 calves at farm E affected hyena like disease. Severe diarrhea in the neonatal stage occurred at farms A-C and the preparations of vitamin ADE had been used for treatment and prevention of the calfscour. The same vitamin preparation was administered for 6 to 9 months old calves at farm E. A disorder of skeletal development in these farms did not occur after stopped use of the preparation. In a result of virological examination, persistent infection of BVD-MD virus was revealed in case NO.4. Ten calves of same group of NO.4 were checked BVD-MD virus and the antibody, but persistent infection was only NO.4. Case NO.6 in farm E was negative both BVD-MD virus and the antibody.

Clinical signs: All 6 calves exhibited a low growth rate marked in the pelvic limbs and defective locomotion which became apparent at 2 to 4 months old in case NOs. 1-4. Metatarsus bended to outside in NO.1 of the most severe case. Knuckling at the hind fetlock, insuficient stretch at the hock were seen as the common signs. Mild clinical signs were observed in NO.6 which started dosage of vitamin ADE from six months old.

Haematological and pathological findings: The avereges of hematological values in six cases were as follows. PCV: 32.3±3.2%, WBC: 11,500±2,200/μl, BUN: 4.6±2.0 mg/100 ml, GOT: 72±17 KU, LDH:2440±435 Wrob.U, ALP:14.4±3.2 KA.U, Ca:4.7±0.2 mEq/l, P: 6.8 ±0.9 mg/100 ml, Vitamin A: 171±123 ng/ml. Serum vitamin A concentration of NO.6 (291 ng/ml) was higher than the other cases. Vitamin A concentration of 5 calves in same group of NO.6 was average 387±44 ng/ml, and 237±32 ng/ml in 4 control calves.

Autopsy revealed shortening of long bones especially in femur and tibia. The shortened femur was mostly accompanied with tipped head, and twisting of trochlea ossis femoris. In sagittal section, the epiphyseal cartilaginous plates of the femur, tibia and humerus partially disappeared or iregularly changed.

Histologically, the growth plate was irregular and sometimes isolated masses in the sponge bone. Chondrocytes in the metaphyseal regions decreased in number and the proriferative zone disappeared.

Table 1. Natural cases of Hyena disease in Hokkaido.

Farm	Type of Farm	Case NO.	Sex	Age (months)	Administration of Vitamin Age	Duration	Total volume** Vit.A	Vit.D*	BVD-ND Virus CP non-CP Antibody titer
A	Dairy	1	Female	6	1 week	10 days	5×10^6	5×10^6	NT NT < 2
B	Beef	2	Male	8	1 week	1 day	1×10^6	1×10^6	NT NT 512
C	Dairy	3	Female	15	1 week	?			- - 1,024
		4	Female	22	1 week	?			- - < 2
D	Dairy	5	Female	30	?	?			+ - < 64
E	Dairy	6	Female	10	6 months	3 months	3.6×10^7	7.2×10^6	- - < 2

* : All Holstein-Fliesian. ** : Oral administration (IU). NT : Not tested.

Table 2. Experimental cases caused by vitamin A and/or D.

Group	Calf No.	Sex	Age (days)	Body weight (kg)	Treatment Duration (days)	Route	Total volume (IU) Vit.A	Vit.D	Disorder of skeletal development
ADE	1	Male	13	42.5	10	oral	2.6×10^7	2.5×10^6	+
	2	Female	31	46.0	17	oral	4.25×10^7	4.25×10^6	+
A	3	Male	324	502.0	10	sc.	0	0	-
	4	Male	324	535.0	17	oral	5.1×10^7	0	+
D	5	Female	324	483.0	10	sc.	0	1.5×10^6	+
	6	Female	30	61.0			0	0	-
control	7	Female	23	53.0			0	0	-
	8	Female	29	41.0			0	0	-

* : All Holstein Fliesian

We diagnosed the six cases as hyena disease based on the obtained results and previous reportes (4,6,8), and suspected overdoses of vitamin A and D as a etiological agent.

Experimental cases

NO.1 administered vitamin ADE showed clinical signs of hyena disease after 2 months beginning the treatment. Autopsy of NO.1 revealed same lesions of metaphyses in natural cases. NO.2 of ADE group died on day 27 after the treatment, and severe lesions in the metaphyseal regions were already formed. the clinical signs caused by skeletal abnormarity were not observed in NO.3 administered vitamin A. NO.4 administered approximately 10 time volume vitamin A of NO.3 showed typical symptoms and pathological findings of hyena disease. NO.5 administered vitamin D showed arch of back and partial ossify in the growth plate of radius. All control calves did not show any clinical and pathological abnormalities.

It was considered that excessive doses of vitamin A, D and ADE in the neonatal cattle produced hyena disease by destruction of epiphyseal cartilage.

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SUMMARY.

In the epidemiological observation, the cases of hyena disease in Hokkaido had been administered high level of vitamin A ($1\times10^{7}\sim3.6\times10^{7}$ IU) and D ($1\times10^{6}\sim7.2\times10^{7}$ IU) in the growing stage. Hyena disease did not occurred after stopped overdoses of vitamin ADE. One of six cases was intact from BVD-MD virus which suspected as a etiological agent.

Therefore we tried to reproduce the disease by overdoses of vitamin A (5.1×10^{7} IU), D (1.5×10^{7} IU) and ADE (A:2.5×10^{7}, D:2.5×10^{6}) to the neonatal calves. Abnormalities of skeletal development resembling that of hyena disease were reproduced by the treatment.

It was proposed that excessive use of vitamin A and, or D in the neonatal cattle caused hyena disease.

HYPOTRICHOSE ASSOCIEE A DE L'HYPOTHYMIE CHEZ LE VEAU

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INTRODUCTION

L'absence complète ou partielle de pilosité (HYPOTRICHOSE) a été décrite dans de nombreuses espèces animales domestiques ou sauvages.
Cette anomalie apparaît soit seule, soit comme élément d'un syndrome (anodontie, hypothymie,...) et l'on connaît aujourd'hui 7 types d'hypotrichoses chez le bovin dont les éléments sont donnés dans le tableau 1.
Nous avons eu l'occasion d'observer et d'étudier divers cas d'hypotrichose congénitale depuis 1986 et le présent article est la description d'un de ces cas survenu dans une exploitation où 2 autres cas similaires avaient déjà attiré l'attention de l'éleveur.

EXAMEN CLINIQUE

A la naissance, le veau mâle de race pie-noir Holstein pesant environ 40 kg, était d'apparence normale mais dépourvu de pilosité hormis un fin duvet sur le sommet du crâne, les joues, le bout de la queue et les boulets.
Au moment de l'examen, l'animal alors âgé de 8 jours souffrait de polyarthrite et de diverses plaies de décubitus.
Le veau est mort au 15ème jour d'une septicémie engendrée par les lésions mentionnées ci-dessus.

EXAMEN NECROPSIQUE

A l'ouverture de la cavité thoracique, nous avons constaté que le thymus était réduit à une masse rudimentaire d'environ 5 cm^3 située dans le médiastin antérieur à l'entrée de la cavité thoracique.
Les autres organes lymphoïdes ne présentaient aucune anomalie macroscopique.
Les examens histologiques ont porté essentiellement sur le thymus.

EXAMENS HISTOLOGIQUES

Le thymus présentait un cortex homogène et dense bien différencié d'une médullaire moins riche comportant de nombreux corpuscules de Hassal de petite taille.
L'image est plutôt celle d'un thymus hypoplasique ou d'un thymus atrophique avec régénération.

DISCUSSION

L'association de l'hypotrichose et de l'hypothymie est tout d'abord intéressante d'un point de vue embryologique puisque le thymus possède une origine ecto-endodémique. En effet, une fraction endodermique dérivée de la troisième portion entobranchiale est enveloppée par une portion ectodermique qui s'ébauche à partir de la vésicule cervicale issue de la troisième fente ectobranchiale (11).
Deux autres cas survenus au cours des deux dernières années dans l'exploitation présentaient des tableaux similaires avec en outre, pour l'un des veaux femelle, une hypertrophie notable du clitoris.
Le pédigrée des veaux concernés est repris à la figure 1 afin de tenter de trouver une possible explication génétique à l'incidence élevée de cette anomalie dans l'exploitation. Dans l'ascendance des veaux anormaux, on retrouve une grand-mère maternelle commune et, pour 2 des veaux, un père commun.

Tableau 1 - Types d'hypotrichose

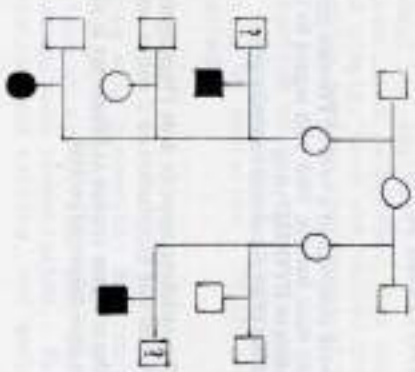
TYPE		HEREDITE	RACE	DESCRIPTION-CARACTERES
1. Hypotrichose partielle léthale		Autosome récessif		
Mohr et Wriedt	(1928)		Holstein-Friesian	Poils sur le mufle, sourcils, oreilles queue et paturons
Eisele	(1936)			
Shibata et Ishibara	(1949)		Pie-Noire (D)	
			Bétail indigène Japonais	
2. Semi-nudité		Autosome récessif		
Craft et Blizzar	(1934)		Polled Hereford	Peau et poils fins à la naissance ; apparition de poils par endroits avec l'âge.
Kidwell et Guilbert	(1950)			
3. Hypotrichose avec anodontie		Lié au sexe Récessif		
Drieux et al.	(1950)		Maine Anjou	Complètement nu à la naissance ; Poils fins après quelques semaines. Sans dents.
4. Hypotrichose partielle viable		Autosome récessif		
Hutt et Saunders	(1953)		Guernsey	Absence de poils partielle ou totale à la naissance. Thymus présent.
Becker et al.	(1963)			
Rosenberger	(1939)		Pie-Noire (D)	
Surrager	(1943)			
Mc Gavin et Alexander	(1961)			
Pirchner et Grünberg	(1970)		Pinzgau	
5. Hypotrichose avec absence d'incisives		Dominant		
Cole	(1919)		Holstein Friesian	Minimum d'hypotrichose. Absence d'incisives ; peau normale avec l'âge.
6. Nudité en stries		Lié au sexe dominant facteur A 28		
Eldridge et Atkeson	(1953)		Holstein Friesian	Lignes verticales sans poils.
7. Autres cas		?		
Denis et al.	(1975)		Normande	Alopécie totale avec malformations des oreilles et de la tête.

Aucun cas de maladie des muqueuses et aucun épisode diarrhéique imputable au virus BVD-MD n'ont été relevés dans l'exploitation au cours des dernières années et la sérologie pratiquée sur une partie du cheptel s'est avérée négative.
Signalons toutefois que différents auteurs (9,14) ont pu reproduire des alopécies totales ou partielles chez le bovin après inoculation du virus BVD-MD.

CONCLUSION

Au vu des différents éléments évoqués ci-dessus et de cas similaires observés dans d'autres exploitations depuis 1986, nous pouvons exclure l'intervention d'un agent tératogène comme le virus BVD-MD et privilégier la thèse d'un gène autosome récessif responsable d'une hypotrichose partielle viable décrite par divers auteurs (1,8,12,15,16,18).
Le tableau clinique ne correspondait pas aux anomalies de même nature causées par un gène dominant à pénétrance partielle.

Figure 1 : Relation entre les veaux affectés.



Légende :

- mâle normal
- femelle normale
- mâle identique utilisé à 2 reprises
- mâle touché
- femelle touchée

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SUMMARY

3 cases of hypotrichosis were observed in a herd of Friesian cattle. The author describes the case of an hypothymic male born in 1988 with regard to the pedigrees of affected animals and special attention to BVD-MD virus.
This study suggests the action of an autosomal recessive gene.

RESUME

L'auteur décrit un cas d'hypotrichose associée à de l'hypothymie chez un veau pie-noir holstein mâle né en 1988 dans une exploitation où 2 autres cas semblables étaient déjà survenus.
L'examen des pédigrées des animaux atteints et l'étude sur le virus BVD-MD permet de retenir l'intervention d'un gène autosome recéssif.

BOVINE CONGENITAL DEFECTS ASSOCIATED WITH DROPSY OF FETAL MEMBRANES

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INTRODUCTION

The dropsy of fetal membranes is the condition of excessive accumulation of fluids in the fetal sacs of pregnant animals. They are hydraminios and hydrallantois. The former is characterized by a gradual enlargement or filling of the amniotic cavity that is associated with a genetic or congenitally defective fetus(5). The latter occurs in 85 to 90% of cases of fetal dropsy(5). It is usually associated with a diseased uterus in which most of the caruncles in one horn are not functional and the rest of the placentomes are greatly enlarged and possibly diseased(5). It is seen quite commonly in cattle carring twin fetuses(2). Generally the fetus with hydrallantois are normal but smaller.

They can be differenciated each other, but usually they are not done clinically. And simply, they are called the dropsy of fetal membranes or hyrallantois because the excess of fluid is usually found in the allantoic sac(2).

During the investigation of the congenital defects in calves, some cows had the history of distension of abdomen by the excess of fetal fluid in the later stage of gestation. In this paper, their incidence and defects were examined.

MATERIALS AND METHODS

Seven hundred calves with congenital defects were collected during 1981 and 1989 by Kagoshima University. For the defective calves, the date of birth, condition at birth, sex, body weight, clinical findings and pathological findings were investigated. For their dams, parity, condition during gestation and calving, and gestation period were investigated.

RESULTS

The dropsy of fetal membranes investigated from the history during gestation were found in only 13 cases(1.9%) among 700 cases of bovine congenital defects. Their outlines are shown in Table 1 and the incidence are summarized in Table 2.

Eleven cases were found in Japanese black beef cattle and two were in Holstein Friesian cattle, one of which was accociated with heterozygous twin. Male calves(11 cases) were much more than female ones(3 cases).

Stillbirth and death immediately after birth occupied more than half cases(7 cases, 54%). Even in the case of survival, they are less than 5 days old and always weak. As for the date of birth, one case (abdominal rupture) of Holstein cattle was artificially interuppted at 160 days of gestation because of severe fetal hydrops. One case (dicephalus) was delivered by cesarean section at 13 days before term.

Table 1. Thirteen cases of congenital defects associated with dropsy of fetal membranes found in 700 defective calves collected by Kagoshima University during 1981 and 1989.

Case No.	Breed	Sex	Age (days)	B.W. (kg)	Parity	Calving	Difference from term (days)	Defects
1	JB	female	stillbirth	20	6	dystocia	+154	cyclops, hydranencephaly, cleft palate
2	JB	male	5	22	1	normal	-2	hydranencephaly(Akabane disease)
3	Hol	male	2	43	5	normal	-3	distortion of lumber vertebra
	Hol	female	2	-	5	normal	-3	freemartin (normal growth)
4	JB	male	stillbirth	24	1	dystocia	+25	multiple skeletal defects
5	Hol	male	stillbirth	14	5	induced, dystocia	terminated at 160 days	abdominal rupture
6	JB	male	stillbirth	18	2	normal	+5	cleft palate, rectal stricture, right hydrokidney, cryptorchidism
7	JB	male	stillbirth	26	3	Cesarean section	-13	dicephalus, right cleft palate, left jaw separation, scollosis, distorted hind legs
8	JB	male	1	17	7	normal	+19	separated ileum, cryptorchidism
9	JB	female	2	21	1	dystocia	-4	hydranencephaly, cerebellar hypoplasia (Chuzan virus disease)
10	JB	male	1, dead	34	8	dystocia	±0	dicephalus, cleft palate, ventral septal defect
11	JB	male	3	15	1	normal	+1	arthrogryposis(fore legs), internal hydrocephalus, hypoplastic abdominal wall, cryptorchidism
12	JB	male	2	20	3	dystocia	+9	nervous sign, no other defects
13	JB	male	stillbirth	11	1	dystocia	+17	hairlessness, facial defects, ventral septal defect, hydranencephaly, cryptorchidism

JB: Japanese black beef cattle
Hol: Holstein-Friesian cattle

Table 2. Summary of incidence of dropsy of fetal membranes in 13 defective calves.

Item		Number (%)		
Breed:	Japanese Black	11(84.6)	Holstein Friesian	2(15.4)
Sex:	male	11(78.6)	female	3(21.4)
Viability:	alive	7(50.0)	dead	7(50.0)
Body weight:	normal	2(15.4)	small	11(84.6)
Parity:	first calving	5(38.5)	2-8th calving	8(61.5)
Gestation period:	normal	7(53.8)	interrupted	1(7.7)
	Cesarean section	1(7.7)	retarded	4(30.8)
Defects:	simple	4(30.8)	multiple	9(69.2)

Table 3. Classification of defects in 14 calves.

Defects	Number	%
Skeletal system	7	25.0
Muscular system (joint)	1	3.6
Central nervous system	6	21.4
Ventral septal defect	2	7.1
Alimentary system	2	7.1
Hairlessness	1	3.6
Hydrokidney	1	3.6
Abdominal rupture	1	3.6
Cryptorchidism	4	14.3
Freemartin	1	3.6
Dicephalus	2	7.1
Total	28	100.0

Seven cases were delivered around the term. Remaining 4 cases were retarded birth with more than 300 days of gestation. The longest one (cyclops) was delivered at 448 days of gestation.

Though gestation periods tend to be normal or longer, the body weight were usually small (11 to 26 kg), except for 2 cases with normal size (34 kg for Japanese black and 43 kg for Holstein). The parity of cows were 5 cases in first calving, but other 8 cases were distributed in 2 to 8th birth (0 to 2 cases each). Dystocia (9 cases) was highly

occurred including one case of cesarean secion.

In the classification by anatomical body system(Table 3), multiple defects including skeletal system(7 cases) and central nervous system(6 cases) were predominat and simple defects were found only in 4 cases. Four cases were associated with cryptorchidism. Dicepalus were found in 2 cases. However, remaining 11 cases were different defects each other. Akabane disease and Chuzan virus disease were found in one case each.

DISCUSSION

The source of the amniotic fluids is probably from the amniotic epithelium and from the fetal urine. Its volume is probably regulated by swallowing by the fetus. The source of the allantoic fluids is probably from the allantoic epithelium and from the fetal urine. Its volume is probably regulated by the bladder sphincter(5).

In the pregnant ewes, Alexander et al.(1) found that estrogen and progesterone had some influences on the amounts of allantoic fluid. Reeves et al.(4) and Mellor et al.(3) found that amniotic and allantoic fluids are derived mostly from fetal urine until midgestation. As gestaton advances, the amniotic fluid comes mostly from urine, while the allantoic fluids comes from the transudate of plasma by epithelium.

Spencer et al. reported that a structural, or functional, changes in the chorio-allantoic membrane could be the cause of hydrallantois by the analysis of electrolytes and hormones.

In this research, though hydramnios and hydrallantois were not clearly differentiated from the anamnesis of farmers, most of the cases are believed to belong to the hydroallantois. However, placental lesions of dams were not confirmed.

Roberts reported in his text(5) that genetic or hereditary conditions resulting in defective fetuses often associated with hydramnios are 1) "bull dog" calves in Dexter cattle, 2) small brachygnathic, defective calves in Angus cattle, 3) a muscle contracture monster in Red-Danish cattle, 4) immature defective small fetus with pituitary hypoplasia or aplasia and with prolonged gestation in Guernsey cattle, and 5) hydrocephalic fetuses in Hereford cattle. All of the above defective fetuses have been caused by recessive autosomal genes. Hydramnios is also seen in cattle with congenitally anomalous fetuses such as conjoined twin monters, schistosomus reflexus and some others.

In this research, genetic or hereditary causes were not confirmed because there were no group incidence and the defects were different each other. Viral cause of defects were found in one case of Akabane and in one case of Chuzan. However, more than 100 cases of each defects were found without hydrops among 700 cases collected. Therefore, these cases were thought not to be the result of these virus infection during gestation.

In conclusion, hydrops of fetal membrane are found in the calves with congenital defects, but the incidence rate is very low(1.9%). The incidence was found much more in male fetuses than in female ones. The date of birth showed a tendency to be normal or longer. Newborn calves were usually small either in dead or weak alive, and tended to become dystocia. The various multiple defects were found in hydrops

of fetal membrane and not limited to special organ defects.

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SUMMARY

The defective calves with hydrops were found in 13 cases(1.9%) among 700 defective calves collected during 1981 and 1989 by Kagoshima University. Eleven of them were Japanese black cattle and two were Holstein Friesian cattle, one of which was associated with heterozygous twin. There were male in 11 cases and female in 3 cases.

Stillbirth and death immediately after birth occupied more than half of the cases. The survived calves were less than 5 days of age and always weak. The date of birth were around term(7 cases) or retarded(4 cases), except for two cases of induced abortion and cesarean section. Most of calves were small, but the dystocia highly occurred. As for the parity of dam, first calvings were found in 5 cases and 2 to 8 calvings were found in 0 to 2 cases each.

In the classification of the defects by anatomical body system, the multiple defects including skeletal system(7 cases) and central nervous system(6 cases) were predominant and the simple defects were found only in 4 cases. Only 2 cases were belonged to the same defects(dicephalus), and remaining 11 cases were independently found.

ACTIVE IMMUNIZATION OF COWS WITH A SALMONELLA TYPHIMURIUM MUTANT BACTERIN-TOXOID AND THE PASSIVE TRANSFER OF ANTI-CORE-ANTIGEN ANTIBODIES IN COLOSTRUM

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INTRODUCTION

Gram negative endotoxins have been implicated in the pathogenesis of equine CHO laminitis (1), bovine coliform mastitis (2) and adult and neonatal septicemias (3,4,5,6). Fortuitously, the demonstration that cattle (2,5,6) and horses (3,4) could be protected from various Gram negative endotoxins via anti-core-antigen antibodies offers viable possibilities for immunologically protecting animals from the deadly effects of endotoxemia.

The anti-core-antigen antibody research work conducted prior to investigations in cattle and horses was centered around laboratory animal studies utilizing a mutant Salmonella typhimurium (8) or the J5 mutant of E. coli utilizing laboratory animal and human clinical endotoxemia cases (9). This foundation of knowledge has recently been applied to problems in cattle (2,5,6) and horses (3,4) and has added a new immunological dimension to the possibilities for controlling Gram negative endotoxin mediated diseases.

The specific source or sources of endotoxin involved in a particular case of endotoxemia may be one or more members of the large Gram-negative family Enterobacteriaceae. Because there are hundreds of serotypes, it is impractical to combine sufficient autogenous vaccines to provide broad-spectrum protection. Thus, there is a paramount need for a single source bacterin that provides cross-protection against virtually all Gram-negative endotoxins.

The R-mutants of Gram-negative bacteria are biochemically characterized by their relative absence of oligosaccharide ("O") side chain attachments. The relative degree of "O" side chain absence is designated by the capital letter R accompanied by the small case letters "a" through "e" with Re mutant of S. typhimurium completely lacking "O" side chains. In contrast, the J5 E. coli mutant is characterized as Rc and possess some "O" side chains. Removal of these "O" side chains via mutation allows the core antigen of the cell wall to be presented to the immune system for the subsequent production of cross protective antibodies.

A mutant S. typhimurium bacterin-toxoid was administered to cows late in gestation first) to learn if seroconversion could be achieved in cows, second) to learn if the anti-core-antigen antibody titer in the colostrum was higher in the vaccinated cows than in control cows, and third) to determine whether or not serum anti-core-antigen antibody titers in calves that suckled vaccinated cows were higher than in calves that suckled unvaccinated cows.

MATERIALS AND METHODS

Vaccine

The vaccine (Endovac-Bovi™:IMMVAC Inc., Columbia, MO, USA) used in these experiments contained a killed bacterial Re-mutant of S. typhimurium (bacterin), an immune modulator (endotoxoid), an oil

adjuvant and a protein/lipid binding carrier/adjuvant (dialuminum trioxide) (5). Each cow was vaccinated and boostered within 2 weeks with either the vaccine or a dialuminum trioxide/saline placebo.

Animals

Thirty-seven normal Holstein and Guernsey cows ranging from 2-7 years of age in the last six weeks of gestation were used in this study.

Serum analysis

Serum samples collected from each cow prior to and 4 weeks after the first injection of vaccine or placebo, as well as those collected from the calves at birth and 24 hours after suckling, were analyzed by an ELISA assay adapted from a previously developed radioimmunoassay for measuring specific IgG(t) anti-endotoxin antibody levels (10).

Statistical analysis

Data were analyzed via analysis of variance statistical techniques. The predetermined acceptable probability level was 0.05 or less.

RESULTS

The post-vaccination mean Log_2 12.15 (1:4545 dilution) anti-core-antigen antibody titer of the 19 cows that received S. typhimurium bacterin-toxoid was significantly (P=0.0003) higher than the mean pre-vaccination titer of Log_2 10.73 (1:1698 dilution). The post-vaccination mean Log_2 10.75 (1:1722 dilution) titer of the placebo treated cows was not significantly (P=0.8976) different than the mean pre-vaccination Log_2 10.80 (1:1783 dilution) titer (Table 1).

TABLE 1 Anti-core-antigen antibody mean serum Log_2 titers, least square estimates, standard deviation and standard error values of vaccinated and placebo treated cows are displayed along with the ANOVA derived probability levels comparing specific subpopulations.

	Least square estimates					Statistical tests
	Common slope	Placebo treated		Vaccine treated		Prevac vs. Postvac
		PreVac	PostVac	PreVac	PostVac	
LS mean	-7.08	10.75	10.80	10.73	12.15	Placebo..P ≤0.8976
Std err	±0.09	±0.27	±0.27	±0.27	±0.27	Vaccine..P ≤0.0003
	Simple group statistics					Pre-treatment
N		18	18	19	19	Placebo vs. Vaccinates
Average		10.75	10.80	10.73	12.15	
Std dev		±1.23	±1.31	±0.97	±1.62	P ≤0.9573
Std err		±0.30	±0.32	±0.23	±0.38	Post-treatment
						Placebo vs. Vaccinates
						P ≤0.0006

There were no untoward effects such as abortions or other local or systemic illnesses that arose from vaccinating cows with S. typhimurium bacterin-toxoid.

The mean Log_2 13.18 (1:9281 dilution) core-antigen-antibody titer of the colostrum from 9 vaccinated cows was significantly (P=0.04) higher than the mean Log_2 12.22 (1:4771 dilution) titer of colostrum from placebo treated cows. Calves of the vaccinated cows also demonstrated significantly (P=0.027) higher serum titers of core-antigen-antibodies than did the calves of placebo treated cows, mean Log_2 10.64 (1:1596 dilution) vs. mean Log_2 9.20 (1:588 dilution) respectively (Table 2).

Six calves of vaccinated mothers increased their mean anti-core-antigen serum antibody titers from Log_2 6.7 (1:104 dilution) to Log_2 10.64 (1:1596 dilution). Eight calves from placebo treated cows increased their mean anti-core-antigen antibody titers from Log_2 6.19 (1:73 dilution) to Log_2 9.2 (1:588 dilution) (Table 2).

TABLE 2 Anti-core-antigen antibody mean colostrum and serum Log_2 titers, least square estimates standard deviation and standard error values of vaccinated and placebo treated groups are displayed along with ANOVA derived probability levels comparing subpopulations.

		Least square estimates					
		Placebo treated			Vaccine treated		
	Common slope	Colostrum	Calf suckle Pre	Calf suckle Post	Colostrum	Calf suckle Pre	Calf suckle Post
LS mean	-8.77	12.22	6.19	9.20	13.18	6.70	10.64
Std err	±0.20	±0.33	±0.48	±0.43	±0.33	±0.51	±0.47
		Simple group statistics					
N		11	8	8	11	6	6
Average		12.22	6.19	9.20	13.18	6.70	10.64
Std dev		±1.41	±0.92	±1.43	±0.68	±0.85	±0.81
Std err		±0.45	±0.35	±0.54	±0.22	±0.38	±0.36

Statistical tests		
Within placebo	Presuckle vs. postsuckle . . .	$P \leq 0.0001$
Within vaccine	Presuckle vs. postsuckle . . .	$P \leq 0.0001$
Placebo vs. vaccine	Presuckle	$P \leq 0.4501$
	Postsuckle	$P \leq 0.0269$
	Colostrum	$P \leq 0.0397$

A significant increase in serum anti-core-antigen antibodies was produced in both the cows and calves in this study. The increase in mean serum antibody titer of the cows was due to active immunization and in the calves due to passive immunization via colostrum absorption.

DISCUSSION

The adult pregnant cows responded to an anti-core-antigen vaccination regime by significantly seroconverting. Core-antigen-antibodies have been shown to significantly lower the incidence of coliform mastitis in lactating dairy cows (2). Whether or not cows in this study possessed increased resistance to Gram-negative induced mastitis awaits completion of ongoing epidemiological studies. However, the results of previously conducted studies of S. typhimurium bacterin-toxoid vaccinated calves challenged with E. coli and Pasteurella endotoxins suggested that significant cross protection can be provided by the anti-core-antigen antibodies produced in response to S. typhimurium bacterin-toxoid vaccination (5).

Whether or not the increases in serum anti-core-antigen antibodies in both cows and calves significantly increased their resistance to Gram negative endotoxins can be answered either by long term epidemiologic or direct endotoxin challenge studies. Epidemiological studies are presently in progress.

The results of this study and the investigative work of others (2,6,7) suggest that the application of core-antigen-antibody immune strategies could translate into significant reduction of economic losses in both the dairy and beef cattle industries. The many Gram-negative endotoxin mediated diseases in calves and adult cattle provide a wide potential spectrum of maladies that may be very responsive to this new immunological approach to controlling Gram negative diseases.

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SUMMARY

Thirty-seven normal Holstein or Guernsey cows in their last six weeks of gestation were injected with either a placebo or Salmonella typhimurium mutant bacterin-toxoid. The vaccine stimulated a significant ($P<0.05$) increase in the mean Log_2 anti-core-antigen serum antibody titers from 10.73 to 12.15 while there was no significant increase in serum antibody titers in placebo treated cows.

There was a significant ($P<0.05$) difference between the colostral mean anti-core-antigen antibody Log_2 titers of the placebo, 12.22, and vaccinated, 13.18, cows. The mean Log_2 serum antibody titers of suckled calves at 24 hours of age from the placebo and vaccinated groups were 9.20 and 10.64 respectively and significantly ($P<0.05$) different.

It was concluded that cows vaccinated with bacterin-toxoid in the last six weeks of gestation seroconverted in terms of anti-core-antigen antibodies and that they passively transferred significantly ($P<0.05$) higher levels of these antibodies through colostrum to their calves.

CONCLUSION

Treinta y siete vacas normales , de la raza Holstain y Guernsey , fueron inyectadas en las últimas semanas de la gestación con placebo o con una bacterina-toxoide de un mutante de Salmonella Typhimurium.La vacuna , estimuló un incremento significante($p<0.05$) de los promedios Log_2 de títulos de anticuerpo desde 10.73 hasta 12.15 , mientras , no hubó incremento significante en los títulos de anticuerpo en suero en vacas tratadas con placebo.
La diferencia entre los promedios de los títulos de anticuerpo en calostro , contra la sección central de la membrana celular bacterial Log_2 de el grupo tratado con placebo 12.22 y el grupo vacunado 13.18 fue significativa ($p<0.05$).El promedio de los títulos de anticuerpos contra la sección central de la membrana celular bacterial de terneros inyectados con placebo y de terneros vacunados fue 9.20 y 10.64 respectivamente y significativamente diferente($p<0.05$).
En conclusión , las vacas inyectadas con la bacterina-toxoide mutante en las ultimas seis semanas de gestación , se convirtieron serologicamente en terminos de anticuerpos y transfirieron pasivamente(por medio de el calostro) niveles significativamente mas altos ($p<0.05$).

RÉSUMÉ

Trente sept vaches de race holstein ou guernsey furent injectées avec soit un placebo ou soit un mutant toxoïde bactérien (Salmonella typhimurium) durant les six dernières semaines de gestation. Le vaccin a stimulé un accroissement significatif ($P<0.05$) de la moyenne du titre d'anticorps sérique, après transformation logarythmique en base deux (Log_2), allant de 10.73 a 12.15. Par contre, il n'y avait pas d'accroissement significatif du titre d'anticorps sérique chez les vaches qui ont reçu le placebo.

Après transformation logarythmique en base 2, dans le colostrum les moyennes du titre d'anticorps dirigés contre les antigènes de la membrane interne étaient significativement différentes ($P<0.05$) entre le groupe recevant le placébo (12.22) ou le vaccin (13.18). Les moyennes du titre d'anticorps dirigé contre les antigènes de la membrane interne etaient aussi significativement differentes ($P<0.05$) entre les veaux agés d'un jour issues du groupe placébo (9.20) ou issues du groupe vaccine (10.64), après transformation logarythmique en base 2.

En conclusion, les vaches vaccinés avec le mutant toxoïde bactérien (S. typhimurium) durant les six dernières semaines de gestation ont seroconverties contre les antigènes de la membrane interne. Par l'entremise du colostrum, les veaux de ces même vaches ont reçu un plus haut titre d'anticorps dirigés contre la membrane interne.

SOME CELLULAR ASPECTS OF BOVINE COLOSTRUM.
DISTRIBUTION OF LYMPHOCYTE SUBSETS

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INTRODUCTION

Bovine offspring are born agammaglobulinaemic because of thick placenta barrier and are thus particularly susceptible to neonatal infections.

The newborn calf only acquires immunity through colostrum feeding.

The colostrum ingestion and normal intestine function is important since the absorption of immunoglobulins (Ig) occurs in the small intestine of the calf immediately post birth up to 48 hours.

The Ig G_1 is the dominant isotype in colostrum and in milk, while the IgM, IgA and Ig G_2 are present in reduced amounts.

Recent work carried out on bovine species (1) rodents (2, 3) and humans (4, 5) also indicate that passive transfer of cellular immunity may have an important immunoregolatory role in the immune responsiveness of the newborn calf.

Colostrum, infact, contains besides soluble factors such as lysozyme, lactoferrin, complement and lactuperoxidase, also cellular factors. Between 80-90% of cells in milk are polymorphonuclear leucocytes and macrophages and these about 10% are B and T lymphocytes.

T and B cells, whose number decreases from first to third day of lactation, are able to pass digestive processes reaching the gastrointestinal tract in a viable immunoreactive condition.

Aim of present work was to compare the distribution of peripheral blood and of colostrum lymphocyte subpopulations in cows and to verify whether a selective migration of lymphocyte subsets occurs from mother to newborn calf at parturition.

MATERIALS AND METHODS

Experimental Animals

15 healty adult cows were selected for this study. Complete clinical examination and particularly of the mammary gland was carried out on each animal on sampling days.

Blood and colostrum were collected within 3 days post-partum.

Separation of peripheral blood lymphocyte

10cc of peripheral blood was collected by jugular venipuncture from the cows on the same day as for sampling colostrum.

The lymphocyte were isolated according to the Böyum method (6).

Separation of colostrum lymphocytes

Samples of about 1 litre of colostrum were processed for lymphocyte isolation, according to Duhamel et al. (7).

The colostrum was diluted 1/1 with phosphate buffered saline (PBS), centrifuged at 1300 g for 20 min. at 4 °C, then, the pelletted cells were resuspended in 50 ml of PBS centrifuged at 1000 g for 15 min. at 20 °C. After this wash, the pellet was resuspended into 10 ml of TRIS buffered saline solution and layred on lymphocyte separation medium (Flow Laboratories, Milan), then centrifuged for 35 min. at 400 g. The mononuclear cells collected at the interfacie were then washed twice with Hank's salt (Flow Laborato-

ries).

The viability was determined by the trypan blue exclusion method.

Monoclonal Antibiodes and immunofluorescence analysis

The monoclonal antibodies used to characterize perypheral blood and colostrum lymphocytes were ILA42 (peripheral T), ILA12 (T helper/inducer), ILA51 (T suppressor/cytotoxix), ILA30 (B lymphocytes) all from the International Laboratory for Research on Animal diseases (ILRAD) (8, 9, 10). 50 ul of monoclonal antibodies were added to tubes containg $1x10^6$ cells and incubated in ice for 30 min. the cells were then washed and incubated with goat anti mouse Ig conjugated with FITC (Becton Dickinson) for 30 min. in ice.

The washed cells were resuspended in PBS and analyzed epiluminescence microscopic (Leitz).

Statistical Analysis. Data obtained were analyzed by the student t-test.

RESULTS and DISCUSSION

In this study the colostrum of 15 cows was examined in order to investigate the presence and the distribution of lymphocyte subpopulation in colostrum.

Data show in TAB. 1, demostrate that most colostrum lymphocytes are T cells and the presence in colostrum of T helper/inducer and T suppressor/cytoxic subpopulations.

From statistical analysis it results that the T cells of colostrum are significantly increased ($P < 0.01$).

TAB. 1. DISTRIBUTION OF LYMPHOCYTES IN COLOSTRUM.

NUMBER	PERCENTAGE OF CELLS REACTIVE WITH			
OF	ILA42	ILA12	ILA51	IL30
COWS	(T)	(TH)	(TS)	(B)
15	56.9 ± 6.5^c	42.7 ± 7.0^c	26.0 ± 6.3^c	24.8 ± 10.6^c

c significant difference in comparison to peripheral blood.

Results also show a significant increase in percentage of T helper and T suppressor subpopulations in colostrum ($P < 0.01$).

No significant variation in T helper/T suppressor ratio is present in comparison to that in the blood of tested subjects (TAB. 2).

Furthermore, no fluctuations exist between distribution of lymphocyte subset in the blood of cows from which colostrum was drawn and the blood of non pregnant cows.

Results obtained in this study confirm data obtained by Duhamel;et al. (7) in bovine species.

TABLE 2. RATIO ILA12/ILA51 LIMPHOCYTE SUBSETS IN COLOSTRUM, BLOOD AND IN BLOOD FROM NON PREGNANT COWS.

LYMPHOCYTE FROM	RATIO ILA12/ILA51 (T HELPER/T SUPPRESSOR)
COLOSTRUM	1.67 ± 0.3
PERIPHERAL BLOOD OF POST-PARTUM COWS	1.82 ± 0.6
PERIPHERAL BLOOD IN NON PREGNANT COWS	2.08 ± 0.7

The novelty of our study consist in the observation that cells with phenotype helper/inducer and cells with phenotype suppressor/cytotoxic are present in colostrum, as already verified in humans by Richie et al. (11).

Having demo,strated that the ratio T helper/T suppressor is the same in blood excludes the possibility of preferential migration of lymphocyte subset.

The presence of both subsets in colostrum suggests that both of them could have an influence on newborn calves.

Further research is necessary, therefore, to better characterize the origin and functional features of lymphocyte subpopulations in the mammary gland.

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SUMMARY

The aim of this work was to compare the lymphocyte subpopulations distribution in

bovine colostrum and jugular blood and to verify whether a selective migration of lymphocyte subset occurs at parturition.

In 15 cows within 3 days post-partum, blood and colostrum were collected. Results show higher percentages of T lymphocyte in colostrum in comparison to blood and lower percentages of B lymphocytes. In colostrum the presence of T helper/inducer and T suppressor/cytotoxic subpopulations was demonstrated.

Both subsets result increased in percentage terms, while TH/TS ratio is unchanged, and are there isn't a preferential migration of a subset from blood to mammary gland.

RESUME'

Observations sur les population lymphocitaries dans le colostrum de la vache.

L'objective de cette experimentation est connaître la distribution des subpopulations lymphocitaries dans le colostrum et dans le sang jugulaire des vaches et de verifier s'il y a migration selective des fractions lymphocytaries à la mise bas.

Le sang et le colostrum ont été prelevez dand 15 vaches jusque à trois jour du post-partum.

Les resultats démontrent la presence d'une plus grand quantité de T lymphocite dans le colostrum par rapport au sang, au contraire les B lymphocite sont plus bas.

Dans le colostrum a été démonstré la presence de populations de T adiuvant/inducteur et T suppressor/cytotoxic.

Les deux fractions sont percentuellament augmenté, au contraire le rapport TH/TS ne change pas il n'y a pas de migration préférentiel de quelque fraction lymphocitaire du sang au colostrum.

ZUSAMMENFASSUNG

Dieses Studuim hat als das Ziel den Vergleich mit der Verteilung der Unterbevölkerungen von Lymphocyte in Colostrum und in peripherischen blut der kuh und das prüfen, ob sich eine selektive von Unterbevölkerung der Lymphozyten beim Gebären ereignet.

Von 15 Kühen werden Blut und Colostrum binnen 3 tagen nach dem Gebären estnommen. T Lymphozyten und niedrigere Prozentsätze von B Lymphozyten in Colostrum in Vergleich mit dem Blut. In Colostrum wurde die Anwesenheit der Unterbevölkerungen T Helper/Inducer und T Suppressor/Cytotoxic bewiesen. Beide Unterbevölkerungen sind nach den Prozentsätzen angewachsen, dagegen bleibt die Beziehung TH/TS unverändert.

Ausserdem gibt es keine selektive Vanderung einer Unterbevölkerungen vom Blut zur Mutterbrust.

ETIOPATHOGENIE DES TACHYCARDIES NEONATALES BOVINES

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INTRODUCTION

Il s'agit d'affections apparaisant le plus souvent dans les 24 heures suivant la naissance chez des veaux typiquement très bien conformés et souvent extraits par césarienne.

Ces veaux présentent une tachycardie extrême (f>150) de rythme régulier, accompagnéede polypnée et d'anorexie secondaire car leur essoufflement est tel qu'ils ne peuvent plus téter.

Le traitement habituel de ces veaux fait appel aux digitaliques, à l'inosine et au sélénium. Un traitement de 5 à 7 jours s'accompagne le plus souvent de la guérison et les animaux traités ne semblent pas garder de séquelles ultérieures puique leur croissance s'avère normale par la suite.

Différentes étiologies peuvent être suspectées à priori:

- Origine fonctionnelle : hypercatécholaminergie, hypersécrétion de catécholamines à la suite de "stress" du vêlage. Trouble de la conduction au niveau du tissu nodal qui ne filtre plus les influx en provenance du "pace-maker".
- Origine lésionnelle : malformatiou congénitale (CD) correspondant probablement à une partie des cas. Les malformations graves débouchent sur la mort du sujet tandis que certaines peuvent s'amender spontanément avec l'âge (persistance du trou de Botale). Insuffisance cardiaque vraie : peu probable compte-tenu de l'évolution favorable observée classiquement après un traitement conventionnel médical de courte durée. Déficit en Se et/ou Vit.E. : l'habitude thérapeutique inclut bien souvent l'apport de ces facteurs car le syndrome évoque la myopathie dyspnée. Le but de cette étude est de clarifier l'étiopathogénie des troubles "cardiaques" observés chez le veau nouveau-né et d'en définir les caractéristiques électrocardiographiques. Compte-tenu des hypothèses formulées deux approches thérapeutiques sont proposées.

MATERIEL ET METHODES

Les animaux

Les 30 sujets retenus pour l'essai sont des veaux nouveaux-nés (<1 semaine) présentant une tachycardie constante (f>150) non reliable à une pathologie intercurrente entrainant une polypnée et une anorexie partielle ou totale. Les animaux sont comparés à 30 veaux sains contemporains issus des mêmes exploitations.

Conduite des traitements expérimentaux

Les 60 animaux de l'essai sont répartis en 3 lots : lot de 30 veaux témoins. Lot de 15 veaux traités au Propranolol (Avlocardyl ND), β bloquant doué de propriétés bradycardisantes sur les tachycardies adrénodépendantes. Il est utilisé chez le veau à la dose de 5mg IV à renouveler. Lot de 15 veaux traités au Disopyramide (Rythmodan ND) antiarythmique du groupe IA utilisé dans le traitement des tachycardies ventriculaires et supraventriculaires. Il est utilisé chez le veau à la dose de 50 à 100 mg IV à renouveler.

Examens réalisés

Electrocardiographie : un contrôle électrocardiographique est réalisé avant la mise en place du traitement et aux temps de 5 minutes, 24 heures, 5 jours après traitement. L'ECG de référence a été déterminé préalablement sur 22 veaux sains. L'électrocardiogramme est recueilli au moyen de CARDIMAX FX102M, les électrodes étant placées à la racine de chaque membre. On enregistre les dérivations en DI, DII, DIII, aVR, aVL et aVF.

Examens paracliniques : pour chaque animal on a effectué avant le traitement le bilan suivant: sur le veau malade et contemporain, prise de sang pour dosage de CPK, LDH, TGO, Cu et Zn. Sur la mère du veau malade : prise de sang pour dosage du Cu et du Zn. Prise de lait pour dosage du Se (5 à 7 jours après le vêlage). Sur la mère du veau contemporain : prise de sang pour dosage du Cu et de Zn.

Examens cliniques : le devenir des animaux est suivi à l'aide de tracés ECG et de leur examen clinique : disparition ou maintien de l'anorexie et de la polypnée.

Etude statistique : Les calculs présentés sont effectués selon les cas par les tests t pour séries indépendantes ou appariées ou par le test de Wilcoxon en cas d'inégalités des variances. Les effectifs sont comparés par le test chi-deux avec correction de Yates.

RESULTATS

Evolution clinique

Evolution clinique de l'effectif : l'essai a porté sur 30 animaux traités. 2 veaux sont exclus a posteriori car ils ont été reconnus atteints de malformations congénitales confirmées à l'autopsie. 4 animaux sont exclus pour traitement complémentaire . Sur 24 animaux retenus on obtient un pourcentage de guérison similaire de 70 % dans les deux traitements.

Evolution électrocardiographique : tracé électrocardiographique normal. L'électrocardiogramme des animaux se présente comme un tracé isodiphasique ou à complexe R en D1, avec onde T de signe instable, avec des ondes négatives de grande amplitude en D2, D3 et aVF, ondes T de signe opposé, d'amplitude également importante, avec en aVR et aVL un complexe majoritaire qui est positif et l'onde T négative. L'onde P est positive en D1, D2, D3 et aVF et négative en aVR et aVl. Elle peut-être diphasique ou crochetée sans pour autant être anormale, avec absence d'arythmie sinusale respiratoire, avec une fréquence basale de 135 coups par minute dans les conditions de l'enregistrement, et donc a priori en état hyper-catécholaminergique.

Trouble cardiaque initial : Chez les 24 animaux de l'essai on observe une fréquence cardiaque initiale de 145.6 ± 26.9. Il y a donc une légère tachycardie puisque les tracés obtenus antérieurement sur 22 veaux sains enregistrés dans les mêmes conditions ont fourni une fréquence moyenne de 135 ± 34.5 coups/minute. Toutefois cette différence de fréquence n'est pas significative. Le tracé obtenu est généralement de type sinusal et présente les caractéristiques suivantes :
En cas de tachycardie, on peut caractériser le tracé par : D1 inchangé ou apparition d'ondes négatives. D2, D3 et aVF avec une augmentation de l'amplitude des complexes majoritaires. Cette remarque est valable également pour aVR et aVL. Un raccord ST sus ou sous dénivelé et curviligne concave ou convexe en fonction du complexe le plus volté. Une sub-fusion TP logique du fait de l'augmentation de la fréquence.
En cas de manifestations pathologiques telles que les anoxies post-partum, malformations cardiaques avérées, le peu de recueils dans ces différentes situations permet de conclure avec beaucoup de prudence à : augmentation de l'amplitude de R en D1 ou alternance électrique de type 2/1, avec raccord ST ou ST de type PARDE, en début d'évolution. Le même type d'anomalie peut-être observé en D2, aVF, toujours en début d'évolution. Un catalogue de variations morphologiques très protéiformes non interprétables actuellement du fait du très petit nombre des observations. Fréquence de 150 à 200 coups par minute.

Evolution électrocardiographique : On constate une baisse hautement significative de la fréquence cardiaque dans le lot propranolol seulement (143.8 ± 20.2 vs 122.3 ± 21.9). Dans le lot disopyramide, aucune différence n'est constatée (140.8 ± 16.8 vs 138.4 ± 16.7). Dans le lot propranolol, la fréquence 5 minutes après injection (122/') devient même inférieure à celle notée chez les veaux sains (135/'). En fin de

traitement et sur 17 animaux guéris, la fréquence cardiaque moyenne s'établit à respectivement 148.1 ± 13.5 et 159.9 ± 34.6 pour les animaux traités au propranolol et au disopyramide pour des valeurs initiales respectives de 139.1 ± 16.4 et de 144 ± 17.5. Globalement sur l'ensemble des animaux guéris, quelque soit leur traitement, la fréquence cardiaque finale est supérieure à celle qui a été enregistrée à J0. Cette différence est significative (p=1.51%).

Résultats paracliniques

Dosages enzymatiques : l'analyse porte sur les 28 sujets conformes aux critères d'inclusion. Les valeurs obtenues dans le lot malade sont significativement différentes des témoins pour les paramètres : CPK : 993.8 ± 2722 U/l vs 70.47 ± 92.12 U/l - LDH : 1183 ± 749.6 U/l vs 836 ± 175.4 U/l - TGO : 53.9 ± 34.4 U/l vs 30.3 ± 11.68 U/l. On a vérifié à ce propos que les animaux traités n'avaient pas reçu d'injections antérieures au prélèvement qui auraient pu expliquer une hausse de l'activité CK.

Oligo-éléments : L'analyse porte sur les mêmes 28 animaux. Les taux sanguins de Cu sont comparables entre les deux lots tant chez les veaux (44.64 ± 23.47 vs 48.43 ± 17.6) que chez les mères (67.89 ± 24.96 vs 73.03 ± 16.56). Ils sont néanmoins très faibles dans l'absolu, en particulier chez les veaux puisque l'on considère comme normales des valeurs de 80 à 120 µ/100ml. Il y a donc globalement dans la population étudiée une carence en Cu. Les taux sanguins de Zn des veaux malades et de leurs mères sont par contre significativement inférieurs à ceux des veaux et de leurs mères témoins (76.36 ± 32.92 et 63.11 ± 15.42 vs 106.7 ± 38.86 et 77.03 ± 20.43 : p = 0.31 et 1.04%). Dans l'absolu, la zincémie est basse (sauf chez les veaux témoins) puisque les valeurs normales vont de 80 à 120 µg/100ml. Il y a donc chez les veaux malades un état de subcarence en zinc. Le taux lacté de Se est en moyenne de 0.09 mg/kg M.S., ce qui peut-être considéré comme le niveau limite de la carence, la myopathie apparaissant pour des teneurs de l'ordre de 0.05 mg/kg M.S. En résumé, on est donc en présence d'une population carencée en Cu, subcarencée en Se et de surcroît subcarencée en Zn chez les sujets malades seulement.

DISCUSSION

L'essai mené sur les cardiopathies néonatales bovines a mis en évidence :

Sur le plan étiologique : des taux de CPK, TGO et LDH anormalement élevés chez les sujets malades. On peut interpréter ces élévations d'activités enzymatiques comme étant les témoins d'une myopathie, sans qu'il soit possible de préciser si le muscle cardiaque fait l'objet ou non des lésions en l'absence du typage des isoenzymes tissulaires. A l'origine de cette souffrance musculaire, il est classique d'évoquer, en particulier dans la zone d'élevage charolais, une carence en oligo-éléments. Les analyses effectuées dans ce but ont révélé une carence en Cu, une subcarence en Se et une hypozincémie chez les veaux malades. Combinées entre-elles, ces carences peuvent d'après LAMAND (communication personnelle) favoriser spécialement dans le lot malade, l'apparition de pathologies musculaires et/ou myocardiques.

Sur le plan cardiaque : le trouble purement cardiaque paraît relativement benin. L'analyse des tracés électrocardiographiques n'a permis de caractériser aucun trouble du rythme ou de la conduction ; les tracés ne diffèrent de ceux obtenus sur veau sain que par un léger degré de tachycardie sinusale. De plus les animaux cliniquement guéris présentent une fréquence cardiaque significativement supérieure à leur fréquence cardiaque initiale. Enfin les diagnostics thérapeutiques tentés n'ont pas confirmé l'origine fonctionnelle des troubles cardiaques. Le résultat obtenu avec le bêta-bloquant ne doit pas surprendre mais relève d'un effet symptomatique lié au stress de l'examen ECG, ce qui explique que des valeurs relevées 5 minutes après l'injection soient inférieures à celles que l'on obtient chez les sujets réputés normaux. L'effet orexigène observé sur ces animaux pourrait s'expliquer de la même façon.

L'affection clinique constatée paraît donc être à composante essentiellement musculaire au moins dans le cas des animaux présentant de fortes activités enzymatiques. Chez ces animaux, il ne semble pas exister de myocardiopathie invalidante. Dans ce contexte, la polypnée s'explique dans le cadre du syndrome "myopathie-dyspnée". Des conclusions similaires ont été rapportées par KENNEDY (1) sur un modèle expérimental de carence en Se-Vit E ou en dépit de lésions histologiques majeures, le fonctionnement cardique, évalué par les tracés électrocardiographiques, n'apparaissait pas gravement perturbé. KENNEDY (2) mentionne toutefois la présence constante d'anomalies électrocardiographiques dans un autre essai. Ce fait n'a pas été confirmé par nos essais, peut-être en raison du degré moindre de la carence en Se.
Cet essai n'a pas permis d'explorer le débit cardiaque dont NAVETAT (4) évoque l'insuffisance comme pouvant être à l'origine de la symptomatologie en raison de la disproportion entre le coeur et le volume musculaire chez certains animaux exceptionnellement conformés. On peut également évoquer à la lumière des résultats précédents la possibilité de lésions myocardiques sans traduction électrocardiographique, mais capable d'obérer les performances cardiaques. Toutefois, s'il existe ce déficit n'a probablement pas une importance majeure dans la pathogénie de l'affection compte-tenu du caractère relatif de la tachycardie constatée et de l'absence de troubles de la conduction (confirmée à l'électrocardiographie). Dans le cas des animaux présentant des faibles activités enzymatiques et des taux normaux d'oligo-éléments, on pourrait être en présence d'anomalies cardiaques congénitales bénignes et spontanément réversibles (communication interauriculaire ...) n'ayant pas de traduction électrocardiographique et qui entraîneraient une polypnée par déficit de l'hématose.

CONCLUSION

Dans la région de l'essai, les veaux nouveaux-nés présentant un syndrôme polypnée-anorexie, communément réputés "cardiaques" apparaissent le plus souvent carencés en Cu, Zn et Se et atteints d'un syndrôme de myopathie-dyspnée et/ou rarement d'anomalies cardiaques, les unes mineures et spontanément réversibles, les autres gravissimes et fatales à court terme. Le plus souvent chez les animaux carencés, le fonctionnement cardiaque en lui-même n'apparait pas gravement perturbé.

D'un point de vue thérapeutique, les traitements tonicardiaques usuels peuvent avoir un effet de soutien, mais le traitement du trouble cardiaque pour lui-même paraît hors de propos ou irréaliste dans le cas des lésions congénitales. On peut par contre envisager la correction des carences minérales et un traitement de soutien énergétique et/ou électrolytique visant à corriger les troubles induits par les lésions musculaires et les troubles métaboliques (acidose par hyperlactacidémie).

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RESUME

Les tachycardies néonatales bovines se manifestent cliniquement par de la polypnée. Afin de préciser, soit l'origine fonctionnelle (hypercatécholaminergie - immaturité du tissu nodal), soit l'origine lésionnelle (malformations congénitales - cardiomyopathie d'origine carentielle) 30 veaux malades et 30 veaux témoins ont fait l'objet d'un suivi clinique (évolution électrocardiographique), paraclinique (dosages d'enzymes et d'oligoéléments) et thérapeutique (disopyramide et propranolol). L'analyse des tracés électro-cardiographiques montre une légère tachycardie sinusale ; les diagnostics thérapeutiques n'ont pas permis de confirmer l'origine fonctionnelle des troubles cardiaques. Les variations enzymatiques observées (CPK, TGO, LDH) associées à des subcarences en oligoéléments (Cu - Zn - Se) font penser à une cardiomyopathie.

En dehors d'une malformation congénitale, la tachycardie du veau à la naissance ne paraît pas s'accompagner de troubles cardiaques majeurs.

SUMMARY

Neonatal, bovine tachycardia finds clinical expression as polypnea. In order to determine whether the disorder is of functional origin (hypercatecholaminergic - immaturity of the nodal tissue) or due to a lesion (congenital malformations - cardiomyopathy caused by deficiency), 30 sick calves and 30 control calves underwent clinical follow-up (electrocardiographic monitoring) and paraclinical follow- up (assays of enzymes and trace elements) as well as receiving medication (disopyramide and propranolol). The ECG traces revealed slight sinus tachycardia ; clinical diagnoses did not confirm the functional origin of these heart disorders. Changes in the enzyme levels observed (CK, SGOT, LDH) associated with trace element deficiencies (Cu - Zn - Se) suggest the possibility of cardiomyopathy.
In the absence of congenital malformation, tachycardia of the newborn calf does not appear to be accompanied by any major cardiac disorders.

RESUMIR

Las taquicardias neonatales bovinas se manifestan clínicamente por polipnea. Con el fin de precisar el origen funcional (hypercatecolaminergia-inmadurez del tejido nodal carencial) se sometieron 30 terneros enfermos y 30 terneros testigo a un control clínico (evolucion electrocardiográfica), paraclínico (evaluacion de enzimas y de oligoelementos) y terapéutico (disopiramide y propanolol). El análisis de los trazados electrocardigráficos muestra una ligera taquicardia sinusal ; los diagnosticos terapéuticos no permitieron confirmar el origen functional de los trastornos cardiacos. Las variaciones enzimáticas observades (CK, TGO, LDH) combinadas con subcarencias de oligoelementos (CU - Zn - Se) hacen pensar que se trata de una cardiomiopatia.
Con excepcion de una malformacion congenital, la taquicardia del ternero al nacer no parece que vaya acompanada con trastornos cardiacos mayores.

TREATMENT OF INFECTIOUS DIARRHOEA IN CALVES USING THE LACTOPEROXIDASE SYSTEM AND LACTOFERRIN

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INTRODUCTION

An entirely new therapeutic approach to neonatal calf diarrhoea (scouring) has now been developed; it is based on the antibacterial activity of the lactoperoxidase system (LP-s) and lactoferrin (LF) (5). LF and lactoperoxidase (LP) occur naturally in bovine thermically non-treated milk and belong to the non-immunoglobulin protective proteins in milk. In vitro the LP-s, consisting of LP, thiocyanate ion and hydrogen peroxide temporarily inhibits lactic acid bacteria but kills and eventually lyses Gram-negative organisms such as E.coli. In both instances the metabolic and synthesizing activity is inhibited within minutes of exposure to the LP-s (1,5). LF, naturally occuring in important quantities in human milk, was proved to inhibit temporarily the growth of E.coli in the medium. A temporary suppression of the growth of E.coli by LF in vitro has also been confirmed (5).

The aim of this study was to test the clinical effectiveness of a preparation produced by Synfina-Oleofina Co. (Brussels, Belgium) under the commercial name Safilac ® (Coopers Co). This contains both LF and LP-s and was used for the therapy of infectious neonatal calf diarrhoea provoked by enterotoxigenic strain of E.coli (ETEC - Study I) and mixed intestinal infection (Study II), including Cryptosporidia spp., rotavirus and ETEC.

MATERIALS AND METHODS

Experimental animals and their management

47 Colostrum fed dairy calves were purchased from different, ETEC-and Salmonella spp. - free farms in Belgium. No vaccination against calf gastrointeatinal pathogens was performed on the farms. The calves belonging to two different age groups were subdivided at random into experimental and control groups after their arrival at the experimental centre (Table 1). The calves were housed in individual pens and fed milk, reconstitued from a commercial lactoreplacer (Cremo-elevage, Sanders Co) twice a day. Recommended high standards of environmental hygiene and nutrition were maintained throughout the experimental period (7).

Infection of calves

23/30 Calves of study I and 13/17 calves of study II were given per os 10ml of suspension containing 15 x 10^{10} colony forming units of ETEC, strain 510, at the beginning of experiments (Table 1). The strain (0101: K30, K99, F5, F41, non - mobile) had been proven to produce the heat stable enterotoxin STa (2) and to provoke severe diarrhoea in neonatal calves under experimental conditions (8). This strain had been also found to be

sensitive to LP-s and LF in vitro (1). Culture of the strain grown on BHI agar (Difco Co) for 18 hours at 37°C and washed off with 0.9% physiologic saline solution (pH 6.8) was used for the infestation of the calves.

Treatment

Calves of the treatment groups (Table 1) were given 10g of a preparation (Safilac®) containing LF (35mg), constituents of LP-s (19.3mg of LP, 19.3 mg of thiocyanate, 1.55mg of glucose oxidase, 1g of glucose) and a vehiculum (9.9g of lactoserum) for each litre of ingested milk since appearance of diarrhoea. The treatment was continued for six consecutive days without any modification of environmental parameters or nutrition.

Clinical procedures

All calves were examined clinically 4-times a day throughout the experimental period. Rectal temperature was measured once a day (morning). The gastrointestinal disorders were rated as follows: Normal faeces (no apparent abnormalities), mild diarrhoea (more frequent emissions of bulky and apparently softer faeces), severe watery diarrhoea (frequent and voluminous emissions of the malodorous watery faeces). The degree of dehydration and clinical depression were evaluated as recommended by Sherman et al (9). The calves were weighed before the beginning of the experiment and then every three days during the experimental period.

Identification of infectious agents

Faecal shedding of four different gastrointestinal pathogens of the calf (10) was recorded daily in all the calves. E.coli, strain 510, was identified using three different methods for each sample: ELISA technique, direct immunofluorescence and agglutination. Rotavirus, coronavirus and Cryptosporidia spp. were diagnosed by the ELISA technique.

Evaluation of the immune status of calves

The immune status of calves was estimated by the level of serum immunoglobulins (Ig) using the laser immunonephelometry. Values lower than 8mg of Ig per ml of blood serum were considered to be insufficient and recorded as hypogammaglobulinaemic status (3).

Statistical analysis of results

Chi-square test and one-way analysis of variance were used.

RESULTS

Table 2 summarises the results. ETEC (strain 510) was significantly involved in the precipitation of diarrhoea in calves of study I and Cryptosporidia spp. and rotavirus were the major gastrointestinal pathogens in study II, as confirmed by the faecal shedding of the microorganisms during experiments. A comparison between the results obtained in ETEC infected/treated and ETEC infected/non-treated calves suggests that the treatment with Safilac® significantly reduced the occurence of severe watery diarrhoea (lasting two or more days), the duration of the diarrhoea and the mortality among calves of the first study ($p<0.05$). The clinical status (degree of dehydration and clinical depression) was better in treated than non-treated ETEC infected calves of Study I ($p < 0.05$). In contrast, the treatment did not significantly improve the above mentioned parameters in calves in the second study ($p > 0.05$). Live-body mass was not significantly improved by the treatment in either study ($p > 0.05$).

DISCUSSION

ETEC, rotavirus, coronavirus and Cryptosporidia spp. are considered as important pathogens causing neonatal calf diarrhoea. They induce scouring alone or in various combinations (10). ETEC (Study I) and Cryptosporidia spp. and rotavirus (Study II) were found to be the major gastrointestinal pathogens in this trial. The characteristics of the diarrhoea and the age of calves were also typical for the syndrome (7,9,10,).

Results of study I suggest a preparation containing LP-s and LF to be effective in the treatment of enteric colibacillosis in calves. This is in agreement with data of other studies in calves confirming the bacteriocidal effect of LP-s on abomasal E.coli (5) and clinical efficacy of the treatment using LP-s to manage the experimentally induced enteric colibacillosis (4). There are no available data concerning the use of LF to treat the calf diarrhoea. The relative inefficiency of LP-s and LF against certain protozoal and viral intestinal species could be hypothetically involved in a partial failure to treat the neonatal calf diarrhoea provoked by Cryptosporidia spp. and rotavirus in Study II of this trial. However, no in virtro studies were conducted to confirm the hypothesis.

The protective milk proteins, including LP-s and LF, play an important role in protection of the newborn against intestinal infection. The antibacterial function of the proteins is limited especially to Gram-negative intestinal bacteria including the pathogenic strains (5). In vitro inhibition of Gram-positive intestinal commensals, such as Clostridia and Lactobacilli, by LP-s and LF is significantly less important (1,5). This relatively selective antibacterial action of LP-s and LF could be therapeutically advantageous in improving the disturbed balance between predominantly proteolytic (E.coli) and saccharolytic (Lactobacilli) intestinal microflora, that occurs in certain types of bacterial diarrhoea, such as enteric colibacillosis.

The therapeutical effect of LP-s and LF is similar to that of peroral antibiotic drugs. Certain peroral antibiotics used to treat bacterial diarrhoea in calves are known to induce multiresistance among the intestinal commensals and pathogens. Some of these drugs were also proven to provoke a malabsorption/ diarrhoea syndrome in healthy calves (6). The therapeutic use of the protective milk proteins, including LP-s and LF, can be an interesting alternative the peroral administration of antibiotics. This can be true espically when calves are fed by reconstitued milk, which does not contain any active LP and LF or other non-immunoglobulin and immunoglobulin protective systems of the fresh (thermically untreated) cow milk. Some other biological functions of these proteins (such as the effect of LF on the intestinal absorption of iron (5)) could be important in improving the health of calves, especially in the preventive programmes.

SUMMARY

Lactoperoxidase system and lactoferrin are two non-immunoglobulin protective (antimicrobial) systems of milk of several mammalian species. A product (Safilac" - Coopers) based on the two systems was used to treat neonatal calf diarrhoea produced by enterotoxigenic Escherichia coli (Study I) and coronavirus and Cryptosporidia spp. (Study II). The treatment significantly reduced the prevalence of severe watery diarrhoea (lasting two or more days), the duration of the diarrhoea and the mortality rate among the calves in study I ($p<0.05$), but not in study II ($p > 0.05$). The general clinical status was improved by the treatment in the calves of study I ($p<0.05$). The product is recommended for treatment of enteric colibacillosis in calves.

RÉSUMÉ

Le systéme de la lactoperoxydase et la lactoferrin sont classés parmi les systémes protectifs (antibactériens) non-immunoglobuliniques du lait maternal des multiples espéces mammiféres. Un produit (Safilac" - Coopers) basé sur les deux systémes fut cliniquement testé, sous des conditions contrôlées, pour le traitement d'une diarrhée néonatale du veau produit par une souche entérotoxigénique d'Escherichia coli (1'étude I) et par des rotavirus et Cryptosporidia sp. (1'étude II). Le traitement a réduit significativement la prévalence des diarrhées liquides séveres (d'une durée de ≥ 2 jours), la durée de la diarrhée et la mortalité des veaux de 1'étude I ($p < 0.05$), mais pas dans le cas de 1'étude II ($p > 0.05$). L'état clinique significativement amelioré par le traitement dans 1'étude I ($p < 0.05$). Le produit est récommandé pour le traitement de la diarrhée du veau provoquée par des Escherichia coli entérotoxigeniques.

SUMARIO

Lactoperoxidasa y lactoferrina constituyen dos sistemas de proteccion antimicrobiana que no pertenecen al grupo de los proteinos non-immunoglobulinos, presentes en la leche de muchas especies de mamiferos. En este trabajo se utilizo un producto (Safilac" - Coopers) cuya formulacion se basa en los dos sistemas mencionados, en el tratamiento de la diarrea neonatal de los terneros, producida por Escherichia coli (Experimento I) y Coronavirus y Cryptosporidia (Experimento II). En los aninales tratados del Experimento I se pudo apreciar una significativa reduccion de la severa diarrea acuosa que fuera observada por 48 h o mas ($p<0.05$). La duraction de la diarrea y el indice de mortalidad de los terneros del Experimento I fue significativamente menor ($p <0.05$), pero lo mismo no ocurrio con los animales del Experimento II ($p>0.05$). La condicion general de los animales pertenecientes al Experimento I mejoro con el tratamiento ($p<0.05$). El producto es recomendado para el tratamiento de colibacilosis entericas en terneros.

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Table 1: Characterization of experimental calves and their distribution into groups

	Study I			Study II		
	enteric colibacillosis			mixed infectious diarrhoea		
Age of calves at the beginning of experiment	8 - 36 hours			7 days		
Duration of experiment (days)	6			21		
Experimental groups	infected ETEC/ treated	infected ETEC/non-treated	non-infected ETEC/non-treated	infected ETEC/ treated	infected ETEC/ non-treated	non-infected ETEC/ non-treated
n of calves	12	11	7	9	4	4
Distribution of immunodeficient calves n/ % of group	5 (45)	5(42)	0(0)	4(44)	2(50)	2(50)

Table 2: Evaluation of clinical status and mortality rate in calves

	Study I enteric colibacillosis			Study II mixed infectious diarrhoea		
Experimental group	infected ETEC treated	infected ETEC non-treated	non-infected ETEC non-treated	infected ETEC treated	infected ETEC non-treated	non-infected ETEC non-treated
Prevalence* of -diarrhoea -severe watery diarrhoea n of calves (% of group)	12 (100) 1 (8)	10 (91) 5 (45)	3 (43) 0 (0)	8 (89) 3 (33)	3 (75) 1 (25)	4 (100) 3 (75)
Duration of - mild & severe watery diarrhoea - severe watery diarrhoea days x ± SD	1.8 ± 1.3 0.4 ± 1.3	2.6 ± 1.7 1.6 ± 1.3	0.4 ± 0.5 0 ± 0	8.6 ± 2.3 2.3 ± 1.0	12.0 ± 6.8 5.5 ± 4.5	9.0 ± 6.0 4.0 ± 2.2
General clinical deterioration** n of calves (% of group)	1 (8)	8 (73)	0 (0)	2 (22)	0 (0)	2 (50)
Live-body mass changes per calf during the total experimental period (kg) x ± SD	-0.7 ± 1.6	-1.1 ± 2.6	+0.5 ± 1.0	+9.0 ± 2.6	+8.3 ± 3.4	+7.5 ± 1.5
Mortality rate n of calves (% of group)	1 (8)	4 (36)	0 (0)	3 (33)	0 (0)	2 (50)

* diarrhoea = number of calves showing diarrhoea during ≥ 1 day
severe diarrhoea = number of calves showing severe watery diarrhoea during 2 or more consecutive days

** severe watery diarrhoea accompanied by mild to severe dehydration and depression, mortal cases.

DEVELOPMENT OF A COMPUTERISED MULTIFUNCTIONAL HERD CONTROL SYSTEM

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INTRODUCTION

Dairy herd management and health programmes should improve production efficiency and animal health. In many countries computerised herd management and health programmes are used. In West Germany some efforts have been made to introduce the VAMP-programme *(Noordhuizen and Baumann, 1984)* and a small version of the PANACEA-programme *(Pflug and James, 1989)* in veterinary practice.
The objective of this paper is to present a computerised herd control and information system, which is developed for Bavarian dairy herds. The average herd size in Bavaria averages to 22 cows and therefore the programmes must be adapted to such a situation.

MATERIALS AND METHODS

- *Data recording*

A pilot project for 28 dairy herds was started in March 1988, to introduce recording of disease data. After the first year, a herd health and management programme was implemented. The programmes are of modular structure and are running on a mainframe or personal computer. As programming language and data analysis system SAS is used. An essential tool in a computerised management and health system is an integrated data recording system to increase acceptance of the programme and to improve data quality. Therefore all data, which seem useful for a herd control programme and which are already collected by the official milk recording organisation and A.I.-stations in Bavaria, can be transfered and used in the programme.
The basic information for cows is provided by the official milk recording organisation. Data exchange can be performed on-line or by disc using a standardized interface. In particularly, following data are transfered automatically on a weekly basis: herd and cow identity, breed of cow, sire and mother of the cow, birth date, calving date, calving performance, calf viability, date of milk recording and milk yield, fat content, protein content and somatic cell counts, date of culling and culling reason. Pedigree data up to six generations, 305-day milk records and breeding values are updated each year. Fertility data are also stored in an EDP system by the official milk recording organisation (MRO), but due to the rather slow data flow from the inseminator to the data base updating can be performed only on a two to three monthly basis. An accelerated data flow can be achieved if the traditional organisation of data storing and processing at A.I.-stations can be changed.
At present time data on insemination, heat detection, drying off, calving and calf health must be additionally recorded by the farmer on a worksheet on a weekly basis and are stored in the data base for the herd management and health programme. Data registered by the farmer are tested for consistency and, if necessary, are completed by the data of MRO or in the case of inconsistencies a list for data correction is produced by the computer programme.

Veterinary data are recorded by the practitioner on a special form, because on farm data input is not possible at the farms involved in this project and seems too time consuming in a dairy practice with a lot of small farms, which are not participating in a herd health and management programme. The most common disease entities are listed on the form, but the veterinary practitioner can also give a more detailed description of the disease. Symptoms, therapy and results of specialised examinations like metabolic parameters, infectious pathogens, can be also entered in the data base.
Economic and feeding data as well as a basic description of the management, housing and economic situation of the farm can be processed by the developed programme. For the extension service in feeding a ration calculation programme installed on a portable computer (Epson HX-20) can be used by the veterinary practitioner. If more severe or longer lasting feeding problems exist in a dairy herd, the official agricultural extension service and the veterinary practitioner solve the feeding problems in cooperation.

- *Information retrieval*

In herd management and health programmes information can be presented in following ways:
- action lists, which remember the farmer and veterinary practitioner on certain examinations in animals
- survey or index lists for animals, herds, seasonal trends or special events in order to visualise by simple statistics and an efficient data presentation the actual status and comparisons for selected groups
- diagnostic lists for animals, herds, seasons, regions or other groups in order to analyse for example the importance of diseases on milk production and insemination success or to analyse factors which might be responsible for low production, low insemination success or low production efficiency
- planning and optimization programmes in order to use results of data analysis to draw conclusions in herd management and health for the future.

Based on weekly intervals action lists are produced by the programme. In figure 1 and 2 examples of the action list for the veterinary practitioner and farmer, respectively, are displayed. The programme-output for the veterinary practitioner and farmer can be separated in 4 parts:
(1) animals for veterinary check (vet. practitioner) or management control (farmer)
(2) animals which will calve within one week
  - with reported diseases in previous lactation
  - without reported diseases in previous lactation
(3) animals affected by diseases in the last 8 to 14 days
(4) animals with an indication of energy or protein imbalance

The preconditions for selecting animals in the programmes can be changed according to the demands of the veterinary practitioner or farmer. The most important preconditions are listed in following:

calf	- abnormal birth type, but calf is still alive
	- newborn diseases of the calf
	- vaccination of calves/mothers, if a herd problem exists for specific infectious pathogens
	- selection of calves as breeding or fattening animals
heifers/cows	- abnormal birth type or stillbirth
	- abortion
	- check for overdue
	- regular veterinary check of reproductive system and udder 8-12 days p.p.
	- heifers having no heat reported until an age of 15 months
	- cows having no heat reported until 35-40 days after calving/abortion
	- heifers and cows showing abnormal heat symptoms
	- heifers and cows showing abnormal types of estrus cycles

- heifers having no insemination/mating reported until an age of 18 months
- cows having no insemination/mating reported until 80 days p.p.
- heifers/cows to be examined for pregnancy
- heifers/cows to be checked for estrus after insemination/mating
- heifers/cows to be examined due to infertility (repeat breeders, more than 140 days open)
- heifers/cows which were affected by more than two severe diseases
- heifers/cows for veterinary check a.p. (reproductive system, udder)
- cows to be dried off.

Survey lists are provided for animals, herds, seasonal trends or other groups which can be freely selected. Some examples are given in figures 3 to 6. For each animal a cow card showing all or only the desired (milk production, fertility, breeding values, disease histories, udder health, energy/protein balance, economic value) information can be listed. Also animals of certain or all herds can be selected and summarized according to the desired criteria as e.g. milk production, fertility status and history, disease status and history. Milk production can be characterized by milk yield, fat-%, protein-%, fat-yield, protein-yield as predicted or realised 100-day-, 305-day-production or as milk production until the actual date or as daily milk production with or without correction for days open. A.I.-parameters preferentially used in this programme are days to first heat/insemination, days open, days between first insemination and conception, insemination/conception rate after 90/120/150 days p.p., problem cows (not conceived after 140 days p.p.), average days between two estrus cycles. Disease frequencies can be grouped according to disease complexes or chronically primary diseases. Disease frequencies can be computed as by feeding days weighted averages or unweighted averages. Incidence rates of diseases can be defined as lactational (percent cows diseased per lactation) or monthly/seasonal (percent cows/animals diseased in relation to total number of cows/animals producing in this month/season) frequencies. An example for selecting problem cows is given in figure 3. Herd and seasonal trends for A.I.-parameters are shown in figure 4 and 5. Grouping of cows according to primary diseases and the resulting days open are illustrated in figure 6. The data presented can be corrected for systematic influences like herd, age of animal, breed of animal, season of calving and other effects. The user is supported by the programme in definition and selecting systematic effects. All figures can be displayed also in tables and can be standardized on mean and standard deviation to allow more objective comparisons among parameters used.

Diagnostic lists are useful in searching causes for animals or grouped animals, which fail in reproduction or show low production or are severely and/or very often affected by diseases. The programmes list all possible factors ordered by their probability in contributing to infertility, low production or diseases. Another application of these programmes is the analysis of production loss by diseases and of the expected production, fertility and disease frequency. In table 1 the estimation procedures are outlined in general terms. An example for herd fertility is illustrated in figure 7. After the analysis of A.I.-parameters, disease and treatment frequencies as well as treatment efficiency (kind of drugs, application) have to be checked in certain herds by the computer programme.

RESULTS AND DISCUSSION

Preliminary results on the success of the herd management and health programme in terms of A.I.-parameters are shown in table 2. Insemination results are improved significantly applying the herd programme. Seasonal trends in A.I.-parameters are regarded in these figures by using insemination results of herds from the same community with similar milk production level and the same breed.

Herd management programmes seem to be also useful in small sized herds. Data analysis, presentation and recording must be adapted to such a situation. Integrated data recording and analysis are essential tools in a successful approach to improve cost effectiveness in herd management programmes.

Figure 1: Weekly action list for the veterinary practitioner
List of heifers/cows for veterinary check

Running number	Herd	Identity	Lactation	Last management/event	Days p.p.	Action	Symptoms GE SYM KON OVAR CER	UDDER 1 2 3 4
1	111	180 Blanka	1	8/12/89	9	Postpartum control		
2	111	95 Viola	2	15/11/89	36	Heat control		
3	111	109 Babsi	2	2/12/89	19	Lochia-last heat		
4	111	109 Babsi	2	29/11/89	57	Protein deficiency?		
5	111	109 Babsi	2	21/11/89	49	Endometritis II Ubrucellose Lat.		

List of heifers/cows to be dried off or with expected calvings without reported diseases

Running number	Herd	Identity	Breed	Lactation	Last calving	Days open	expected calving	pregnancy duration	ZKZ	Service site	Action
1	111	97 Otto	01	3	15/8/88	211	24/12/89	281	496	Redax 17601	calving

List of heifers/cows affected by diseases in the last 8-14 days

Running number	Herd	Identity	Breed	Lactation	Days p.p.	Calving date	Disease	Actual Milk yield	Fat %	Protein %
1	111	94 Berta	01	4	13	16/9/89	Endometritis III			
2	111	94 Berta	01	4	33	16/9/89	Endometritis I Ovary cyst	15/30	4.00	3.17

Figure 2: Weekly action list for the farmer
Herd: 112 List of heifers/cows for management control

Running number	Identity	Lactation	Last management/event	Days	Animal history	Action	Date for Action	Actual/Previous lactation milk yield	Fat %	Protein %
1	109 Eule	2	15/12/89	8	Calf before 6/10 days	Lochia? Udder?	23/12/89	7221	4.87	3.40
2	164 Erna	5	05/12/89	61	Heat before 17 days	Insemination in 4 days?	26/12/89	1160	4.15	3.30
3	188 Trude	8	28/11/89	75	Insemination before 18	Heat in 2/4 days?	25/12/89	1420	3.74	3.06

Herd: 112 List of heifers/cows to be dried off or with calvings with reported diseases

Running number	Identity	Breed	Lactation	Last calving	Days open	expected calving	pregnancy duration	ZKZ	Service site	Action	Disease
1	147 Wanda	01	3	15/8/88	211	24/12/89	281	496	Redax 17601	calving	Mastitis acuta 24/9/88 Ovary cyst 6/10/88

Figure 3: Selection of problem cows in survey lists
Herd: 115

Running number	Identity	Lactation	Last management event	Days p.p.	Animal history	Actual milk yield	Fat %	Protein %
1	170 Beta	2	14/11/89	197	No insemination until 80 days p.p.	3980	3.73	3.36
2	187 Olla	3	30/8/89	211	Not pregnant/more than 2 A.I.	3980	4.37	4.00
3	192 Elsa	9	16/9/89	13	Endometritis	-	-	-
4	192 Elsa	9	16/9/89	33	Acyclia	1380	4.02	3.20
5	192 Elsa	9	16/9/89	44	Ovary cyst, Endometritis	1840	4.00	3.17

Figure 4 Herd trends for days open and disease frequencies (standardized as mean and standard deviation) in survey lists

Figure 5 Seasonal trends for days to first breeding in survey lists

Figure 6 Days open in dependence of grouped primary diseases

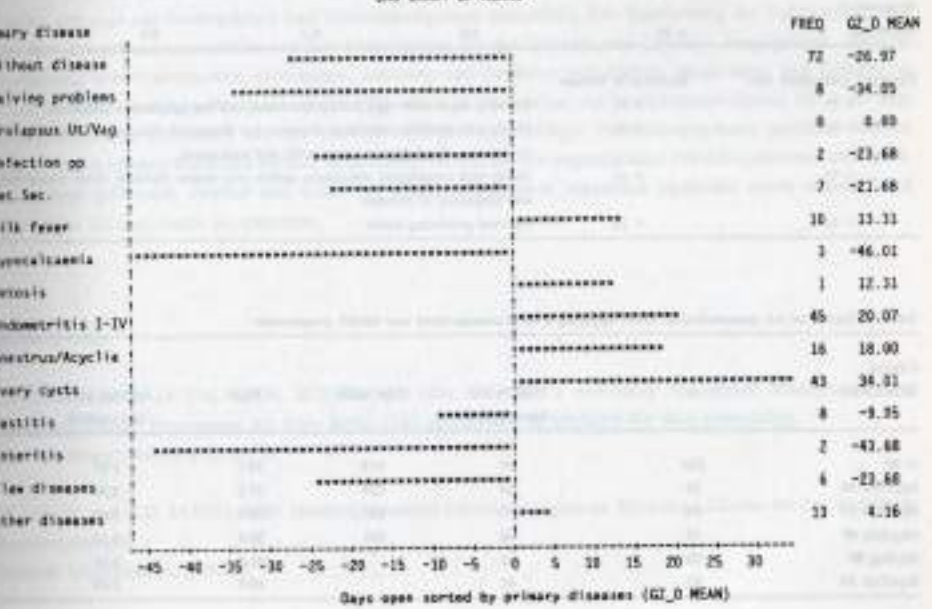

Table 1: Estimation of expected production and production loss by statistical procedures

I. $Y0 = E(Y0) + e = Xb + e$;

II. $Y1 = E(Y1) + e = E(Y0) + E(D) + e$;
Xb = expectations for herd, stage of lactation, genetic factors, ... (= systematic components);
E(D): expected effects of the disease on systematic influences like herd, genetic factors, ...;
Y0 : production record of a healthy cow;
Y1 : production record of a cow being diseased;
E(.) : expectation for (.); e : error component;

III. Reaction to disease
$= E(Y0) - Y1 = E(Y0) - E(Y0) - E(D) - e = -E(D) - e$;

IV. Disease frequency under production conditions
$Y = E(Y) + e$;
E(Y) expected disease frequency regarding the effects of herds, lactation number, milk yield, size,...;

Figure 7: Analysis of expected production and production loss on herd basis for conception rate

Conception rate		Reaction to disease in standard deviations		
		< 1s	± 1s	> 1s
Expected conception rate in standard deviations	< 1s	0.0	5.8	3.8
	± 1s	6.9	65.4	7.3
	> 1s	4.6	6.2	0.0

Expected conception rate	Reaction to disease		
< 1s	> 1s	:	Problem herds with high production losses and low production potential
< 1s	< 1s	:	Herds with possible subclinical diseases, not diagnosed diseases or unsufficient treatments; check disease frequencies and treatments
> 1s	> 1s	:	Herds with unsufficient treatments and/or very severe diseases; check treatments and frequency of diseases
> 1s	< 1s	:	Optimal producing herds

Table 2: Trends of A.I.-parameters in herds applying a herd management and health programme

Calving Month/Year	n	Days to first insemination	Days open	CR120	Average lactation number
< 89	239	97	143	33.5	2.87
Jan/Febr 89	94	64	124	37.2	2.90
Mar/Apr 89	84	65	121	42.9	3.07
May/Jun 89	73	66	101	35.6	3.58
Jul/Aug 89	98	62	*	41.8	3.41
Sept/Oct 89	92	61	*	40.9	2.65

* Data not yet complete; CR120: Conception rate until 120 days p.p.

SUMMARY

A pilot project, in which 28 dairy herds participate, was started in March 1988. The first step in developing a herd management system is the implementation of an appropriate and efficient data recording system and the organisation of the data flow. Therefore all data, which are already computerised by the milk recording organisation and A.I.-station, are used in this system. The herd management system is based on milk recording, A.I., calving, feeding and economic data. The programmes are of modular structure and are running on a mainframe or PC. As programming language and data analysis system SAS is used. Based on weekly intervals action lists are provided for the veterinary practitioner and the farmer in order to optimize reproduction, health status of cows, heifers and calves, culling and replacing of cows by superior heifers and milk production of cows in a quota system. A main component of information retrieval in this programme is the analysis of production loss. A procedure was developed by which the production loss caused by genetic and environmental factors can be estimated. This procedure enables to detect herds or cows which produce inefficiently and/or respond poorly to veterinary treatments.

ZUSAMMENFASSUNG

In einem Pilotprojekt mit 28 Milchviehherden wurde ein Herdenbetreuungsprogramm durchgeführt. Die Datenerhebung wurde so aufgebaut, daß alle bereits vom LKV Bayern e.V. und den Besamungsstationen erfaßten Daten, soweit wie möglich, von dem Programm verwendet werden.
Die Datenbasis für das Herdenbetreuungsprogramm stellen die Daten aus der Milchleistung, der Besamung und der Fütterung dar. Zusätzlich werden noch ökonomische Daten erhoben. Die Programme sind von modularer Struktur und sind auf Großrechnern und Personalcomputern ablauffähig. Die Bearbeitung der Daten erfolgt mit SAS. In wöchentlichen Intervallen werden Aktionslisten für den Tierarzt und Landwirt bereitgestellt, um eine Optimierung von Fruchtbarkeit, Gesundheit, Merzung und Selektion von Kühen, Jungrindern und Kälbern zu erreichen. Eine wesentliche Komponente des Programms ist die Analyse von Produktionsverlusten. Es wurde eine spezielle Prozedur entwickelt, mit der umwelt- und genetischbedingte Produktionsverluste geschätzt werden können. Damit können Kühe und Herden identifiziert werden, die ein ungenügendes Produktionsniveau aufweisen. Die Analyse hilft auch, Herden und Kühe mit subklinischen, nicht erkannten klinischen sowie unzureichend behandelten Erkrankungen zu erkennen.

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THE APPLICATION OF PLANNED ANIMAL HEALTH AND PRODUCTION TO DAIRY FARMS : DAISY - THE DAIRY INFORMATION SYSTEM

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INTRODUCTION

It is estimated that in the UK, on the 100,000 full-time farms, there are nearly 10,000 microcomputers in use. Approximately 2000 of these farms have a dairy recording program on a computer incorporated into the milking parlour or in the office.

The development of dairy recording systems on microcomputers has been taking place for over ten years. The system described (DAISY - The Dairy Information System) was developed from a mainframe herd health system, in use in 1968 at the University of Melbourne, and developed at the University of Reading (UK) from 1972 onwards. DAISY accepts any type of individual cow record (identity, life events, health, fertility, milk yield and quality etc.,). No records are lost and all entries are thoroughly validated. The user chooses how much detail to enter.

The output is available in a range of sizes of printout and on screen, covering action lists, feed lists, analysis of health and fertility, recording forms and integrated management reports.

If the records are supplied via ASCII files, the system can integrate with a National Milk Recording system via floppy diskettes. Information automatically recorded in the milking parlour can be accepted directly into DAISY and instructions can be computed for operating feeders. The cow data can be transferred into a database to create inter-farm comparative data, league tables and a database for research purposes, and can be used to develop indices and parameters to help predict performance and allow farmers to take cost-effective preventive measures.

The lack of integrated systems from the central sector meant that in the early days many farmers were willing to use software that had not been properly tested. The software available today is likely to be chosen on the basis of whether it will make the farmer more efficient and profitable. (Nowell,1978). While suppliers of centralised services concentrate on the average requirement, there will be a small specialised market sector looking for highly developed information systems. This may only constitute some 2.5% of the farmers, but these farms may be larger than average and responsible for 7.5 to 10% of the milk produced.

The affect of computers can only be felt if the farmer makes and applies a good decision based on improved

information (Carmi 1987).

In the UK, over the last ten years, the price of on-farm computers has dropped twentyfold in real terms. In general, up to this year, dairy profits have been fairly static so farmers have not had extra income for dairy automation or computerisation.

Gradually, in the UK, the centralised services from the milk recording agency have developed with the inclusion of a Viewdata (telephone line and TV) service and the addition to the milk records of a limited amount of health and fertility data.

Computers are only a tool and interpretation of reports are essential. Advice may be supplied by extension agents visiting the farm, so long as they understand the program, the principles of economic animal production, and providing quality data is entered. Work is available for local bureau services where advice is part of the scheme, and a new type of facility is arising where travelling specialists offer a service, achieving and maintaining proper levels of data entry and report production on the farmers' own computer.

THE REQUIREMENTS OF A DAIRY INFORMATION SYSTEM - A SUMMARY

Simple method to start up a herd on the system
Data validation and error checking of records
Data can be entered in any order from many sources
All types of record to be kept for cows and youngstock
Ease of editing records
No record, once entered, to be overwritten or discarded
Speed of response and operation to match the users need
Quality of presentation of output
Any coding system ; alpha not numeric, extendible to suit the user without spoiling the integrity of the records kept

ORIGINS OF DAISY THE DAIRY INFORMATION SYSTEM

DAISY is designed for computers with a hard disk and needs 7 MB of space including the operating system, and up to 3 MB for work areas depending on herd size.

The original DAISY program was written for dairy cows with Youngtock and herd costings being added later. One of the reasons for the programs' survival is its link with farmers and veterinarians, and its flexibilty and extendability.

In 1981, the opportunity was taken to devise a completely new system that was useful at all levels, infinitely extendible, validated by the program and that provided a way for commonality of coding across DAISY users.

DAISY now operates at over 140 sites in 33 countries around the world. The program is used for all sizes of herds from small-holders with three or four cows to farms

with 5000 cows. Users include farmers; veterinarians and consultants running bureau services; education establishments using DAISY for teaching, as well as research farms and pharmaceutical companies recording trial data. In some countries the program is used on state farms, and in some developing countries DAISY is used as a basis for the national milk recording (NMR) system.

Other dairy programs which have been developed include those from Cornell (Rasmussen 1986), Minnesota (Williamson 1988), Michigan (Mather & Bartlett 1984), Saskatoon (Radostits and Blood 1985) and California (Rywater and Goodger 1984).

STRUCTURE OF DAISY, COW RECORDS PROGRAM

DAISY has been designed in a modular form (Fig 1). The central component is Dairy Cow Records. Milk Yield Manager calculates individual cow concentrate feed requirements. The Youngstock program can be linked to the DAISY Cow Records, or can be indepent to manage the records of male or castrated calves. Two other programs run independently, the ration calculation and herd costings programs.

1) Data Management

a) Herd Identity

A new herd is started by entering a herd number, and the farmer's name and address. The user chooses how to record cow identity from one of four ways; either all alpha, all numeric, alpha-numeric with four numbers preceded or followed by a letter.

b) Cow Data Entry

Once the cow identity, current lactation number and calving date have been entered, further data can be added. The user enters the cow number, the reason the cow has been recorded and the date. Reasons cover all the events in the cow's life and are classified in two categories. The first type is the system-defined Reasons that include Calving (CA), Heats (HE), Services (SE), Pregnancy Diagnosis (PD) and Drying Off (DO). The other Reasons are the ones added by the user or by the DAISY group to suit the purpose of the site involved, and can include such diseases as Mastitis, Lameness, Vulval Discharge and Milk Fever. When a Reason is recorded, any additional information to do with the Reason can be added. These are classified in DAISY as Findings (F), Diagnoses (D) and Treatments (T) (Fig 2).

DAISY will operate with a minimum of information and under a Calving only the elements marked with a * are essential (Fig 3). Data validation is incorporated checking cow numbers, reasons and grouping. Taking calving as an example, the program automatically checks that the cow was recorded in calf, had been dried off, and is not calving more than ten days early.

Comments (C) and Errors (E) are incorporated into the data entry routine and appropriate messages appear on the screen. A Comment can be overwritten, but an Error means

that alteration is necessary to this particular data entry, or another part of the cows record.

A Reason can be up to four alpha-numeric characters long. Up to five Events can be entered. When an Event is entered the code list checks for its presence and the full description is given under "Description" (Fig 3). When entering data there is a facility to "View" the cows whole record for this lactation.

Having entered a calving, calf details can be included, covering identity, sex, breed, weight and fate.

Data can be entered in any order and hence taken from a single or several recording books kept on the farm, a log being printed by DAISY of all data entered.

It is possible to transfer records automatically to another herd on DAISY, which is often necessary where one owner operates several herds.

DAISY REPORTING SYSTEM

The output from DAISY was designed to suit a wide range of users, and is available in a variety of formats, sizes and contents. The output is always available on the printer and, where appropriate, on the screen.

The reports cover six main areas - Action Lists, Data Review, Performance Analysis, Feeding and Integrated Management Reports, Events Analysis and Recording Forms.

The eleven Action lists are split into three categories; the first set covers the fertility cycle of the cow; the second set has lists of animals needing attention from the herdsman or veterinarian on a time crucial basis, and the third set lists cows that have been "flagged" for a specific purpose - to be culled or for a "revisit".

The Data Review Reports give full or condensed information on each cow, or categories of cows, in the herd, and can be used in the parlour or office.

The Milk Yield Management Reports give a one line summary for each cow, of age, calving date, services, bull used, cases of mastitis and lameness, summarised and last three yield records, and the feed required by each cow. Poorly performing cows can be highlighted by the adviser/manager to help the herdsman alter, for example, the feeding for specific cows.

Performance Analysis reports examine the herd over a period of weeks, months or years. PI looks at the fertility of the herd for up to three years, showing, by month of calving, the main fertility indices. This report allows the advisor to see if the levels of husbandry are being achieved, and to intervene to improve the levels to set the herd back on target.

The other reports give an overview of health, fertility and yield for the year, in bar charts and histograms. Health events can be examined separately or in many combinations, as can production performance.

Further analysis can be run when any of these reports

show under-performance. Bar charts, tables and cumulative sums can be produced for heat detection and conception rates, and can look at the overall herd or groups of cows, bulls used, stockman, days of the week, age of the cow, days since calving etc.

THE SUCCESSFUL APPLICATION OF INTEGRATED DECISION SUPPORT SYSTEMS

The application of this type of information needs a particular type of adviser, who can define the objectives at the outset of the operation, and will have realistic targets to aim for.

The program is in use in 25 leading practices in the UK who operate bureau services for up to 40 herds in each practice. Schemes revolve around raw records kept on the farm in a self duplicating note book, the top copy (containing all the health, fertility and cow events) being sent or taken to the practice on a regular basis. The relevant reports are then returned or picked up.

The output for the veterinarian may differ from that of the farmer, with more detailed action lists and analytical reports, giving the practitioner an overview of the herd performance and trends of fertility on the farm.

As technology improves, the data management system will play a larger part in delivering near-model standards of fertility. Formula health management, knowledge based, expert or decision support systems are arising faster in animal health than in the medical field.

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Fig 1
The Suite of DAISY Programs :

DAISY (including BDS operating system)
Milk Yield Management
Youngstock
Costings
Feed Package
Special Data Entry Facility
Individual Cow Margins

Fig 2
Event Codes - Examples of Types Used in DAISY

Reason	CA	Calving
F	ABTN	Abortion
F	RFM	Retained Foetal Membrane
F	Torn	Torn Vaginal Tissue
D	MF	Milk Fever
T	TR1	Traction Level 1

Fig 3
Example of Data Entry for Calving - A System Defined Code

Cow: 292A* Reason: CA* Date: 20/9/88*

Number of Calves: 1* Group: YD12
Live or Dead: Calf 1: L*
Comments on Calving: (Calved in Dry Cow Yard)
Number of Weeks before Revisiting: 1
Cow Weight: Cow Score:

Events	Type	Code	Description
	F	Diff	Difficult Calving
	T	TR2	Traction Level 2
	F	RFM	Retained Foetal Membrane
	T	UTOC	Utocyl Pessaries

SINTEL : AN INTEGRATED AUTOMATED VETERINARY MANAGEMENT PROGRAM FOR DAIRY FARMS

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INTRODUCTION

The quota system has been applied to the European milk sector since 1984. It has completely changed the economic context for the producers. Their past development scheme was to increase their production. They now have to reduce their production costs. For this reason, the producer is demanding new advice for the management of his herd, integrating health of the cows and optimizing the economic results of the farm.

To face this new demand, the veterinarian has to adapt as well as the total product he offers to the farmers' market (8). He has already turned over his practice from individual medicine to collective herd health programs. The objectives of the new veterinary herd management program, in order to follow the actual economic goals of the farmer, should be not only to increase the reproductive performance of the cattle but also to improve their milk productivity and, in fact, the economic profitability of the farm. The evolution of the herd management program is then to use a whole farm approach more frequently (6,13).

This approach requires knowing of the weak points of the farm in order to adapt the advice, and a flexible and individualized system of data collection and interpretation. An intregrated automated veterinary management program for dairy farms is presented in this paper. This program is implemented by a series of regular visits during which the veterinarian or the team of advisors control the diseases of the animals and continuously monitor the performance of the herd.

BASIC ORGANISATION

This system is designed to support management decision making at two levels : strategic planning and day-to-day or month-to-month decision making.

The first module used for strategic decision is based on the interpretation of the whole farm assessment (7). This assessment is derived from a comprehensive study of the herd, which is regarded as a production system. The system results from the combined effect of four subsystems called "blocks" : economics (profitability and financial results), productvity (production and reproduction performances), diseases (identification of the dominant diseases) and animal breeding (feeding, housing and general management) (fig 1). Each block is characterized by a finite number of variables selected for their relevance and reliability. Analysis of these variables when compared to regional norms shows the weak points of herd management under the given circumstances. Factors which limit the functioning of the herd are ranked in order feasibility and economic profitability. A program for surpervision is then suggested to the farmer for overcoming the weak points detected. This program consists of feeding, housing, management economic advice and systematic intervention

(usually monthly) by the veterinarian. At the end of each year, a second herd assessment is conducted and compared to the first one in order to measure the profitability and the efficiency of the program. The result is used to plan the new management and health program for the following period of reproduction. The whole farm assessment could help the advisor to find the main direction of his action in the farm.

The second module is used to support month-to-month decisions that have to be made by the farmer and the veterinarian during the regular visits. This programIt is designed as a production, economic and health monitoring system based on the use and interpretation of a succession of herd reports and reminders for implemantation. The comparison between two successive reports shows the improvement or the deterioration of the herd situation.

DATA INPUTS

The farmer can enter the data on his own computer and give the floppy to the veterinarian, send his documents by mail or give the data by phone. The data are entered by the secretary at the veterinary clinic. There are four main sources of data : the farm diary, the milk recording systen, the dairy industry and the veterinarian.The program is conceived in the form of unrolling menus and functions on all compatible IBM computers. Some of the data have to be collected once a year, others every ten days or every month, before and during each veterinary visit. In the reproduction sub-routine the data are entered on a daily basis. It is possible, also, to enter the events for each cow. The entry of production data can be made on an individual and on a collective basis. The economic events concern the total milk production for the month, the milk price and the different components which comprise the quality of the milk.

DATA INTERPRETATION

The data outputs have been chosen to help the advisor and the farmer make strategic and tactical decisions. These data are presented in the form of charts and graphs as often as possible (13).

The strategic decisions are taken according to the general assessment of the farm. The weak points of the herd management are pointed out by assigning a grade to each "block" (Fig. 2). This grade is transformed into a percentage of the optimum grade the farm can reach in its particular condition. All the results are presented in a single chart that is easy to read and which can be compared to the previous and following results. In this way, the advisor can see the progress that has been made and the necessary steps to be undertaken for improvement.

The tactical decisions depend on the interpretation of the productivity and economic reports as calculated from observing the overall situation of the farm. The reports reflect the actual situation of the herd in its production and reproductive performances, its mastitis and other main disease status and its economic results. They can be printed as often as the veterinarian wants and for the time periode he has chosen. One of the original aspects of the system is that it provides assistance for interpreting the reports and for clarifying the relationship between the performance and breeding factors. The milk production, the reproduction results, the loss of milk production, the main causes of health disorders and the variation of the milk price are interpreted in this way. The variation of the milk price can be taken as an example. The farmer who wants to

maximize his gross income has to face two problems : 1) producing the greatest amount of milk when the milk price is the highest which depends on his reproduction policy. 2) getting the best price that depends on the quality of his product. The milk price is a combination of a basic price that is higher in Autumn than in Spring and rewarded or penalized according to the quality of the milk (proteic and fat rate, somatic cell count, microbial contamination rate). The computer studies the periods of milk production in relation to the price of milk and the differences between the basic price and the observed price (Fig 3). It then shows which components of the milk quality explain the price differences . The advisor has access to several graphs(such as Figure 3) and can analyse the different results.

For each cow, the individual reports combine information about its reproductive status, milk production performance and its diseases since calving.

The implementation lists are similar to those produced by other computer systems (2,9,12). Two implementation lists are avalaible : The "examination list" (cows due for prebreeding check, due for pregnancy diagnosis, not observed in oestrus, with irregular cycles and cows which have been inseminated too close to the calving), and the "cows for special care" list (cows with an early pregnancy diagnosis, cows in their third heat and cows over the third insemination). The implementation lists are used by the veterinarian to prepare his regular visits.

Moreover, the veterinarian can calculate the results and perform statitical analysis on all the farms in his practice to which the program has been applied. The presentation of these annual results increases the farmers' interest in the integrated program and improves the image of the practitionner (6,12).

CONCLUSION

The objective of the program is not only to optimise the health status of the herd but to optimise the economic return to the farmer (1). The system described above pernits the veterinarian to achieve a classical herd health program but also to monitor the economic and production results of the farm (3,4). In addition, it can measure the cost-effectiveness of the veterinary program.

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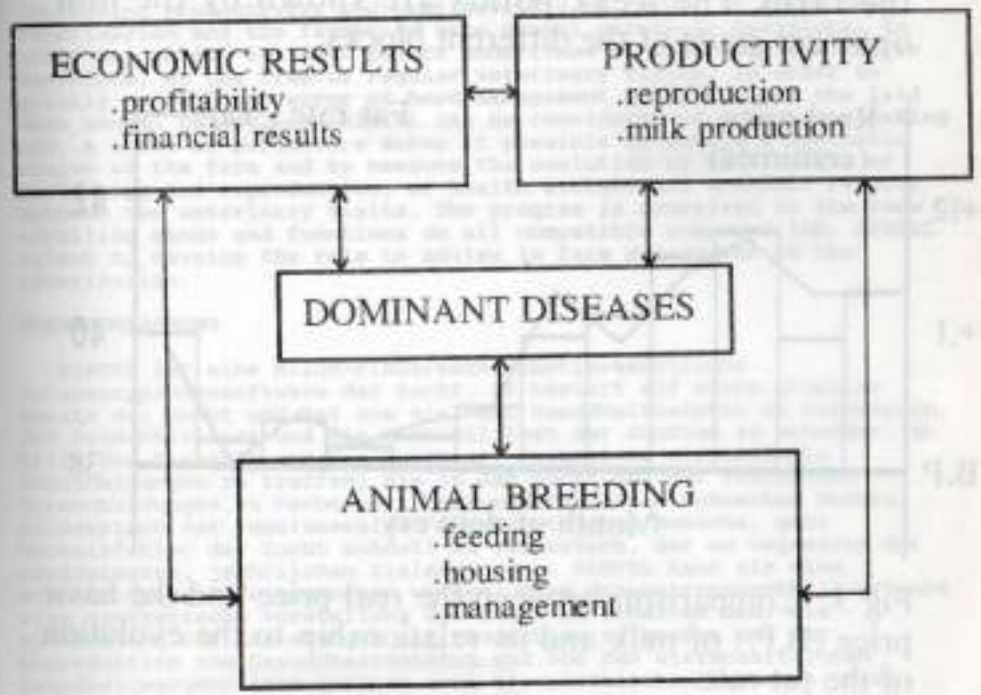

Fig 1 : the organisation of the farm in four "blocks".

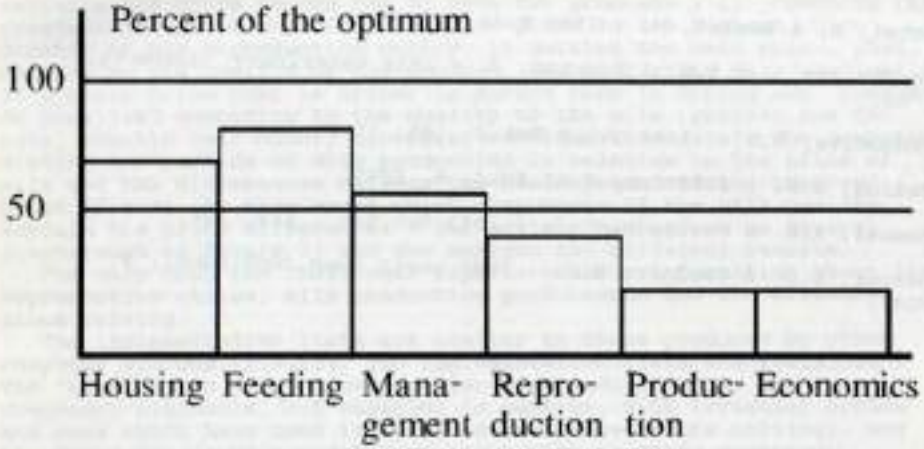

Fig 2 : This chart represents the general assessment of the farm. The weak points are shown by the non-optimum grade of the different blocks.

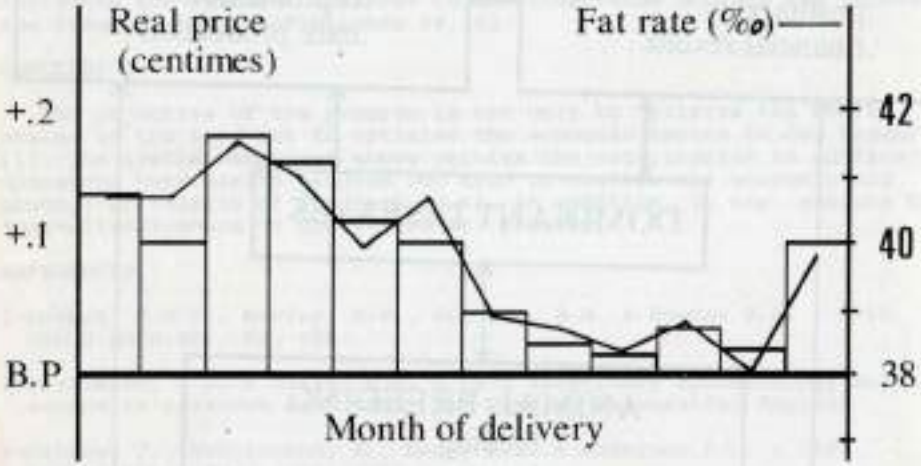

Fig 3 : Comparison between the real price and the basic price (B.P.) of milk and its relationship to the evolution of the fat rate.

RESUME

SINTEL est un logiciel de suivi vétérinaire d'élevage bovin laitier. Il repose sur une approche globale de l'élevage et a pour objectif d'améliorer le statut sanitaire, d'augmenter la productivité et la rentabilité des élevages. Il aide le vétérinaire et l'éleveur à prendre des décisions stratégiques annuelles, afin de corriger les points faibles mis en évidence dans l'élevage, et des décisions tactiques, lors de visites vétérinaires régulières, afin de corriger rapidement toute erreur de technique d'élevage allant à l'encontre des objectifs annuels fixés. SINTEL peut être considéré comme une aide à la décision. Un ensemble de paramètres permet d'obtenir une vision synthétique de l'élevage et de mesurer l'évolution des performances de production et de reproduction, du statut sanitaire et des résultats économiques entre deux visites vétérinaires. Le programme est conçu sous la forme de menus déroulants et fonctionne sur tous les ordinateurs compatibles IBM. SINTEL permet de développer le rôle de conseiller en élevage du vétérinaire.

SUMMARY

SINTEL is a veterinary dairy herd management program. It rests on a whole farm approach and it aims at improving the health statute and increasing productivity and profitability of farms. It helps the veterinarian and the farmer to make annual strategic decisions, in order to correct the weak points underlined in the farm, and tactic decisions, at the time of regular veterinary visits, in order to quickly correct any error of herd management going against the laid down annual objectives. SINTEL can be considered as a decision-making aid. A whole of parameters makes it possible to obtain a synthetic vision of the farm and to measure the evolution of the results of production and reproduction, of health statute and economic results between two veterinary visits. The program is conceived in the form of unrolling menus and functions on all compatible computer IBM. SINTEL allows to develop the role to advise in farm management of the veterinarian.

ZUSAMMENFASSUNG

SINTEL ist eine Milch-rinderaehnlichetieraerztliche Folgemassnahmensoftware der Zucht. Er basiert auf einem globalen Ansatz der Zucht und hat zum ziel das Gesudheitsstatut zu verbessern, die Produktivitaet und die Rentabilitaet der Zuchten zu erhoehen. Er hilft dem Tierarzt und dem Zuechter, jaehrliche strategische Entscheidungen zu treffen, die in der Zucht und der taktischen Entescheidungen zu verbessern herausgestrichenen schwachen Punkte, anlaesslich der regelmaessigen tieraerztlichen Besuche, ganz Technikfehler der Zucht schnell zu verbessern, der an Gegensatz der festgelegten, jarhrlichen Zielserzungen. SINTEL kann als eine Entscheidungshilfe angesehen werden. Eine Parametergesamtheit erlaubt eine synthetische Vorstellung der Zucht zu erhalten und, die Entwicklung der Leristungen der Produktion zu messen und der Reproduktion vom Gesundheitsstatut und von den wirtschaftlichen Gesudheitsergebnissen zwichen zwei tieraerztlichen Besuchen. Das Program in der Form der Menues stattfindenden geplanter Osten und Punktioniert auf allen kompatiblen Rechnern IBM. SINTEL erlaubt, die Rolle zu entwickeln, in Zucht des Tierarztes zu beraten.

XVI CONGRESSO MUNDIAL DE BUIATRIA
VI CONGRESSO LATINO AMERICANO DE BUIATRIA

TOMO I