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YPOLOGIE DE L'ELEVAGE BOVIN EN AFRIQUE INTER TROPICALE : PRODUCTIVITE ET PATHOLOGIE.

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NTRODUCTION

Avec un cheptel de plus de 140 Millions de têtes de bovins (7), l'Afrique intertropicale est assurément une région d'élevage potentiellement prospère, une région où l'élevage est, au-delà d'une source de richesse, une activité importante surtout dans les zones sub-humides du Sahel et des savanes arborées des plateaux de l'Afrique centrale et orientale. Dans ces régions, en effet, l'activité pastorale contribue :

1.à la correction du déficit de la couverture des besoins en protéines animales des populations ;

2.à la fourniture de la traction animale comme mode de transport ou de façons culturales dans les systèmes d'exploitation agricole ;

3.à la valorisation du milieu dans des zones "interdites" aux cultures parce que peu fertiles.

Mais l'examen des traits principaux de l'élevage traditionnel fait apparaître rapidement son caractère principal, son faible niveau de productivité, lequel est lié à la structure même de cet élevage.

LES TRAITS PRINCIPAUX DE L'ELEVAGE BOVIN TRADITIONNEL

Les traits principaux de l'élevage bovin peuvent être identifiés avec des paramètres simples à analyser : la fertilité, la composition du troupeau, la mortalité des jeunes.

S'agissant de la fertilité, on retiendra d'abord un âge élevé au premier velage (4½ à 6 ans) en liaison avec la nécessité pour la génisse d'atteindre le poids adulte (180 à 220 kg) auquel elle pourra être fécondée. En effet, le gain moyen quotidien ne dépasse guère les 350 g en période d'abondance et les animaux subissent des pertes de 20 à 30 % à chaque saison sèche de sorte que la moyenne annuelle du GMQ se situe autour des 100 g. Par ailleurs, l'intervalle entre velages est très long (en moyenne de 20 mois) en raison du sevrage tardif et du cycle obligé des fécondations. La vache arrive alors à l'âge de réforme (10-12 ans) après avoir fait seulement 3 ou 4 veaux (10).

La composition du troupeau aggrave le déficit de fertilité car seulement 25 % de l'effectif est constitué par des femelles en production, les autres étant composés d'animaux improductifs (40 % de mâles et surtout 35 % de femelles n'ayant pas encore atteint l'âge (ou le poids) au premier velage. Il y a là une charge non productive incompatible avec la réduction croissante du disponible fourrager.

A ces deux paramètres s'ajoute un troisième, le taux de mortalité des jeunes qui se situe entre 25 % et 45 % chez les veaux de moins d'un an alors que ce taux est de 1 à 2 % dans les pays européens (4, 8). Ces très fortes mortalités sont liées pour beaucoup à un poids à la naissance trop faible (12 à 16 kg), à un niveau de lactation insuffisant des mères (1 à 3 L de lait/j) et à une pression parasitaire et infectieuse mal maîtrisée ; 10 à 20 % de mortalités sont dues au parasitisme gastro-intestinal (6).

Au total, les facteurs fort complexes de l'infécondité aggravés par l'importante mortalité des jeunes et la composition aberrante du troupeau traduisent le faible niveau de productivité numérique du cheptel bovin en Afrique. Mais au-delà de ce constat, il faut en identifier les causes profondes dans la tétralogie que constituent les hommes, le bétail, le milieu et les moyens.

Y-a-t-il insuffisance du savoir-faire des hommes ? On a souvent dit et à juste titre que "le paysan ou l'éleveur africain n'est ni un paresseux ni un imbécile car, compte tenu des moyens dont il dispose, il ne paraît guère le plus souvent de faire beaucoup mieux". Peu d'éleveurs dans le monde sauraient faire aussi bien que les Massaïs et les Peulhs dans les conditions difficiles de l'élevage africain. Ils ont en tous cas une bonne connaissance des animaux et des ressources des parcours. Mais la bonne gestion du troupeau implique une indépendance du système de production vis-à-vis du disponible fourrager hélas lié au cycle des saisons et de la végétation.

Faut-il conclure à l'insuffisance des races ? Il n'y a pas de bonnes ou de mauvaises races dès lors qu'on se place dans un système peu éloigné de la cueillette. Une race est ou n'est pas adaptée aux conditions du milieu. En Afrique, les races sont rustiques et une sélection naturelle a engendré des races adaptées à différents types écologiques comme par exemple les petites races trypanotolérantes des régions humides. De nombreuses études ont d'ailleurs montré que les races africaines peuvent être performantes quand la gestion du troupeau est améliorée (1, 5).

Tout semble donc tourner autour du milieu c'est-à-dire les conditions dans lesquelles est placé le bétail africain. L'élevage se fait en général dans des zones sub-humides ou semi-arides, des zones la plupart du temps non favorable aux cultures et où la nature peu clémente offre un disponible fourrager souvent réduit. Mais il serait trop facile de s'en remettre à la nature car les moyens utilisés ne sont pas toujours à la hauteur des exigences de cette nature. Ces moyens varient selon le type d'éleveur, le système et le mode d'élevage... en un mot, de la structure de l'élevage.

LA STRUCTURE DE L'ELEVAGE BOVIN

Les types d'éleveurs

Les éleveurs et agro-pasteurs qui gèrent l'élevage africain peuvent être regroupés en 3 catégories (hormis l'Etat et les Sociétés privées).

- La 1ère catégorie est celle que nous qualifierons d'Eleveur-héritier. C'est le type dominant, l'éleveur par tradition de père en fils. Ce sont les pasteurs Peulhs du Sahel et des moyens plateaux de l'Afrique de l'Ouest et du Centre, les Massaïs de l'Afrique Orientale. Si ces éleveurs ont le mérite de connaître leur métier, ils sont aussi les moins perméables au modernisme.
- La 2ème catégorie, c'est l'agriculteur-éleveur ou exploitant agricole qui utilise un système intégré de production avec une diversification des spéculations. Il y a, chez lui, une association agriculture-élevage par une utilisation des sous-produits agricoles pour l'alimentation du bétail.
- La 3ème catégorie est faite d'une nouvelle race d'éleveur spéculateur ou épargnant constitué par les fonctionnaires (actif ou retraité). Il dispose parfois d'une ferme d'élevage ou d'une ferme agricole en système intégré. Souvent, il confiera ses animaux à un homme du métier (le pasteur peulh) qui se chargera de conduire ses animaux souvent avec ceux d'autres fonctionnaires. Dans ce dernier cas, le système de rémunération est très variable, parfois en espèce, souvent en nature par donation des veaux mâles par exemple. Dans tous les cas, le peulh reste tacitement le propriétaire du lait (au détriment du veau qui est ainsi très fragilisé).

A cette 3ème catégorie, on peut rattacher une forme d'élevage qui est une non activité : c'est l'élevage domestique ou "élevage de case", beaucoup plus pratiqué pour les petits ruminants et la volaille. Il n'est pas rare cependant de voir des gens qui achètent 1 ou 2 veaux durant les périodes vulnérables en vue de les engraisser autour des cases pour une future cérémonie coutumière ou simplement pour la revente. C'est souvent le 1er pas pour devenir éleveur-spéculateur.

A chaque type d'éleveur, correspond en général un mode ou un système d'élevage.

Le mode ou le système d'élevage

Le système d'élevage prédominant est un système extensif géré par la 1ère catégorie d'éleveurs. Les modes d'élevage sont :

- soit le nomadisme, déplacements anarchiques où les préoccupations qui s'attachent à l'élevage et aux besoins du troupeau l'emportent sur celles que commande la culture du sol ou l'attachement à un terroir (2) ; ils déplacent leur campement dès que les conditions ne sont plus favorables au bétail. Les risques épidémiologiques de maladies sont aussi grands que le hasard qui préside à ses déplacements ;
- soit la transhumance, déplacements pendulaires d'animaux et de pasteurs aux rythmes de l'exploitation du disponible fourrager. Ici encore, les flux migratoires sont des éléments de dissémination de maladie infectieuse et d'entretien des cycles parasitaires.

Les agro-pasteurs pratiquent un élevage "sédentaire" dans des zones de prairies naturelles.

En tout état de cause, la taille de l'exploitation est fortement corrélée avec le mode d'élevage. C'est ainsi que les grands troupeaux (plus de 50 têtes) sont le fait de nommade et de pasteurs alors que les agro-pasteurs ont de petits et moyens troupeaux (20 à 50 têtes).

Il y a eu de nombreuses tentatives d'élevage intensif en milieu pastoral mais toutes se sont soldées par des échecs. L'intensification de l'élevage n'a marché qu'en aviculture où l'on peut maîtriser l'espace et les intrants.

Où sont donc les facteurs limitants au développement de l'élevage bovin ?

LES CONTRAINTES AU DEVELOPPEMENT

Ce sont des contraintes socio-culturelles et des contraintes techniques.

La force de la tradition

L'élevage est un domaine réservé à certaines peuplades pour leur savoir-faire : les Peulhs et les Massaïs, très attachés aux valeurs socio-culturelles de la tradition au point que de les transformer en contraintes au développement.

Chaque société a ses valeurs, ses propres signes de richesse et de réussite. Pour ces pasteurs, l'élevage est un signe de réussite dans la société beaucoup plus qu'une richesse, et la taille de l'exploitation est positivement corrélée avec le niveau de prestige atteint. L'élevage est une forme de thésaurisation ; les ventes ponctuelles interviennent pour couvrir les dépenses de la famille ou du hameau, soit les dépenses quotidiennes de nourriture, soit les dépenses périodiques des grands moments de la vie (mariage ou dot, baptême, deuil...); on comprend donc les limites à l'organisation des circuits de commercialisation qui peut constituer une dynamique à l'amélioration de la productivité.

Par ailleurs, l'ancrage des habitudes et de la coutume fait que ce monde est difficile à convaincre pour des changements à apporter à ce qui est leur "connaissance". Il faut beaucoup de patience d'autant que les Peulhs et les Massaïs sont des peuples très fiers. La fatalité est souvent utilisée comme recours pour expliquer les épidémies et autres mortalités. Ces contraintes socio-culturelles sont confortées par les aléas du climat.

Le cycle des saisons

La notion de saison a une signification très particulière dans les zones semi-arides et sub-humides de l'Afrique où l'élevage est tributaire du disponible fourrager des pâturages naturels dont le rythme d'abondance et de pénurie varie tout au long de l'année avec le rythme des précipitations. Celles-ci, lorsqu'elles existent (500 à 900 mm d'eau) sont irrégulièrement réparties dans l'année, établissant ainsi des saisons (saison des pluies et saison sèche) dont les conséquences sont nombreuses.

- Conséquences alimentaires : le disponible fourrager se raréfie en fin de saison sèche ainsi que les points d'eau obligeant le bétail à des déplacements parfois très longs pour une ration quasi inexistante.
- Conséquences sanitaires : en relation avec la périodicité des pluies, il y a un cycle de parasitisme qui s'établit. Les animaux s'infestent pendant la saison des pluies et ne peuvent tirer meilleur parti du disponible fourrager. C'est pendant cette saison aussi qu'il y a recrudescence des grandes maladies en particulier celles transmises par les tiques et les insectes piqueurs (babésiose, theileiriose, trypanosomose).
- Conséquences sur la fertilité. Il est bien connu que les femelles et les mâles sont plus féconds pendant la saison de l'abondance, pendant que le niveau de la biomasse herbacée est à son apogée et que, en raison de cela les périodes de mise-bas se situent pendant la saison sèche où l'herbe se raréfie. Or, cette période de pâturage quantitativement et qualitativement déficitaire correspond à la phase des besoins alimentaires élevés pour les vaches en fin de gestation et en allaitement (croissance du veau, dépenses énergétiques liés aux déplacements de plus en plus longs vers les pâturages)(10).

Les cycles de sécheresse, sans modifier la structure profonde de la productivité, ont

plutôt accentué les traits de l'élevage même s'ils ont poussé certains éleveurs à vendre précipitamment bon nombre de leurs animaux dans un marché très ouvert.

Les carences du marché

Les carences du marché se situent sur le plan organisationnel et sur le plan des motivations (9).

Il n'existe pas de circuits organisés et parfois même, ce sera un simple troc. Le manque d'organisation est un atout pour les spéculateurs qui utilisent des pressions de toutes sortes :

- pressions politiques, les déplacements d'animaux se faisant souvent sans se soucier des frontières entre états ;
- pressions juridiques, par des menaces d'applications de réglementation souvent méconnues par l'éleveur (terres mises en défends...)

Au niveau des motivations, le prix payé aux éleveurs n'est souvent qu'un prix moral de propriété et non le prix du capital transformé.

Il y a donc un malaise de l'élevage traditionnel qu'il faut savoir corriger.

LES PERSPECTIVES

Trois séries d'action peuvent être envisagées face aux facteurs limitants.

- former les hommes aux méthodes nouvelles
- dompter la nature avec les moyens disponibles
- organiser le marché.

Deux approches sont possibles :

- la 1ère est une approche séquentielle, c'est-à-dire celle qui, par une cascade d'actions achevées, conduirait d'emblée, et à plus ou moins long terme, à l'amélioration de tout le système de production (fig. 1)

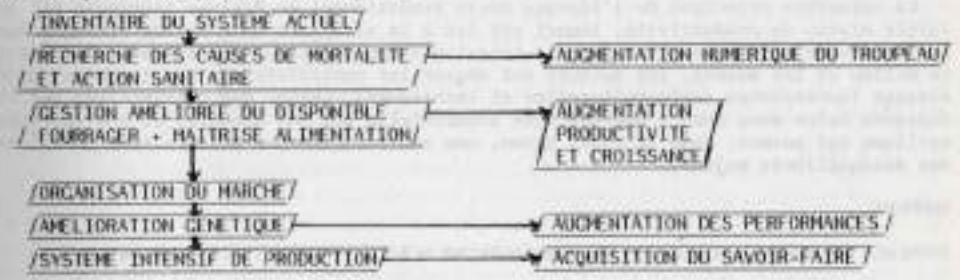

Figure 1 : APPROCHE SEQUENTIELLE

- la 2ème est une approche cyclique qui permet, dans le court terme, une amélioration, par paliers, des unités du système sans entraîner des déséquilibres majeurs ; il s'agit de gérer à la fois le temps et les implications nouvelles (facteurs de déséquilibre) résultant des modifications apportées au système à chaque stade (fig. 2).

Figure 2 : APPROCHE CYCLIQUE

Au total, il faut combiner gestion technique, gestion sanitaire et gestion économique du troupeau tel qu'il existe. Mais pour cela il faut former l'homme-éleveur et ce n'est pas la tache la plus facile. Lui faire éviter le nomadisme pastoral et le surpâturage, éléments générateurs de désertification en lui préconisant le "mixed farming", c'est le convaincre de la nécessité de pratiquer un élevage rationnel intégré avec les moyens et le matériel adapté à nos sols à la place des parcours de culture. Cette démarche exige, en dehors de la décision politique, une approche pluridisciplinaire où sont interpellés les hommes d'horizon divers, du socio-ethnologue à l'économiste, de l'agronome au vétérinaire... L'important enjeu du développement est à ce prix.

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RESUME

TYPOLOGIE DE L'ELEVAGE BOVIN EN AFRIQUE INTERTROPICALE : PRODUCTIVITE ET PATHOLOGIE

La caractère principal de l'élevage bovin traditionnel en Afrique tropicale est son faible niveau de productivité, lequel est lié à la structure même de cet élevage. Après avoir analysé cette structure dans la tétralogie que constituent les hommes, le bétail, le milieu et les moyens, les auteurs ont dégagé les contraintes au développement dudit élevage (contraintes socio-culturelles et techniques). Enfin, des perspectives ont été dégagées selon deux axes : une approche séquentielle qui est globale, et une approche cyclique qui permet, dans le court terme, une amélioration par paliers sans entraîner des déséquilibres majeurs.

SUMMARY

TYPOLOGY OF BOVINE PRODUCTION IN SUB-SAHARIAN AFRICA : PRODUCTIVITY AND PATHOLOGY.

The main characteristic of bovine production in sub-saharian Africa is its low productivity which is ascribed to the very structure of this production. The authors have highlighted the social, cultural and technical constraints to the development of bovine production after they have analysed its structure in the tetralogy that is made up or man, livestock, environment and methods in the ecosystem. Finally plans for futur actions have been laid down in two approaches : a sequential approach which is global and a cyclical approach which will allow in short run to improve productivity progressevely without any major disprution of the overall system.

RESUMEN

TIPOLOGÍA DE LA CRÍA DE GANADO BOVINO EN EL AFRICA INTERTROPICAL : PRODUCTIVIDAD Y PATOLOGÍA.

La característica principal de la cría de ganado bovino tradicional en el Africa tropical es su bajo nivel de productividad, el cual esta relacionado con la estructura misma del desarrollo ganadero. Al analizar dicha estructura en los cuatro elementos que la constituyen : el hombre, el ganado, el medio ambiente y los medios de producción,

los autores han entresacado las limitaciones del desarrollo de dicha cría(limitaciones socio-culturales y tecnicos). Finalmente, se han también destacado las perspectivas alrededor de dos ejes : un enfoque que alcanzaría el mejoramiento de todo el systema de producción a un plazo mas o menos largo, y un segundo enfoque que permitiría, a corto plazo, un mejoramiento por etapas sin que ello implique desequilibrios mayores.

RESUMO

TIPOLOGÍA DA PECUÁRIA BOVINA EM AFRICA INTER-TROPICAL : PRODUCTIVIDADE E PATOLOGÍA.

O carácter fundamental da pecuária tradicional bovina em Africa Tropical é de baixo nivel de productividade, ligado á propria estructura da referida pecuária. Depois de analisar esta estructura no quadro da tetralogia que constituem os homens, o gado, o meio ambiente e os meios, os autores salientaram as difficuldades inerentes ao desenvolvimento desta pecuaria (difficuldades socio-culturais e técnicas). Enfin destacaram perspectivas em dois positos : uma percepção global conseguente e uma percepção que permite atingir no cunto prazo melhoramentos por etapa sem provocar grandes desequilibrios.

AVALIAÇÃO DE UM SURTO DE INTOXICAÇÃO AGUDA CAUSADO PELA INGESTÃO DE SAMAMBAIA (Pteridium aquilinum, L. Kuhn) EM BOVINOS CRIADOS EM PARAÍBUNA/S.P. - ASPECTOS CLÍNICO-HEMATOLÓGICOS.

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INTRODUÇÃO

Os relatos da ocorrência de intoxicação crônica por samambaia, no Brasil, são numerosos, sendo raros no entanto, os que referem-se à intoxicação na sua forma aguda e, particularmente, abordando os aspectos clínicos e anátomo-patológicos (1, 14). Análises mais detalhadas sobre alterações do quadro hematológico e plaquetemia em bovinos acometidos pela intoxicação aguda são praticamente inexistentes na literatura nacional, razão pela qual faz-se necessária a presente avaliação.

MATERIAL E MÉTODOS

Diagnóstico da intoxicação aguda por samambaia

A ocorrência do surto, de intoxicação aguda de bovinos por ingestão de samambaia(P. aquilinum), relatado neste trabalho deu-se ao final do inverno, em uma propriedade da região de Paraíbuna, Estado de São Paulo. A fazenda apresentava solo arenoso, com pastagens nativas de péssima qualidade e altamente invadidas por samambaia(Pteridium aquilinum, L. Kuhn). O rebanho afetado, constituia-se de 18 bovinos mestiços da raça holandesa preta e branca, fêmeas e machos, com idades variando entre 6 meses e 5 anos, imunizados há dois meses contra carbúnculo hemático, provenientes da mesma região e, mantidos no local a cerca de dois anos sob regime de criação extensivo. A história clínica obtida, revelava que os animais afetados apresentavam emagrecimento progressivo, hiporexia e alguns com apatia. O exame clínico revelou entre os bovinos avaliados, além das referidas manifestações, um apresentando mucosas pálidas e outro com sangramento de pele e mucosas a qualquer ferimento leve ou picada de inseto(hematidrose), epistaxis, eliminação de fezes com sangue vivo e leve taquicardia. No exame necrocópico deste último animal, que morreu após 7 dias de evolução do quadro clínico, observou-se: mucosas perláceas; sangue ao redor do ânus; petéquias e sufusões da pleura e peritôneo visceral de intestinos e rúmen; hemorragias sub-pleural, sub-capsular dos rins e em pequenas áreas do fígado; intestino grosso com conteúdo sanguinolento, com coágulos de sangue, além de conteúdo serosanguinolento no útero. Com base nestes dados e nos resultados dos exames hematológicos estabeleceu-se o diagnóstico de diátese hemorrágica causada por intoxicação aguda pela ingestão de samambaia.

Animais e delineamento experimental

Os animais utilizados para análise do quadro hematológico e plaquetemia foram aqueles que no momento do exame clínico não apresentavam os sintomas de diátese hemorrágica.

As análises (hemograma e contagem de plaquetas) foram efetuadas em 17 amostras de sangue obtidas no período de 1 a 3 semanas após a observação de sintomas de intoxicação nos animais do rebanho.

Os resultados do hemograma foram distribuídos em quatro grupos conforme os valores do número de plaquetas existentes nas amostras: Grupo I - nº plaquetas/mm^3 $\geq 300 \times 10^3$; Grupo II - nº plaquetas/mm^3 $\geq 140 \times 10^3$ e $< 300 \times 10^3$; Grupo III - nº plaquetas/mm^3 $\geq 100 \times 10^3$ e $< 140 \times 10^3$ e Grupo IV - nº plaquetas/mm^3 $< 100 \times 10^3$.

Métodos

As determinações do hemograma foram efetuadas conforme técnicas e padronizações estabelecidas classicamente(3, 12). Para a contagem diferencial dos leucócitos e verificação de plasmoses sanguíneas os esfregaços de sangue foram corados pelo método de Rosenfeld(3). A contagem do número de plaquetas executada pelo método de Oxalato de amônia tamponado (12).

RESULTADOS

Os valores médios e desvios padrões das determinações do eritrograma e do leucograma e das contagens do número de plaquetas estão consignados na Tabela 1. Deve-se assinalar que em nenhum dos esfregaços examinados foram observados hemoparasitas(Anaplasma sp.; Babesia spp.) e que um animal no grupo I e outro no grupo IV apresentaram anemia leve a moderada, não regenerativa e do tipo normocrômica normocítica.

DISCUSSÃO

A trombocitopenia é um dos fatos marcantes nas modificações dos valores hematológicos de bovinos intoxicados de forma aguda pela samambaia (1, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16), assinalando-se sua ocorrência, mesmo em animais intoxicados que não manifestem quadro clínico de diátese hemorrágica (11, 15). Tal evidência foi constatada em 13 das amostras de sangue examinadas, considerando-se como normal o valor médio do nº plaquetas/mm^3 do Grupo I, sendo o fato justificado pela ingestão de volume variado da samambaia pelos animais no rebanho ou pela variação da resposta orgânica frente ao agente tóxico (4, 11, 15).

A leucopenia é também outra constatação ressaltada na literatura como característica desta intoxicação (1, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16). Considerando-se o número total de leucócitos observados (tabela 1), os seus valores médios foram menores nos animais dos Grupos III e IV, evidenciando-se em cada um destes, uma amostra leucopênica (respectivamente 4,8 e 5,0 x 10^3 leucócitos/mm^3).

A diminuição do número total de leucócitos, conforme evidencia-se nos resultados obtidos, é reflexo da queda dos valores médios relativos e absolutos dos polimorfonucleares neutrófilos, os quais demonstram neutropenias relativa (Grupos III e IV) e absoluta (Grupo IV). No Grupo IV constatou-se em quatro de sete amostras, valores relativos dos neutrófilos menores ou iguais a 10%, variando estes entre 2 e 10%. Nesse mesmo grupo os valores absolutos destas células foram menores que 1,0 x 10^3/mm^3 em 5 amostras, caracterizando-se, assim, a granulocitopenia por diminuição de neutrófilos relatada em várias pesquisas (1, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16). O comportamento dos valores dos polimorfonucleares eosinófilos e basófilos, no geral, pode ser considerado como dentro da normalidade, quando confronta-se os resultados com aqueles estabelecidos em outras pesquisas para animais sadios (2). No entanto, os valores relativos e absolutos maiores dos Eosinófilos nos grupos III e IV não encontram assinalamento similar em outras pesquisas (1, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16) podendo ser evidência da depressão seletiva medular que atinge particularmente as linhagens neutrofílicas e megacariocíticas determinada pelo princípio tóxico da samambaia (5, 6, 11) e que ao contrário, em relação a linhagem de polimorfonucleares eosinófilos, teria este referido princípio tóxico, em determinado momento da intoxicação, um efeito estimulador.

Tabela 1 - Valores médios e desvios padrões ($\bar{X} \pm S$) das determinações do eritrograma, leucograma e número de plaquetas de bovinos distribuídos pelos quatro grupos estabelecidos segundo a plaquetemia.

Determinações / GRUPOS ($\bar{X} \pm S$ do nº plaquetas x10^3/mm³)	ERITROGRAMA					
	Hemácias x10^6/mm³	Hematócrito %	Hemoglobina g%	VCM µ³	HCM µµ	CHCM %
I (350± 6,8) n= 4	6,6±1,5	35,0±7,0	11,8±2,8	53,9±6,9	18,0±2,1	33,5±1,6
II (171±26,2) n= 3	6,3±0,7	31,7±4,0	10,5±1,1	49,9±2,1	16,6±0,5	33,3±0,6
III (106± 1,5) n= 3	6,3±1,2	30,7±4,2	10,3±1,5	49,3±4,3	16,6±1,5	33,6±0,5
IV (78±22,0) n= 7	5,8±1,4	29,4±4,3	9,7±1,5	51,9±8,9	17,2±3,0	33,1±1,6

Determinações / GRUPOS ($\bar{X} \pm S$ do nº plaquetas x10^3/mm³)	LEUCOGRAMA					
	Leucócitos x10^3/mm³	Neutrófilos % (absoluto)	Eosinófilos % (absoluto)	Basófilos % (absoluto)	Linfócitos % (absoluto)	Monócitos % (absoluto)
I (350±6,8) n= 4	12,0±3,1	26,5±8,2 (3071,7 ± 976,3)	4,0±2,9 (413,0 ± 231,1)	0,7±1,0 (96,2 ± 139,6)	67,7±11,3 (8307,5± 3203,0)	1,0±1,4 (110,5 ± 140,8)
II (171±26,2) n= 3	11,0±1,5	26,7±14,4 (3041,7 ± 1824,7)	2,7±4,6 (258,7 ± 448,0)	1,0±1,0 (116,3 ± 127,1)	69,0±10,6 (7499,33± 479,1)	0,7±1,1 (84,0 ± 145,5)
III (106±1,5) n= 3	8,8±3,5 *	14,7±2,1 (1306,0 ± 564,4)	11,0±5,3 (1042,3± 719,0)	0,7±0,6 (54,0 ± 56,7)	73,3±6,3 (6431,0± 2496,8)	0,3±0,6 (16,0± 27,7)
IV (78±22,0) n= 7	8,8±2,5 *	9,0±5,5 (856,7 ± 704,0)	7,1±10,2 (627,7 ± 1018,8)	0,0±0,0 (0,0 ± 0,0)	83,7±13,4 (730,43 ± 2107,4)	0,1±0,4 (12,6 ± 33,3)

- Grupo I - nº plaquetas ≥ 300.000; Grupo II - nº plaquetas ≥ 140.000 e < 300.000 ; Grupo III - nº plaquetas ≥ 100.000 e < 140.000 e Grupo IV - nº plaquetas < 100.000/mm³

* Grupos com uma amostra Leucopênica

Os valores absolutos de linfócitos não apresentaram-se alterados, constatando-se somente uma linfocitose relativa no grupo IV, havendo amostras com até 98% destes nos nucleares. Tal achado é concordante com os de outros autores (5, 6, 10, 11), todavia não observando-se neste relato linfopenia absoluta (6).

Alterações do eritrograma não foram evidenciadas na maioria dos animais. Apenas um bovino do grupo IV apresentou anemia normocrômica normocítica não regenerativa associada à trombocitopenia e leucopenia por neutropenia, e que poderia ser atribuída à ação tóxica da samambaia. Esta variação do eritrograma era esperada, pois o quadro anêmico somente poderia estar presente, nas formas agudas da intoxicação pela samambaia em concomitância com os fenômenos hemorrágicos (6, 11, 13, 16).

CONCLUSÕES

- As variações do quadro hematológico e plaquetemia são manifestações que podem fundamentar o diagnóstico de intoxicação aguda por samambaia mesmo em ausência de sinais hemorrágicos.

- As variações hematológicas que podem subsidiar o diagnóstico clínico das intoxicações agudas por ingestão de samambaia são: trombocitopenia - nº menor que 140 x 10^3 plaquetas/mm³; leucopenia - nº total de leucócitos menor que 8,8 x 10^3/mm³; neutropenia relativa e absoluta - nº de neutrófilos, respectivamente menores do que 15% e 1300/mm³; linfocitose relativa - nº de linfócitos relativo maior que 73% e anemia não regenerativa.

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RÉSUMÉ

On a evalué les manifestations cliniques, surtout les variations des paramètres hématologiques, presentées pour des animaux d'un troupesu où il y a eu un foyer d'intoxication alguée par la fougère (**Pteridium aquilinum**, L. Kuhn). Les animaux étaient élevés en Paraibuna dans l'état de São Paulo. Dans les animaux examinés et qui ne presentaient pas des symptomes de diathèse hémorragique on a trouvé que l'alteration plus caractéristique est la thrombocytopénie et, puis, la leucopénie pour neutropénie relative et absolute associée à une lymphocytose relative. Cettes deux variations gardaient une rélation étroite avec l'intensité de la diminuition du numéro des plaquettes. Entre les animaux examinés, seulement un a démontré anémie pas régénerative attribuée à l'intoxication.

SUMMARY

Clinical and hematological studies were done on cows belonging to a herd which broken fern intoxication has been reported. The animals were raised in Paraibuna, São Paulo. Although no bleeding signs were observed, trombocytopenia was the major hematological abnormality found. Leukopenia, relative and absolute neutropenia and relative limphocytosis were also observed, with high relationship to the trombocytopenia. Only one case of non regenerative anemia due to the poisoning was observed among the animals studied.

SUMÁRIO

Avaliou-se as manifestações clínicas, principalmente as variações dos parâmetros hematológicos, apresentadas por animais pertencentes a um rebanho onde ocorreu um surto de intoxicação aguda por samambaia (**Pteridium aquilinum**, L. Kuhn). Os animais eram criados em paraibuna, estado de São Paulo. Nos animais examinados e que não apresentavam sintomas de diátese hemorrágica observou-se que a alteração mais característica é a trombocitopenia, destacando-se a seguir a leucopenia por neutropenia relativa e absoluta associada a uma linfocitose relativa. Estas duas últimas variações do leucograma mantinham magnitude estreitamente relacionada com a intensidade da diminuição do nº de plaquetas. Entre os animais examinados sòmente em um constatou-se anemia não regenerativa atribuível à intoxicação.

ZUSAMMENFASSUNG

Die klinischen Befunde, insbesondere die Veränderungen des Blutbildes einer Rinderherde mit akuter Adlerfarnvergiftung (**Pteridium aquilinum**, L. Kuhn) wurden untersucht.

Die Tiere sind in Paraibuna, São Paulo, gezüchtet worden. Bei den untersuchten Rindern, die keine Blutungserscheinungen hatten, wurden die Thrombozytenzahlen stark abgesunken. Es wurde auch Leukozytopenie, relativ und absolut Neutropenie, und relativ lymphozytose festgestellt. Diese zwei letzten Befunde des weissen Bluthildes hatten eine enge Beziehung zu der Verminderung der Thrombozytenzahlen.

Es wurden von den untersuchten Tieren nur eins mit Anämie gefunden durch die Vergiftung von **Pteridium aquilinum**.

A STUDY ON LEPTOSPIRAL INFECTION OF COWS BY BLOOD CULTURE AND MICROAGGLUTININ TEST OF SERUM IN A KOREAN RURAL AREA

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INTRODUCTION

In Korea epidemic pulmonary hemorrhagic fever, as a new clinical syndrome by experienced clinicians since 1975 epidemic, has been occurring sporadically from year to year yet without identification of the cause despite the continuous efforts(1).

In the middle of September 1984 following flood there was another epidemic of the disease, still sporadic in nature but resulting in several deaths from the disease.

One of the authors above(Kim) was commissioned at that time to investigate the cause of the epidemic, and it was proved to be leptospiral infection through a series of field and laboratory studies(2).

Infection of domestic animals by leptospires is an important problem in two aspects; one is as a possible source of human infection and the other as an economic loss due to frequent abortion of infected cow(3,4).

This study of leptospiral infection of cows was carried out preliminarily to determine a possible role of the cow as a source of sporadic human infection, and the magnitude of economic loss owing to abortions caused by leptospiral infection among the pregnant cows.

MATERIALS AND METHODS

Area Characteristics

The area studied is an ordinary and typical rural area covering 31.7 Km². There were 1,097 households with 4,315 population. At the time of the study the total number of cows being raised was 2,172 heads.

Sampling Procedure

Blood samples of 459 cows in May and 445 heads in November of 1989 were collected aseptically in vacutainer, labelled with code number of the cow, sex and age, packed in board box and transported to the laboratory on the day of collection.

Culture and Serological Test

Blood sample in vacutainer was centrifuged at 3500 rpm for 30 minutes and then serum was separated into serum vial and kept in refrigerator for MAT(microagglutination test). The sediments of the blood were inoculated to EMJH media in two to three drops in duplicates and kept in 28°C incubator. The culture tubes were examined for ring formation macroscopically, and the inoculum under dark field with X400 microscope for leptospires in every week for two months(5).

Eight standard strains and three locally isolated strains of leptospires from human were used as antigen for the MAT. Separated sera were inactivated in 56°C water bath for 30 minutes before the test. The critera for positive test was 1:80 (5).

Table 1 presents the strains of antigen used in this study. These locally isolated strains are identified as C. canicola, I. hemorrhagiae and I. lai by cross absorption test. The standard strains were obtained from either Pasteur Institute of France or CDC of U.S.A.

Table 1. Strains used for MAT

Serogroup	Serovar	Strain	Source
Autumnalis	autumnalis	Akiyama A	CDC
Bataviae	bataviae	V Tienen	CDC
Icterohaemorrhagiae	ndahamboukuje	Ndahamboukuje	pasteur
Louisiana	louisiana	LSU 1945	pasteur
Pomona	pomona	pomona	CDC
Sarmin	sarmin	Sarmin	pasteur
Sejroe	hardjo	Hardjoprajitno	pasteur
Tarassovi	tarassovi	Mitis Johnson	pasteur
Gp-858-2 (c. canicola)*			
2-18-111-2(I. copenhageni)*			
87K-71-7 (I. lai)*			

* Locally Isolated strains in Korea

RESULTS

Isolation of Leptospires

Only one strain of leptospires was isolated out of 985 cow blood samples cultured. This one strain isolated from the May sample reacted most strongly with Pomona antiserum.

Positive Rate of MAT

The blood samples collected in November showed much higher positive rate than that collected in May, 26.2% versus 12.0%. This finding corresponds with the seasonal difference in positive rate among human population. The positive rate by serogroup of the antigen used revealed rather an even distribution as shown in Table 2.

Table 2. Positive Rate of MAT on 985 (1st 459, 2nd 446)Cattle Sera by Serogroup of Antigen

Serogroup	No. positive		Positive rate (%)	
	1st Survey (May):459	2nd Survey (Nov.): 446	1st Survey (May)	2nd Survey (Nov.)
Autumnalis	3	4	0.7	0.9
Icterohaemorrhagiae	20	11	4.3	2.5
Pomona	ND	12	ND	2.9
Sarmin	-	9	-	2.0
Sejroe	-	14	-	3.1
Tarassovi	3	20	0.7	4.5
Gp-858-2 (C. canicola)	15	16	3.3	3.6
2-18-111-2 (I. copenhageni)	5	8	1.1	1.8
87K-71-7 (I. lai)	9	22	2.0	4.9
Total	55	117	12.0	26.2

In European countries and American continents several serogroups of leptospires had been isolated such as L. sejroe and L. pomona most frequently(4,5). This was the first case of leptospiral isolation from a cow, however, in Korea. A few seroepidemiological studies on cows in Korea showed a wide rage of positive rate; for example Choi(7) reported 3.8%, Suh et al(8) 8.6% and Suk et al 13.1%(9). On the other hand the seropositive rate of cows in tropical countries like Malaysia and Thailand was reported to be much higher than the positive rate we obtained. The positive rate among Malaysian cows was 27%(10) and that of Thailand was 48.5% (11).

Table 3, 4, and 5 shows the positive rates of cows tested by age, sex and a kind of cow. There was a tendency of increase in positive rate as cows grow older, male cows had significantly higher positive rate, but there was no difference in positive rate between dairly cattle and Korean cattle.

Table 3. Seropositive Rate by Age

Age	No. tested	No. positive	Positive rate (%)
1m - 3m	17	2	11.8
4m - 12m	208	41	19.7
13m - 3y	290	49	16.9
4y - 6y	267	39	14.6
7y - 9y	64	18	28.1
10y+	11	0	0.0
Unknown	48	11	22.9
Total	985	160	17.7

Table 4. Seropositive Rate by Sex

Sex	No. tested	No. positive	Positive rate (%)
Male	54	14	25.9
Female	787	138	17.5
Unknown	64	8	12.5
Total	985	160	17.7

Table 5. Result of Serological Test for Leptospirosis in Dairy and Korean Cattle

Animals tested	No. tested	No. positive	Positive rate (%)
Dairy Cattle	628	115	18.3
Korean Cattle	213	37	17.4
Unknown	64	8	12.5
Total	985	160	17.7

Table 6 is two by two table to see association between abortion history of the past one year and the result of the MAT, and in this study it failed to demonstrate the correlation of leptospiral infection to the history of abortion.

Table 6. Result of MAT and History of Abortion

MAT	Abortion		Total
	Yes	No	
Positive	17	143	160
Negative	53	692	745
Total	70	835	985

P>0.05

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"ENGLISH ABSTRACT"

The study of leptospiral infection among cows was carried out to find out possible role of this domestic animal as an infection source for humans, and the magnitude of economic loss due to abortions caused by leptospiral infections among the domestic animal.

Blood samples of 459 cows in May and 446 heads in November 1989 were collected from 19 villages of Kyunggi Province. These samples were cultured and serologically tested.

Results obtained are as followings:

1. Only one strain of leptospires, most strongly reactive to Pomona antibody, was isolated from duplicated cultures of 905 cow blood samples in EMJH medium.

2. Serological test by MAT with seven reference strains and three locally isolated strains was positive in 12% of the sample in May and 26% in November samples; positive rate increased slightly as the age of cows increased and male cows had higher positive rate, however, there was no difference in positive rate by type of cow and area.

3. There was no statistically significant association between abortion history among cows(one year-period) and positive MAT.

Thus it was concluded that the leptospiral infection of cows may play a role for human infection, and the leptospiral infection of cow may not be the major cause of abortion although further study is necessary for definite conclusion.

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LES METAUX OLIGO - ELEMENTS THERAPEUTIQUES

LEURS EXIGENCES DE PREPARATION , CONDITIONS DE LEUR EFFICACITE

Comme l'indique le titre de cette intervention,son but est d'évoquer l'usage des métaux en thérapeutique animale , mais ceci dans un cadre délimité : celui du processus catalytique emprunté par certaines réactions biochimiques ,en utilisant pour ce faire les métaux à doses infinitésimales.

La thérapeutique , mais aussi la diététique qui en découlent usent de formules qui n'ont rien à voir avec l'improvisation.

Elles résultent des enseignements acquis par la recherche fondamentale qui sont suivant de leçons que nous sollicitons ; elles ont reçu aussi l'apport de l'expérimentation clinique , dont le pragmatisme fut souvent confirmé par la confrontation avec les notions théoriques.

Ce sont ces règles de "bonne fabrication " et leur justification que je vais s'efforcer d'exposer afin que soit exploité au mieux de leur efficacité l'effet biocatalytique des métaux.

La première loi , pour ainsi dire , la révélation fut publiée dans les Comptes de l'Académie des sciences de Paris en 1897 par Gabriel Bertrand[1]qui remarquait que le ferment soluble oxydant issu de l'arbre à laque : la laccase, donne, par incinération, des cendres relativement riches en Oxyde de Manganèse. Il apprécie le pouvoir oxydant des échantillons de laccase sur l'hydroquinone qui change de couleur et se cristallise proportionnellement au degré d'oxydation.

Il effectua plusieurs essais comparatifs , en se basant sur la quantité d'Oxygène absorbé par une solution d'hydroquinone dans laquelle on ajoutait du Mn. ou de la laccase , ou encore les deux associés le pouvoir oxydant s'apprécie en fonction de la quantité d'Oxygène consommé.

Les résultats sont exprimés ci dessous.

1° avec le Mn.seul.....0,30 ml
2° avec la laccase seule.....0,20 ml
3° avec laccase additionnée de Mn.....6,30 ml

C'était inopinément la découverte de la catalyse biologique révélant à l'occasion de la laccase et du Mn.ce qui était déjà connu depuis longtemps en chimie minérale:l'accélération et l'augmentation des rendements d'une réaction , qui en l'absence du métal catalyseur, n'aurait pas lieu ou aboutirait à de mauvais rendements , le métal ne jouant que par sa présence est restitué à la fin de la réaction.

C'est ainsi que les métaux après avoir été dans la nuit des temps utilisés contre les carences, ou dans le traitement de pathologie exigeant de doses massives : Iode dans les actynomycoses,Ca et Mg dans les éclampsies vitulaires etc, se révélaient disposant d'un créneau d'action non utilisé, sans oublier

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leur incorporation à des concentrations , elles aussi infinitésimales dans des noyaux biologiques : le Fer dans la structure tétrapyrolique de l'hemoglobine , ce qu'est d'ailleurs le Magnésium à la chloropylle, le Cobalt à la cyanocobalamine le Cuivre à la turacine , pigment rouge des plumes de certains oiseaux, et à une hemocyanine du sang d'escargot,le Zinc à la sycotipyn,pigment du sang des mollusques.

Les travaux dans ce domaine se multiplièrent et en 1925 BERTRAND et MACHEBOEUF, publièrent un article sur la teneur relativement élevée du pancréas en Nickel et Cobalt.(2)soit en Nickel pour les boeufs, veaux , chevaux, respectivement 715, 800 et 500 agrs par Kg de matière sèche, et en Cobalt 350, 357 et 500 agrs par Kg de matière sèche.

Ces travaux furent à l'origine du second enseignement qu'il convenait d'utiliser:la quantité des oligo - éléments décelés dans les tissus ou ils se rencontrent se rassemblent autour du Mu Gr, µ Gr en alphabet grec ou 10^-6 ,ou p.p.m: ceci pour la plupart des métaux , à l'exception de quelques uns tels l'Iode, le Chrome , le Nickel qui atteignent un niveau plus faible du nano Gr, soit 10^-9 ou p.p.b

Cela signifiait que l'activité catalytique s'exercait entre 10-6, et 10^-9 et indiquait que les préparations conçues à titre thérapeutique devaient tenir compte des normes physiologiques : le µ Gr par ml étant la concentration adaptée à la dynamique de la catalyse biologique

Troisième remarque : les dosages révélaient des différences considérables dans le poids des métaux évalués dans des échantillons variés ,ces écarts se retrouvant toujours dans des proportions identiques chez les métaux considérés.
Il est évident qu'un élément comme le Lithium dont la masse atomique (proton + neutron) égale 7,a moins de "corpulence" que l'Antimoine de masse atomique 120.
Il fallait donc trouver un caractère présent dans tous les métaux mais variable numériquement d'un métal à l'autre.

Ce caractère commun servant à identifier chaque élément et à définir l'unité pondérale présente dans l'unité de volume de l'excipient , soit le ml.

Le proton,présent dans chaque élément suivant un multiple variant d'un élément à l'autre,repondit à ces exigences et fut choisi.

La Masse Atomique : 2 exprime le nombre de protons du corps considéré et se trouve être le numéro de la Classification Périodique des Eléments de MENDELEIEV; il exprime la valeur pondérale présente dans l'unité de volume de la présentation.

C'est ainsi qu'un ml contient 25 µ gr de Mn., 42 µ gr de Mo 29 µ gr de Cu , 30 µ gr. de Zn. 51 µ gr.de Sb.

Cette notion de microdose se déduit des dosages effectués sur divers tissus ou les divers éléments sont rencontrés dans des concentrations du millionème de Gr. Et il a été possible de constater effectivement qu'à des concentrations éloignées du

µ gr.l'efficacité thérapeutique s'estompait pour disparaitre.

Le Sélénium possède entre autres fonctions, celle d'accroitre l'immunité non spécifique , propriété qui s'apprécie par l'augmentation du P.C.F.(Plaque Forming Cellules)et par l'hémaglutination. Le P.C.F.augmente 4 fois sa valeur de base pour une distribution partant de 0 et atteignant 1,25 µ gr/Gr d'aliments distribués.Si l'on excède cette quantité optima, l'effet immunitaire chute avec le P.C.F.(3).

Même constatation avec le Manganèse.La concentration du Mn hépathique est supérieure chez les animaux recevant un aliment faiblement enrichi en métal,soit 4 µ gr que chez ceux en recevant 1000 µ gr (4) LA PRESENCE DU Mn.est 15 fois supérieure chez les premiers par rapport à ce qu'elle est chez les seconds.

En résumé l'absorbtion croit lors d'apport en faibles quantités, et diminue si ces apports deviennent considérables. Il en est de même pour le Cuivre.

Ces normes sont valables quelques soient les voies d'administration: il peut s'agir d'un métal alimentaire , ou du même métal sous forme pharmaceutique administrable per-os ou parentéral

C'est aussi l'expérience et les méthodes de la"Médecine Expérimentale",pour reprendre le titre de Claude Bernard ,qui nous apprennent que si l'on recherche l'efficacité des ions catalytiques , et que l'on applique pour ce faire les règles précédemment citées,il ne faut pas perdre de vue les autres éléments métalliques présents dans la formule , même si l'on n'attend pas de leur part un effet thérapeutique: c'est entre autre le cas de certaines formules ou un métal tient le role d'excipient, ce qui est complètement aberrant.Même à ce titre ils doivent en toute nécessité se conformer aux règles citées plus haut: présence correspondant au numéro du tableau de la classification périodique 10-6 gr/ml.

La présence à forte concentration d'un métal peut inhiber totalement l'activité biologique d'une série d'autres métaux. Ce qui s'explique par la possibilité pour une protéine transporteuse de recevoir plusieurs métaux compétitifs pour occuper le même site de liaison.C'est en particulier ce qui se présente dans la liaison Cu-Protéine au niveau du duodénum des poulets , cette protéine pouvant aussi bien voir son site de liaison occuper par le Cu , que par le Ca ou le Zn.
Il y a compétivité qui peut nuire à l'équilibre physiologique

Les exemples d'inhibition de l'action catalytique par dominante d'un élément abondent et expliquent bien des échecs de certaines diététiques ou thérapeutiques correctrices.

Les excès de Fer inhibent l'absorbtion du Manganèse , et inversement : de même que l'abondance de Calcium diminue la solubilité du Manganèse .L'excrétion foecale du Mn administré par voie parentérale est plus importante et la rétention hépathique plus faible avec 1 % de Ca.dans le régime qu'elle l'est, avec 0,6 %.

Le Cuivre , le Calcium et le Phosphore phytique limitent l'absorbtion du Cuivre .

Par contre la coopération synergique se rencontre fréquemment. Des rats privés de Nickel réduisent leurs réserves de Fer de 87 à 46 %.Le doublement de l'apport de Fer de 50 à 100 µgr ne suffit pas à restaurer le niveau normal du Fer dans les tissus pour prévenir l'anémie (5)l'apport de Nickel par contre supprime ces mêmes signes d'anémie.

C'est à de telles synergies que se rattachent les conclu-sions de l'expérimentation de FILMER et UNDERWOOD (6)qui guérirent un type de diarrhée imputée à une carence en Fer L'adjonction de ce métal au régime n'apportait aucune amélio-ration , alors que les mêmes animaux paturants sur d'autres herbages attusaient une très nette amélioration. L'analyse du sol de ces Herbages permettaient de déceler la présence de métaux qui ne semblaient pas se trouver dans les patures précédentes . Expérimentalement la guérison fut obtenue par l'administration d'une association dite"Groupe Zinc" , conte-nant du Zinc , du Manganèse , du Nickel et du Cobalt

Cette collaboration peut encore s'exercer non plus dans le sens synergie , mais en opposition , c 'est l'exemple de la"diarrhée teart"due à un excès de Molybdène traitée par le Cuivre, l'inverse pouvant aussi être appliqué , le tout réside encore une fois dans l'équilibre ; et l'homéostasie peut être obtenue parfois , par une entente complaisante entre deux facteurs dont l'un cède la place à l'autre , ou l'autre supplée à la défaillance de l'un. La parakératose du poulet illustre cette règle des bons usages , cette affection se rencontre à la suite d'une déficience en Zinc ou en Nickel, un apport de ce dernier supprime la parakératose , il entraine en même temps une chute du taux du Zinc , et l'inverse peut aussi avoir lieu (7).

Les Vitamines et les hormones semblent aussi en certaines circonstances tributaires, pour accomplir leur rôle biologique,de la"présence disponible"de métaux toujours dans le même ordre de concentration
La vitamine K intervient,dans la longue chaine de réactions aboutissant à la coagulation,en faisant appel au Manganèse. Cette même Ménadione parait aussi assumer le role de transpor-teur d'électron dans les phosphorylations oxydatives au niveau des phénomènes respiratoires (8) Une expérimentation clinique réalisée depuis deux ans en différentes régions de France a confirmé que lors d'infections pulmonaires accompagnées de dypsnée l'apport de l'association Vitamine k - Mn mettait fin à ces troubles que les antibiotiques traitant la phase infec-tieuse n'avaient pas amendés.

Relation inattendue : les déficiences en Cobalt sont responsables d'une baisse du taux de Thiamine et d'Acide ascorbique (9).

Le Manganèse offre un exemple caractéristique des rela-tions: métaux catalyseurs-hormones stéroides , que celles-ci soient sexuelles ou corticoides.L'anoestrus des bovins en est un exemple . Les injections d'oestrogène même renouvelées, ne pro-voquent pas toujours l'oestrus recherché,en y associant le Mn, à la concentration de 25 µgr/ml par voie parentérale on obtient l'état physiologique recherché

Le Zinc,semble,lui,polariser son action par un tropisme dirigé vers l'appareil génital mâle.De tous les organes , la prostate concentre le maximum de Zinc, dont le taux baisse à la suite de la castration.Par contre la concentration du métal augmente par administration de testostérone ou de gonadotrophine

Les deux cas suivants vont nous permettre d'exposer ce que l'on peut appeler l'effet Mn gr

S'inscrivant toujours dans le cadre des actions cata-lytiques , mais agissant seul ou affecté de l'aide d'un assis-tant dont ignore la nature, tel est le cas du Phosphore agis-sant dans une affectation décrite comme étant d'origine pseudo para-thyroidienne(10),d'autres auteurs semblent avoir évoquer la même affection sous la rubrique"Polydypsie,Polyurie"ce qui peut prêter à confusion. Le trouble a été observé sur des chiens et des bovins, on constate une soif intense , accompagnée de mictions rares mais abondantes , caractérisées par une urine décolorée, pale comme de l'eau de roche,accompagnée de diarrhée du type entérite chronique.Les animaux atteints sont gourmands de Phosphate de Sodium et non du Chlorure , ce qui indiquait une pathologie mettant en cause le Phosphore , et une thérapeutique dépendante du même métalloide.

J'utilisai en tant que traitement le Phosphore, sous forme de Phosphate disodique à la concentration de 15 µgr/ml En début d'expérimentation j'injectai 50 ml aux bovins et 10 ml aux chiens de taille moyenne.Les résultats furent favorables , avec un taux de guérison dépassant les 90 % et au demeurant 10 ml suffi-saient pour traiter efficacement les chiens et 20 pour des bovins de 500 Kgs , effectivement en catalyse il suffit d'apporter l'élément approprié en l'état de disponibilité biocatalytique

Le cas clinique se semble intéressant , mais plus l'est encore son aspect biochimique.Le dosage du Phosphore dans le sang de bovins en bonne santé atteint la valeur de 45 mg au litre(10). En cours de maladie on dose jusqu'à 55 et 85 mg. au litre , après traitement la concentration retombe à 37 et 45 mg. au litre

Mêmes types de résultats acquis avec le Fer , dans certaines anémies identifiées par un hématocrite descendant à 20 , avec un Fer sérique atteignant 2,7 mg/litre (normal 1,60). Une injection de 10 ml de Fer à la concentration de 26 µgr/ml permit de ramener le taux sérique à 1,33 mg/litre , sans que l'hématocrite augmente pas plus d'ailleurs que le nombre de globules rouges qui ne dépassaient pas 4.200.000.Ce n'est qu'à la suite de l'ad-tration du groupe Zn, dont il a été fait mention précédemment (Zn.Mn.Ni.Co.)que l'hématocrite remonta à 28 et les globules rouges à 5.800.000.

Ces différentes observations semblent pouvoir nous permettre de conclure que les métaux catalytiques agissent

suivant le mode exponentiel , la fonction Phosphore , la fonction Cuivre, la fonction Cobalt etc sont affectées d'un exposant , variable,indéterminé,ou inconnu appelé pour cette raison exposant.

Le P agit avec comme exponant la vitamine B2 , la vitamine D et on ne sait si c'est seul ou en coordination avec d'autres facteurs qu'il amende les signes cliniques et ramène la taux de P sérique à la normale dans la pseudo-hypo-parathyroïdie

Le Zn s'intègre à plus de 200 types d'enzymes entretenant des relations avec des exponants métalliques , hormonaux , vitaminiques .Il en est de même du Mn qui en rapport avec la vitamine K collaborent dans les réactions conduisant à la coagulation, et à certaines pathologies respiratoires

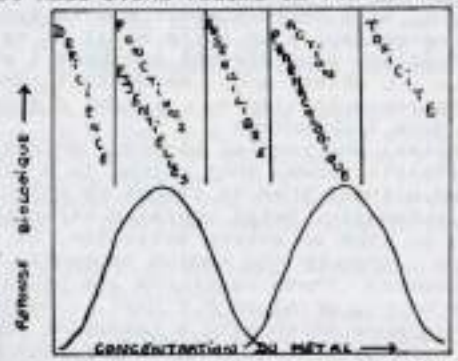
S'ajoutant aux règles à observer précédemment citées, la notion de spécificité du catalyseur paraît stricte et rigide dans certains cas : la chlorophylle et l'hémoglobine disposent d'une stucture identique : l'hème tétrapyrollique au centre duquel se situe un Fer bivalent pour le pigment sanguin et un Magnésium chez le végétal , mais il ne saurait être question d'interchanger les métaux.Par contre il existe des réactions biocatalytiques exigeant la présence d'un ion bivalent , celui-ci pouvant sans dommage être remplacé par un autre à condition qu'il soit bivalent aussi.C'est ce qui se passe au niveau de la Properdine, qui dans le processus de voie alterne d'activation du complément nécessite la présence d'ion Magnésium , remplacable par le Manganèse ou le Cobalt.

Arrivé à ce stade se pose une question majeure , l'explication de ce qui semble être une incohérence dans le déroulement de faits biologiques. A savoir le défaut d'un métal ou d'un métalloïde catalyseur entraine des troubles physiologiques caractéristiques d'un système diastasique auquel sont intégrés métal ou métalloïde. Mais de nombreuses autres perturbations devraient apparaitre si le dit élément catalyseur appartient à plusieurs systèmes et c'est par exemple le cas du Zinc dont dépendent 200 métalloenzymes

Plusieurs explications se proposent: la participation à un édifice particulièrement stable , tel le Co dansla cyannocobalamine la liaison carboxypeptidase - zinc est plus faible que celle de l'ADH hépathique (alcool déshydrogénase)Aussi,les troubles consécutifs à chacun des enzymes ne se manifestent pas en général de façon simultanée. l'activité enzymatique peut s'effectuer par le truchement de "canaux ioniques "des membranes cellulaires . Les ions Potassium sont associés à tous les processus d'excitations nerveuses , musculaires,ou glandulaires L'ion K est présent partout ou l'acétylcholine transmet l'influx nerveux; Il intervient là en tant que catalyseur de médiateurs chimiques(13)

En resumé tout métal ou métalloïde présent dans un organisme végétal ou animal peut y tenir plusieurs roles . C'est ce qu'expose Thomas G.SPIRO (11),reprenant ce qu'il qualifie lui même les lois de G.BERTRAND (12), considère que les

différents états du Zinc comme des autres métaux ,vont des effets physiologiques aux doses toxiques par une progression continue Il en résulte deux courbes l'une partant quand l'autre s'interrompt, cet ensemble délimitant trois zones : la zone de déficience , celle de fonctions essentielles correspondant à la catalyse, une zone ou l'élément est présent mais indifférent, puis apparait la zone à effet pharmacologique suivit de phase toxique.Prenons comme exemple l'Iode absent totalement des régions montagneuses , puis le catalyseur infinitésimal qui associé au Mn traite les hypothyroïdies avec agalaxie, suivi d'une présence inerte , précédent l'activité pharmacodynamique dans l'actynomicose, pour terminer par la phase toxique de d'iodisme et nous avons résumé la vie du métal.



Je terminerai par une recommandation d'une extrême importance , en provenance toujours du même auteur G.BERTRAND datant de 1895 (14).L'auteur compare les quantités d'Oxygène absorbée par l'hydroquinone en présence de différents sels de Mn sur une durée de vingt quatre heures .Plus la quantité d'Oxygène absorbée est considérable , plus la catalyse est efficace.Il en découle un enseignement majeur qui ne laisse aucun doute sur le type de sel à utiliser pour dynamiser au maximum les réactions catalytiques , or, dans de nombreux cas cette loi ne semble ni connue , ni appliquée

Avec le	Nitrate de Mn	1,5 cc
d°	Sulfate de Mn	1,6 cc
d°	Chlorure de Mn	1,8 cc
d°	Formiate de Mn	7,4 cc
d°	Benzoate de Mn	15,3 cc
d°	Acetate de Mn	15,7 cc
d°	Salicylate de Mn	16,3 cc
d°	Lactate de Mn	17,6 cc
d°	Gluconate de Mn	21,6 cc (*)
d°	Succinate de Mn	22,1 cc

* Des cristaux de quinhydrone ont apparus après deux heures (t° 18°)Le salicylate détersine aussi la production de quinhydrone , mais beaucoup plus lentement, avec les autre sels , même le succinate, il ne s'en est pas formé.La nature de l'acide semble agir à la fois sur l'intensité et sur le sens de l'oxydation.

Résumé: Les métaux et métalloïdes disposent en biologie animale et végétale d'un pouvoir de catalyse qui permet à une réaction biologique de s'effectuer alors que sans eux, elle n'eut pu le faire, ou se fut réalisée beaucoup plus lentement et ce avec moins d'intensité. Le métal agit soit en étant intégré à un substrat, soit comme activateur externe, soit encore au niveau de canaux ioniques par l'intermédiaire de récepteurs membranaires neuronaux ou hormonaux. Ces réactions sont obtenues à des concentrations du µgr ou ppm/ml, soit la Masse Atomique : 2 µgr/ml.
Chaque métal agit le plus souvent dans le cadre d'une spécificité catalytique, mais parfois aussi par la synergie de plusieurs métaux permettant à un autre métal de se révéler. De fortes concentrations d'un élément inhibent l'effet catalytique d'autres métaux. L'effet µgr. se définit comme le pouvoir d'un métal à la concentration du µgr/ml d'éliminer un excès de ce même métal dans l'organisme.

SUMMARY - In animal and vegetal biology, metals and metalloids dispos of a catalytic power that allows to a biologic reaction to be effectued, without them it cannot be effected or slowwer and with less intensity. Metal operates other being integrated to a substrat, or like an extern activator, or at the level of ionic drains, through the medium neuronic hormonaux membranous receptors. These reactions are obtained by concentrations of µgr/ml or P.P.M/ml., weither Atomic Mass: 2 = µgr/ml. This more often with a catalytic specificity, but sometimes by synergy of several metals allowing to an other metal to be revealed. Strong concentration of one element inibit the catalytic effect of an other one. MU gr effect (µgr) has been defined as a power of a metal at the concentration of µgr/ml to eliminate

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ASPECTOS CLÍNICOS E EPIDEMIOLÓGICOS DA FEBRE CATARRAL MALÍGNA.

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INTRODUÇÃO

A Febre Catarral Maligna no Brasil é uma enfermidade dos bovinos e bubalinos, causada provavelmente por um vírus, de transmissão desconhecida.

A síndrome clínico-patológico da ocorrência esporática, apresenta alta mortalidade com baixa moderada morbidade, e está sempre associado à presença de ovinos nas propriedades-focos.

A enfermidade foi notificada na África, no século XVIII, contudo só em 1832, Auker na Suiça fez a sua 1a. comunicação científica. Até 1963 muitos países da Europa, assim como USA, Canadá, África, Indonésia, Rússia e outros notificaram a presença da referida enfermidade, sem no entanto determinar o seu agente.

Plowright em 1963, estudando a F.C.M. conseguiu isolar um vírus, do grupo Herpes ao qual atribuiu a responsabilidade etiológica da F.C.M. africana.

No Brasil a Febre Catarral Maligna é citada pela primeira vez em 1924 pelo professor Torres, com o apelido de "mal do chifre" ou "coriza gangrenoza dos bovinos". Após 24 anos é novamente citada com ocorrência no Rio de Janeiro. Porém só em 1957 Doberainer e Tokarnia fazem uma detalhada comunicação dos achados clínicos e anátomos-patológicos de casos ocorridos no Rio Grande do Norte. Outros focos foram notificados através da literatura científica: Rio de Janeiro (Sampaio - Sampaio Dacorso/1970) São Paulo (Correa, Gottscjalk, Correa, Zezza / 1971) Rio Grande do Sul (Barros, Santos e Barros/1973), estes com ricos detalhes histopatológicos. Bahia (Oliveira, Figueiredo, Resende / 1978), Pernambuco (caso clínico), (Tabosa/1981). Novamente Bahia (Figueiredo, Oliveira, Firmo/1983). São Paulo (Marques, Alessi e Tomás / 1983) e Sergipe (Figueiredo, Oliveira e Carvalho/1984).

Aqui chamamos atenção que durante o período 1983/89, 5 focos eclodiram em 3 diferentes Estados, demonstrando que esta doença emergencial caminha à passos largos, advertindo-nos de evolução semelhante ocorrida nos Estados Unidos na década de 70.

MATERIAL E MÉTODOS

Foram estudadas, pelo período variáveis de duração de cada surto, a F.C.M. nos estados da Bahia-BA, Sergipe-SE e Paraíba-PB.

Surto BA/1978 - Município de Riachão do Jacuípe.
Efetivo de 400 bovinos convivendo com 28 ovinos.
Surto BA/1983 - Município de Alagoinhas.
Efetivo de 600 bovinos convivendo com 40 ovinos.
Surto SE/1984 - Município de Itaporanga/Sergipe.
Efetivo de 610 bovinos convivendo com 30 ovinos entre 2 a 6 anos.
Surto PB/1988 - Município de Taperoá-Paraíba.
Efetivo de 967 bovinos convivendo com 610 ovinos.
Surto BA/1989 - Município de Riachão do Jacuípe.
Efetivo de 82 bovinos convivendo com 32 ovinos.

Todos os surtos foram acompanhados pelo período da sua existência, onde todos os animais enfermos eram clinicamente examinados com anotações em fichas próprias (iguais em todos os surtos). As necrópsias foram realizadas pelas mesmas técnicas do decúbito lateral com anotações em fichas próprias e coleta de material imediata, para os exames histopatológicos.

Foram levantados dados epidemiológicos, na região de cada surto.

RESULTADOS

Surto BA/78.
Dos 400 bovinos existentes na propriedade, 35 (8,7%), animais adoeceram e morreram entre 3 a 10 dias num período decorrido de 4 meses- ver tabela I, quadro I.

Surto BA/83.
Dos 600 bovinos, 15 (2,5%) animais morreram num período de 6 meses entre 72 horas a 40 dias.

Surto SE/84.
Dos 610 bovinos, 68 (11,15%) morreram num período de 11 meses com evolução de até 10 dias.

Surto PB/88.
Dos 967 bovinos, 33 (3,4%) morreram num período de 6 meses com evolução de até 5 dias.

Surto BA/89.
Dos 82 bovinos, 26 (31,7%) morreram com evolução de até 6 dias num período de 2 meses.

Achados clínicos

As observações aqui descritas, dizem das alterações comuns aos animais examinados de todos os surtos.

Estado nutricional: regular a bom. Estado de saúde: ruim, agudamente doente. Comportamento: prostração e depressão. Temperatura: 39.5° a 41°C.

Pele e anexos: Pelos: eriçados sem brilho. Conjuntivas e mucosas: nasal, vaginal e oral, congestas. Linfonodos: cervical superficial e sub-iliaco (2X), aumentados de volume. Pele de úbere hiperêmica com rachaduras.

Digestivo: Inapetência. Boca: odor fétido, mucosas edemaciadas e congestas, salivação excessiva. Esôfago: sensibilidade na região laringo-esofágica. Rúmen: palpavel, movimentos: 1:2 fracos. Fezes: pastosas a diareicas, escuras para achocolatadas e fétidas.

Respiratório: Narinas: acúmulo de muco catarral amarelado nas superficies externas e internas. Corrimento uni e bilateral, mucoso-rançoso. Sensibilidade à palpação e percussão dos seios para-nasais com tons de surdo à sub-maciço. Sopro hiper-fonético. Dispnéia inspiratória com extertores unidos, variáveis. Frequência: 30 a 60 por minutos.

Aparelho circulatório; Taquicardia ritmica (até 120 BC/minutos).

Aparelho genito-urinário: vulva edemaciada e congesta. Hiperemia com peq. erosões da mucosa vaginal. Urina xaroposa, coloração vermelho sanguinolento em alguns.

Locomotor e nervoso: marcha forçada, incordenada até cambaleante, evolução para paraplégia, paralisia com opistótomo em decúbito lateral. Sensibilidade positiva, hiperestesia e tremores musculares (alguns animais).

Órgãos do sentido: audição aguçada.

Olhos: discreta exoftalmia. Lacrimejamento bilateral de aspecto seroso a mucu purulento. Congestão e edema de pálpebras. Conjuntivas hiperemicas. Injeção dos vasos da esclerótica. Opacidade de córnea com acúmulo de líquido sanguinolento na câmara anterior, de alguns animais. Cegueira.

Exames complementares

Sangue: Leucopenia ($\bar{X} = 4850\ mm^3$)

Urina: Micção expontânea: quantidade diminuida.
Cor - amarelo escuro a vermelho sanguinolento.
Aspecto - turvo
Proteínas - média de ++
Hemácias - presentes (hematúria) 2 animais

Alterações macroscópicas

As alterações mais constantes ocorreu nas vias aéreas superiores. A mucosa nasal mostra intensa hiperemia com ulcerações de tamanhos variáveis, na maioria das vezes, recobertas por membranas cuprosas de coloração amarelada, que se desprendem facilmente. Pelas fossas nasais flue um exsudato espesso mucopurulento de cheiro fétido, daí o nome de Coriza Gangrenosa pelo qual é conhecida a enfermidade. No focinho geralmente aparecem crostas que ao se desprenderem deixam a mostra tecido ulcerado.

A mucosa bucal está avermelhada e as gengivas, face interna dos lábios e plato exibem áreas de inducto fibrinoso recobrindo superfícies ulceradas. Alterações semelhantes podem também ser encontradas na faringe. As vezes há inflamação acentuada no cório dos chifres que se encontra hiperêmico e edemaciado.

A mucosa traqueobrônquica está hiperêmica e com petéquias. Os pulmões estão congestos e edematosos, podendo exibir áreas de enfisema.

Há corrimento ocular mucopurulento com hiperemia da conjuntiva e esclerótica e opacidade da córnea, sempre presente.

Na mucosa prepucial observam-se lesões fibrino-necróticas semelhantes as encontradas nas fossas nasais e vulvo vaginal.

No esôfago podem ser encontradas alterações erosivas geralmente na porção anterior. No abomaso a mucosa está hiperêmica e edemaciada. O intestino revela mucosa edemaciada com petéquias e conteúdo catarral.

O fígado está discretamente aumentado, e pode ser observado em alguns casos, um pontilhado branco que corresponde aos acúmulos de células mononucleares. Os linfonodos estão muito aumentados especialmente os superficiais que exibem superfície de corte úmida e avermelhada. Há um aumento sensível do líquido cefaloraquidiano. As meninges usualmente estão úmidas, congestas com petéquias e nos sulcos, observa-se uma certa turvação.

Alterações microscópicas

A alteração considerada patognomômica da enfermidade é uma vasculite necrótica acompanhada de acúmulos de células mononucleares na adventícia. Estas alterações vasculares podem ser encontradas em qualquer órgão e são a base patogenética da maior parte das alterações macroscópicas. Elas são localizadas e podem atingir tanto a média como a adventícia ou a intima.

Dados epidemiológicos

Constatou-se a presença de ovinos em todas as fazendas/surtos estudados. O aparecimento esporádico da doença aconteceu sempre 2/3 meses após alguns partos de ovelhas. O intervalo mais curto entre um novo surto na região problema aconteceu após 2 anos e o mais longo até então foi de 10 anos com um percentual de morbidade bem maior (no 1º surto BA/78 8,7%, BA/89 31,7%).

As ovelhas do rebanho de um surto tiveram um elo de ligação reprodutiva com animais de outros surtos, mesmo sendo de Estados diferentes. Animais dos surtos BA/83 e SE/84, foram comprados da região do surto BA/78 que por sua vez adquiriram animais da região do surto PB/88. Os animais da fazenda/surto BA/89 foram comprados da fazenda/surto BA/78.

Animais bovinos enfermos foram transportados para outras fazendas onde morreram em convivio com outros bovinos,não sendo notificado nenhum caso da doença na fazenda receptora. A Clínica de Bovinos da Escola de Medicina Veterinária da Bahia, atualmente desenvolve pesquisa objetivando a transmissibilidade da FCM-bovino/bovino ,com resultados parciais positivos.

DISCUSSÃO E CONCLUSÃO

Considerando os achados descritos e analisando as descrições e achados de outros autores da literatura consultada,concluimos os quadros e a tabela abaixo.

QUADRO I - EVOLUÇÃO CLINICA DA FEBRE CATARRAL MALIGNA (surtos BA/SC/PB)

Super aguda	Morte até 48 horas
Aguda	Morte até 10 dias
Sub-Aguda	Morte até 40 dias

QUADRO II - MANIFESTAÇÕES E SINAIS CLINICOS DA FEBRE CATARRAL MALIGNA. (surtos BA/SC/PB)

Temperatura elevada
Lacrimejamento com oftalmia
Mucosas hiperêmicas
Descarga nasal
Gânglios visiveis aumentados
Enterite
Marcha lenta
Depressão
Incoordenação evolutiva
Paralisia/opstotomo/morte

TABELA I - RELAÇÃO: efetivo,morbidade,letalidade da Febre Catarral Maligna/BA.

VARIAVEIS	SURTOS				
	BA/78	BA/83	SE/84	PB/88	BA/89
Rebanho	400	600	610	967	82
Mortes	35	15	68	33	26
% Morbidade	8.7	2.5	11.15	3.4	31.7
% Letalidade	100	93.4	100	100	100

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RESUMO

São descritos os achados clínicos e dados epidemiológicos de surtos da Febre Catarral Maligna nos estados da Bahia,Sergipe e Paraiba.

A evolução clínica dos surtos estudados,variou de super aguda com morte até 48 horas,aguda com morte até 10 dias e sub-aguda com morte até 40 dias.

O quadro sintomatológico observado em todos os surtos e com manifestações variáveis em alguns animais,se estabeleceu na seguinte evolução: temperatura elevada,lacrimejamento com oftalmia,mucosas hiperêmicas,descarga nasal,gânglios visiveis aumentados, enterite, marcha lenta,depressão, incoordenação evolutiva, paralisia/opstotono e morte.

Foi observado a presença de ovinos, em convívio permanente com os bovinos de todas as fazendas/surto.

A tabela abaixo esclarece as relações epidemiológicas dos surtos.

TABELA I - Relação: efetivo,morbidade,letalidade,da Febre Catarral Maligna.

VARIAVEIS	BA/78	BA/83	SE/84	PB/88	BA/89
Rebanho	400	600	610	967	82
Mortes	35	15	68	33	26
% Morbidade	8.7	2.5	11.15	3.4	31.7
% Letalidade	100	93.4	100	100	100

ZUSAMMENFASSUNG

Die klinischen uns epidemischen Daden des ploetzlichen Auftauschens des boesartigen Catarral-Fiebers in den Staaten Bahia,Sergipe und Paraiba werden beschrieben.

Die klinischen Entwicklung der studierten Faelle,variierten von Super-Akut,die bis zu 48 Stunden danach zum Tode fuehen,Akut mit bis zum Tode fuehren.

Das beobachtete Krankheitsbild in aleen Faellen und mit veraenderlichen Anzeichen in einigen Tieren,wurde durch folgende Entwicklung festgestellt: hohe Temerpatur,Traenen mit Augenentzuendung,Hiperemische Schleimhaut, Nasale Catarro, sehbar grosserwerdende Nevernknoten, Enteritiden, Laugsam gang, Depression, Incordenarsion Lahmung-Opstotono und Tod.

Man beobachtete die Anwesenheit von schaf in stetem Zusammenleben mit den Rindern in allen "Fazendas"/Krankheitsfaellen.

Die folgende Tabelle erklaert die epidemischen Verhaeltnisse Faelle.

TABELLE I - Verhaeltnis: effektiv, Zahl der Krankheitsfaelle, Zahl der Todesfaelle des Catarralfiebers.

VERAENDERLICH

	BA/78	BA/83	SE/84	PB/88	BA/89
Herde	400	600	610	967	82
Todesfaelle	35	15	68	33	26
% Krankheitsfaelle	8.7	2.5	11.15	3.4	31.7
% Todesfaelle	100	93.4	100	100	100

RESUME

Sont décrits les aspects cliniques et les données épidémiologiques d'attaques de Fièvre Catarrhale Maligne dans les Etats de Bahia,Sergipe et Paraiba.

L'evolution clinique des cas étudiés à varié de super-aigue,avec la mort jusqu'en 48 heures, aigue avec mort jusqu'en 10 jours et sub-aigue avec mort jusqu'en 40 jours.

Le cadre syntomatique observé dans tous les cas,avec des manifestations diverses chez quelques animaux a presenté l'évolution suivante : température élevaã,larmoiement avec ophtalmie,muqueuses hyperêmiées , écoulement nasal,ganglions visibles augmentés,entérite,marche lente,dépression,perte progressive de coordênation,paralysie opstotone,congestionnées et mort.

On a observé la presence d'ovins en contact permanente avec les bovins dans toutes les fermes/cas.

Le tableau suivant e'claire les relations épidemiologiques des cas.

TABLEAU I - Relation: effectif,morbidité,létalité de la Fièvre Catarrhale Maligne.

VARIABLES

	BA/78	BA/83	SE/84	PB/88	BA/89
Cheptal	400	600	610	967	82
Morts	35	15	68	33	26
% Morbidité	8.7	2.5	11.15	3.4	31.7
% Letalité	100	93.4	100	100	100

INTOXICAÇÃO EXPERIMENTAL DE BEZERROS (Bos taurus, LINNAEUS 1758) POR AFLATOXINA B1.

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INTRODUÇÃO

O fígado é um órgão de muitas e diversas atividades metabólicas sendo, entretanto, difícil estabelecer a ocorrência de uma disfunção hepática. Este fato é conseguinte a sua grande reserva funcional e de sua capacidade de regeneração (5).

Segundo Rosenberger (1983b), as enzimas intracelulares, são liberadas após a disfunção celular e sua presença no soro sanguíneo pode fornecer informações sobre a natureza do dano celular, de acordo com a magnitude de variação. Entre os exames utilizados para a avaliação laboratorial das lesões hepáticas em bovinos, devem ser destacadas, por serem sensíveis e específicas, as determinações séricas de gama-glutamil-transferase, aspartato amino transferase e bilirrubinas (direta, indireta e total).

Inúmeras intoxicações que acometem os animais domésticos são produzidas por micotoxinas, isto é, toxinas produzidas por fungos contaminantes de grãos ou forragens. Existe uma grande variedade de fungos produtores de micotoxinas, como é o caso do **Penicilium citrinum**, cuja sua micotoxina é denominada de citrina; do **Claviceps purpurea**, causador do ergotismo e do **Pitomyces chartarum** produtor da esporodesmina (7).

A micotoxina mais conhecida e cuja intoxicação é mais intensamente pesquisada é a aflatoxina, sendo produzida pelo **Aspergillus flavus** dentre outros.

Este trabalho tem-se como objetivo avaliar as manifestações físicas e de alguns parâmetros bioquímicos séricos de bezerros intoxicados experimentalmente.

MATERIAL E MÉTODOS

Neste estudo utilizou-se 11 bezerros (**Bos taurus**, LINNAEUS 1758) da raça holandesa preta e branca, de idade entre 15 a 30 dias, com o peso corporal oscilando entre 22 e 50 kg.

Após a ingestão do colostro, estes animais foram alimentados com uma ração constituída por 4 litros de sucedâneo lácteo comercial, 2 vezes ao dia; ração concentrada, além de capim, ferro e água **ad libitum**.

GRUPOS EXPERIMENTAIS

O primeiro grupo foi constituído por quatro animais, sendo que dois, receberam por via subcutânea, doses diárias de aflatoxina B1 de 0,0016mg por kg/P.V. durante 30 dias; de 0,0027 mg de aflatoxina B1 por kg/P.V. durante mais 30 dias e de 0,0045 mg de aflatoxina B1 por kg/P.V. nos últimos 30 dias em um total de 21 mg de aflatoxina B1.

O segundo grupo também foi composto por quatro bezerros sendo que dois permaneceram como controle e dois receberam aflatoxina B1 por via subcutânea durante 90 dias, sendo as seguintes doses empregadas: 0,035; 0,0060 e 0,0090 mg por quilograma de peso vivo, no primeiro, segundo e terceiro mês, respectivamente, sendo o total administrado em cada animal de 42 mg de aflatoxina B1.

O terceiro grupo foi constituído por 3 bezerros, dos quais, um permaneceu como controle, e os demais receberam doses únicas de aflatoxina B1

de 0,8 e 1,8 mg por quilograma de peso vivo.

Antes do início do tratamento todos os animais foram submetidos à exame clínico diário e colheita de sangue e soro para a realização das provas laboratoriais. Após o início do experimento todos os animais continuaram sendo examinados diariamente quanto às funções vitais (temp.; freq. cardíaca e respiratória) sendo as colheitas de amostras de sangue para a elaboração do hemograma completo e das provas de função hepática realizadas semanalmente. Nos animais do terceiro grupo também foram realizados exames clínicos diários, sendo que as amostras de sangue destinada a realização do hemograma e das provas bioquímicas foram colhidas, inicialmente, em dias alternados e após 4 colheitas, diariamente, devido a piora das condições dos animais.

A aflatoxina, usada no presente estudo, foi adquirida na SIGMA CHEMICAL COMPANY, St. Louis, USA cod. 6636. Sua concentração foi confirmada de acordo com a técnica preconizada (15). Após a determinação da concentração de Aflatoxina B1, alíquotas de 1. 2; 4 e 8 mg foram acondicionadas em frascos de aproximadamente 30 ml de capacidade. Após a evaporação de todo o diluente, foi imediatamente re-suspensa em 10 ml de Dimetil-sulfóxido (DMSO).

As amostras de sangue foram colhidas por punção da veia jugular externa, sendo as amostras colhidas em frascos contendo EDTA (Etileno diamino-tetracético-di-sódio), na proporção de 5 mg/ml de sangue, para a realização dos valores do hemograma: contagem do número de hemáceas e leucócitos, valor da hemoglobina, determinação do hematócrito e dos índices hematimétricos (VCM, HCM e CHCM), e os esfregaços sanguíneos foram confeccionados com sangue "in natura", com vistas a contagem diferencial dos leucócitos e a avaliação morfológica e tintorial das hemáceas, (1). Em outro tubo, de capacidade de 15 ml, foi colhido sangue sem anticoagulante e mantido em temperatura ambiente até a formação do coágulo. Os soros assim obtidos foram mantidos à temperatura de -20ºC, até o momento da realização das provas bioquímicas, a saber:

- Proteína sérica total - Método do Biureto, de acordo com a técnica preconizada (8).
- Bilirrubina (Direta, Indireta, Total) - Método colorimétrico baseado na prova de Van Den Bergh (Reactoclin).
- Aspartato amino transferase (AST) - Monotest Got optimado - Merck S.A. Indústrias Químicas.
- Gama glutamil transferase - Monotest GGT - Merck S.A. - Indústrias Químicas.

Após o período experimental de 3 meses, todos os animais do primeiro e do segundo grupo foram sacrificados e submetidos ao exame necroscópico. Os animais do terceiro grupo, com exceção do controle, tiveram morte natural, sendo de uma semana no animal que recebeu 1,8 mg por quilograma de peso vivo e de duas semanas no bezerro que recebeu 0,8 mg de quilograma de peso vivo de aflatoxina B1, sendo o animal controle sacrificado no final da 2 semena, sendo todos os 3 animais também submetidos a exame histopatológico.

Os órgãos estudados foram fixados em formol a 10% e corados pela hematoxilina eosina.

Na interpretação estatística dos resultados foi utilizada a análise de variância com um critério de classificação segundo o estabelecido (14). O controle entre médias foi efetuado utilizando-se o teste pre estabelecido (6), sendo que o nível de significância adotado neste estudo foi de 5%.

RESULTADOS

Nenhum dos animais intoxicados com aflatoxina B1, nas dosagens totais de

21 mg e 42 mg, apresentaram até o momento em que foram sacrificados, alterações das funções vitais, enquanto que nos animais intoxicados com dosagem única desta micotoxina, um deles, o que recebeu 1,8 mg por quilograma de peso vivo, após 8 dias da administração, morreu repentinamente e o outro bezerro, que recebeu 0,8 mg por quilograma de peso vivo, apresentou a partir do 9º dia, após a administração da Aflatoxina B1, hiporexia, pelos arrepiados, incoordenação dos movimentos dos membros posteriores, prostração, desidratação moderada, intensa apatia revelada pela dificuldade em manter a cabeça elevada, seguida de morte no 14º dia após a administração da micotoxina.

Dos resultados dos exames laboratoriais realizados, nos animais do primeiro e segundo grupo, o único que demonstrou diferença estatisticamente significante foram os valores obtidos da determinação de gama glutamil transferase, apresentadas na tabela 1.

Enquanto que os resultados obtidos a partir das determinações séricas dos animais do terceiro grupo, estão apresentadas na tabela 2.

Não foram observadas alterações anatomo-patologica no exame necroscópico ou na avaliação histopatológica nos órgãos dos animais intoxicados experimentalmente, com doses totais de 21 mg e de 42 mg de aflatoxina B1.

No bezerro intoxicado de forma aguda com 0,8 mg de aflatoxina B1 por quilograma de peso vivo, com dose unica, o exame histopatologico do fígado demonstrou degeneração vacuolar de hepatocitos, necrose hemorragica centro-lobular, infiltrado inflamatorio misto junto as áreas de necrose. No outro animal ao qual foi administrada dose unica de 1,8 mg de aflatoxina B1 por quilograma de peso vivo. Observou-se, macroscopicamente, ascite fibrinosa, aderencia do baço ao diafragma e repleção de vesícula biliar. O fígado apresentava-se com uma coloração amarelada, sendo muito friável a palpação; histologicamente detectou-se ocorrencia de cilindros biliares junto aos canalículos biliares, degeneração vacuolar de hepatocitos, severa necrose hemorrágica centro lobular e periportal e infiltrado inflamatorio misto junto as áreas de necrose.

DISCUSSÃO

Quanto aos testes de função hepática, vários autores concordam com sua utilização para avaliarmos a integridade deste órgão (2; 3; 10; 16), mas nos animais intoxicados por aflatoxina B1 na dose total de 21 mg, não foi constatado aumento dos níveis séricos de bilirrubinas (direta, indireta e total), aspartato amino transferase e gama glutamil transferase ou diminuição dos valores séricos de proteína total, enquanto que nos bezerros intoxicados com dose total de 42 mg de aflatoxina B1, houve um aumento estatisticamente significante nos níveis séricos de gamaglutamil transferase, nas fases finais da intoxicação, concordando com ROSENBERGER (1983b), que nos bovinos, a elevação significativa dos teores séricos desta enzima indica colestase.

A ausencia de lesões macro ou microscópicas no fígado dos animais em que se procurou determinar uma microstoxicose crôncia, pode significar que as doses empregadas não tinham sido suficientes para determinar quais quer das lesões citadas na literatura nas intoxicaçoes por aflatoxina (4; 9; 11; 17).

O animal intoxicado com 1,8 mg de aflatoxina B1 por quilograma de peso vivo, dose única, não manifestou sinais clínicos evidentes, todavia sua morte subita indica uma condição aguda de intoxicação, semelhante com o que foi encontrado (10), já o bezerro no qual aplicou-se dose única de aflatoxina B1 de 0,8 mg por quilograma de peso vivo, dose única, manifestou um quadro clínico semelhante ao que foi relatado (12), que salienta comprometimento gradativo do estado geral, porem sem sinais caracteristicos de um quadro específico. Os níveis séricos de aspartado amino trans-

TABELA 2 - Variação dos valores médios e desvios padrões das provas de função hepática do soro sanguíneo de bezerros submetidos à intoxicação em dose única de 0,8mg/kg de peso vivo de Aflatoxina B1 e do animal controle (3), São Paulo, 1989.

Provas de função hepática / Animal	GGT (µ/l)	AST (µ/l)	BILIRRUBINAS (mg/dl) BD	BI	BT	PT (g/dl)
1	47,53 ± 4,25^b	26,18 ± 3,85^b	0,14 ± 0,05^a	1,13 ± 0,10^b	1,23 ± 0,12^b	4,62 ± 0,08^a
2	69,20 ± 1,82^a	54,77 ± 28,29^a	0,47 ± 0,18^a	4,35 ± 1,70^a	4,82 ± 1,87^a	4,46 ± 0,06^a
3	21,10 ± 0,98^c	16,59 ± 1,48^b	0,14 ± 0,11^a	0,53 ± 0,10^b	0,60 ± 0,05^b	4,65 ± 0,08^a

Médias contendo letras diferentes nas colunas, revelam diferenças significativas pelo teste de DUNCAN (p 0,05)

TABELA 1 - Variação dos valores médios e desvios padrões dos teores de gama glutamil transferase (UI/l) do soro sanguíneo de bezerros submetidos a duas dosagens de Aflatoxina B1 (1-21 mg e 2-42 mg). São Paulo, 1989.

Grupos Experimentais	FASES DA INTOXICAÇÃO			
	Antes da Intoxicação	Durante a Intoxicação		
		1º Mês	2º Mês	3º Mês
1 Intoxicado	$7,3 \pm 0,75^{b}$	$10,3 \pm 1,24^{1}$	$17,1 \pm 2,80^{a}$	$16,5 \pm 2,13^{2}$
1 Controle	$11,0 \pm 0,10^{ab}$	$12,1 \pm 1,00^{ab}$	$9,1 \pm 0,46^{b}$	$9,2 \pm 1,06^{b}$
2 Intoxicado	$10,9 \pm 0,05^{bc}$	$11,2 \pm 1,60^{bc}$	$17,0 \pm 1,66^{a}$	$20,5 \pm 0,87^{a}$
2 Controle	$7,6 \pm 1,60^{c}$	$10,3 \pm 0,57^{bc}$	$10,3 \pm 0,57^{bc}$	$12,5 \pm 0,84^{b}$

Médias contendo letras diferentes, revelam diferenças significativas pelo Teste de DUNCAN (P 0,05).

ferase e gama glutamil transferase tiveram um aumento estatisticamente significante, fato que, nesta intoxicação aguda, representa forte evidência de lesão hepatocelular e de ductos biliares.

Na determinação das bilirrubinas séricas, demonstrou-se uma hiperbilirrubinemia, consequente ao aumento da bilirrubina de reação indireta, de acordo com a literatura (5), que afirma que nos bovinos as hiperbilirrubinemia são decorrentes do aumento da bilirrubina de reação indireta.

A avaliação dos teores séricos de proteína total, não demonstrou alterações significativas nestes animais.

Ao exame histopatológico do fígado são semelhantes aqueles encontrados na literatura (4), sendo as lesões mais severas no animal que recebeu maior dose de aflatoxina B1.

CONCLUSÕES

A intoxicação por aflatoxina B1 não demonstrou, em bezerros, ter poder acumulativo capaz de causar uma disfunção hepática na dose total de 21 mg e 42 mg aplicada fracionada e diariamente durante 3 meses.

As doses de 0,8 e 1,8 mg de aflatoxina B1 por quilograma de peso vivo determinaram uma intoxicação aguda e letal para os bezerros.

A avaliação da atividade de gama glutamil transferase sérica foi a prova mais sensível para indicar a existência de um processo hepático com comprometimento do sistema biliar.

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RESUMO

Com o objetivo de se avaliar os efeitos da intoxicação crônica e aguda por aflatoxina B1, seis bezerros foram intoxicados com doses diferentes desta micotoxina. Os animais tratados com pequenas doses e de forma crônica, apenas apresentaram alterações nos valores bioquímicos séricos de algumas enzimas indicadoras de disfunção hepática, enquanto que os animais que receberam maior dose e de forma única, apresentaram modificações do seu estado geral com morte; além dos valores de enzimas séricas indicadoras de disfunção hepática e também lesões histopatológicas indicativa de lesão por esta micotoxina.

SUMMARY

The objective of this study was appraise the consequence of agute and chronic aflatoxin B1 intoxication in six calves with different doses level of this mycotoxin animals with small doses and chronic form only showed modification in value of serum biochemical of the liver function tests, while the animals that was administrat larges doses and simple dose showed clinic manifestation with dead, modification in value serum biochemical of the liver function and the liver histopathologic examination showed characteristic lesion by aflatoxin.

RÉSUMÉ

Pour faire, une évaluation des effets de l'intoxication chronique et aiguÏ pour aflatoxine B1, six veaux out été intoxiques avec differentes doses de cetle mycotoxine. Les animaux traites avec petites it plusieurs doses out presente seulement des altérations dans les valeurs biochemies de certaines enzymes sériques indicatrices de troubles hepatiques,tandis que les animaux qui out reque grandes et uniques doses out presente des modifications de son état général et mort. Les valeurs des enzymes sériques et l'examen histopathologique out demontré, dans ces animaux, la lésion du foil.

AVALIAÇÃO DO LEUCOGRAMA DE FÊMEAS DA RAÇA HOLANDESA BRANCA E PRETA NATURALMENTE INFECTADAS PELO VÍRUS DA LEUCOSE BOVINA

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INTRODUÇÃO

Talvez tão antigo quanto as primeiras notificações da ocorrência da Leucose Enzoótica Bovina (LEB) (1) seja o conhecimento da intensa proliferação linfocitária que acompanha essa doença. No início deste século foi citado ainda que esta alteração hematológica não era apenas quantitativa,mas também qualitativa, ou seja, nos animais acometidos pela LEB eram encontrados vários linfócitos com morfologia atípica (2,3). Outra importante averiguação foi o fato das alterações hematológicas não ocorrerem apenas em animais com tumorações, sendo também detectadas em animais aparentemente sadios (4). Baseado nestes fatos o primeiro programa de controle da doença foi estabelecido considerando se os valores do leucograma como parâmetro de diagnóstico da infecção pelo vírus da Leucose Bovina (VLB) (5), dando-se o nome de "Chave Leucométrica" ao conjunto destes parâmetros (6, 7, 8). Desde que VLB foi isolado (9) foi possível estabelecer-se uma nova metodologia de diagnóstico baseada na detecção de anticorpos séricos desenvolvidos durante a infecção, através da prova de imunodifusão em gel (IDLB) (10). Desde então, o diagnóstico hematológico vem sendo desconsiderado face às suas limitações de sensibilidade e praticidade (11). No Brasil, entretanto, ainda não existe a produção do antígeno para a realização da IDLB e face à ausência de trabalhos sobre o comportamento hematológico dos animais com LEB (12, 13, 14), decidiu-se apresentar estudos das variações do quadro leucométrico de bovinos naturalmente infectados pelo VLB.

MATERIAL E MÉTODOS

797 fêmeas bovinas da raça holandesa branca e preta, criadas na região da bacia leiteira de Campinas-SP, em regime semi-intensivo, foram divididas em dois grandes lotes de animais: reagentes e não reagentes à prova de IDLB. Em cada lote os animais foram distribuídos em grupos segundo as faixas etárias detalhadas na Tab. 1.

Tab. 1 - Grupos experimentais, caracterizando-se o número de animais examinados, distribuídos segundo a faixa etária e a reação frente a prova de imunodifusão em ágar gel. São Paulo. 1990.

Faixa etária em anos			Nº de amostras reagentes	Nº de amostras reagentes	Total
-	de	1	59	108	167
1	a	2	77	88	165
2	a	3	94	63	157
3	a	4	60	38	98
4	a	5	47	28	75
5	a	6	36	20	56
+	de	6	50	29	79
TOTAL			423	374	797

Após a colheita das amostras de sangue dos animais, por punção da veia ugular, utilizando-se EDTA como anticoagulante foram realizadas a contagem global de eucócitos em câmara hematimétrica (15), a contagem diferencial de leucócitos em esfre-aços sanguíneos corados (15) e a pesquisa de anticorpos séricos anti-VLB através da imu odifusão radial dupla em gel de agar com antígeno glicoprotéico gp51 (Rinderleukose-An igen da Behring - RFA) (16).

ESULTADOS

Os resultados encontrados estão expostos nas Tabs. 2, 3 e 4.

ISCUSSÃO

O maior número de leucócitos (leucocitose) encontrado no sangue dos animais reagen-es à IDLB implicou necessariamente quetal variação deveu-se exclusivamente ao aumento o número absoluto de linfócitos, posto que não se encontrou qualquer diferença entre as édias do número absoluto dos polimorfonucleares granulócitos (neutrófilo, eosinófilo e asófilo) ou dos monócitos. Essa observação coincide com as afirmações apresentadas por úmeros autores (5, 6, 11, 16).

Com relação aos aspectos morfológicos dos linfócitos notou-se que Sombras de Gumpre-ht, linfócitos com núcleos marcados, linfócitos com núcleo picnótico, linfócitos com anulações azurrófilas e células de Türk não possuem importância decisiva no diagnósti o hematológico da leucose Bovina. Todavia, deven-se ressaltar os resultados encontra-os para duas atipias que demonstraram significância no diagnóstico hematológico desta nfecção, ou seja, os linfócitos monocitóides (Células de Riedel) e os linfócitos com úcleo duplo (células tetraplóides).

Os resultados obtidos na presente pesquisa demonstram que a avaliação hematológica a equada continua sendo um potente recurso no diagnóstico da Leucose Enzoótica Bovina.

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RESUMO

Desde o início do século já era conhecido que os animais afetados pela Leucose Enzoó tica dos Bovinos (LEB) apresentavam uma leucocitose por linfocitose. Tal aspecto tem si do muito usado como recurso no diagnóstico clínico da LEB através do emprego das chama-das "Chaves leucométricas". O presente estudo procurou avaliar o perfil do quadro leuco citário de fêmeas bovinas da raça holandesa branca e preta naturalmente infectadas pelo vírus da Leucose Bovina (VLB) dando-se particular atenção à morfologia dos linfócitos . Para tanto, realizou-se o leucograma de 797 amostras de sangue colhidas de 423 bovinos reagentes e 374 não reagentes à prova de imunodifusão em gel para a Leucose Bovina (IDLB). Os resultados obtidos mostraram que, ao contrário do que ocorria com os polimor fonucleares e monócitos, os linfócitos apresentaram diferenças estatisticamente signifi cativas, sendo os valores encontrados nos animais reagentes à IDLB maiores do que os ob tidos nos animais não reagentes. No tocante ao aspecto morfológico dos linfócitos con cluiu-se que os linfócitos monocitóides e os linfócitos com núcleo duplo apresentaram contagens significativamente maiores nos animais reagentes.

SUMMARY

Lymphocytosis caused by Enzootic Bovine leukosis (EBL) has been know since the beginning of the century. This characteristics has been largely utilized for the EBL clinical diagnosis through the use "hematologic keys". The objetive of this paper was to evaluate the hematologic condition of black and white holstein cows infected by Bovi-ne Leukosis Virus (BLV) with special attention to lymphocytic morfology. 797 blood samples were collected from 423 cows reagents in Agar Gel Immodiffusion Test (AGID) and from 374 no reagent cows, for hematologic examination. The results showed that no statistical differences between reagent and no reagent cows were found in polymorphic nuclear leucocyte counts and monocyte counts but the AGID-reagent cows showed higher lymphocytes counts than the no AGID-reagent cows. When the lymphocytic morphologic was analysed, the results showed that only the monocytic cell counts and the double nucleus lymphocyte counts were higher in AGID-reagent animals.

RÉSUMÉ

Depuis le début du siècle on savait que les animaux avec Leucose Enzootic Bovine (LEB) avaient leucocytose pour lymphocytose. Cet aspect a été beaucoup utilisé comme recours dans le diagnostic clinique de la LEB employant les "clés leucometriques". Ce travail a eu le but d'évaluer les altérations leucocytaires dans femelles bovines natu-ralment infectées pour le Virus de la Leucose Bovine (VLB) avec particulaire préocupa-tion à la morphologie lymphocytaire. Dans ce but, 797 portions du sang on été obtenues de 423 animaux positifs et 374 négatifs à la immunodiffusion en gélose (ID). Les résul-tats ont démontré qu'il y a différences statistiques entre les animaux ID-positifs et ID-negatifs seulement dans le numero de lymphocytes. Dans ce qui concerne à la morpholo gie lymphocytaire, on a trouvé une élévation du raconte des lymphocytes monocytés et des lymphocytes avec le noyau double les animaux ID-positifs.

Tabela 3 - Valores médios e desvios padrões do número total de linfócitos, de linfócitos atípicos e de linfócitos típicos (grandes e pequenos) por mm^3 de sangue de fêmeas bovinas da raça holandesa branca e preta distribuídos segundo a faixa etária dos animais e a reação frente ao antígeno cápsula (gp51) do vírus da Leucose Bovina. São Paulo. 1990

FAIXA ETÁRIA EM ANOS	Nº TOTAL DE LINFÓCITOS		Nº TOTAL DE LINFÓCITOS ATÍPICOS		Nº TOTAL DE LINFÓCITOS TÍPICOS		LINFÓCITOS GRANDES		LINFÓCITOS PEQUENOS	
	r	nr	r	nr	r	nr	r	nr	r	nr
- de 1	14786	11683	3211	2597	11575	9085*	3230	2406	8345	6679*
	7871	5828	2632	1834	5998	4775	3105	2245	5496	4634
1 a 2	16530	12473*	3590	2450	12941	10024*	4264	2818*	8676	7205*
	6834	5298	2458	1308	5575	4490	4114	2842	4977	4269
2 a 3	13702	10086*	2335	2119	11367	7967*	2194	2073	9172	5893
	6834	4316	1341	1194	6267	3787	1906	1687	6094	3460
3 a 4	15203	10578*	2875	2207*	12328	8370*	2827	1964*	9501	6407*
	7414	5094	1889	1192	6217	4492	2438	1312	5258	4536
4 a 5	12138	10580	2070	2055	10068	8525	1627	2025	8441	6501
	6922	6813	1244	1390	6221	5865	1269	1601	5912	5972
5 a 6	13659	7557*	2117	1436*	11542	6121*	2594	1557	8948	4564
	14780	2759	1640	608	13799	2652	2953	1267	13027	2593
+ de 6	12259	7300*	2199	1263*	10059	6037*	2389	1746	7670	4291*
	11058	4414	2196	487	9128	4241	3180	1303	6794	3262
TOTAL	14233	10844*	2698	2236*	11535	8608*	2799	2277*	8735	6331*
	8639	5447	2049	1442	7432	4614	2958	2124	6649	4348

* diferença estatisticamente significante para alfa = 5%; r = reagentes; nr = não reagentes

Tabela 2 - Valores médios e desvios padrões dos elementos constituintes do leucograma (em células por mm^3 de sangue) de fêmeas bovinas da raça holandesa branca e preta distribuídas segundo a faixa etária dos animais e a reação frente ao antígeno capsular (gp 51) do vírus de leucose Bovina. São Paulo. 1990

FAIXA ETÁRIA EM ANOS	LEUCÓCITOS		NEUTRÓFILOS		EOSINÓFILOS		BASÓFILOS		MONÓCITOS		LINFÓCITOS	
	r	nr	r	nr	r	nr	r	nr	r	nr	r	nr
- de 1	19731	16066*	4155	3655	513	475	103	67	171	190	14786	11683*
	8229	6635	2278	2523	646	587	157	118	161	190	7871	5828
1 a 2	21932	17669*	4069	3667	1081	1195	84	79	224	219	16530	12473*
	7225	6152	1885	1822	873	914	140	136	238	212	6834	5298
2 a 3	19257	15533*	3965	3797	1235	1320	83	89	292	275	13702	10086*
	7065	4846	1761	1497	826	1061	135	112	414	260	6834	4316
3 a 4	20622	16113*	3765	4145	1278	1079	91	75	239	243	15203	10578*
	7607	5571	1445	3421	1043	659	168	105	251	193	7414	5094
4 a 5	17383	16004	3817	3443	1213	1656	64	107	162	252	12138	10580
	7542	7517	2125	1287	684	1284	116	130	162	251	6922	6813
5 a 6	19247	12050*	3766	3033	1367	1145	83	64	322	262	13659	7557*
	15138	3151	1842	1374	1069	564	182	106	390	172	14780	2759
+ de 6	16806	12586*	3205	3609	1080	1406	72	101	188	187	12259	7300*
	11434	5051	1638	1933	752	1042	167	161	168	141	11058	4414
TOTAL	19505	15869*	3859	3679	1103	1044	84	80	231	225	14233	10844*
	8998	6113	1868	2164	879	944	150	124	286	210	8639	5447

* = diferença estatisticamente significante para alfa = 5%; r = reagentes; nr = não reagentes

Tabela 4 - Valores médios e desvios padrões dos números absolutos (por mm^3 de sangue) dos diferentes tipos de linfócitos atípicos de fêmeas bovinas da raça holandesa branca e preta distribuídas segundo a faixa etária dos animais e a reação frente ao antígeno capsular (gp 51) do vírus da Leucose Bovina. São Paulo, 1990.

FAIXA ETÁRIA EM ANOS	LINFÓCITOS MONOCITÓIDES		SOMBRAS DE GUMPRECHT		LINFÓCITOS COM NÚCLEO MARCADO		LINFÓCITOS COM NÚCLEO DUPLO		LINFÓCITOS PICNÓTICOS		LINFÓCITOS COM GRANULAÇÕES AZURÓFILAS		CÉLULAS DE TURK	
	r	nr	r	nr	r	nr	r	nr	r	nr	r	nr	r	nr
- de 1	1066	905	1142	792	102	113	233	112	288	329	238	214	142	132
	892	650	2011	1468	168	177	461	293	389	392	304	250	217	164
1 a 2	1048	796*	1341	695*	111	151	377	124*	284	362	328	237	99	84
	647	614	2134	719	184	212	508	325	343	458	358	245	154	132
2 a 3	762	747	590	638	72	75	218	87*	377	252*	226	256	100	75
	530	602	807	981	139	169	332	146	450	300	293	243	179	116
3 a 4	815	697	917	617	133	63	332	138*	369	266	256	334	60	91
	597	510	1454	702	363	99	679	267	401	269	299	328	112	123
4 a 5	601	694	462	445	144	107	172	182	332	265	298	336	61	36
	410	539	643	653	206	172	227	442	339	331	260	271	104	64
5 a 6	697	474	388	400	235	67	147	119	237	205	352	122*	61	48
	755	592	633	348	817	170	244	241	286	237	281	125	147	82
+ de 6	771	368*	459	333	100	64	275	93	227	141	284	246	71	27*
	686	240	917	385	179	122	627	157	144	166	245	171	122	56
TOTAL	839	692*	804	641	117	104	261	118*	310	291	277	246	89	87
	692	591	1454	1008	313	176	476	282	372	364	300	251	158	133

* = diferença estatisticamente significante para alfa = 5%; r = reagente; nr = não reagente

ENTWICKLUNG UND PRUFUNG EINES GERATES ZUR PANSENSAFTENTNAHME UND -UBERTRAGUNG SOWIE ZUR EINGABE FLUSSIGER ARZNEIMITTEL BEIM ERWACHSENEN RIND

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Die Pansensaftentnahme kann zu diagnostischen und zu therapeutischen Zwecken genützt werden (3,4). In der BRD ist die Pansensaftentnahme beim erwachsenen Rind bisher aber nur in geringen Umfang in die tierärztliche Praxis eingeführt. Der Grund dafür dürfte nicht zuletzt darin zu suchen sein, daß weder die mancherorts dazu verwendeten Nasenschlundsonden, noch die bisher speziell dazu im Handel befindlichen Geräte (1,2,9) die Anforderungen, die an ein solches Gerät zu stellen sind (6), hinreichend erfüllen. Wir haben daher ein neues Gerät entwikkelt und geprüft (5,6,7,8).

MATERIAL UND METHODE

Dieses Gerät besteht aus einer Maul-Pansen-Sonde (Prinzip SØRENSEN & SCHAMBYE) (Abb.1), einer Saugpumpe (Abb. 2) und einem Fülltrichter. Über einen Schnellverschluß kann die Sonde zur Pansensaftentnahme an die Saugpumpe, zur Eingabe flüssiger Arzneimittel oder zur Pansensaftübertragung an den Fülltrichter und zur Reinigung an einen Wasserhahn angeschlossen werden. Die Saugpumpe besteht aus einer Punpeinheit und einem Auffanggefäß. Zur Aufnahme kleinerer Volumina Pansensafts zu Untersuchungszwecken steht ein 1-l-fassende Gefäß, zur Aufnahme größerer Volumina Pansensafts zu Behandlungszwecken ein 3-l-fassende Gefäß zur Verfügung.

Abb. 1: Maul-Pansen-Sonde

Abb. 2: Saugpumpe mit angeschlossenem 3-l-Gefäß, 1-l-Gefäß

A. PRUFUNG DER SONDE AN KUHEN MIT PANSENFISTELN

Ein Vorgängermodell (5,7) der aktuellen Sonde wurde an 3 Kühen der Rasse Deutsche Schwarzbunt (Tab. 1) geprüft.
Versuchsanordnung:
1. Um herauszufinden, wie weit die Sonde eingeschoben werden muß, damit ihr Sondenkopf in den ventralen Pansensack gelangen kann, wurde

bei den 3 Versuchstieren die Entfernung von den Schneidezähnen zum ventralen Pansensack ermittelt. Dazu wurde mit Hilfe der Sonde jeweils die Entfernung von den Schneidezähnen zum hintersten Punkt des ventralen Pansenendblindsacks und die Entfernung von den Schneidezähnen zum hintersten Punkt der linken 9. Rippe (vorderer Bereich des ventralen Pansensacks) gemessen und daraus die Mittelwerte errechnet.

2. Prüfung der Sonde (je 30 Einsätze vor der morgendlichen Fütterung): Es wurden folgende Kriterien berücksichtigt: Zum Einschieben der Sonde erforderliche Zeit, Volumen des "Spontanabflusses" innerhalb von 2 Minuten, Anzahl der Verstopfungen des Saugschlauchs und Anzahl der verstopften Sondenkopföffnungen bei der Entnahme von 2 l Panseninhalt, zur Eingabe von 2 l Pansensaft - der mit dieser Sonde gewonnen wurde - bzw. von 2 l Wasser (je 15 Einsätze) über Fülltrichter und Sonde erforderliche Zeit.

3. Manuelle Ermittlung der Lage des Sondenkopfes im Hauben-Pansenraum über Pansenfistel, vor der morgendlichen Fütterung und 3 bis 4 Stunden nach der morgendlichen Fütterung (je 30 Einsätze).

4. Untersuchung der mittels Maul-Pansen-Sonde gewonnenen Pansensaftproben auf sondierungsbedingten Speichelzufluß: Im Zuge von Speichelbeimengungen kommt es zu meßbaren Veränderungen des pH-Werts sowie des Na- und K-Gehalts im Pansensaft (Anstieg der pH-Werts und des Na-Gehalts, Absinken des K-Gehalts). Anhand der Unterschiede in per Maul-Pansen-Sonde und per Pansenfistel entnommenen Proben kann sondierungsbedingter Speichelzufluß ermittelt werden (10). Es wurden Proben vergleichend untersucht, die per Maul-Pansen-Sonde und per Stabsonde über Fistel vom gleichen Ort des Panseninnern entnommen wurden, wobei die Entnahme der Probe per Maul-Pansen-Sonde zum einen vor und zum anderen nach der Entnahme der Probe per Stabsonde erfolgte (je 15 Einsätze).

ERGEBNISSE PRÜFUNG DER SONDE AN KÜHEN MIT PANSENFISTELN

Tab. 1: Alter (Jahre), Körpermasse (kg), Stockmaß (cm), Entfernung von den Schneidezähnen zum hintersten Punkt des ventralen Pansenendblindsacks (a), Entfernung von den Schneidezähnen zum hintersten Punkt der linken 9.Rippe (b), Mittelwerte x aus a und b (cm) der Versuchstiere M, K, F.

Tier	Alter	Masse	Stockmaß	a	b	x
M	6	630	135	227	182	204
K	4½	610	134	224	182	203
F	5	567	133	226	180	203

1. Die Sonde muß bei erwachsenen Kühen der Rasse Deutsche Schwarzbunt auf eine Länge von 1,8 bis 2,25 m eingeschoben werden, damit ihr Sondenkopf in den ventralen Pansensack gelangen kann (Tab. 1). Für die weiteren Untersuchungen wurde die Sonde jeweils auf eine Länge von 2,0 m eingeschoben.

2. Siehe Tab. 2.

3. Vor der morgendlichen Fütterung gelangte der Sondenkopf in 2 Fällen (7%) in den Schleudermagen und in 28 Fällen (93%) in den ventralen Pansensack. 3 bis 4 Stunden nach der morgendlichen Fütterung gelangte der Sondenkopf in 3 Fällen (10%) in den Schleudermagen, in 2 Fällen (7%) in die Pansenmitte und in 25 Fällen (83%) in den ventralen Pansensack.

Tab. 2: Mittelwert x, Maximalwert xMax und Minimalwert xMin der zum Einschieben der Sonde erforderlichen Zeit tE (Sek.), des Volumens v (l) an "Spontanabfluß" von Pansensaft innerhalb von 2 Minuten, der Anzahl der Verstopfungen des Saugschlauchs nS und der Anzahl der verstopften Sondenkopföffnungen nö bei der Entnahme von 2 l Panseninhalt sowie der erforderlichen Zeit zur Eingabe von 2 l Pansensaft tP (Sek.) und der zur Eingabe von 2 l Wasser tW (Sek.) über Fülltrichter und Sonde.

	tE	v	nS	nö	tP	tW
x	41	1,2	0	7	50	52
xMax	75	2,4	0	60	60	72
xMin	25	0,4	0	0	45	40

4. Pansensaftproben, die per Maul-Pansen-Sonde entnommen wurden, unterschieden sich hinsichtlich pH-Wert, Na- und K-Gehalt nicht signifikant von Proben, die per Stabsonde über Fistel vom gleichen Ort des Panseninnern gewonnen wurden. Die Reihenfolge der Entnahme (zuerst Maul-Pansen-Sonde, dann Stabsonde bzw. umgekehrt) hatte keinen Einfluß auf den Unterschied (t-test) (Tab.3).

Tab. 3: Mittelwert x und Streuung (s) der Differenz von Pansensaftproben bezüglich pH-Wert, Na- und K-Gehalt (mmol/l), die jeweils per Maul-Pansen-Sonde bzw. per Stabsonde über Pansenfistel vom gleichen Ort des Pansenraums gewonnen wurden, unterteilt nach Entnahmefolge in 2 Probegruppen: P1 (zuerst Maul-Pansen-Sonde dann Stabsonde), P2 (zuerst Stabsonde dann Maul-Pansen-Sonde).

	x		s	
Proben gruppe- Parameter	P1	P2	P1	P2
pH	0,03	-0,01	0,07	0,17
Na	1,97	-1,84	9,46	13,31
K	-0,69	-1,18	11,12	5,61

B. PRÜFUNG DES GERÄTS AN GESUNDEN KÜHEN

Das in den Untersuchungen an Kühen mit Pansenfisteln verwendete Vorgängermodell wurde danach abgeändert (8) und das so entstandene aktuelle Modell der Sonde zusammen mit der Saugpumpe an 106 gesunden Kühen der Rasse Deutsches Fleckvieh geprüft. Besonderes Interesse galt dabei der Frage, ob mit diesem Gerät 2 l Vormageninhalt zu gewinnen sind und wann der günstigste Zeitpunkt zur ihrer Entnahme ist (8).

Versuchsanordnung:

Jedem Versuchstier wurde, vor oder 1 bis 7 Stunden nach der morgendlichen Fütterung, die Sonde auf eine Länge von 2,0 m eingeschoben und

Pansensaft mittels Saugpumpe abgepumpt. Protokolliert wurden die zur Entnahme von 2 l Vormageninhalt erforderliche Zeit und die dazu vorgenommenen Lageänderungen der Sonde. Die Zeitmessung erfolgte ab Pumpbeginn. Lageänderungen der Sonde wurden vorgenommen, sobald trotz Pumpbewegungen kein Panseninhalt mehr in das Auffanggefäß einströmte. Dazu wurde die Sonde um 30 cm bis einen Meter herausgezogen und erneut auf eine Länge von 2.0 m eingeschoben.

ERGEBNISSE DER PRÜFUNG DES GERÄTS AN GESUNDEN KÜHEN

Tab. 4: Zur Gewinnung von 2 l Pansensaft nötige Zeit (Mittelwert t, Maximalwert tMax, Minimalwert tMin) (Sek.) und dazu vorgenommene Lagekorrekturen (Mittelwert k, Maximalwert kMax, Minimalwert kMin) der Sonde während n Probenahmen in Abhängigkeit vom Fütterungszeitpunkt (h: Stunden nach Abschluß der morgendlichen Fütterung, h=0: vor der morgendlichen Fütterung).

h	0	1	2	3	4	5	6	7
n	5	15	15	14	14	15	15	13
t	69	144	111	97	101	129	136	158
tMax	105	210	165	165	180	180	210	255
tMin	30	90	60	60	60	90	60	90
k	0,8	2,6	1,6	1,2	1,2	1,7	2,0	3,0
kMax	1	4	4	3	2	4	4	5
kMin	0	0	0	0	0	0	0	1

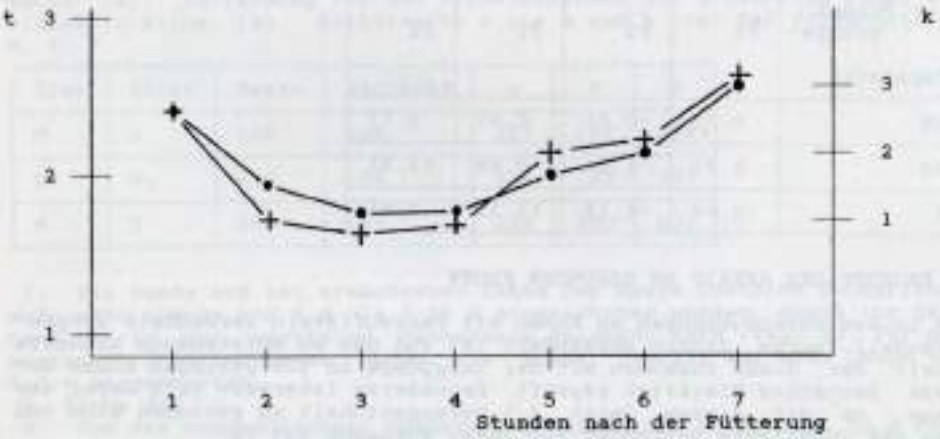

Abb. 3: Zur Gewinnung von 2 l Pansensaft erforderliche Zeit t (Minuten) (+) und Anzahl der dazu vorgenommenen Lagekorrekturen k (•) der Sonde, in Abhängigkeit vom Fütterungszeitpunkt.

BESPRECHUNG

A. Um in den ventralen Pansensack gelangen zu können, sollte die hier vorgestellte Sonde bei Kühen der Rasse Deutsche Schwarzbunt auf eine Länge von 2,0 m eingeschoben werden. Nach Einschieben auf eine Länge von 2 m gelangte der Sondenkopf des Vorgängermodells der aktuellen Sonde mit hinreichender Sicherheit in den ventralen Pansensack. In den so gewonnenen Pansensaftproben konnte kein sondierungsbedingter Speichelanteil nachgewiesen werden, da sich Proben, die mit dieser Sonde entnommen wurden, hinsichtlich der zum Nachweis von Speichel im Pansensaft geeigneten Parameter pH-Wert, Na-und K-Gehalt nicht signifikant von Proben unterschieden, die per Stabsonde über Fistel entnommen wurden. Verstopfungen der Sonde behinderten den Entnahmevorgang nicht. Sie eignete sich auch zum Abhebern von Pansensaft. Außerdem können mit dieser Sonde, bei angeschlossenem Fülltrichter, wässrige Lösungen und Pansensaft, der zuvor per Sonde entnommen wurde, eingegeben werden.

B. Sonde (aktuelles Modell) und Saugpumpe haben sich zur Entnahme therapeutisch nutzbarer Volumina Vormageninhalts (2 l) als zweckdienlich erwiesen. Der günstigste Zeitpunkt zur Gewinnung von 2 l Panseninhalt ist vor der morgendlichen Fütterung und etwa 3 Stunden nach Abschluß der morgendlichen Fütterung. Mittels Lageänderungen der Sonde konnten Abflußstörungen beim Abpumpen von Panseninhalt behoben werden.

Die Aussagen der Prüfung des Vorgängermodells der Sonde an Kühen mit Pansenfisteln können auf die hier vorgestellte aktuelle Sonde übertragen werden, da sich beide Sonden nicht wesentlich unterscheiden (8). Somit eignet sich das hier vorgestellte Gerät nicht nur zur Entnahme repräsentativer Pansensaftproben zu Untersuchungszwecken, sondern auch zur Entnahme und Eingabe von Pansensaft zu Behandlungszwecken (Pansensaftübertragung). Außerdem können über Fülltrichter und Sonde in Wasser gelöste Arzneimittel eingegeben werden, z.B. im Anschluß an eine Pansensaftentnahme und -untersuchung, wobei die Sonde während der Untersuchung in der Patientin verbleibt.

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ZUSAMMENFASSUNG

Es wurde ein Gerät zur Entnahme und zur Übertragung von Pansensaft sowie zur Eingabe flüssiger Arzneimittel beim erwachsenen Rind entwickelt und geprüft. Das Gerät besteht aus einer Maul-Pansen-Sonde, einer Saugpumpe und einem Fülltrichter. Über einen Schnellverschluß kann die Sonde entweder an die Saugpumpe (zur Pansensaftentnahme) oder an den Fülltrichter (zur Pansensaftübertragung, Eingabe in Wasser gelöster Arzneimittel) angeschlossen werden.

Nach den Ergebnissen der Prüfung an Kühen mit Pansenfisteln und an gesunden Kühen eignet sich das Gerät sowohl zur Entnahme repräsentativer Pansensaftproben zu Untersuchungszwecken, als auch zur Entnahme grösserer Volumina Panseninhalts für die Pansensaftübertragung, wobei der günstigste Zeitpunkt zur Entnahme vor bzw. etwa 3 Stunden nach Abschluß der morgendlichen Fütterung ist.

SUMMARY

A instrument for acquisition and transfer of rumen fluid and for administration of liquid medicaments in adult cattle was developed and tested. The device consists of an oro-ruminal probe, a suction pump and a funnel. The probe can be connected to the suction pump (for acquisition of rumen fluid) or to the funnel (for administration of rumen fluid or liquid medicaments).
Following the results of the testing in fistulated cows and healthy cows the device is suitable for the acquisition of representative samples of rumen fluid for diagnostic purposes and for the collection of forestomach content for transfer purposes. The most favorable time for collection of forestomach content via probe and pump is before and about 3 hours after the cows were fed in the morning.

RESUMEN

Un instrumento para la extracción y la transfusión de liquido ruminal y para administrar medicamentos liquidos en el bovino adulto fue desarrollado y probado. El instrumento consiste en una sonda oro-ruminal, una bomba aspirante y una tolva. Se junta la sonda con la bomba para la extracción de liquido ruminal. Para la transfusion de jugo ruminal o para administrar medicamentos liquidos se junta la sonda con la tolva.
Segun la prueba del instrumento en vacas con fistula ruminal y en vacas sanas el instrumento es apropiado para la obtencion de muestras representativas de jugo ruminal (intención: evaluación del jugo ruminal) y para la extraccion y transfusion de cantidades terapeuticas del liquido ruminal. El tiempo mas favorable para la extracción de liquido ruminal es antes y 3 horas despues la alimentation de las vacas en la mañana.

Vertreiber des Gerätes
distributor of the instrument
distribuidor del instrumento

HEILAND, Albert-Schweitzer-Ring 5, BRD-2 Hamburg 70, Tel. 040/66987100

TRATAMENTO INTERMITENTE DA TUBERCULOSE BOVINA COM ISONIAZIDA

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INTRODUÇÃO

A hidrazida do ácido isonicotínico (isoniazida) reúne um conjunto de propriedades que a torna um quimioterápico eficaz e prático para o combate da tuberculose bovina em países que não podem adotar o abate e a indenização dos reagentes. Em doses altas a isoniazida exerce ação bactericida sobre o Mycobacterium bovis e em doses menores atua como bacteriostático, levando a micobactéria a um estado de latência com progressiva perda de patogenicidade e morte quando a administração é prolongada (Kleeberg 1967a). Em culturas de M. bovis (amostra BCG) foi demonstrado que a isoniazida inibe a síntese do DNA, RNA e proteína. A inibição do DNA provoca perda da viabilidade da cultura (McClatchy 1970).

A isoniazida pura, em forma de sal cristalizado, quando fornecido ao bovino, por via oral, misturado na ração ou dissolvido na água ou no leite, em doses adequadas alcança níveis terapêuticos no soro-sanguíneo, nos tecidos e se difunde rapidamente nas lesões causadas pela tuberculose, mantendo-os durante 18 a 24 horas (Kleeberg & Weyland 1961, Cerruti et al. 1961, Straka 1968). Kleeberg et al (1966) demonstraram bacteriologicamente a cura estéril em 78%.

A posologia utilizada no tratamento da tuberculose bovina variou muito nos últimos três decênios. A dosagem inicialmente utilizada de apenas 4 mg/kg p.v. passou a ser até de 30 mg e as vias subcutânea e intramuscular foram substituídas pela via oral. A duração da medicação variou de 30 dias até 30 meses e as percentagens de curas cresceram progressivamente até 96,4% (Langenegger et al. 1988).

Predominantemente o regime de medicação da isoniazida, tanto na medicina humana quanto na da veterinária, consistia na administração contínua, diária, por períodos longos. Em tratamentos mais recentes foram usadas dosagens maiores nos primeiros meses do tratamento e a duração foi sendo reduzida até 6 meses.

No tratamento da tuberculose bovina a medicação intermitente se limitou a alguns ensaios, em que períodos de administração contínua por 15 ou 60 dias eram interrompidos por 15 dias de descanso (Rosati & Tini 1955, Nai & Crespi 1958, Nai & Perini 1960, D'Ascani & Micozzi 1961).

Langenegger et al (1981a) vinham utilizando um regime misto em que nos primeiros 60 dias a isoniazida era administrada diariamente e depois num período de 4 meses e 21 dias o medicamento era fornecido em dias alternados, ou mais precisamente, nas 2as, 4as e 6as feiras, em doses de 25 mg/kg p.v., obtendo-se com esta posologia, em média, 95% de curas estéreis dos animais tratados nos últimos 15 anos.

Na presente pesquisa avaliou-se a eficácia do tratamento intermitente desde o início do período de medicação.

MATERIAL E MÉTODOS

A experimentação foi realizada num rebanho leiteiro da raça holandesa preto-e-branca, com 254 animais, que acusou a presença de 55 reagentes positivos e 13 suspeitos para tuberculose. O diagnóstico alérgico da tuberculose foi feito com tuberculina PPD bovina, contendo 5.000 UI por dose e tuberculina PPD aviária com 2.500 UI. Os critérios de interpretação das reações obedeceram às normas oficiais adotadas pelo Ministério da Agricultura (Langenegger et al. 1981b)para a tuberculinização comparativa simultânea conforme mostra o Quadro 1.

Quadro 1. Chave de interpretação da tuberculinização comparativa simultânea.

Testes simultâneos		Diferença mm	Resultado em relação à tuberculose
TB[(a)]	menor que TA[(b)]	-	Negativo
TB	maior que TA	0,1 a 1,9	Negativo
TB	maior que TA	2,0 a 2,9	Suspeito
TB	maior que TA	3,0	Positivo

(a) TB = Tuberculina bovina.

(a) TA = Tuberculina aviária.

A anamnese revelou a existência da tuberculose em anos anteriores e a inspeção clínica comprovou apenas a existência de tosse curta e seca em alguns animais.

Os bovinos reagentes positivos e suspeitos foram isolados dos demais animais da fazenda em pasto e abrigo próprio e o peso corporal de cada animal foi avaliado por estimativa.

O tratamento da tuberculose foi feito com a hidrazida do ácido isonicotínico (isoniazida), sob forma de sal cristalizado, fornecida por via oral aos bovinos misturada na ração concentrada, individualmente, em doses de 25 mg/kg p.v. nas 2ªs, 4ªs e 6ªs feiras, durante 6 meses perfazendo um total de 80 doses.

Durante o período de aplicação da medicação não foi feito nenhum teste alérgico.

Passados 90 dias após o término da medicação, foram realizadas 4 tuberculinizações com intervalos de 3 meses para avaliar o índice de cura através da dessensibilização alérgica.

RESULTADOS

O tratamento intermitente com 25 mg/kg p.v. de isoniazida, durante 6 meses, seguido do monitoramento alérgico de 3 em 3 meses, revelou progressiva dessensibilização dos animais conforme mostra o Quadro 2.

A julgar pelas reações negativas nos testes de tuberculinização, verificou-se maior índice de cura (31 casos), até os 90 dias após o término da medicação e que aos 360 chegou a 63. Paralelamente diminuiram os reagentes positivos que aos 360 dias pós-tratamento passou a zero. As reações suspeitas permaneceram numa faixa intermediária como ilustra a Figura 1. A persistência de apenas 5% de animais com reação suspeita, equivale a 100% de cura, pois a experiência já demonstrou que estas reações suspeitas tendem a regredir com o tempo.

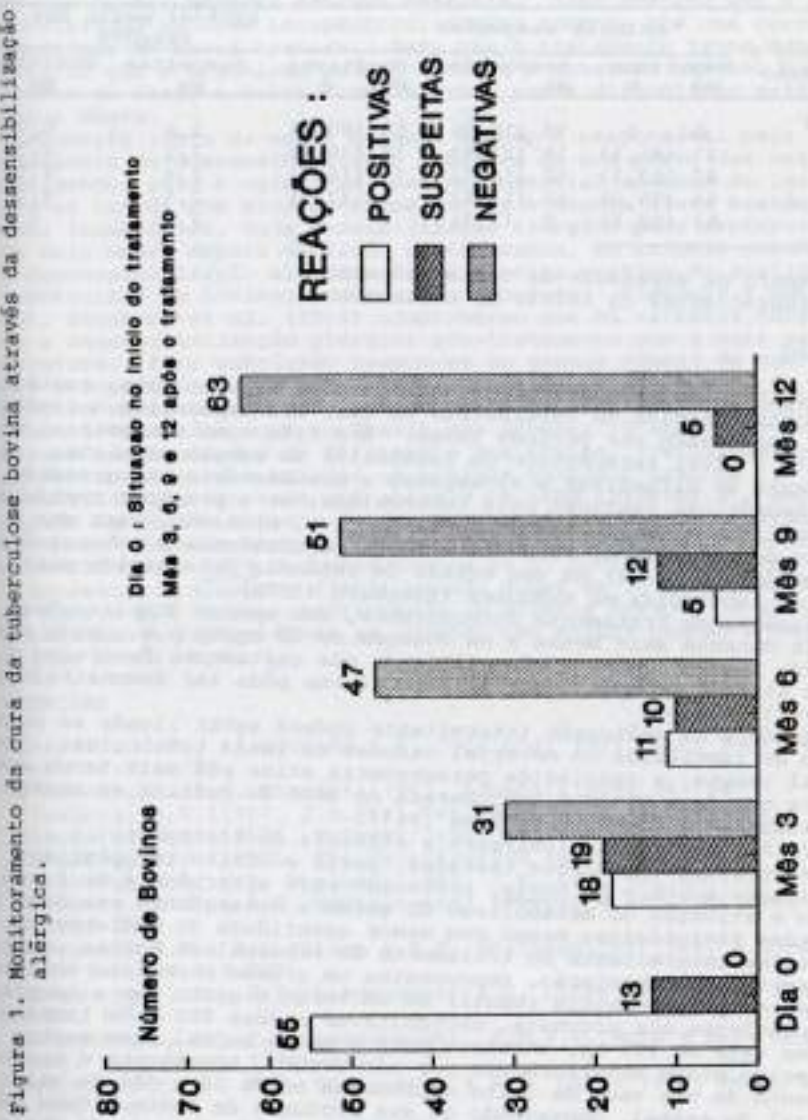

Figura 1. Monitoramento da cura da tuberculose bovina através da dessensibilização alérgica.

Quadro 2. Resultado da dessensibilização alérgica após a medicação com isoniazida.

Dias após a medicação	Animais reagentes						AEDP(a) médio das reações	
	Negativos		Suspeitos		Positivos		Suspeitas	Positivas
	Nº	%	Nº	%	Nº	%	mm	mm
0(b)	0	0	18	(19,2)	55	(80,8)	2,6	6,0
90	31	(45,5)	19	(27,9)	18	(26,4)	2,3	4,7
180	47	(69,1)	10	(14,7)	11	(16,1)	2,4	4,7
270	51	(75,0)	12	(17,6)	5	(7,3)	2,1	4,3
360	63	(92,6)	5	(7,3)	-	0	2,1	-

(a) Aumento da espessura da dobra da pele.
(b) Situação antes do início do tratamento.

DISCUSSÃO

O resultado do presente trabalho trouxe um aspecto novo, contestando a posologia clássica da tuberculose na qual era imperativo o tratamento diário, continuado por período longo. Era tido como fundamental manter constante o nível terapêutico da isoniazida no sangue para que: a) o medicamento se difundisse e alcançasse a micobactéria nas grandes massas necróticas causadas pela tuberculose; b) a presença contínua da droga suprisse a multiplicação do M. bovis e, c) ainda, para que a presença do medicamento vencesse a longa persistência e sobrevivência do germe (até 6 meses) em seu estado de latência determinado pelo efeito da isoniazida em subdoses (Kleeberg 1967a).

A eficácia do tratamento intermitente, com apenas 3 aplicações semanais durante seis meses e na dosagem de 25 mg/kg p.v., pode ser atribuída à alta concentração utilizada que certamente favoreceu a terapia através da ação bactericida conforme pôde ser demonstrado por Kleeberg (1967a,b).

A eficácia da medicação intermitente poderá estar ligada ao possível acúmulo de isoniazida no material caseoso da lesão tuberculosa. Neste material inerte, a isoniazida permaneceria ativa por mais tempo como acontece quando a droga é incorporada ao meio de cultura em testes de sensibilidade (Kleeberg & Weyland 1961).

Outra hipótese admissível para a eficácia do tratamento intermitente, nas condições testadas, seria o efeito terapêutico tindalizante sobre o M. bovis, provocado pela alternância de fases de redução e ativação do metabolismo do germe e conseqüente exaustão das atividades fisiológicas mesmo com menor quantidade do medicamento.

O regime intermitente do tratamento da tuberculose bovina, empregado na presente experimentação, representou um grande benefício econômico ao produtor pois permitiu reduzir em um terço o gasto com a isoniazida. Com a posologia ora proposta, necessita-se apenas 800 g de isoniazida para uma vaca de 400 kg, enquanto para o mesmo animal eram gastos 1200 g no regime misto anteriormente por nós usado. Atualmente o custo do tratamento de uma vaca de leite representa entre 10 e 15% do valor comercial do animal, dependendo da sua produção de leite. Como não há condições de obter indenização para os reagentes, a opção do abate representaria uma perda média de 50% do valor comercial. Este é um argumento forte que leva o produtor a optar pelo tratamento.

A duração de 6 meses no tratamento da tuberculose não pode ser reduzida por causa da lenta absorção das grandes massas caseosas das

lesões tuberculosas. Em experimento anterior verificou-se que em lesões com mais de 2 cm de diâmetro, após 2 meses de medicação contínua, ainda havia Mycobacterium bovis vivos e patogênicos no centro da lesão, ao passo que lesões menores estavam estéreis. Isto mostrou que a difusão da isoniazida, em dose terapêutica, ocorre somente até uma certa profundidade da massa caseosa. Para que o tratamento tenha êxito, é necessário que o organismo animal absorva o material caseoso da periferia da lesão e desta forma a droga possa difundir-se mais para o interior desta.

A absorção lenta da massa caseosa também é responsável pelo retardamento da dessensibilização alérgica de boa parte dos animais após o tratamento, pois o animal ao absorver material caseoso da lesão depara-se também com micobactérias mortas e diante disso mantém armado o sistema imunológico, cuja sensibilização alérgica pode manter-se até 12 ou mais meses depois de findo o tratamento, em animais curados.

A dessensibilização alérgica é uma maneira prática de avaliar a cura da tuberculose em bovinos embora seja demorado. Kleeberg & Worthington (1963), Kleeberg et al. (1966) comprovaram que há estreita correlação entre a dessensibilização alérgica pós-tratamento com a cura estéril da tuberculose. Esta conclusão baseou-se em grande número de necrópsias e exames bacteriológicos e longa convivência de grande número de bovinos curados em rebanhos indenes. Levado pelo monitoramento de muitos rebanhos já tratados aqui no Brasil nos últimos 15 anos, foi possível confirmar os resultados de Kleeberg e sua equipe. Verificou-se que podem ser considerados curados os bovinos quando o grau de dessensibilização decresce até atingir um AEDP inferior a 3 mm, que é alcançado na grande maioria dos casos aos 12 meses após o término do tratamento. Baseado neste critério, considerou-se que no presente experimento houve 100% de curas dentre os 55 bovinos reagentes positivos e 13 suspeitos. Kleeberg (1961) usando doses de 10 mg/kg/dia durante 8 meses obteve 92% de cura monitorando os animais durante 36 meses.

O tratamento intermitente se revelou tão eficaz quando o regime misto que vinha sendo usado.

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RESUMO

Foi adotado um regime de tratamento intermitente da tuberculose com isoniazida em 55 bovinos reagentes positivos e 13 suspeitos. A isoniazida em forma de sal cristalizado foi fornecida aos animais misturada em ração concentrada, em doses de 25 mg/kg p.v., nas 2as, 4as e 6as feiras, durante 6 meses. A cura da tuberculose foi avaliada pela dessensibilização alérgica, após o término do tratamento através de tuberculinizações comparativas realizadas de 3 em 3 meses, durante um ano. Este monitoramento revelou progressiva redução de sensibilidade alérgica até o desaparecimento definitivo. O tratamento intermitente com 3 dosagens de isoniazida semanais foi tão eficiente quanto o regime contínuo. O regime intermitente reduziu de 120 para 80 o número de doses administradas e reduziu o custo em 40%.

SUMMARY

An intermittent treatment regimen for bovine tuberculosis with isoniazid was used. Fifty five positive and 13 suspect reactors were treated orally with cristalized isoniazid (25 mg/kg bw) mixed in a concentrated ration given 3 times a week, during 6 months. The cure of the tuberculosis was evaluated after the treatment by allergic desensitization with comparative tuberculinizations performed in 3 months intervals, during a year. The monitoring showed progressive reduction of the allergic sensitivity until definitive disappearance. This experiment showed that the intermittent 3 week dosages was as good as the continuous regimen with the advantage of about 40 percent cost reduction.

ZUSAMMENFASSUNG

Ein intermittierendes Behandlungsschema mit isoniazid wurde in einer Rinderherde mit Tuberkulose angewandt, indem man 55 positiven und 13 verdächtigen Tieren oral kristallisiertes Isoniazid (25 mg/kg Körpergewicht), in ein Futterkonzentrat gemischt, 3 mal wöchentlich während 6 Monaten verabreichte. Die abheilung der Tuberkulose wurde nach Ende der Bechandlung durch allergische Dessensibilisierung mit, Tuberkulintesten in Abständen von 3 Monaten während eines Jahres beurteilt. Die Teste zeigten eine progressive Abnahme der allergischen Empfindlichkeit bis zu ihren völligen Verschwinden. Dieser Versuch hat gezeigt, dass intermittierende, 3 mal wöchentlich angewandte Dosierungen sich als ebenso gut erwiesen wie eine kontinuierliche, tägliche Behandlung, mit dem Vorteil einer Verringerung der Kosten um 40%.

INFLUÊNCIA DO FATOR ETÁRIO NO ERITROGRAMA DE BOVINOS LEITEIROS CRIADOS EM SÃO PAULO

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INTRODUÇÃO

Apesar da importância que os bovinos representam na agropecuária brasileira, inúmeras condições fisiológicas e patológicas podem influir sobre a produção destes animais e, sem possibilidade de contestação, pode-se afirmar que os parâmetros fisiológicos do gado holandês, há muito tempo adaptado às condições ambientais de manejo e alimentação das regiões sub-tropicais de nosso país, não foram ainda definitivamente estabelecidos ou, ainda, adequadamente estudados. Para tanto, nesta pesquisa, tomou-se cuidado em padronizar o material e a precisão das técnicas laboratoriais utilizadas para controlar ou eliminar a atuação de diferentes fatores de variabilidade, alguns considerados como passíveis de influenciar o eritrograma. Neste aspecto e com o objetivo de se verificar a influência do fator etário sobre o quadro eritrocitário foram analisadas amostras sangüíneas de animais da raça holandesa preta e branca, criados em cinco granjas produtoras de leite tipo B, no Estado de São Paulo. Os bovinos, todos do sexo feminino, foram divididos em grupos experimentais, cujas idades variavam do nascimento a mais de 72 meses de vida e eram criados na mesma região geográfica, submetidos a alimentação e manejo criatório zootecnicamente equivalentes. Antes da colheita das amostras eram submetidos a exame clínico, para se aquilatarem suas condições de saúde, sendo somente incluídos na presente pesquisa os animais considerados sadios, livres de hemoparasitas, brucelose e tuberculose. Além disso, não eram reagentes ao antígeno do vírus da leucose bovina na avaliação sorológica realizada.

MATERIAL E MÉTODOS

Constituição dos grupos experimentados

Os 321 animais foram distribuídos em sete grupos experimentais. O primeiro era formado por 27 animais lactentes com idades variando de um dia até 3 meses de vida. O segundo grupo experimental era composto por 24 animais desmamados de 3 a 6 meses de idade. O terceiro grupo constituia-se de 37 bezerras com idade variando de 6 a 12 meses, criadas em piquetes, algumas já púberes. O quarto grupo experimental era composto de 75 novilhas de 12 a 24 meses de idade. No quinto grupo incluiam-se 89 animais de 24 a 48 meses de vida, sendo vacas de primeira cria, com algumas lactantes e/ou gestantes. O sexto grupo experimental compunha-se de 43 vacas com idade compreendida entre 48 e 72 meses, lactantes e em fase de reprodução plena. O sétimo e último grupo experimental, com animais cuja idade era superior a 72 meses, era composto de 26 vacas consideradas zootecnicamente excepcionais, mantendo alta capacidade reprodutora e de produção.

Colheita das amostras de sangue

As amostras de sangue eram colhidas no período da manhã, por punção da veia jugular externa, sem garroteamento excessivo do vaso, evitando-se as manobras que pudessem excitar os animais. O material, nos

bovinos adultos, era colhido com agulhas 40 x 20 mm e nos animais até 6 meses de idade com agulhas 30 x 15 mm, devidamente montadas em seringas com 10 a 20 ml de capacidade. O sangue para a hematimetria era colocado em tubos de vidros siliconizados, providos de rolhas de borracha contendo 0,05 ml de uma solução aquosa de 10% de EDTA (6). O volume colhido era de 5 ml de sangue para as contagens do número de hemácias, determinação dos teores de hemoglobina, volume globular e contagem de reticulócitos. Para a sorologia o material era colhido em tubos de vidro, de 10ml de capacidade, adaptáveis a centrífuga apropriada, sendo o soro para a imunodifusão obtido após a retração do coágulo e separação por centrifugação. O material colhido era acondicionado em recipiente de isopor e mantido refrigerado durante o transporte até o laboratório, onde as provas eram realizadas antes de decorridas 6 horas de conservação.

Contagem de hemácias

Após as amostras serem homogeneizadas adequadamente por agitação mecânica, o sangue era aspirado em pipetas hematimétricas e, a seguir, diluido em líquido de Gower, obtendo-se uma suspensão de 1/200. Depois da homogeinização em agitador para pipetas tipo "Arthur Tomas", durante 1 minuto, desprezava-se 1/3 do conteúdo da pipeta e preenchia-se o hemocitômetro. As contagens foram efetuadas sobre áreas de 1/5 de mm^2, em câmara tipo Neubauer modificada com 1/10 de milímetro de altura (2).

Dosagem de hemoglobina

A dosagem de hemoglobina foi feita pelo método da cianometahemoglobina, empregando-se reativo comercial do laboratório da LABTEST, com leitura em espectofotômetro. Os resultados foram obtidos diretamente comparando-se os valores das provas com a leitura de padrão, cujo teor de hemoglobina era conhecido (2).

Determinação do volume globular

O volume globular foi determinado pelo método do hematócrito, empregando-se microtécnica, com uso de tubos capilares, centrifugados com aproximadamente 11.000 rpm, durante 5 minutos, aplicando-se uma força real de centrifugação equivalente a 13.000 g e leitura em cartão apropriado (2).

Cálculo dos índices hematimétricos absolutos

O volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM) e a concentração hemoglobínica corpuscular média (CHCM) foram calculados conforme preceitos clássicos (8).

Contagem de reticulócitos

Os reticulócitos foram pesquisados em esfregaços sangüíneos, inicialmente corados com solução alcoólica a 1% de azul cresil brilhante e posteriormente com o corante de Rosenfeld. A diferenciação foi pesquisada sobre 1.000 hemácias em campos microscópios homogêneos.

Imunodifusão para Leucose Bovina

A seleção de animais não reagentes ao antígeno do vírus da leucose bovina, foi realizada através de avaliação sorológica, utilizando-se a prova da dupla difusão, em placa de ágar de Ouchterloney empregando-se antígeno glicoproteico gp 51*.

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Análise estatística

Para calcular os valores da média aritimética, do devido padrão, desvio padrão da média e a análise de variância dos constituintes do eritrograma foi utilizado o programa BMDP - Biomedical Statistics Sofware, 1982 da Universidade da Califórnia. Realizou-se a comparação das médias obtidas aplicando o teste de Bonferroni (1).

RESULTADOS

O delineamento experimental utilizado na presente pesquisa permitiu uma avaliação segura da influência do desenvolvimento etário sobre o eritrograma de bovinos fêmeas da raça holandesa preta e branca, criadas em São Paulo, pois na população examinada inclue material obtido de animais perfeitamente estratificados segundo a idade, contendo cada grupo etário substancial número de amostras. Os resultados denotam haver a influência da idade sobre o eritrograma dos bovinos estudados sendo os maiores valores médios do número de hemácias, volume globular e taxa de hemoglobina observados nos animais jovens, refletindo-se esta variação de modo significativo, no cálculo dos índices hematimétricos absolutos. No número de hemácias do sangue circulante observou-se uma diminuição gradativa do nascimento até os 24 meses de idade, com os valores estabilizando-se após este período. O volume globular, por sua vez, também sofre influência significativa do fator etário ocorrendo diminuição até os 12 meses de vida, aumentando gradativa e significativamente entre esta faixa etária até os 72 meses de vida, para se estabilizar a seguir. Neste experimento, a variação do volume globular é inusitada, não se encontrando explicação baseada nos princípios da hematologia, quer relacionada a eritropoiese ou às falhas técnicas. O fato, provavelmente, relaciona-se ao manejo ao qual os animais são submetidos nas fazendas, onde os animais após o desmame e recria inicial em piquetes, são submetidos a regime de criação inadequado, quer no aspecto alimentar ou sanitário. Na oportunidade os animais infestam-se por carrapatos ou premunem-se contra Babesia sp e Anaplasma marginale, podendo, na dependência de estresse intenso adoecerem. Nesta fase da vida, as bezerras são frequentemente acometidas por babesiose ou anaplasmose. Na população que constitui a amostragem desta pesquisa, mesmo em exames microscópicos extenuantes, não se encontrou os referidos hemoparasitas, mas não se pode eliminar a possibilidade de alguns animais estarem em fase de convalescença, sem demonstração de parasitemia. Por outro lado, a taxa de hemoglobina sangüínea não apresentou variações significativas que pudessem ser atribuídas ao desenvolvimento etário. Em consequência às variações desproporcionais dos parâmetros do eritrograma durante o desenvolvimento etário, os índices hematimétricos absolutos sofreram significativa influência, ocorrendo um aumento gradativo do volume corpuscular médio e da hemoglobina corpuscular média. A concentração hemoglobínica corpuscular média demonstrou variações bem definidas atingindo valores máximos nos animais de 12 a 24 meses de vida. Na presente pesquisa não foi observada a presença de reticulócitos no sangue circulante dos 321 animais examinados. Os valores de referência, em termos médios, para a população bovina examinada foram: hemácias 6,42 ± 1,06 x $10^6/mm^3$; volume globular 30,12 ± 2,72%; taxa de hemoglobina 10,09 ± 0,98 g/dl; volume corpuscular médio 47,75 ± 6,69 μ^3; hemoglobina corpuscular média 16,03 ± 2,56 $\gamma\gamma$ e concentração hemoglobínica corpuscular média 33,54 ± 2,20%.

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RESUMO

Estudou-se o eritrograma de bovinos leiteiros, da raça holandesa preta e branca, do sexo feminino, objetivando reconhecer a influência do fator etário. Os 321 animais, criados na bacia leiteira de Campinas, no Estado de São Paulo, foram divididos em 7 grupos experimentais, cujas idades variavam do nascimento a mais de 72 meses de vida. Os animais eram sadios, não reagentes ao vírus da leucose bovina, livres de hemoparasitas, brucelose e tuberculose. Os resultados mostram haver influência da idade sobre o eritrograma dos bovinos estudados, sendo os maiores valores médios do número de hemácias, volume globular e taxa de hemoglobina observados nos animais jovens, refletindo-se esta variação, de modo significativo, no cálculo dos índices hematimétricos absolutos. Os valores de referência, em termos médios, para a população bovina examinada foram: hemácias 6,42 ± 1,06 x 10^6/mm³; volume globular 30,12 ± 2,72%; taxa de hemoglobina 10,09 ± 0,98 g/dl; volume corpuscular médio 47,75 ± 6,69μ³, hemoglobina corpuscular média 16,03 ± 2,56γγ e concentração hemoglobínica corpuscular média 33,54 ± 2,20%. Não se observou qualquer reticulócito nas lâminas examinadas.

SUMMARY

This research was conducted in order to investigate the effect of age on the eritrogram of 321 female Holstein cattle at Campinas dairy region, São Paulo State. All the animals used in this assay were healthy and free of Leucosis, Tuberculosis, Brucellosis and blood parasites. The animals were divided into 7 experimental groups ranging from birth to more than 72 months of age. The results showed an influence of age on the eritrogram. The highest mean values of erythrocyte count, packed cell volume and hemoglobin were obtained in calves, reflecting significantly upon the erythrocyte indexes. The average reference values for all the animals studied were: erythrocyte count 6,42 ± 1,06 x 10^6/mm³; packed cell volume 30,12 ± 2,72%; hemoglobin 10,09 ± 0,98 g/dl; mean corpuscular volume 47,75 ± 6,69μ³; mean corpuscular hemoglobin 16,03 ± 2,56γγ e mean corpuscular hemoglobin concentration 33,54 ± 2,20%. No reticulocytes were observed in any of the stained preparations examined.

RESUMEN

Se estudio el eritrograma de bovinos lecheros de la raza Holstein, del sexo femenino, procurando conocer el efecto de la edade sobre las constantes sanguíneas. Los 321 animales procedentes de la región lechera de Campinas, del Estado de São Paulo, fueron divididos em 7 grupos experimentales, cujas edades variaban entre el nacimiento hasta los 72 meses de vida. Los animales eram sanos, sin reacción al vírus de la leucosis bovina, libres de parásitos sanguíneos, brucelose e tuberculose. Los resultados muestram que hay influencia de la edad sobre el eritrograma de los bovinos estudados, siendo que los mayores valores medios del número de eritrócitos, volumen globular y la tasa de hemoglobina fueron observados en los animales jóvenes, se reflejando esta variación de manera significativa, en el cálculo de los índices hematínicos absolutos. Los valores de referencia en media, para la población examinada, fueron los seguintes: eritrócitos 6,42 ± 1,06 x 10^6/mm³; volumen globular 30,12 ± 2,72%; tasa de hemoglobina 10,09 ± 0,98 g/dl; volumen corpuscular medio 47,75 ± 6,69μ³; hemoglobina corpuscular media 16,03 ± 2,56γγ y concentracion corpuscular media de hemoglobina 33,54 ± 2,20%. No se observó ningun reticulocito en las láminas exaninadas.

FOTOSENSIBILIZACION EN VACAS LECHERAS POR INGESTA DE PARDOS CONTAMINADOS POR PITHOMYCES CHARTARUM

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INTRODUCCION

Diferentes agentes han sido mencionados como fotosensibilizadores en bovinos. Dentro de ellos, existen micotoxinas capaces de producir severas pérdidas en el ganado bovino. Tal es el caso del hongo Phitomyces Chartarum el cual está ampliamente distribuido y es causa de fotosensibilidad en el cuadro clínico conocido como exzema facial en bovinos y ovinos de diferentes partes del mundo (1,2,3,4,5). La toxina de dicho hongo, esporodesmina, se concentra en las esporas del hongo, produce una severa necrosis del tracto biliar (6). La presencia de condiciones climáticas ideales (temperatura y humedad elevadas) provocan sobre un sustrato de abundante materia vegetal muerta, una rápida proliferación de dicho hongo (2). Si bien los cuadros de fotosensibilización en bovinos por ingesta de pasturas contaminadas con P. Chartarum no son infrecuentes, la presencia por ingesta de heno contaminado no es común. En el presente trabajo se describe un brote de fotosensibilización por el consumo de heno en vacas lecheras de un tambo del noreste de la Provincia de Buenos Aires.

MATERIALES Y METODOS

El problema se presentó en un rodeo lechero compuesto por cuatrocientas vacas Holando Argentino en ordeñe mecánico ubicado en el partido de Salto, Provincia de Buenos Aires, Argentina. Los animales pastoreaban en un lote de avena con buena disponibilidad forrajera y diariamente recibian un suplemento a base de ocho kilogramos de maíz quebrado, cuatro kilogramos de expeller de girasol, y cinco kilogramos de heno mezcla de pasturas (gramíneas y leguminosas), de buena calidad, por animal. El promedio de la producción diaria, sobre la base de dos ordeñes, era de 19 litros por cabeza.

En el mes de mayo, al agotarse el heno suministrado, se lo sustituyó por una nueva partida de heno mezcla de pastura (alfalfa, trebol rojo, cebadilla, y pasto ovillo) el cual fué utilizado en similar volumen a lo mencionado. El resto de los componentes de la ración (concentrado) no sufrieron modificación siendo administrado como se citó anteriormente. Luego de 48 horas de administrar el nuevo heno comenzaron a observarse animales con prurito generalizado, caída en la producción, anorexia y decaimiento. Clínicamente los animales presentaron fiebre (40 a 41°C), constipación, ictericia, lagrimeo y conjuntivitis, dolor abdominal a la palpación, marcha vacilante con incordinación del tren posterior, orina de color oscuro, edema en morro, párpados, área submaxilar, ubre, vulva y periné. En algunos casos, las lesiones de piel eran muy severas con dermatitis. Los cuartos mamarios estaban dolorosos lo que dificultaba su ordeñe. Existió edema en el área de los flexores en los miembros posteriores, con sperganinamiento de la piel en la zona del garrón. A los diez - quince días de iniciado los síntomas las dermatitis fueron más severas con necrosis en las áreas de piel despigmentada, la cual se levantaba en forma de costras en morro, cuello, grupa, ubre y miembros posteriores. Uno de los animales más afectados muere a los 14 días de iniciado el brote. Se extrajeron muestras del heno problema para su análisis micológico.

Se indicó un tratamiento sintomático local a base de cremas humectantes con corticoides (triancinolona) y sistémicos con hepatoprotectores (acido tióctico al 0,5%), solución glucosada hipertónica al 50%, diuréticos (furosemida), antihistamínicos (clorhidrato de difenhidramina), tetracidina de acción prolongada y pomos antimastitis a base de penicilina y estreptomicina. Dicho tratamiento se instauró durante quince días, teniendo los animales sombra a su disposición. El heno problema fué eliminado de la ración luego de aparecer los primeros síntomas. Los animales siguieron en pastoreo en avena y recibieron dos kilogramos de concentrado por animal por día.

RESULTADOS

El curso del brote fué de aproximadamente 30 días. Durante dicho lapso, la producción láctea diaria decayó en 12,5%.

La morbilidad del brote fué del 11% y la mortalidad del 2.2%. El 15% de las vacas afectadas preñadas abortaron (fetos de 3 a 6 meses). Algunas de dichas vacas presentaron endometritis como complicación secundaria. El 52,3% de las vacas afectadas de fotosensibilización se secaron siendo la mamitis la complicación más severa observada.

Se observó una adecuada respuesta al tratamiento instituído.

El análisis del heno problema confirmó la presencia de un número elevado de esporas de Phitomyces Chartarum (> 100.000 / gramo). Dicho análisis fué gentilmente realizado por el Instituto de Patobiología del INTA, Castelar, Argentina.

Las lesiones presentes en el animal necropsiado evidenciaron una severa coloración ictérica en las mucosas, tejido subcutáneo y tejido adiposo. Las zonas despigmentadas de la piel afectadas estaban necróticas con formaciones costrosas. El hígado presentaba una marcada hepatomegalia y de consistencia firme, bordes engrosados y de color ocre al corte del órgano. Los canalículos biliares demostraban abundante retención de pigmento, la vesícula biliar se encontraba pletórica, con contenido biliar viscoso de color más intenso, que lo normal. Los riñones presentaban tinte ictérico al corte, mientras que la orina presente en la vejiga urinaria era de color ocre. Las heces presentes en el recto eran de color intenso con severo grado de deshidratación.

DISCUSION

El presente caso de fotosensibilización de origen hepatógeno debido a la presencia del hongo Phitomyces Chartarum en heno de pasturas contaminadas pone en evidencia las severas pérdidas económicas cuando el brote se instaura en un rodeo.

La acción de la toxina esporodesmina sería la responsable del cuadro clínico patológico descripto en el presente y coincide con lo mencionado por otros autores (1,2,5,6). Las lesiones hepatobiliares debido a la excreción de la toxina sin conjugar en bilis y su concentración allí junto a las dificultades en la metabolización de la filoeritrina por parte del hepatocito la cual pasa a los tejidos quedando expuesto a la acción fotodinámica de la luz solar (longitud de onda de 540 a 600nm.) originarían las reacciones fotoxidativas responsable del cuadro del fotosensibilización (1,6). Resulta de interés destacar que la mayoría de los brotes descriptos se originan por el consumo directo de pasturas contaminadas. En el presente caso, el origen del brote fué debido a la ingesta de heno contaminado. Una búsqueda informativa posterior permitió establecer que el mismo se elaboró bajo condiciones de

abundantes lluvias y temperaturas cálidas, con abundante materia vegetal muerto proveyendo un sustrato adecuado para la proliferación de las esporas. Los recuentos de esporas realizados arrojaron valores que son mencionados como tóxicos (2). La persistencia de la toxina en las esporas del forraje contaminado depende de la exposición a la luz solar, produciéndose la detoxicación luego de una prolongada exposición solar (2).

Lamentablemente, la carencia de tomas de muestras séricas en los animales convalescientes así como el análisis histopatológicos de los tejidos del animal necropsiado nos priva de una información complementaria de valor.

Las pérdidas económicas producidas en el presente brote se consideran como serias, si bien la mortalidad fué baja, las complicaciones secundarias por mastitis, pérdidas por secados y abortos avalan lo expuesto.

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RESUMEN

En un tambo de 400 vacas Holando Argentino en el partido de Salto Provincia de Buenos Aires, Argentina, se comprobó un brote de fotosensibilización 48 hs. después de la ingesta de un heno contaminado por Phitomyces Chartarum. La morbilidad del brote fué del 11% y la mortalidad del 2,5%. La producción lactea diaria decayó un 12,5% y el 15% de las vacas afectadas preñadas abortaron. El 52,3% de las vacas con fotosensibilización se secaron. Al suprimir el heno problema y realizar tratamiento medicamentoso a los animales afectados el brote fué superado.

SUMMARY

An outbreak of photosensitizacion in a dairy farm of 400 Argentine holstein cows located in SALTO county, Buenos Aires Province, Argentina is described. 48 hs. after the herd consumed contaminated hay with Pithomyces Chartarum the condition was present. Morbility was 11%, mortality 2.5%. The daily milk production dropped to 12.5% and abortion was present in 15% of pregnant affected cows. 52% of photosensitised affected cows suffered an early drying off.

When the contaminated hay was suppresed and the sistematic treatment was instituted to the animals the outbreak was avercome.

RESUME

A Salto, Province de Buenos Aires, Republique Argentine, on a verifié dans une laiterie de 400 vaches Hollande Argentin une manifestation de Photosensibilisation 48 heures après d'avoir mangé un foin contaminé avec Phitomyces Chartarun.

En ce cas la morbidité a été d'un 11% et la mortalité d'un 2,5%. La production lactée par jour est tombée d'un 12,5% et le 15% des vaches pleines affectées sont abortées.

Le 52,3% des vaches avec Photosensibilisation est laissé d'être fecond. Quand on a supriné le foin contaminé qui avait provoqué ce problème et on a realisé le traitement avec les medicaments convenables aux animaux affectés, cette manifestation de Photosensibilisation a été superé.

ESTUDO SOBRE A OCORRÊNCIA DE CÁRIE EM BOVINOS LEITEIROS

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INTRODUÇÃO

A frequente indicação de que o aparecimento de doenças dentais e periodontais são responsáveis pela diminuição ou alterações na produção de leite dos bovinos nos demonstram que os dentes deveriam merecer, por parte dos pesquisadores veterinários e afins, maior atenção. Entretanto na literatura compulsada por nós e ao nosso dispor notamos que poucos são os dados relativos àquelas estruturas notadamente os relativos aos aspectos morfológicos e principalmente os epidemiológicos.

As clássicas descrições encontradas na literatura se restringem ao relato da forma e fórmulas dentárias nesta espécie animal salientando particularmente o papel relevante desempenhado pelos dentes na apreensão e secção dos alimentos, em colaboração com a língua.

Nas considerações exaradas por tratadistas e pesquisadores, cremos que as mais relevantes, apesar de serem pouco substanciadas e de brevíssimo relato, são as de GETTY (3) que nos informa que em bovinos o esmalte dos dentes incisivos, devido ao atrito é desgastado na face oclusal da coroa, expondo assim uma dentina escurecida, até que os dentes consistam apenas em uma raiz e uma coroa sendo que os dntes incisivos decrescem de tamanho dos médios para os cantos.

SANTOS (5) afirma que os dentes incisivos podem sofrer desgaste excessivo em consequência de alimentação seguida de vegetais de fibras grosseiras.

BOLZ et alii (2) fazem referência a encurtamento de dentes, diastema senil e outras entidades nozológicas devido à retenção de restos de alimentos.

SIEGMUND et alii (6) relatam que em bovinos os sinais de uso dos dentes apresenta pouca segurança para o cálculo da idade dos animais já que o desgaste e o aparecimento das cáries determinan alterações da morfologia dental.

BATISTON (1) e JARDIM (4) informam que os bovinos apresentam afastamento das pinças e dos primeiros médios a partir dos 11 anos de idade e que a partir dos 12 anos todos os dentes ficam afastados e gastos até o colo e consequentemente tendem a apresentar cáries dentária.

Com objetivo de contribuirmos com a verificação através de estudo epidemiológico, das alterações nos dentes incisivos de bovinos leiteiros da região do Triângulo Mineiro - MG, Brasil, buscamos observar e assinalar a ocorrência de cárie dentária em animais com diferentes tipos de manejo e idade.

MATERIAL E MÉTODO

Para esse estudo valemo-nos do exame de 342 animais da espécie bovina com características de produção leiteira, adultos, fêmeas, sem raça definida com idade comprovadamente acima de 5 anos, e submetidas a diferentes tipos de manejo e oriundas dos Municípios de Uberlândia, Uberaba, Tupaciguara, Frutal, Iturama, Canápolis e Prata - Estado de Minas Gerais - Brasil.

Os animais foram divididos em três grupos de faixas etárias que compreendiam:

GRUPO A : 152 animais (44,44%) com idade de 5 a 7 anos
GRUPO B : 142 animais (41,52%) com idade entre 8 e 10 anos
GRUPO C : 48 animais (14,04%) com idade acima de 11 anos

Utilizamos como representação esquemática dos dentes incisivos a denominação recomendada pela Nomina Anatômica Veterinária, isto é: I_1, I_2, I_3, I_4 combinada com a indicação da hemiarcada direita com a letra "D" e para a hemiarcada esquerda com a letra "E".

Para a realização dos exames da cavidade bucal, procedíamos a contenção do animal em tronco imobilizador e a abertura da cavidade bucal com o auxílio de compressas cirúrgicas. As explorações odontológicas foram feitas com o auxílio de espelho de BARASCH, cureta de STARLET e YENAX e extratores de tártaros de TENAX.

Os dados relativos aos exames das arcadas dentárias foram tabulados e tratados estatisticamente, respeitando-se a divisão em grupos.

RESULTADOS

Dos 342 bovinos leiteiros examinados, pudemos observar que 18 (5,26%) animais apresentavam dentes cariados, sendo que 7 (2,05%) animais compunham o Grupo A, 8 (2,34%) bovinos compreendiam o Grupo B e as restantes 3 vacas (0,87%) o Grupo C.

Os animais que apresentavam dentes cariados com maior frequência foram aqueles que compreendiam o Grupo B, com 27 dentes cariados, seguidos dos animais que compunham o Grupo A com 19 dentes cariados e dos bovinos do Grupo C, com 13 dentes cariados.

Das 18 vacas que apresentavam cárie dentária, identificamos 59 dentes comprometidos com a referida entidade, sendo que mais frequentemente observamos cárie no I_1D - 13 vezes, I_1E - 10 vezes, I_2D - 8 vezes, I_2E - 7 vezes, I_3E - 7 vezes, I_3D - 6 vezes, I_4E - 5 vezes e I_4D - 3 vezes.

Os dentes que mais frequentemente foram notados com cárie no Grupo A foram: I_3D, I_1D, I_1E, I_2E, I_3E - 3 vezes; seguido do I_2D - 2 vezes, I_4D, I_4E - 1 vez.

No Grupo B, a cárie dentária foi vista mais frequentemente nos órgãos dentais I_1D - 8 vezes, I_2D, I_1E - 5 vezes cada; I_3D, I_2E, I_3E, I_4E - 2 vezes cada; I_4D - 1 vez.

No Grupo C registramos cáries mais frequentemente nos dentes I_1D, I_1E, I_2E, I_3E, I_4E - 2 vezes.

TABELA 1 - Ocorrência de cárie em dentes incisivos de bovinos, de acordo com o grupo etário correspondente. MG, 1989.

DENTE \ GRUPO	A	B	C
I_4D	1	1	1
I_3D	3	2	1
I_2D	2	5	1
I_1D	3	8	2
I_1E	3	5	2
I_2E	3	2	2
I_3E	3	2	2
I_4E	1	2	2

DISCUSSÃO

Os comentários atinentes a ocorrência de cárie dentária em bovinos leiteiros, principalmente aqueles oriundos de confrontação de dados, mostram-se prejudicados pela escassez de referências na Literatura, o que reforça ao nosso ver, a proposta inicialmente colocada para esse trabalho, ou seja aquela que objetivava a coleta de dados relativos aos rebanhos nacionais, e que poderiam facilitar a indicação do manejo e de outras técnicas de trato de rebanho que diminuissem ou mesmo impedissem a diminuição da produção leiteira que se dá comumente nos referidos rebanhos e que por falta de informação e de dados epidemiológicos tem responsabilizado genéricamente as alterações dentárias como causa da queda de produtividade, sem contudo indicar a frequência do aparecimento das referidas doenças aos órgãos dentais.

De acordo com os resultados obtidos podemos notar que independentemente do grupo etário a que pertencem os animais, o dente que mais frequentemente foi acometido de cárie foi o I_1D, seguido pelos dentes I_2D e I_1E, ou seja aqueles que ladeiam o primeiro, sugerindo assim que nesta região bucal, há predisposição para o aparecimento de doenças dentais sugerindo também que a secção e a apreensão de forragens se faz com maior frequência com o auxílio direto desses dentes o que determinaria maior desgaste e maior ação mecânica do que sobre os demais órgãos dentais. Já os dentes I_4D, I_4E, são os que menor índice de cárie apresentam, valendo lembrar que essas estruturas mostram-se deslocadas do eixo mediano, e consequentemente sugerindo menor ação mecânica.

Estudos relativos ao tratamento das cáries dentárias nesta espécie de animais, são ao nosso ver, de fundamental consubstanciação para a retomada da produção leiteira, apesar da baixa incidência da doença. Entretanto cabe lembrar que antes da caracterização da cárie propriamente dita, o órgão dental passa por um processo de diminuição da sua capacidade funcional o que pode determinar a diminuição da ingestão de alimentos e consequente alteração na produção leiteira. Assim, estudos mais amplos relativos as doenças dos dentes ou de estruturas a eles relacionadas devam ser efetuadas com maior frequência principalmente considerando variáveis

como raça, tipo de alimentação, etc.

CONCLUSÕES

A análise dos resultados nos permite concluir que:

1- A cárie dentária em bovinos leiteiros da região do Triângulo Mineiro MG, Brasil, ocorre em cerca de 5,26% dos animais.
2- A cárie dentária foi mais frequentemente observada nos dentes I_1D (22,03%) seguido de I_2D (13,55%) e I_1E (16,95%).
3- O grupo etário que percentualmente apresentou maior frequência de cáries dentárias anotadas foi aquele compreendido por animais com idade acima de 11 anos (27,08%).

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RESUMO

Estudou-se a cárie nos dentes incisivos em 342 bovinos, fêmeas em lactação, sem raça definida e com idades variadas e superiores a 5 anos.

O exame da cavidade bucal permite concluir que:

- a cárie em dentes incisivos ocorre em 5,26% dos bovinos leiteiros;
- a cárie dentária foi mais frequentemente observada em I_1D (22.03%) seguido de I_1E (16,95%) e I_2D (13,55%);
- o grupo etário que percentualmente apresentou maior frequência de cáries dentárias foi aquele compreendido por animais com mais de 11 anos.

SUMMARY

It was studied the caries of the incisor teeth in 342 crossbred cows over 5 years old in lactation.

The examination of the cavity of the mouths allowed the following conclusions:

- in dalily cows the caries of the incisor teeth occurs in 5.26%;
- the preponderant incidence of caries was observed in the right first incisor (22.03%) followed by the left first incisor (16.95%) and the right second incisor (13.55%);
- related to the age, the greatest frequence of dental caries ocurred in animals over 11 years old.

ZUSAMMENFASSUNG

Die Untersuchung der Kanes der Incisive Zahne der 342 Küne, die ohne bestimmte Rasse und mehr als 5 Jahre alt sind, hat beweistet:

- Die Karies der Incisive Zahne kommen zum 5,26% der Lakatationsrinder Vorschein;
- Die karies hat häufiger in I_1D (22,03%), I_1E (16,95%) und I_2D (13,55%) beobachtet;
- Die Gruppe, die mehr Karies hat, ist older als il Jahre.

RESUMEN

En el presente trabajo se estudío la ecistencia de carie en los dientes incisivos en 342 bovinos, hembras en lactatación, sin raza definida y con varias edades superiores a 5 años

El examen de la cavidade bucal permite concluiyir:

- La carie en los dientes inciaivos ocurre en el 5,26% de los bovinos lecheros;
- La carie dentaria fuí mas frequentamente observada en I_1D (22,03%) seguido de I_1E (16,95%), I_2D (13,55);
- En porcentaje, la mayor frequencia de caries dentarias fuí encontrada en los animales con mas de 11 años de edad.

CONSERVAÇÃO DO SUCO DE RÚMEN; AVALIAÇÃO DE ALGUMAS PROVAS FUNCIONAIS.

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INTRODUÇÃO

No interior dos pré-estômagos dos ruminantes os nutrientes são degradados e transformados bioquimicamente por ação da micropopulação de bactérias e protozoários, sendo necessário para tal determinadas condições como: umidade, anacrobiose, pH adequado e temperatura quase constante.

A mudança de hábito alimentar visando maior produtividade (JOHNSON et alii, 1956; TELLER et alii, 1977 e ORTOLANI et alii, 1980) ou tal vez a passagem brusca de lactente para ruminante (CHURCH, 1974) tem alterado as condições normais do rúmen levando a distúrbios digestivos.

A introdução do exame do suco de rúmen na clínica possibilitou conhecer as funções bioquímicas e microbianas normais e patológicas dos pré-estômagos, auxiliando assim, no diagnóstico dos distúrbios gástricos (ROSENBERGER, 1963 e DIRKSEN, 1981). Sendo a atividade microbiana avaliada através de determinadas provas como: digestão da celulose, fermentação da glicose, redução de nitrito, redução do azul de metileno, etc... (JOHNSON et alii, 1956; HOLTENIUS et alii, 1959; DIRKSEN, 1969 e CAMPOS NETO, 1977).

DIRKSEN & WOLF (1963) DIRKSEN (1981) e BRAUN et alii (1988) recomendaram o uso do suco de rúmen nas indigestões primárias de origem alimentar. Porém seu uso esbarra no inconveniente do mesmo ter suas qualidades terapêuticas diminuidas poucas horas após a colheita.

Tentativas para conservação do suco ruminal foram desenvolvidas por: DIRKSEN & WOLF (1963); PHILLIPS et alii (1975); BLANCOU (1976); ORTOLANI et alii (1980) e SINGH & SUD (1980).

O presente trabalho teve como objetivo avaliar o suco de rúmen de bovinos recém-abatidos, baseando-se no pH e nos tempos de redução do nitrito e do zaul de metileno, desejando-se, ainda, desenvolver um método prático e de baixo custo para sua conservação, visando seu uso terapêutico nas indigestões dos bovinos.

MATERIAL E MÉTODOS

Este trabalho foi desenvolvido no Centro de Desenvolvimento da Pecuária (CDP) - Fazenda Experimental da Universidade Federal da Bahia localizado no distrito de Oliveira dos Campinhos, município de Santo Amaro da Purificação, Bahia.

Utilizou-se 50 amostras de suco de rúmen obtidos de bovinos recém-abatidos no matadouro de Oliveira dos Campinhos, onde os animais eram submetidos a um jejum inferior a 12 horas.

Foram colhidos em torno de seis litros de cada amostra de suco de rúmen em baldes plásticos com torniz e das 50 amostras colhidas e analisadas 20 foram escolhidas de modo intencional, sendo cada uma dessas 20 amostras divididas em porções de 80 ml e separadas em dois grupos: A-Natural e B-com 10% de uma solução saturada de açúcar contendo 10% de ovo, os quais foram conservados em três diferentes temperaturas (a= ambiente ± 27ºC; b = resfriamento ± 5ºC e C= congelamento -18ºC).

As amostras mantidas em temperatura ambiente e resfriadas foram analisadas nos períodos de 6,12,18,24,36,48,60 e 72 horas e a partir de então nos 79, 159 e 309 dias, enquanto congelados as análises se davam com 1,2,3,7,15 e 30 dias, perfazendo um total de 56 porções.

As provas realizadas foram pH, através do papel indicador universal tempo de redução de nitrito segundo recomendações de HOLTENIUS et alii (1959) utilizando-se para tal 0,2 ml de nitrito de potássio (KNO2) a 0,025% e tempo de redução do azul de metileno a 0,03%.

Os dados foram submetidos a análise estatística utilizando o pacote para microcomputadores SAEG (Sistema de análise Estatística e Genéti - ca) da Universidade Federal de Viçosa.

As significâncias entre as fontes de variação foram submetidas ao teste F (SNEDECOR & COCHRAN, 1974) aos níveis de 5% e 1%.

Para as comparações entre os médios de tratamento utilizou-se o teste de DUNCAN a 5% de significância.

RESULTADOS

a) pH

Os valores de pH encontrados no presente trabalho variaram de 6,4 a 7,0, tendo como valor médio 6,8 valores estes também encontrados por SOSEMBERGER (1963). DIRKSEN (1981) e ROSEMBERGER (1983) que citam valores semelhantes. AMSTEL (1982) relata valores de 6,52 a 7,02. CAMPOS NETO et alii (1976), LUCCI et alii (1982) e ORTOLANI et alii (1982) encontraram valores aproximados aos desta pesquisa, porém mais exatos pois procederam a medição com pH metro. Já CAMPOS NETO et alii (1978) descreveu valores mais elevados.

As amostras com açúcar + ovo mantidas em temperatura ambiente acidificaram; Nas demais amostras adicionadas de açúcar + ovo congeladas ou resfriadas o pH mostrou-se mais estável, particularmente nestas últimas, onde permaneceu praticamente inalterado até o 15º dia. ORTOLANI et alii (1980) descrevem que amostras resfriadas e congeladas sofrem alcalinização enquanto as amostras em temperatura ambiente tornam-se ácidas. Já SINGH & SUD (1980) relatam que o pH permanece inalterado por 10 dias com o congelamento.

b) Redução do azul de metileno

O tempo de redução do azul de metileno variou de um a seis minutos. Tempo semelhante foi encontrado por ROSEMBERGER (1983).

DIRKSEN (1969) obteve tempo inferior a três minutos e CAMPOS NETO et alii (1978) relatam tempo de dois a cinco minutos. Já AMSTEL (1982) cita tempo de três a cinco minutos em dietas mistas e menos de três minutos nas dietas ricas em carboidratos.

As amostras mantidas em temperatura ambiente ao natural tiveram seu tempo de redução aumentado já nas primeiras horas e as com açúcar + ovo perderam a capacidade de redução. As amostras resfriadas mantiveram o tempo de redução até 12 horas quando ao natural e até 18 horas quando adicionadas de açúcar + ovo, sofrendo aumentos significativos após 24 horas, Já as amostras congeladas mostraram uma ligeira queda de tempo de redução após 24 horas, o qual tendem a subir após 48 horas nas amostras ao natural, sendo que ao adicionar-se açúcar + ovo tal aumento não mostrou-se significativo até 7 dias.

c) Redução de nitrito

Observou-se o tempo de 10 minutos para a redução de 0,2ml de KNO2 . HOLTENIUS et alii (1959) obteve tempo de 5 a 10 minutos. HOSOYA et alii (1963), ZAMBRANO (1975) e ROSEMBERGER (1983) relatam tempos inferiores ao encontrado neste trabalho.

Já nas primeiras horas nos diversos métodos de conservação percebeu-se aumentos significativos do tempo de redução do nitrito, sendo tal aumento menos intenso nas amostras congeladas. DIRKSEN & WOLF (1963) citam que baixas temperaturas mostran melhores resultados quanto a redução de nitrito.

CONCLUSÕES

1. O pH do suco ruminal aumentou paralelamente com o tempo quando mantido em temperatura ambiente, resfriado ou congelado.

2. A adição de açúcar + ovo mantêm o pH do suco ruminal mais estável quando o mesmo é resfriado ou congelado, sendo que quando resfriado o pH mantem-se quase inalterado por 15 dias.

3. O tempo de redução do azul de metileno no suco ruminal mantêm-se por 12 horas quando resfriado e por 24 horas quando congelado.

4. A adição do açúcar + ovo no suco ruminal mantêm o tempo de redução do azul de metileno até 7 dias nas amostras congeladas.

5. O tempo de redução de nitrito mostrou aumentos consideráveis já nas primeiras horas, sendo tais aumentos intensos no congelamento.

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RESUMO

Objetivou-se neste trabalho, avaliar o desempenho de algumas provas funcionais:pH, redução do azul de metileno e redução do nitrito no suco de rúmen, natural e com aditivo.

O suco rumenal de bovinos recém abatidos era coletado e separado em duas porções; A= natural e B= adicionado açucar + ovo, as quais eram conservadas sob diferentes temperaturas (a= ambiente + 37ºC; b=resfriado + 5ºC e c= congelado -18ºC) e submetidos às provas laboratoriais pelos períodos de 6, 12, 18, 24, 36, 48, 60 e 72 horas e à partir de então nos 7º, 15º e 30º dias.

Concluiu-se que as amostras Bc apresentaram-se com melhores resultados às provas citadas e as amostras Bd mantiveram o pH inalterado até o 15º dia.

ZUSAMMENFASSUNG

Die prufungen waren: Die Messung der pH-wert,die Methylen blauprobe und die Nitritreduktionsprobe. Unmittelbar nach dem Schlachten der tiere wurde der Pansensaft gewonnen uns in zwei Portionen unterteib . Portion A= Pansensaft ohne zusatz und Portion B= Pansensaft mit zusatz von Zucker und Ei.

Die Pansensaft proben wurden bei den Aufbewahrungs temperaturen a) +27º, b) + 5º und c) - 18º nach 6, 12, 18, 24, 36, 48, 60 und 72 Stunde sowie nach 7, 15 und 30 Tagen untersucht.

Es kann zusammengetasst werden, dass die Probe Bc die besten Ergebnisse zeigten und die Probe Bb unverandert sich nach 15 Tagen.

SUMMARY

The purpose of this work was to evaluate the performance of some functional prooves; pH, methyleneblue reduction and nitrite reduction of ruminal juice without and additive.

The bovine ruminal juicefrom recent slaughtered animais was collected and separated into two parts: A= natural and B= sugar + egg added, which were maintained under different temperatures (a= +27º; b= +5º and c= -18º) and were submited to laboratorial prooves at 6, 12, 18 , 24, 36, 48, 60 and 72 hours and thereafter on 7th, 15th and 30th days.

We concluded that the Bc samples showed the best results on those parameters and Bb ones remained with unaltered pH values till 15th day.

NÚMERO DE BACTÉRIAS DOS GÊNEROS *Staphylococcus* E *Streptococcus* EM AMOSTRAS DE LEITE DE VACAS COM MASTITE SUBCLÍNICA.

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INTRODUÇÃO

Vários são os agentes etiológicos da mastite bovina, tendo sido relacionadas na literatura cerca de 43 espécies de microorganismos pertencentes a 25 gêneros. Entretanto a investigação etiológica das formas clínicas e subclínicas desta enfermidade em diversos países do mundo, tem evidenciado a predominância de bactérias dos gêneros *Staphylococcus* e *Streptococcus*.

O elevado número de casos de mastite subclínica em bovinos aliado a sua importância epidemiológica no controle desta doença, bem como a redução da quantidade e o comprometimento da qualidade do leite secretado pelos quartos afetados, tem determinado a realização de inúmeras investigações com a finalidade de avaliar a eficiência dos vários métodos auxiliares de diagnóstico.

Apesar da determinação do número de bactérias patogênicas no leite constituir-se em um importante parâmetro para a caracterização das formas subclínicas da mastite, poucas são as informações disponíveis a esse respeito. A INTERNATIONAL DAIRY FEDERATION-FIL-IDF -(1980), menciona apenas as contagens de *Staphylococcus aureus* e de *Streptococcus agalactiae*.

Tendo em vista o exposto, idealizou-se o presente trabalho, objetivando-se conhecer o número de bactérias dos gêneros *Staphylococcus* e *Streptococcus* em amostras de leite de vacas com mastite subclínica.

MATERIAL E MÉTODOS

O California Mastitis Test - C.M.T. (SCHALM & NOORLANDER, 1957), foi realizado em 398 vacas lactantes aparentemente sadias em cinco propriedades situadas em Itaguaí-RJ. Entre as 279 fêmeas positivas ao teste (C.M.T. ++ e +++), 64 foram escolhidas ao acaso, das quais colheram-se assepticamente 142 amostras de leite dos quartos reagentes.

O isolamento, a identificação e a contagem das bactérias dos gêneros *Staphylococcus* e *Streptococcus* foram realizados a partir da semeadura em placas de Petri contendo agar Baird-Parker e agar sangue, respectivamente, de acordo com as recomendações contidas no STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS (1953) e AMERICAN PUBLIC HEALT ASSOCIATION (1976).

RESULTADOS E DISCUSSÃO

Os resultados obtidos estão inseridos nas Tabelas 1 e 2.

Os dados apresentados na Tabela 1 mostram que entre 142 amostras de leite examinado, o *Staphylococcus aureus* foi isolado de 41 (28,9%), o *Staphylococcus epidermidis* de 30 (21,1%), o *Streptococcus agalactiae* de 34 (23,9%) e o *Streptococcus dysgalactiae* de 31 (21,8%). Os dados desta Tabela evidenciam ainda, a ocorrência de cultivos sem crescimento em 6 (4,2%) amostras.

Na Tabela 2 são apresentados os valores obtidos nas contagens das bactérias dos gêneros *Staphylococcus* e *Streptococcus*, isoladas das 142 amostras de leite examinado. Observa-se que as contagens de *S. aureus* varia

ram de 90 x 10^3 a 1,5 x 10^5/ml, sendo que a média aritmética destas contagens foi de 2,6 x 10^4/ml. Por outro lado, as contagens de *S.agalactiae* variaram de 10 x 10^3 a 1,0 x 10^6/ml, sendo a média aritmética destas contagens 1,9 x 10^5/ml. Tais achados diferem daqueles assinalados pela INTERNATIONAL DAIRY FEDERATION(1980), segundo os quais as contagens de *S. aureus* e de *S. agalactiae*, variam de 2,1 x 10^3 a 7,8 x 10^4/ml e de 2,7 x 10^3 a 7,8 x 10^5/ml, respectivamente.

Na tabela 1 pode-se constatar ainda, que as contagens de *S. epidermidis* variaram de 6,2 x 10^3 a 1,5 x 10^5/ml, sendo a média aritmética destas contagens 3,6 x 10^4/ml, enquanto que as contagens de *S. dysgalactiae* variaram entre 7,9 x 10^3 e 4,0 x 10^5/ml com média aritmética de 8,6 x 10^4/ml. A ausência de informações a respeito das contagens destas duas espécies, impossibilita a confrontação dos achados no presente trabalho.

Diante do exposto, acredita-se que sejam necessárias novas investigações desta natureza, com a finalidade de trazer subsídios que possam orientar a utilização do número de bactérias patogênicas do leite, como método auxiliar direto para o diagnóstico das mastites subclínicas em bovinos.

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RESUMO

Número de bactérias dos gêneros *Staphylococcus* e *Streptococcus* em amostras de leite de vacas com mastite subclínica.

Foram efetuadas contagens de bactérias dos gêneros *Staphylococcus* e *Streptococcus* em 142 amostras de leite procedentes de 64 vacas aparentemente sadias, porém reagentes ao California Mastitis Test. O *Staphylococcus aureus* foi isolado de 41 (28,9%) amostras, o *Staphylococcus epidermidis* de 30 (21,1%), o *Streptococcus agalactiae* de 34 (23,9%) e o *Streptococcus dysgalactiae* de 31 (21,8%). As médias aritméticas foram de 2,6 x 10^4/ml, 3,6 x 10^4/ml, 1,9 x 10^5/ml e 8,6 x 10^4/ml, respectivamente.

SUMMARY

Number of *Staphylococcus* and *Streptococcus* in samples of milk coming cows with subclinical mastitis.

Countings of *Staphylococcus* and *Streptococcus* were done in 142 samples of milk coming from 64 cows with healthy aspect, but positive to de California Mastitis Test. *Staphylococcus aureus* was isolated in 41 (28,9%) samples, *Staphylococcus epidermidis* in 30 (21,1%), *Streptococcus agalactiae* in 34 (23,9%) and *Streptococcus dysgalactiae* in 31 (21,8%). The arithmetic averages of the countings were 2,6 x 10^4/ml, 3,6 x 10^4/ml, 1,9 x 10^5/ml and 8,6 x 10^4/ml respectively.

RESUMEN

Numero de bacterias de los generos *Staphylococcus* y *Streptococcus* en muestras de leche de vacas con mastitis subclinica.

Han sido realizadas recuentes de bacterias de los generos *Staphylococcus* y *Streptococcus* en 142 muestras de leche que provenian de 64 vacas consideradas normales poro reaccionantes al California Mastitis Test. El *Staphylococcus aureus* ha sido aislado de 41 (28,9%) muestras, el *Staphylococcus epidermidis* de 30 (21,1%), el *Streptococcus agalactiae* de 34 (23,9%) y el *Streptococcus dysgalactiae* de 31 (21,8%). Los promedios de los recuentos de de estas bacterias han sido de 2,6 x 10^4/ml, 3,6 x 10^4/ml, 1,9 x 10^5/ml y 8,6 x 10^4/ml respectivamente.

Tabela 1. Distribuição das bactérias dos gêneros *Staphylococcus* e *Streptococcus* isoladas das 142 amostras de leite reagentes ao CMT.

Agentes etiológicos	nº de amostras	(%)
Staphylococcus aureus	41	28,9
Staphylococcus epidermidis	30	21,1
Streptococcus agalactiae	34	23,9
Streptococcus dysgalactiae	31	21,8
sem crescimento	6	4,2
Total	142	100,0

Tabela 2. Distribuição da contagem de bactérias dos gêneros *Staphylococcus* e *Streptococcus* isoladas das 142 amostras de leite reagentes ao C.M.T.

Agentes etiológicos	Mínimas	Contagem/ml máximas	Médias
Staphylococcus aureus	$9,0 \times 10^2$	$1,5 \times 10^5$	$2,6 \times 10^4$
Staphylococcus epidermidis	$6,2 \times 10^3$	$1,5 \times 10^5$	$3,6 \times 10^4$
Streptococcus agalactiae	$1,0 \times 10^4$	$1,0 \times 10^6$	$1,9 \times 10^5$
Streptococcus dysgalactiae	$7,9 \times 10^1$	$4,0 \times 10^5$	$8,6 \times 10^4$

DETERMINATION DES VALEURS DE REFERENCE ECHOCARDIOGRAPHIQUES CHEZ LE VEAU FRISON EN CROISSANCE EN TANT QU'OUTIL DE DIAGNOSTIC DES CARDIOPATHIES

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INTRODUCTION

L'échocardiographie est une technique qui a connu une expansion considérable ces dernières années, entraînant ainsi sa large utilisation en médecine humaine et dans plusieurs espèces animales (8). Cependant, son application en médecine bovine est encore limitée à l'heure actuelle par manque de valeurs de référence. Or, pour pouvoir détecter des modifications de la structure ou de la fonction du coeur produites par une pathologie, il est indispensable de déterminer au préalable avec précision l'effet de la croissance normale sur les mesures échocardiographiques des dimensions et de la fonction cardiaque (2). Les seuls articles publiés à ce jour en médecine bovine dans le domaine décrivent les structures échocardiographiques associées à un état pathologique et vérifiées à l'autopsie (8). Aucun de ces articles ne fournit de valeurs échocardiographiques quantitatives. L'établissement des valeurs de référence chez des bovins sains pourraient donc élargir de façon importante le champ d'application de l'échocardiographie dans cette espèce.

Par conséquent, le but de cette étude était de déterminer les valeurs échocardiographiques chez le veau sain non anesthésié et d'en investiguer la relation avec la croissance corporelle.

MATERIEL ET METHODE

Animaux

17 veaux (poids moyen 70.9 ± 5.0 kg ; âge moyen 64.0 ± 5.9 jours) ont régulièrement subi un examen échocardiographique au cours de leur croissance, ce qui a permis de récolter un total de 53 protocoles. Tous les animaux utilisés pour cette étude ont subi un examen clinique préliminaire approfondi incluant un examen électrocardiographique, une auscultation et un test d'effort, permettant d'affirmer qu'ils étaient tous sains, et ce particulièrement sur le plan de leur système cardio-vasculaire. Parmi ces animaux, 5 étaient de sexe femelle et les autres de sexe mâle.

Protocole échocardiographique

Les échocardiogrammes ont été récoltés selon 2 modes, temps mouvement (TM) et bidimensionnel (2D), au moyen d'un échocardiographe (Sono-Layer, modèle SAL 77B, Toshiba, Tokyo, Japan) couplé à une sonde sectorielle d'une puissance d'émission de 5 ou de 3 MHz selon la taille du patient investigué.

Pour l'examen échocardiographique, après une tonte préalable de la région parasternale droite, les veaux étaient maintenus dans un travail en position debout, le membre droit étendu en avant. Le transducteur couvert de gel conducteur était placé au niveau du 4ème ou du 5ème espace intercostal droit, parallèlement aux côtes bordant celui - ci et à hauteur du coude du patient. Le réglage de la pente de compensation du gain était ajusté de façon à optimaliser la visualisation des surfaces endo- et péricardiques. La terminologie, les plans utilisés et les techniques de mesures étaient sélectionnés selon les critères adoptés et recommandés par l'American Society of Echocardiography.

Le transducteur était d'abord orienté de sorte à obtenir dans le mode 2D une vue parasternale droite selon le long axe du coeur (LAx) permettant de visualiser le ventricule gauche (VG) et les valvules mitrales. Le curseur TM était alors dirigé à travers la cavité du VG au niveau des cordages tendineux, à hauteur du point d'excursion diastolique des valvules mitrales. Comme pour toutes les autres mesures réalisées dans le mode TM, l'attention était portée sur l'orientation du curseur TM afin que celui - ci croise les structures cardiaques perpendiculairement plutôt qu'obliquement. Dans cette vue LAx du coeur en mode TM (TMLAx), l'épaisseur du septum interventriculaire et de la paroi libre du ventricule gauche (IVS et LVFW respectivement) ainsi que le diamètre interne du VG et du ventricule droit (LVID et RVID respectivement) étaient mesurés en systole et en diastole.

La sonde était alors amenée dans une position légèrement plus cranio-caudale jusqu'à visualisation du tractus d'éjection du VG, ce qui nécessitait parfois de placer le coude du veau en adduction et/ou d'amener la sonde un espace intercostal plus en avant que pour la vue précédente. Quand une image satisfaisante de la racine de l'aorte était obtenue, l'image était stoppée pendant la

fermeure diastolique des valvules aortiques. Dans cette vue LAx et en mode 2D (2DLAx), le diamètre interne de l'aorte était mesuré à 3 niveaux : (1) au point d'attachement des valvules aortiques sur la paroi de l'aorte (Ao_{VA}), (2) au niveau du diamètre maximal des sinus de Valsalva (Ao_{sinus}) et (3) au niveau du diamètre minimal de l'aorte juste en aval des sinus de Valsalva (Ao_{min}). La mesure du diamètre de l'oreillette gauche (LA) incluait l'épaisseur de la paroi postérieure de l'aorte, excluait l'épaisseur de la paroi de l'oreillette gauche et était réalisée pendant la fermeture diastolique des valvules aortiques. Le curseur TM était alors dirigé à travers les valvules aortiques. Dans cette vue TMLAx, le diamètre de l'aorte (Ao) était mesuré en incluant l'épaisseur de la paroi antérieure de celle - ci et en excluant l'épaisseur de sa paroi postérieure, et LA était mesurée comme décrit ci - dessus.

Une rotation de la sonde à 90 degrés dans le sens des aiguilles d'une montre permettait d'obtenir ensuite une vue parasternale droite selon un axe perpendiculaire au cœur (SAx). En faisant légèrement pivoter le transducteur vers le haut et vers le bas, une visualisation du VG en coupe transversale à hauteur des muscles papillaires était recherchée. L'image tomographique ainsi obtenue était considérée comme valable quand le bord externe du VG apparaissait circulaire dans le mode 2D. Dans cette vue SAx et dans ce mode 2D (2DSAx), l'échocardiogramme était stoppé en systole puis en diastole et IVS, LVFW, LVID étaient mesurés. Les surfaces interne et externe du VG (LVIS et LVES respectivement) étaient obtenues par planimétrie.

Le curseur TM était alors dirigé perpendiculairement au diamètre du VG et dans la vue TMSAx ainsi obtenue, IVS, LVFW et LVID étaient à nouveau mesurés en systole et en diastole.

Chacun des paramètres était mesuré 5 fois sur 5 cycles cardiaques différents et la valeur moyenne sur ces 5 mesures était retenue. La répétabilité et la fiabilité des mesures échocardiographiques décrites ci - dessus avaient été préalablement testées dans notre laboratoire.

Calculs

A partir des mesures en vue LAx du tractus d'éjection du VG, le rapport LA/AO était calculé en divisant LA par Ao_{sinus} ou Ao dans les vues 2DLAx et TMLAx respectivement.

Dans les vues TMLAx, 2DSAx et TMSAx, les paramètres suivants ont été calculés : le pourcentage d'épaississement du septum interventriculaire (% Δ IVS) = [IVSs - IVSd / IVSd].100, où s = mesure effectuée en systole et d = mesure effectuée en diastole ; le pourcentage d'épaississement de la paroi libre du VG (% Δ LVFW) = [LVFWs - LVFWd / LVFWd].100 ; la fraction de raccourcissement du VG (% Δ LVD) = [LVIDd - LVIDs / LVIDd].100 ; la fraction de raccourcissement du ventricule droit (% Δ RVD) = [RVIDd - RVIDs / RVIDd].100 et le rapport entre IVSd et LVFWd (IVS / LVFW).

La variation systolique de la surface interne du VG (FAC) était obtenue selon la formule [LVISd - LVISs / LVISd].100. La surface du myocarde en systole (LVMSs) et en diastole (LVMSd) était obtenue en soustrayant LVISs ou LVISd de LVESs ou LVESd respectivement. Le pourcentage de variation systolique de la surface du myocarde du VG (% Δ SM) était calculé selon la formule [LVMSs - LVMSd / LVMSd].100.

Analyse des résultats

La relation entre chacun des paramètres mesurés ou calculés avec le poids d'une part et l'âge des veaux d'autre part a été testée par une régression logarithmique. L'équation de régression allométrique et le coefficient de régression ont ainsi été calculés pour chacun de ces paramètres échocardiographiques. Pour les paramètres qui n'étaient pas significativement influencés par l'âge et le poids des animaux, la moyenne, l'erreur standard et la déviation standard ont été calculés sur l'ensemble des 53 protocoles récoltés.

RESULTATS

Tous les paramètres échocardiographiques d'estimation de la morphologie cardiaque, à l'exception de RVIDs, augmentaient significativement ($p \leq 0.001$) avec l'âge et le poids des animaux. Les équations de prédiction pour chacun de ces paramètres en fonction de l'âge et du poids des veaux ainsi que les coefficients de régression de ces équations sont donnés dans le tableau 1.

Lorsqu'un paramètre était mesuré dans différents modes et/ou plans, les équations de prédiction obtenues dans ces différents modes et/ou plans étaient remarquablement comparables entre elles. La majorité des paramètres présentaient des régressions étroites avec l'âge et le poids des animaux.

Les paramètres échocardiographiques d'estimation de la fonction cardiaque ainsi que les rapports IVS/LVFW et LA/Ao n'étaient quant à eux pas influencés de façon significative par l'âge ou le poids des animaux, comme en témoignaient des équations de régression non significatives ($p > 0.05$) et des coefficients de régression faibles. C'est pourquoi les valeurs de ces paramètres n'ont pas été données sous forme d'équations prédictives, mais plutôt sous forme de moyennes ± erreur standard sur l'ensemble des 53 protocoles récoltés (Tableau 2).

Tableau 1. Equations et coefficients (r^2) de régression de divers paramètres échocardiographiques en fonction du poids et de l'âge chez 17 veaux frisons en croissance (53 données).

Paramètres	Mode et vue	Régression avec poids (x en kg) Equation	r^2	Régression avec l'âge (x en jours) Equation	r^2
IVSs (mm)	TMLAx	$y = 4.1 . x^{0.36}$	0.79	$y = 8.7 . x^{0.19}$	0.67
	2DSAx	$y = 4.5 . x^{0.32}$	0.76	$y = 9.4 . x^{0.16}$	0.52
	TMSAx	$y = 4.1 . x^{0.36}$	0.79	$y = 9.1 . x^{0.18}$	0.59
IVSd (mm)	TMLAx	$y = 3.4 . x^{0.30}$	0.71	$y = 6.2 + x^{0.16}$	0,64
	2DSAx	$y = 3.2 . x^{0.30}$	0.74	$y = 6.2 . x^{0.15}$	0.56
	TMSAx	$y = 3.3 . x^{0.29}$	0.71	$y = 6.4 . x^{0.15}$	0.55
LVIDs (mm)	TMLAx	$y = 17.8 . x^{0.11}$	0.11	$y = 21.4 . x^{0.07}$	0.14
	2DSAx	$y = 18.2 . x^{0.11}$	0.09	$y = 21.0 . x^{0.08}$	0.14
	TMSAx	$y = 17.7 . x^{0.10}$	0.06	$y = 20.1 . x^{0.07}$	0.10
LVIDd (mm)	TMLAx	$y = 16.4 . x^{0.26}$	0.69	$y = 28.7 . x^{0.14}$	0.55
	2DSAx	$y = 16.3 . x^{0.25}$	0.71	$y = 27.4 . x^{0.13}$	0.58
	TMSAx	$y = 16.9 . x^{0.25}$	0.72	$y = 29.1 . x^{0.12}$	0.58
LVFWs (mm)	TMLAx	$y = 3.9 . x^{0.38}$	0.77	$y = 9.2 . x^{0.19}$	0.56
	2DSAx	$y = 3.5 . x^{0.38}$	0.77	$y = 7.9 . x^{0.19}$	0.59
	TMSAx	$y = 3.3 . x^{0.41}$	0.66	$y = 8.6 . x^{0.19}$	0.45
LVFWd (mm)	TMLAx	$y = 2.8 . x^{0.34}$	0.46	$y = 5.8 . x^{0.18}$	0.62
	2DSAx	$y = 3.0 . x^{0.31}$	0.71	$y = 6.0 . x^{0.15}$	0.46
	TMSAx	$y = 2.6 . x^{0.33}$	0.52	$y = 5.8 . x^{0.15}$	0.32
RVIDs (mm)	TMLAx	$y = 5.7 . x^{0.12}$	0.04	$y = 7.0 . x^{0.08}$	0.07
RVIDd (mm)	TMLAx	$y = 5.4 . x^{0.27}$	0.44	$y = 11.0 . x^{0.11}$	0.27
Ao va (mm)	2DLAx	$y = 5.7 . x^{0.26}$	0.35	$y = 11.4 . x^{0.10}$	0.16
Ao sinus (mm)	2DLAx	$y = 10.1 . x^{0.27}$	0.79	$y = 18.2 . x^{0.14}$	0.64
Ao min (mm)	2DLAx	$y = 7.5 . x^{0.27}$	0.76	$y = 13.2 . x^{0.14}$	0.62
Ao (mm)	TMLAx	$y = 10.4 . x^{0.26}$	0.74	$y = 18.8 . x^{0.13}$	0.52
LA (mm)	2DLAx	$y = 8.5 . x^{0.26}$	0.74	$y = 14.2 . x^{0.15}$	0.71
	TMLAx	$y = 8.6 . x^{0.26}$	0.71	$y = 14.7 . x^{0.14}$	0.59
LVISs (cm2)	2DSAx	$y = 2.1 . x^{0.23}$	0.10	$y = 3.5 . x^{0.12}$	0.08
LVISd (cm2)	2DSAx	$y = 2.6 . x^{0.45}$	0.69	$y = 7.5 . x^{0.21}$	0.42
LVESs (cm2)	2DSAx	$y = 4.7 . x^{0.47}$	0.74	$y = 12.5 . x^{0.25}$	0.61
LVESd (cm2)	2DSAx	$y = 5.1 . x^{0.49}$	0.83	$y = 15.4 . x^{0.24}$	0.56
LVMSs (cm2)	2DSAx	$y = 3.2 . x^{0.52}$	0.89	$y = 9.3 . x^{0.27}$	0.66
LVMSd (cm2)	2DSAx	$y = 2.5 . x^{0.53}$	0.81	$y = 7.88 . x^{0.26}$	0.59

Tableau 2. Valeurs de référence de divers paramètres d'estimation de la fonction cardiaque mesurés par échocardiographie chez 17 veaux frisons en croissance (53 données).

Paramètres	Mode et vue	Moyenne ± erreur standard
% Δ IVS (%)	TMLAx	57.7 ± 2.1
	2DSAx	54.8 ± 1.8
	TMSAx	60.6 ± 1.6
% Δ LVD (%)	TMLAx	41.6 ± 1.0
	2DSAx	35.9 ± 1.2
	TMSAx	42.3 ± 1.2
% Δ RVD (%)	TMLAx	43.2 ± 1.9
% Δ LVFW (%)	TMLAx	66.4 ± 2.6
	2DSAx	59.4 ± 2.6
	TMSAx	78.1 ± 2.8
% Δ SM (%)	2DSAx	25.8 ± 2.1
FAC (%)	2DSAx	65.3 ± 1.2
IVS/LVFW	TMLAx	1.01 ± 0.02
	2DSAx	1.06 ± 0.01
	TMSAx	1.09 ± 0.02
LA/Ao	2DLAx	0.80 ± 0.01
	TMLAx	0.81 ± 0.01

DISCUSSION

L'analyse du tableau 1 permet d'affirmer que pour pouvoir déceler des anomalies de dimensions cardiaques chez le veau, il était indispensable dans un stade préliminaire de relier ces dimensions, mesurées par échocardiographie, aux dimensions corporelles des animaux. En effet, les résultats montrent que chez le veau, il existe une relation linéaire entre le poids corporel et l'augmentation des dimensions du coeur. Une telle régression a été également démontrée chez l'homme (4, 14, 6) et dans plusieurs espèces animales telles que le chien (2, 12, 9), le chat (7), le cheval (15), le porc (5) et le mouton (11).

Chez le mouton, RVID était non significativement modifié par la croissance (11). Dans notre étude, c'est également le cas pour ce paramètre mesuré en systole. Par contre, sa mesure en diastole montre une augmentation significative ($p \leq 0.001$) avec le poids et l'âge des animaux. De la même façon, les coefficients de régression de la relation décrivant l'évolution de LVID mesuré en diastole étaient plus étroits que ceux décrivant l'évolution de LVID mesuré en systole en fonction de la croissance. Les mêmes résultats avaient été obtenus par d'autres auteurs (15, 11, 5, 9,) ce qui peut probablement être expliqué par une plus grande variabilité interindividuelle des mesures de diamètres des cavités cardiaques lorsque celles-ci sont mesurées en systole plutôt qu'en diastole. Pour déceler une dilatation cavitaire, il sera dès lors préférable de se référer aux dimensions cavitaires mesurées en diastole plutôt qu'en systole.

Les résultats obtenus par d'autres auteurs montrent en général de meilleures régressions lorsque les paramètres échocardiographiques sont étudiés en fonction du poids corporel plutôt qu'en fonction de l'âge ou de la surface corporelle des patients (12, 4, 6, 15, 5, 9). Dans la présente étude, cette constatation ne se vérifiait pas dans tous les cas. En effet, une supériorité de la régression entre un paramètre échocardiographique avec le poids plutôt qu'avec l'âge des animaux n'a été observée que dans 67 % des cas, les 33 % des cas restants donnant des régressions aussi étroites si non meilleures en fonction de l'âge plutôt qu'en fonction du poids des animaux. Ce phénomène peut être expliqué par le fait que dans le groupe des animaux étudiés, la régression entre l'âge et le poids des veaux était très étroite. Notons qu'il est probablement plus fiable et plus aisé de travailler en fonction du poids des animaux plutôt que de leur âge étant donné que le poids d'un animal peut toujours être déterminé avec plus de précision que son âge, surtout chez des animaux de rente tels que les bovins.

Les paramètres d'estimation de la fonction cardiaque mesurés ici n'étaient pas modifiés par la croissance. Cette constatation est conforme à ce qui a été observé dans les autres espèces (2, 15, 12, 11, 6, 7, 4, 9, 5). Les valeurs données au tableau 2 sont donc applicables à tous les veaux, quelle que soit leur taille, puisque le pourcentage de sang éjecté et le pourcentage de raccourcissement des fibres myocardiques et d'épaississement des parois cardiaques restent constant pendant la croissance. Les valeurs de ces paramètres sont par ailleurs comparables à celles obtenues dans d'autres espèces (2, 15, 12, 11, 6, 7, 4, 9, 5).

Il est intéressant de remarquer que dans notre étude, la fraction de raccourcissement du VG était sensiblement comparable à celle du ventricule droit. D'un autre côté, le pourcentage d'épaississement était toujours plus important pour la paroi libre du VG que pour le septum interventriculaire, et ce quelle que soit la vue ou le mode utilisé.

Cette étude démontre également que les rapports IVS/LVFW et LA/Ao, paramètres intéressants à mesurer pour déceler une hypertrophie des parois du VG ou une dilatation de l'oreillette gauche, ne sont pas modifiés au cours de la croissance chez le veau. Le même phénomène avait été constaté dans les autres espèces animales étudiées. D'autre part, les valeurs obtenues pour ces paramètres dans notre étude sont comparables à celle obtenues précédemment par d'autres auteurs dans d'autres espèces animales (13, 15, 10, 1).

En conclusion, les valeurs de référence des paramètres échocardiographiques rapportées dans cette étude peuvent être appliquées pour déceler des anomalies de la morphologie ou de la fonction cardiaque chez le veau frison en croissance. L'utilisateur de ces valeurs de référence aura le choix entre plusieurs vues et modes pour appliquer ces valeurs de référence, dépendant des capacités techniques du matériel dont il dispose.

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RESUME

Au cours des dernières années, l'echocardiographie est devenue un moyen d'investigation non invasif interessant à plus d'un titre pour le diagnostic des cardiopathies en médecine vétérinaire. Son application, à grande échelle, en médecine bovine impliquait nécéssairement une détermination préliminaire des valeurs de référence de différents paramètres échocardiographiques chez des bovins normaux au cours de la croissance.

C'était le but de cette étude, dans laquelle 27 paramètres échocardiographiques (21 morphologiques et 6 fonctionnels) ont été mesurés chez 17 veaux frisons sains, à intervalles réguliers entre 0 et 3 mois d'âge. Les normes de référence de ces paramètres et/ou leurs équations de prédiction en fonction de l'âge et du poids ont ainsi été établies chez le veau frison.

Les paramètres echocardiographiques morphologiques étaient généralement étroitement et positivement corrélés avec le poids et l'âge des veaux, tandis que les paramètres échocardiographiques fonctionnels n'étaient pas modifiés au cours de la croissance des animaux.

ABSTRACT

During the last years, echocardiography has become a non invasive technic more and more interesting for cardiac pathologies diagnostic in veterinary medicine. However, its aplication on a large scale in the bovine medicine inevitably required a preliminar determination of the reference values of echocardiographic parameters during normal growth process in bovine species.

In this study, 27 echocardiographic parameters (21 morphologic and 6 functional) were measured on 17 frisian calves on regular intervals during their growth (between 0 and 3 months of age). The reference values of these parameters and/or their regression equations with age and body weight are given.

The morphological parameters were stongly and significantly correlated with age and body weight, while functional parameters were not altered during the growth process in the frisian calves.

ZUSAMMENFASSUNG

In den letzten Jahren entwickelte sich Echokardiographie zu einer untraumatischen Untersuchungsmethode, die im Rahmen der Diagnose von Herzpathologien bei Tieren unter vielen Gesichtspunkten interessant ist.
Die Anwendung dieser Methode in großen Umfang in der Rinder-Tierheilkunde erfordere eine vorherige Ermittlung der Normalwerte von verschiedenen echokardiographischen Parametern bei gesunden Rindern im Laufe des Wachstums, was zu dieser Untersuchung führte.

In dieser Studie wurden 27 echokardiographische Parameter bei 17 Kälbern in regelmäßigen Zeitabständen von der Geburt bis zum 3. Lebensmonat gemessen.Darunter befanden sich 21 morphologische und 6 funktionelle Parameter. Auf diese Art und Weise wurden die Normalwerte der Parameter und ihre Vorhersagungsgleichungen bei den Kälbern in bezug auf ihr Alter und ihr Gewicht ermittelt.

Die echokardiographischen Parameter morphologischer Art hingen mit dem Gewicht und dem Alter der Tiere im allgemeinen eng und positiv zusammen, während bei den echokardiographischen Parametern funktioneller Art im Laufe des Wachstums der Kälber keine Veränderung festzustellen war.

THE NEW ERA OF RADIOLOGY IN BOVINE MEDICINE

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INTRODUCTION

Bovine radiology has been the most neglected and the least explored area of veterinary radiology. This may be the result of several factors which operate against extensive use of radiology in bovine practice. First, a commercial value alternative has always existed; that is, it was deemed more profitable to slaughter the cow or steer for its meat than to undertake the expense of radiology for exact diagnosis. Second, the logistics involved in radiography of the bovine patient may be time-consuming and complicated, since the animals are often completely untamed compared with dogs or horses. Third, portable generators able to produce an X-ray beam of sufficient magnitude to radiograph body parts other than distal extremities were not available. Finally, but no less important, radiography using portable equipment has been limited because of potential radiation exposure to those performing the examinations.

Over the last several years, changes have taken place both in the economics of bovine farming (both beef and dairy industries) and in the field of radiology, making the use of radiology in bovine medicine today a practical and feasible procedure. One important change is the growing difference between the value of a good, high-producing dairy or beef cow and that of an animal after emergency slaughter. Today it is advantageous to make use of all diagnostic means to ensure exact diagnosis, and hence correct surgical treatment and proper medical care of a bovine patient.

Another change which has taken place is the introduction of excellent, fast-acting sedatives for bovines, which considerably reduces handling problems during radiographic examination. Intravenous injection of a small amount of such an agent makes the animal cooperative within minutes; even intramuscular injection is effective within a relatively short time. These sedatives are useful even in cows in early pregnancy, but are contraindicated in late pregnancy or in patients with known liver disease. Even general anesthesia is a possibility in veterinary hospitals, but is probably not indicated in a practice environment.

Most important changes have occurred within the last several years in radiographic equipment, including X-ray machines as well as recording equipment (i.e. film-screen combinations and processing apparatus). The most revolutionary change in equipment has been the introduction of the rare-earth intensifying screens, which reduces to one-third or one-fourth the amount of X-radiation necessary for production of a radiograph. These rare-earth screens, in conjunction with the commercial introduction of condenser discharge X-ray machines which operate on biphasic and conventional home currents, now make it possible to radiograph many parts of the bovine, such as pelvis, reticulum, cervical spine and thorax, with mobile X-ray machines in field practices.

In view of all these changes, bovine radiography has become an integral part of bovine medicine, both for the practitioner and the academician.

The Change in Radiographic Equipment.

With the marked and rapid changes in radiographic technology in the last decade, both in X-ray machines and processing equipment, general bovine radiography on the farm has become feasible and practical.

Before the 1980's, only the extremities could be radiographed on the farm, and radiographic examination of the neck, head and trunk necessitated transferring the animal to a veterinary hospital. However, it is now possible to obtain good diagnostic radiographs of the bovine patient on the farm premises. This was made possible by three major advances in radiographic technology: 1) the commercial production of condenser discharge type X-ray machines, which are able to produce up to 125 kVp X-rays and up to 40 mAs at maximum kVp load; 2) introduction of the new rare earth intensifying screens which increase the effective mAs on the X-ray film three- to four-fold; 3) the commercial production of table-top electrical automatic film processors. These three technological developments have facilitated the assemblage of radiology laboratory equipment on the farm, thus enabling complete bovine radiological examination to be performed on the premises.

X-Ray Machines.

Two major kinds of X-ray machines, portable and mobile units, are available for radiography on the farm, each having its advantages and limitations.

The portable X-ray machine is a unit which may be packed, moved and reassembled at the side of the animal to be examined. These units are generally capable of producing up to 90 kVp at 10 mA, or alternatively, higher mA (up to 30 mA) at lower kVp. These values limit the usefulness of the machine to radiography of the extremities, although improvisations also permit its use for radiography of parts of the neck and skull. The advantage of the portable unit lies in the possibility of setting it up almost anywhere and positioning it properly wherever the animal is secured. These machines may therefore be considered "cow-side" X-ray units.

The mobile X-ray machine is built in a permanent casing on wheels equipped with a motor, and thus can be moved easily from place to place. These units are much heavier than the portable machines, generally weighing several hundred kilographs. The X-ray tube has a rotating anode, enabling production of higher milliamperage. The machines can produce up to 125 kVp at mAs values up to 45 mAs, and are able to provide these values at an exposure time of several hundredths of a second. The machines are designed with a versatile tube suspension arm, which may be rotated and directed in any direction at any level, thus making the radiographic examination of both standing and recumbent cows relatively easy. These qualities make the mobile unit suitable for radiographing heavier parts of the bovine patient. Excellent diagnostic radiographs may be obtained of the skull, neck, chest and heavier parts of the upper extremities. Good radiographs may also be made of the lumbar vertebrae and the abomasum and reticulum of cows. With the use of rare-earth screens, special techniques such as use of an air-gap to reduce the effect of scatter radiation, and a 24-hour starvation of the patient, it is possible to obtain diagnostic radiographs of lumbar vertebrae of 1000-1200 kg bulls.

The major disadvantage of the mobile unit is that it can only be moved on hard, smooth flooring, such as concrete or tile. Therefore, a bovine patient in a cow-shed or barn with straw or soft bedding must be brought to the machine. This procedure may cause some

complications. Taking into consideration all of these factors, it is advisable for large dairy and beef operators and insemination centers to construct a special area for radiography. This should include a stanchion in a cow-shed with smooth concrete flooring near a convenient power supply. All condenser discharge mobile X-ray machines operate on conventional electrical current found on the average farm or home and need no special generator. This is, indeed, their greatest advantage over most veterinary hospital X-ray units. Some mobile units have even been designed to operate on batteries, and need only the electrical supply of the home for recharging. These capabilities should be considered carefully when purchasing a mobile unit.

Processing Equipment.

An obvious prerequisite for processing of X-rays is adequate darkroom facilities. Processing may be performed using the wet-tank or the automatic processor methods. The wet-tank method involves a considerable amount of manual work, with variable quality of the resulting radiograph.

The wet-tank system is a reasonably reliable processing method for farm use when processing a small or medium number of radiographs. Larger farms or veterinary practices having several cases per week are advised to use an automatic electrical film processor. The new table models of automatic processors are light-weight (no more than 50 kg), and may be easily set up on a table or installed on a countertop. Their major advantages are: 1) no manual work is involved in the processing after the film is removed from the cassette and placed in the processor; 2) consistency in the quality of the resulting radiographs is expected; 3) solutions are always fresh; and 4) films are dry and ready to read within 3 minutes. Automatic processors require a slightly higher financial investment than do tank processors, and are also somewhat more expensive to use. However, the advantages seem to justify the additional expense in large cattle operations, insemination centers, and bovine veterinary practices.

New Applications of Radiology in Bovine Medicine.

In addition to radiography of the extremities of both young and adult cattle, which was the practice for many years when only portable equipment was used on farms, other anatomic areas are now accessible for diagnosis by radiography. Accurate diagnosis of various medical and surgical conditions in the head, thorax, abdomen and spine is now possible.

HEAD:

Radiographic examinations of mandibular and sub-mandibular hard swellings may identify lesions due to actinobacillosis or actinomycosis, or those that are the result of a tooth root infection or abscess. Each may require a different course of management. The paranasal sinuses are another common site of infection, often not exhibiting clear clinical signs. A simple radiographic examination may indicate whether the frontal sinus is impacted, and whether surgery is required for drainage or whether the lesion may be treated medically. Radiography of the skull in neonatal calves suspected of congenital hydrocephalus may aid in confirmation of that condition.

THORAX:

All thorax structures are easily defined by radiography, both in

young and adult cattle. Diseases of the esophagus may be diagnosed by using contrast media (barium sulfate swallow). Pericarditis, especially when involved with cardiomegaly, can be diagnosed even in adult cattle. Pulmonary infections may be diagnosed and other conditions of the lungs, such as caval thrombosis, are recognized radiographically.

ABDOMEN:

Penetrating foreign body is a well known clinical syndrome in cattle. Prior to radiography, only clinical impressions with blood counts substantiated diagnosis and indicated surgery. Radiography is a means of exact diagnosis, making it possible today to determine not only the presence of metal objects in the reticulum, but also whether they are penetrating or free in the lumen. Abomasal sand, or omasal-abomasal impaction by a plastic twine and sand bundle, is a common finding in certain types of feeding regimens.

GENITAL ORGANS:

Radiography has proved to be valuable in examinations of breeding soundness in bulls. Radiography of the testes demonstrates calcified lesions, such as old hematomas or cysts, which block the seminiferous tubules or ductus deferens. Besnoitiosis, a parasitic disease of cattle, can be diagnosed at an early stage by radiography of the testes. A most valuable radiological examination of the genital organs is angiography of the corpus cavernosum penis of bulls suffering from impotentia coeundi. Radiographic examination may differentiate between an arterial-venous shunt and other causes of this condition. Radiography has also been shown to be invaluable as a means for visual demonstration of the level of teat obstruction and its nature.

PELVIS:

The pelvis of the bovine is the site of several disorders. Genetic conditions in bulls such as hip dysplasia will affect their locomotion, thus interfering with their function in the field. Radiography, therefore, is very valuable before selection of beef bulls, or when young bulls start to show hip lameness. Pelvic fractures are not rare. Coxofemoral sub-luxations and total luxation in adult dairy cows are quite common, and can easily be demonstrated by radiography.

SPINE:

Radiography of the spine is especially important in beef and dairy bulls. The spine of these animals is often a source of pain to the bull. Valuable bulls which have reached the stage of approved bull and are valued at many thousands of dollars, become suddenly incapacitated. Various conditions may cause interference with mounting, such as spondylosis, degenerative joint disease of the spine, disc herniation or spinal abscess. Radiography of the spine is not complicated, since only lateral standing radiographs can be made. The information gained by these radiographs are very important for accurate diagnosis and prognosis, often determining the future of a valuable bull.

CONCLUSIONS

Bovine medicine and surgery is entering a new era - the era of equality in diagnostic means to all other species in veterinary

medicine. In addition to hematological, serological, bacteriological and chemical studies, in which bovine medicine was equal to that of other species, it can now be equal to those species in the advantage it can take of radiological examinations of every part of the animal body.

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SUMMARY

The New Era of Radiology in Bovine Medicine

Bovine radiology has been the most neglected and the least explored area in Veterinary Radiology. This was the result of the logistics involved in radiography of the bovine patient, which is not a tamed animal, and the fact that powerful X-ray equipment was only available at Veterinary School Hospitals. Over the last several years many dramatic changes have taken place, which enable the use of radiology in cattle on the farm possible and feasible. These changes are: the introduction of Rompun as a very effective sedative; the introduction of the "condenser-discharge" mobile X-Ray machines; the table

models of automatic processors and the rare-earth intensifying screens. Radiology of the cows and bulls on the farm can now be performed with relative ease, not only on extremities, as was done for many years, but also on the head, the spine, the thorax and the pelvis. As in medicine of other domestic animals, radiology in the bovine patient can now be fully utilized as a powerful means for better diagnosis and prognosis.

ZUSAMMENFASSUNG

Die neue Aera der Radiologie in der Rinderheil - Kunde auf der Farm.

Die Rinderradiologie ist das am meisten vernachlaessigte und das am wenigsten erforschte Gebiet in der Veterinaerradiologie gewesen. Dies war eine Folge des mit dem Roentgen eines Rinderpatienten, der kein zahmes Tier ist, verbundenen Aufwands und eine Folge der Tatsache, dass starke Roentgen-ausruestungen nur an Universitaetskliniken zur verfuegung standen. Wachrend der letzten jahre haben dramatische Aend erungen stattgefunden, welche die Anwendung der Radiologie am Rind auf dem Hof moeglich und durchfuehrbar machen. Diese Aenderungen waren: Die Einfuehrung von Rompun als einem sehr wirksamen Sedativum, die Einfuehrung der mobilen "Condenser-discharge" Roentgengerate, die Tischmodelle von automatischen Entwicklern und die verstaerkenden Seltene-Erde-Filme. Radiologie von Kuehen und Bullen auf dem Hof Konnte nun relativ bequem durchgefuehrt werden; nicht nur an Extremitaeten, wie es seit einigen Jahren getan wird, sondern auch an Kopf, Wirbel-saeule, Thorax und Becken. Wie in der Heilkunde der anderen Haustiere kann die Radiologie des Rinderpatienten nun voll als ein wirksames diagnostisches Mittel fuer eine bessere Diagnose und Prognose eingesetzt werden.

Una Nueva Era Radiologica de Campo en Medicina Bovina

La radiología bovina ha sido el area menos explorada y descuidada en Radiología Veterinaria. Esto fué el resultado de la logística involucrada en la radiografía del paciente bovino, quien no siempre es un animal manso y también de que el poderoso equipo de rayos X se encontrara solamente en escuelas de veterinaria. Durante los últimos años han habido cambios dramáticos que permiten el uso de la radiografía para bovinos a campos. Estos cambios vinieron con la introducción del Rompun como sedático de gran potencia y la introducción del equipo móvil de rayos X del tipo "Condenser-discharge", los modelos de mesa de procesadores automáticos y las pantallas intensificadoras "rare-earth". La radiografía de vacas y toros en el campo puede ahora performarse con relativa facilidad, no solamente de extremidades como se hiciera ya muchos años, sino también de cabeza, espina dorsal, tórax y pelvis. Como en el caso de la radiografía de otros animales domésticos la radiografía bovina puede ahora ser utilizada como un valioso medio de diagnostico para una mejor prognosis y diagnóstico final.

DETECTION OF BOVINE VIRAL DIARRHOEA VIRUS INFECTION BY THE POLYMERASE CHAIN REACTION

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INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is one of the most important pathogens of cattle, causing economic losses of considerable importance throughout the world.

The genome of BVDV is infectious, positive-strand RNA (9), estimated at 2.9 to 4.4 x 10^6 Da in size (7). BVDV is currently classified as a member of the family Togaviridae, genus Pestivirus (17). Propagation of BVDV in cell cultures allows the differentiation of cytopathogenic and of non-cytopathogenic biotypes (cp-BVDV and noncp-BVDV). Both biotypes are pathogenic for cattle (4, 12).

The wide spectrum of diseases associated with BVDV includes subclinical infection, diarrhoea, respiratory disease, immunotolerance, immunosuppression, as well as acute and chronic forms of mucosal disease (1, 6). Transplacental BVDV infection may result in abortion, teratogenic and congenital defects. Cattle immunotolerant to BVDV are persistently infected and viremic. These animals may appear healthy but die prematurely. They have no or low level of BVDV antibodies, and constantly shed the virus.

BVDV is also a frequent contaminant in fetal calf serum, a commonly used component in cell culture systems. This may lead to the BVDV-contamination of biologics, e.g., vaccines (1).

Conventional laboratory diagnosis of BVDV infection is based on virus isolation, immunohistochemistry and on immunoassays. The present methods are either insensitive or are unsuitable for large-scale screening. Sensitive and novel approaches are needed to trace the spread and circulation of BVDV in the nature as well as to study the pathogenesis of the disease.

In recent years efforts have been made to develop direct methods based on the demonstration of the BVDV-RNA by nucleic acid hybridization techniques (5, 14, 15).

In this study, we adapted the method of polymerase chain reaction (PCR) for the detection of the BVDV genome in specimens of cattle as well as in cell cultures. The PCR is an enzymatic method of amplifying gene sequences, e.g., viral nucleic acid sequences in the specimens, to such high amounts which allow their easy detection and identification (13, 16). The PCR is several orders of magnitude more sensitive than the direct hybridization assays.

MATERIALS AND METHODS

Virus strains and cell cultures

In order to estimate the specificity of the PCR method, first we tested various strains of both biotypes of BVDV, grown on bovine turbinate cell cultures. Furthermore, bovine turbinate and bovine kidney cells infected with bovine herpesvirus 1 and 4, with bovine adenovirus 2, and with bovine parainfluenza virus 3 were used as controls of specificity. The inoculated cells were harvested when the cytopathic effect appeared. The noncp-BVDV strains were harvested after 6 days of incubation. The cells were dispersed by trypsinization, whased two times in PBS and the cell number was estimated.

Specimens from cattle

In order to estimate the diagnostic applicability of the PCR in the disease, we tested organ specimens of acutely diseased calves and serum specimens of persistently infected cattle. The organs were homogenized and 10% v/w suspensions were made in PBS. For comparison, the suspensions were tested in parallel by virus isolation and by the PCR.

Conventional diagnosis

Virus isolation from the specimens of cattle was made on bovine turbinate cells, by performing two passages, 6 days each. Subsequently, the presence of BVDV was esimated by indirect immunofluorescence, using monoclonal antibodies prepared by Juntti et al. (11), as well as by an immunoperoxidase method using polyclonal BVDV antibodies.

Preparation of the specimens for the PCR

The cell and the organ preparates were two times frozen at -20°C. Subsequently, 1 ml amounts were centrifuged in an Eppendorf centrifuge with 500g for 5 minutes. The supernatant was recentrifuged with 5,000g for 15 minutes. The pellets were discarded and the supernatants were pelleted at 50,000 rpm in a Kontron TST-50 rotor for 30 min at 15°C. The pellets were collected in TE buffer (10mM Tris pH 7.5 and 1 mM EDTA), containing 1% SDS. Proteinase K was added to have a final concentration of 100 to 200 ug/ml and the specimens were incubated at 55°C for 30 minutes. Subsequently, they were heated to 95°C for 10 min and then 10 ug fresh yeast RNA was added as carrier RNA. The samples were extracted twice with phenol/cholofore and precipitated with ethanol. The precipitates were pelleted at 14,000 rpm in an Eppendorf centrifuge for 20 min at 4°C. The pellets were dissolved in 10 ul 0.5 x TE buffer. Reverse transcription was performed according to standard protocols (8). One of the downstream primers (see below) was used as a reverse transcription primer.

Synthetic oligonucleotides, primers and probe

Four primers were selected, complementary to the published sequences of the gp48 region of the cp-BVDV strain NADL (7). A 21-base long oligonucleotide probe, which corresponded to a region between the two internal primers, was designated. The probe was labeled at the 3' end by adding a tail of biotin-16-UTP (10).

DNA-amplification

The DNA-amplification was performed first in 32 cycles (simple PCR). Each cycle included denaturation at 94°C for 45 sec, primer annealing at 55°C and synthesis at 72°C for 1 min each.

In order to increase the sensitivity of the amplification, trials of double PCR were also made. In these cases pre-amplification was made by using two external primers at an annealing temperature of 55°C in 20 cycles. Then the final amplification was achieved by adding two internal primers to the specimens and continuing the reaction at an annealing temperature of 72°C in 20 cycles. The denaturation and the synthesis were made at the same temperatures as above.

Agarose-gel electrophoresis

To visualize the yield, 10 ul amounts of the PCR products were run on 2.5% agaore gels at 100 V for 45 minutes. The gels were stained with ethidium bromide.

DNA-hybridization

To control the specificity of the amplification, the PCR-products were tested by Southern-blot hybridization and by dot-blot hybridization as previously described (2, 3). Filters were hybridized with the biotinylated BVDV oligonucleotide probe as described elsewhere (2, 3, 8).

RESULTS

The amplification from the BVDV-infected specimens yielded PCR-products which were detected as sharp bands on the gels. When using the external primers in simple PCR, a band of approximately 520 base pairs (bp) was detected. The double PCR with internal primers yielded a band of approximately 430 bp (Fig. 1).

The Southern-blot hybridization and the dot-blot hybridization tests confirmed in each case that the products which yielded the above mentioned bands, contained specific BVDV nucleic acid sequences (not shown).

By the PCR plus hybridization system all the tested BVDV virus strains of both biotypes were detected, but none of the heterologous viruses reacted. The PCR plus hybridization system proved to be suitable for the detection of BVDV nucleic acid sequences in organ suspensions and in serum specimens of infected cattle (Fig. 1). These results are summarized in Table 1.

Figure 1. Electrophoresis of the products of the BVDV PCR assay. Amplification from bovine turbinate cell cultures infected with virus strains NADL (1), Singer (2) and New York (3). Amplification from serum specimens of calves positive in virus isolation (4, 5), and from others, negative in virus isolation (6, 7). PCR of lung (8) and spleen (9) suspensions of an infected calf and the respective organs of a negative control calf (10, 11). Negative control of PCR (water, instead of specimen) is seen in position 12. Positions 8 to 12 show results of double PCR. Size marker is phix174 RF/HaeIII digest DNA (position M).

Table 1. Detection of BVDV in specimens of cattle: number of positive cases versus total number of specimens examined by virus isolation and PCR.

Specimen	Virus isolation	PCR
Spleen	1/4*	1/4
Thyroid gland	0/3	0/3
Lungs	0/2	1/2
Gut	0/1	0/1
Serum	5/8	5/8
Total	6/18	7/18

* Number of positive cases/number of samples examined

As seen in Table 1, a good concordance was found between the results of the conventional virus isolation and of the PCR method. By the PCR, diagnosis was made within 48 hours, in contrast to the 2 weeks' diagnostic time of the virus isolation method.

CONCLUSIONS

The present findings indicate that the PCR has a good applicabilty in the diagnosis of BVDV infection in cattle and in cell lines. PCR combined with a simple, non-radioactive hybridization assay, provides a rapid, specific and highly sensitive direct method for the detection of BVDV infection.

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SUMMARY

The polymerase chain reaction (PCR) was applied to detect bovine viral diarrhoea virus (BVDV) in cell lines, in serum samples of persistently infected cattle and in organ homogenates of acutely infected calves. The primers and an oligonucleotide probe were selected and synthesized from the gp48 gene region of BVDV. Viral RNA was purified from the specimens and transcribed into DNA. Simple PCR was run in 32 cycles, while double PCR was run in two times 20 cycles. The PCR products were analysed by gel electrophoresis and by nucleic acid hybridization. A good concordance was found between the results of the conventional diagnostic methods and of the PCR (out of 18 specimens 6 were positive in virus isolation and 7 in PCR). This indicates that the PCR is a rapid, specific and sensitive method for the detection of BVDV infection in cattle and in cell lines.

ZUSAMMENFASSUNG

Die "Polymerase Chain Reaction (PCR)" wurde angewendet, um das Virus der bovinen Virusdiarrhoe (BVDV) nachzuweisen, und zwar in Zell-Linien, in Serumproben von persistent infizierten Rindern und in Organsuspensionen von akut infizierten Kälbern. Die Primers und eine Oligonukleotid-Sonde wurden gewählt und synthetisiert ausgehend von der gp48-Genregion beim BVDV. Aus den Proben wurde die virale RNA gereinigt und in die DNA transkribiert. Einfache PCR verlief in 32 und die doppelte PCR in zweimal 20 Zyklusen. Die PCR-Produkte wurden analisiert mittels der Gelelektrophorese und Nukleinsäure-Hybridisierung. Zwischen den Ergebnissen der PCR und der üblichen diagnostischen Verfahren herrschte weitgehende Uebereinstimmung, da von 18 Proben sich mittels der Virusisolierung 6 und mittels der PCR 7 positiv erwiesen. Somit ist die PCR ein schnelles, spezifisches und zuversichtliches Verfahren zum Nachweis des BVDV bei Rindern und in Zell-Linien.

RESUMEN

La técnica de "Polymerase Chain Reaction (PCR)" fué aplicada para la detección de virus de la diarrea bovina (BVDV) tanto en cultivos celulares infectados con el virus como en el suero de animales permanentemente infectados y en organos homogenizados de animales agudamente infectados.

"Primers" y sondas de acido nucleico fueron seleccionados y sintetizados a partir de información existente del gen de la glycoproteina gp48. Previa purificación, el ARN viral fué transcrito a ADN. Simple y doble PCR fueron realizados en 32 ciclos y en dos veces 20 ciclos respectivamente. Los productos de PCR fueron analizados en electroforesis en gel y en hybridación del acido nucleico. Los resultados indicaron una alta corelación entre las técnicas convencionales de diagnóstico y la técnica de PCR (de 18 muestras, 6 fueron positivas en aislamiento de virus y 7 en PCR).

Concluyendo, la técnica de PCR ha demosnstrado ser rápida, especifica y sensitiva para la detección de BVDV en animales infectados y en cultivos celulares.

SYSTEMIC MYCOSES IN CATTLE

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INTRODUCTION

Fungal infections are divided into the superficial (dermatomycoses) and the systemic or deep (endomycoses) mycoses. Dermatomycoses are usually caused by a defined population of fungi, whereas the systemic mycoses are caused by a completely different, but also well defined population of fungi. However, in rare cases fungi of both categories may cause the opposite type of mycoses.

In Scandinavia most cases of bovine systemic mycoses are caused by Aspergillus spp., Candida spp., and zygomycetes. In cattle systemic mycotic lesions are predominantly confined to the stomach compartments, intestinal and pulmonary lymph nodes, placenta, and lung [2,5,6]. Generalization from primary foci, frequently located in the forestomachs, intestines, and lung, may be either haematogenous or lymphogenic. The general opinion is that fungi are spread lymphogenic to lymph nodes, whereas the haematogenous route seems to predominate when generalization occurs to other organs, of which the placenta and liver are most frequently affected [2,5,6,7,8].

Isolation of fungi from animal tissues, in which fungi and inflammatory reactions are evident, may be unsuccessful. Moreover, it may be difficult to judge whether an isolate is actually the pathogen, because fungi are common laboratory contaminants. In practice, classification of the fungus in tissue sections using morphological criteria is not always straightforward and the fungi most often involved in bovine systemic mycoses in Scandinavia may be mistaken for each other [1,4,6]. This may be further complicated when the morphology of the fungi in sections indicates one type, while a completely different one is recovered by culture. Additionally, the occurrence of different morphological and atypical fungal forms confuses the diagnostic situation.

These above-mentioned difficulties may, in a wide range of cases, be solved by using immunohistochemical staining of fungi within tissue sections [4,6,7,8,9,13].

The aim of the present paper is to present a connected narrative of our experience with an indirect immunofluorescence staining system used for diagnosis of 208 cases of systemic mycotic lesions in Danish cattle.

MATERIALS AND METHODS

The material comprised mycotic lesions in cattle necropsied during the period 1986-90 at the Department of Pharmacology and Pathobiology The Royal Veterinary and Agricultural University (73 lesions from 33 cattle), and bovine tissues submitted to the National Veterinary Laboratory for diagnosis in the periods 1983-90 (51 placentas and 2 foetuses from 52 cattle) and 1977-89 (98 lymph nodes from 94 cattle). The following methods were employed:

Histopathology: In all cases the diagnosis of mycotic infection was based exclusively on the basis of histological demonstration of fungal elements in tissue sections with characteristic inflammatory reactions. These diagnostic criteria should always be accomplished when dealing with opportunistic pathogenic fungi that are ubiquitous within the environment and/or may be cultured from normal mucous membranes.

Mycology: From all cases of mycotic placentitis (incl. skin plaques on two foetuses) mycological culture was performed on Sabouraud's glucose agar (5 days at 37°C) [10]. In selected cases of mycoses in stomach compartments and lymph nodes mycological culture was performed on Saboraud's glucose agar (7 days at 37 °C and/or 25°C) [5,6].

Immunofluorescence staining: The method used was as previously described [4]. Discrimination between zygomycetes (probably Absidia corymbifera), A. fumigatus, A. flavus, A. niger, and Candida spp. was possible by combination of results obtained by the different antisera with known specificities (Table 1).

Table 1. Immunofluorescence reactivity tested with experimental infections in mice. Homologous and heterologous fungi were stained with heterologously absorbed and diluted antisera raised against somatic antigens from different fungi

	Section with fungal elements				
Antiserum	A.fumigatus	A.flavus	A.niger	A.corymbifera	C.albicans
A.fumigatus	+++	+++	+++	-	-
A.flavus	-	++	-	-	-
A.niger	-	+	++	-	-
A.corymbifera	-	-	-	+++ *)	-
C.albicans	-	-	-	-	+++ **)

Reactivity of immunofluorescence staining: Negative: -. Weak: +. Moderate: ++. Strong: +++. *) Hyphae with positive staining were interpreted as a zygomycete. **) Fungi with positive staining reaction were interpreted as Candida spp..

RESULTS

The various stomach compartments were affected in the following order of magnitude, with the ratio acute - subacute and subacute - chronic in (/): Omasum: 24 cases (20/4); Rumen: 15 cases (11/4); Reticulum: 10 cases (8/2) ; Abomasum: 11 cases (11/0), see Table 2. In six animals with mycosis in the stomach compartments mycosis was found in other organs, too (Table 3). The acute lesions were characterized by pronounced haemorrhage, necrosis, thrombosis with or without vasculitis, and infiltration by predominantly neutrophils. In subacute cases lesions were demarcated from the viable tissues by mononuclear cells, granulation tissue, and in the omasum by simultaneous ingrowth of the epithelial lining from both sides of the laminae omasi. Chronic lesions were characterized by granulomatous tissue formation. In these, connective tissue, mononuclear and specific cells (epithelioid and giant cells), polymorphonuclear cells, necrosis, and dystrophic calcification were regular findings. The pathological and mycological results from most of the cases are being published in detail elsewhere [5]. Based on immunofluorescence staining the fungus distribution given in Tables 2 and 3 was found. Members of the zygomycetes were found in all inflammatory types of lesions, whereas A. fumigatus and Candida spp. were mainly identified in cases of acute mycosis, with A. fumigatus being found only once in a subacute to chronic omasal lesion. Asteroid body formation was only found in chronic lesions and associated with hyphae identified as zygomycetes.

Among 4877 cattle with tuberculosis-like lesions in lymph nodes 94 cases (1.9%) revealed contents of fungal elements. Affected nodes were mesenteric in 84 cases, mediastinal and/or bronchial in 7 cases, and in 3 cases both mesenteric and mediastinal nodes were affected. Eighty-two cases were re-examined histologically and immunohistochemically. All cases of mycotic lymphadenitis were chronic granulomatous in nature and asteroid bodies as well as infiltrations by eosinophils were frequent findings [6]. Evidence of dissemination was obtained in none of the 94 cattle from which the lymph nodes were obtained. The results obtained by immunofluorescence staining are given in Table 3. Mycological culture yielded growth of A. corymbifera from a mesenteric lymph node whereas, in two other cases, mycological cultures were unsuccessful.

Re-examination of sections from 85 cases of bovine mycotic placentitis revealed a continuity of fungi in several tissue sections from 51 placentas and 2 foetuses. Characteristically, in the placentas an acute necrotizing placentitis with disseminated thrombosis, necrotizing vasculitis, and infiltrations by neutrophils were found. Fungi in foetal skin lesions were restricted to epidermis in which hyperkeratosis, necrosis, microabscess-formation, and neutrophilic folliculitis were seen. The results based on immunohistochemical staining are given in Table 3. In the foetal skin plaque hyphal growth predominated in the hair follicles. A detailed comparison of results obtained by mycology, morphology, and immunohistochemical staining will be published elsewhere [8].

Table 2. Immunohistochemical identification of fungi in the stomach compartments of 33 cattle

Immunological identification	Histological location of fungi: Rumen	Reticulum	Omasum	Abomasum	Total
A. fumigatus	3	1	11	3	18
Zygomycetes	9	5	7	4	25
Candida spp.	0	0	0	1	1
A. fumigatus + zygomycetes	0	1	2	2	5
Inconclusive	3	3	4	1	11
Total	15	10	24	11	60

Table 3. Immunohistochemical identification of fungi in mycotic lesions in different bovine tissues from 167 cattle

Site of mycotic lesions	Stomach compartments: Proximal	Terminal	Other organs*	Lymph nodes	Placentas
Total number exanined	25	35	13	82	**53
A. fumigatus	4	14	6	0	**38
Zygomycetes	14	11	1	74	10
Candida spp.	0	1	0	0	2
A. fumigatus + zygomycetes	1	4	0	0	0
Zygomycetes + Candida spp.	0	0	0	1	0
Inconclusive	6	5	6	7	3

Proxinal stomach compartments: Reticulum and rumen. Terminal stomach compartments: Omasum and abomasum. *) All cases had primary mycotic lesions in the stomach compartments, too. The organs involved, numbers in (), were: liver (4), lung (2), kidneys (2), intestines (2), spleen(1), diaphragm (1), and mesenteric lymph nodes (1). **) Including skin lesions in 2 foetuses.

DISCUSSION

In a number of papers it has been suggested, that it is difficult to distinguish between fungi such as Aspergillus spp., zygomycetes, and even Candida spp. in tissue sections based on morphology alone [1,2,4,6]. Even the limited panel of antisera employed in the present survey has extended the possibility of diagnostic discrimination between fungi in tissue sections [4,6].

Our series of bovine systemic mycoses demonstrate that simultaneous infection with different fungi is indeed rare, in particular in cases of metastatic mycotic processes, as seen in lymph nodes and placentas (Table 3). A. fumigatus was found to have a propensity for infecting the terminal stomach compartments, especially the omasum, and placental tissue. Moreover, the omasum was found to be the main site of primary mycotic infection, and the organ is therefore believed to be the main portal of entry for fungi with possible subsequent haematogenous dissemination, including spread to the pregnant uterus resulting in placentitis and abortion. In the stomach compartments the zygomycetes were found more evenly distributed, whereas in the cases of mycotic lymphadenitis the invasive fungi were completely dominated by zygomycetes. Furthermore, it appears that the lymph nodes in cattle are capable of containing zygomycosis, as no evidence of dissemination was obtained in any case of zygomycotic lymphadenitis. Candida spp. were found in a negligible number of cases, which probably reflect a relative high average age of the animals surveyed [5,6], because candidiasis in the stomachs of cattle is predominantly a disease of young calves [3,11,12].

In previous articles we have discussed the predisposing factors for the development of systemic mycoses in cattle [5,6], and from these it can be deducted that intense antimicrobial therapy, feeding with mouldy food stuffs, and metabolic disturbances and/or stress in the puerperium are important factors.

Common antigens have been found in the genera Aspergillus and Candida, and in the order Mucorales, and therefore positive staining of hyphae with the antiserum raised to A. corymbifera should only be used to indicate zygomycetes (order Mucorales) in general. Likewise, fungi positively stained with the anti-C. albicans antiserum should only be judged as Candida spp.. Concerning Aspergillus spp. the immunohistochemical system discriminated between A. fumigatus, A. niger, and A. flavus. However, a number of other Aspergillus spp. are known to be pathogenic to cattle, and until further studies have elucidated the reactivity of the antisera against other Aspergillus spp. it may be more appropriate to use the term "probably" when diagnosing the different Aspergillus spp. by immunohistochemical staining. Moreover, immunohistochemical staining may vary according to strain [13], and expression of different antigens according to state of development [9]. Furthermore, varying stainabilities of fungi in the same section may reflect differences in states of fungal degradation. In conclusion, only cases in which fluorescence staining of fungi was in accordance with the reactions in Table 1 were classified and the remaining considered inconclusive.

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SUMMARY

Immunohistochemical staining of fungi in cases of bovine systemic mycoses demonstrated zygomycetes to occur evenly in the stomach compartments whereas in cases of mycotic lymphadenitis they dominated completely . A. fumigatus was found to have a propensity for infecting the omasum and placenta. Candida spp. were only found in a negligible number of lesions. It was demonstrated that simultaneous infection with different fungi was rare. The omasum is suggested to be the main site for primary mycosis with possible haematogenous spread to the placenta resulting in mycotic placentitis. Furthermore, it appeared that bovine lymph nodes were capable of containing zygomycetes, as no evidence of dissemination was obtained in any case of zygomycotic lymphadenitis.

ZUSAMMENFASSUNG

Bei immunnohistochemischer Färbung von mykotischen Elementen in Fällen boviner systemischer Mykosen hat man Zygomyceten gleichmässig verteilt in allen Magenabschnitten gefunden, während sie in Fällen mykotischer Lymphadenitis total dominierten. A. fumigatus schien ausgeprägt dazu zu neigen das Omasum und die Plazenta zu infizieren. Candida spp. wurden nur in einer unbedeutenden Anzahl observiert. Es wurde nachgewiesen, dass gleichzeitige Infektionen mit verschiedenen mykotischen Elementen tatsächlich selten sind. Es wird angenommen, dass das Omasum der Ausgangspunkt der ursprünglichen Mykosis ist, mit hämatogener Verbreitung zu der Plazenta und mykotischer Plazentitis zur Folge. Ausserdem hat es sich gezeigt, dass boviner Lymphdrüsen Zygomyzeten festhalten, als in keiner Fälle von zygomykotischen Lymphadenitis Verbreitung bewiesen werden konnte.

RÉSUMÉ

Basé sur l'immunohistochimie des mycoses systemiques chez les bovines les zygomycètes se trouvent de facon régulière dans les parties de l'estomac mais dans les cas de lymphadenitis mycosiques ils etaient dominantes. A. fumigatus se trouve avoir une tendance a infecter l'omasum et le placenta. On a trouvé des Candida spp. dans un nombre insignifiant. On a demontré que l'infection simultanée avec des fungiques est en fait rare. On suppose que l'omasum est le terrain principal de la mycose primaire avec dissemination possible au placenta, suivit de inflammation placentaire mycosique. De plus il est apparu que les glandes lymphatiques bovines pouvaient renfermer des zygomycoses parce que on n'a pas trouvé des preuves de dissémination en cas de lymphadenites zygomycosique.

AN EXPERIMENTAL COMPARISON OF THE TRADITIONAL AND STEWART APPROACHES TO ACID-BASE INTERPRETATION IN THE CALF.

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INTRODUCTION

The commonly utilized system of acid/base chemistry is based upon the Henderson-Hasselbalch equation:

$$pH = pK + \log \frac{[HCO_3^-]}{S \times pCO_2}$$

and various interpretation charts and nomographs which have been developed from it.[1] In the Henderson-Hasselbalch equation the partial pressure of carbon dioxide (pCO_2) is considered to reflect the respiratory component and bicarbonate (HCO_3^-) concentration the metabolic component of acid-base balance.

Recently an alternate system of acid-base physiology has been developed and described by Peter A. Stewart.[2,3,4] The method combines electrolyte kinetics, traditional acid-base variables and fundamental laws of chemistry in a more comprehensive model of acid-base physiology. In the Stewart system the Henderson-Hasselbalch equation is only one of six separate equations which must be simultaneously solved to describe acid-base balance in biological fluids.

The Stewart system is based upon independent and dependent variables where the dependent variables can change in concentration only in response to alterations in the concentration of one or more of the independent variables. The three independent variables are as follows:

1. The strong ion difference or SID: This is the difference between the strong cations and the strong anions in the solution. The major strong ions in physiological solution are Na^+, K^+, and Cl^-.

2. The total concentration of weak acids and bases or [ATOT]: These are acids and bases with pKa's in the range of 4 to 12 that are neither totally in the ionized or the salt form at physiological pH and consist primarily of proteins and phosphates. The concentrations of the carbonic acid/bicarbonate/ carbonate system are not included in the [ATOT] as the total concentration of this system varies continuously due to the equilibrium with carbon dioxide.

3. The partial pressure of carbon dioxide in solution or pCO_2. The interpretation for this factor is similar to the classic interpretation.

Dependent Variables

The dependent variables are the concentrations of hydrogen ion, hydroxide ion, bicarbonate, carbonate, and the relative amount of weak acid in the associated and dissociated forms. An experiment was designed to evaluate the Stewart method of acid-base interpretation.

MATERIALS AND METHODS

Experimental Animals

Five Holstein bull calves were obtained at one week of age. The initial health status was assessed by physical examination, complete blood count, biochemistry profile and plasma fibrinogen determination. The calves were housed in individual raised calf stalls and were fed high quality milk replacer (20% protein, 10% fat and 0.25% crude fibre) at 12% of body weight in two daily feedings. Calf starter (20% crude protein) and water was provided to the animals ad libitum. The calves were assessed daily and monitored for appetite, demeanour, temperature, pulse and respiration.

To facilitate arterial blood sampling, surgery was performed under halothane anaesthesia, following a previously described protocol[5] to create a carotid loop.

Experimental Design

The experiment was designed as a latin square,[5,6] with the square chosen randomly from a table of 5x5 latin squares. The calves were infused with five different solutions over a five week period, each animal receiving one infusion per week. The treatments given were: 1) sodium acetate; 2) hydrogen acetate (acetic acid); 3) sodium benzoate; 4) sodium bicarbonate; and 5) a balanced electrolyte solution containing (bicarbonate (28.5 meq/l), chloride (116 meq/l), sodium (140 mEq/l) potassium (4.5 meq/l)). Concentrations of the first four treatments were equal at 250 millimolar based upon the cation component of each solution. The balanced electrolyte solution was included to provide a control to compare the other treatments to.

The calves were examined clinically before each treatment and were haltered and loosely tied in a narrow pen for the experimental trials. They were maintained in a standing position for the infusion and data recording, and were not sedated. One litre of the appropriate solution was given at a constant rate over a one hour period.

Arterial and venous blood samples were collected immediately before and after the period of infusion, then one-half hour, one hour and two hours after the end of infusion. Three mili-liters of arterial and venous blood were collected separately in heparin coated plastic syringes for blood gas and electrolyte determination. An additional 12 ml of arterial blood was collected concurrently by syringe and immediately transferred to heparin coated and serum vacutainers. At 20 and 40 minutes during the infusion period arterial samples were collected for blood gas analysis. Physical data including heart rate, respiratory rate, temperature, central venous pressure and electrocardiogram were continoulsly monitored.

Sample Analysis

Arterial and venous samples blood gas parameters were analyzed on an automated blood gas analyzer. The machine measured pH, pCO_2, and haemoglobin on whole blood. Actual base excess and bicarbonate values were calculated. Arterial heparinized blood was used to determine sodium, chloride, potassium and lactate. Phosphate, magnesium, calcium, total protein and albumin concentrations were determined in serum. The values obtained from these analyses were used to calculate the Stewart independent variables SID and [ATOT].

Data Analysis

The data was analyzed using a three way analysis of variance based upon the latin square design[8] with the analysis determining the

effects of the experimental infusions, individual calf variation and the week of infusion.
The data was analyzed separately for each sample collection period.

RESULTS

The results presented (figure 1) are arterial plasma base excess values thus eliminating non-metabolic variations. There was a significant treatment effect at all sampling time periods from 20 through 180 minutes. The sodium acetate and sodium bicarbonate groups, with the largest base excess values, were not significantly different from each other at any time period. The hydrogen acetate and balanced solution groups were never significantly different from each other. At all times the sodium bicarbonate and sodium acetate groups had significantly greater base excess values than the hydrogen acetate and balanced solution groups.

The sodium benzoate group was intermediate in actual base excess values and had a significantly lower mean arterial plasma base excess compared to sodium bicarbonate at all times, except at 40 minutes. Sodium benzoate had a significantly greater base excess value compared to balanced solution at 40 and 60 minutes. During the treatment period significant differences between groups were seen in SID valves but not in [ATOT] valves.

DISCUSSION

The study was designed to determine if the alkalinizing effect of sodium acetate was due to alterations in the Stewart variables, such as an increase in the SID, or to the metabolism of acetate, as proposed in classical acid-base interpretation. In order to evaluate this objective in a experiment a base similar to acetate, but not metabolizable in the system was sought. Benzoate was chosen as it is a small, non toxic, non metabolizable orgagic anion, similar to acetate in size and dissociation constant.[9] Contrary to acetate it is a metabolic end product and is not further metabolized in a biologic system. The majority of an administered dose of benzoate is excreted unchanged as hippuric acid after conjugation with glycine.[9]

In the traditional acid-base interpretation alkalinization caused by such compounds as sodium acetate have been considered to be due to the metabolism of the base component of infused salt solutions. In the case of sodium acetate it is proposed that the acetate molecule is metabolized, resulting in production of bicarbonate or utilization of hydrogen ions.[10,11]

The increased actual base excess values in the sodium benzoate group, when compared to the group treated with balanced solution, cannot be explained by metabolism of the benzoate molecule, as described above. In the sodium benzoate group a decrease in [ATOT] was noted compared to all other groups over the treatment period, but was statistically nonsignificant. The difference in [ATOT] was appropriate to explain the relative metabolic alkalinization seen in the sodium benzoate group. The [ATOT] is a calculated value and statistical significance is restricted due to the variability introduced in measurement of its component compounds and determination of the physical constants used in its calculation. Treatment with sodium bicarbonate and sodium acetate resulted in greater SID increases than treatment with balanced electrolyte solution. These were appropriate to explain the degree of metabolic alkalinization

with the Stewart method.

The results did not prove that alterations in the Stewart independent variables were responsible for the alkalinization seen with sodium benzoate, however, the differences in the Stewart independent variables between groups were consistent with their relative metabolic acid-base results. Further work is indicated to obtain the physical and chemical constants necessary to calculate more accuarately the Stewart independent variables in each species. It is felt that the use of this system holds considerable promise to further our understanding of the pathophysiology of acid-base disorders as well as to reveal the basic underlying control mechanisms of acid-base and fluid-electrolyte balance.

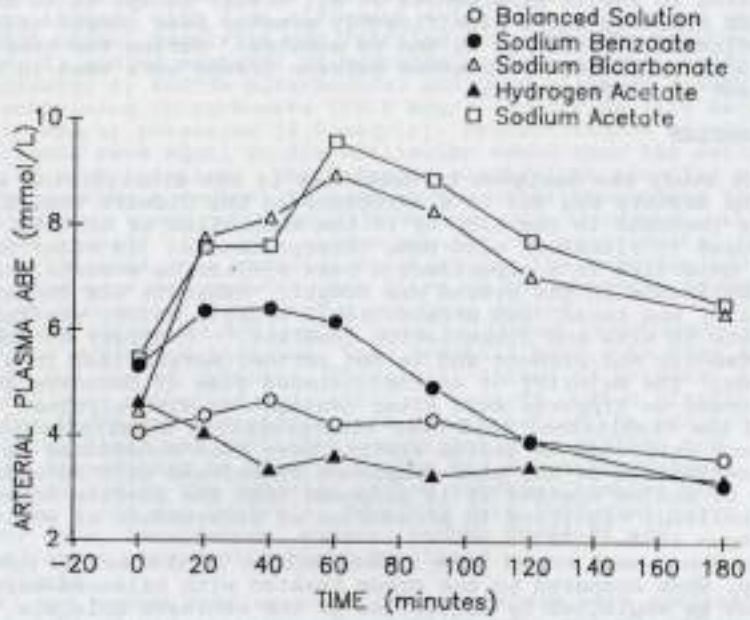

FIGURE 1. Graph of arterial plasma actual base excess versus time with each treatment plotted separately. Each point represents the mean of one treatment at a sampling time. Statistically significant differences are described in the text. (n = 5)

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SUMMARY

An alternate system of acid-base interpretation based upon strong ion difference, total weak acid and partial pressure of carbon dioxide was introduced by Peter Stewart at the end of the 1970's. An experiment involving the use of sodium benzoate, a non-metabolized base, was designed to evaluate the Stewart approach. A metabolic alkalinization was induced by the sodium benzoate which was significantly greater than that induced by a balanced electrolyte solution, but significantly less than that induced by sodium bicarbonate or sodium acetate. The results in all treatment groups were generally consistent with the Stewart approach.

RESUME

Une methode alternative d'interpretation d'acid-base introduit par Peter Stewart vers la fin des années 1970. Cette methode est fondée sur la difference des ions forts, les acides totales faibles et la pression partielle du dioxide de carbon. Une étude experimentale utilisant le benzoate de sodium, une base non-metabolisable fut entreprise afin de comparer la methode tradionelle d'acid-base à celle de Stewart. Une alkalinization metabolique fut observée lors de l'utilisation du benzoate de sodium. Ce dernier produit une alkalinization metabolique beaucoup plus grande que celle induite par une solution d'electrolytes balancés mais beaucoup moindre que celle induite par le bicarbonate de sodium ou d'acetate de sodium. Les résultats n'ont pu être expliqués par la methode traditionelle d'analyse des acides-bases mais étaient compatibles avec la methode d'analyse de Stewart.

ZUSAMMENFASSUNG

Ende der siebziger Jahre beschrieb Peter Stewart eine alternative Interpretationsweise fuer den klinischen Saeure-Base Haushalt. Die wichtigen bestimmenden Parameter, gemaess seiner Theorie sind: Starke Ionen Differenz, Summe der schwachen Saeuren und der Partialdruck von Kohlendioxid. Anhand eines klinischen Experimentes wurde die traditionelle Saeure-Base Interpretation mit der neuen Theorie verglichen. Als nicht metabolisierbare infundierte Base wurde Natriumbenzoat verwendet und mit normaler Elektrolytloesung als Kontrolle, sowie mit metabolisierbaren Alkalose induzierenden Natriumbicarbonat und mit Natriumacetate verglichen. Alle drei Testloesungen induzierten metabolische Alkalose verglichen mit normaler Elektrlytloesung. Die klassische Theorie erlaubte im Gegensatz zur Stewartmethode keine eindeutige Interpretation.

PRIMARY DIAGNOSIS OF FOOT-AND-MOUTH DISEASE USING A HYBRIDIZATION ASSAY

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INTRODUCTION

Foot-and-mouth disease (FMD) is an acute, highly communicable infection of wild and domestic cloven-hooved animals caused by a picornavirus. Control measures adopted by a particular country are dependent on factors such as geographic isolation, type of livestock production, international trade, wildlife population, degree of technological and economic development and prevailing political attitudes.

FMD has never been recorded in New Zealand. To prevent introduction the country has strict quarantine regulations. To detect disease, a nationwide surveillance network is in place, based on private veterinary practitioners who are required by law to report any suspect cases of exotic disease to the Ministry of Agriculture and Fisheries (MAF). If such diseases were detected, an immediate policy of stamping out would be implemented, and the infrastructure and procedures to allow this to happen are in place.

For disease diagnosis, the MAF sends out government veterinarians to investigate all reports of suspect exotic disease. These veterinarians have received specific training in the clinical signs and differential diagnosis of FMD. In the event of a "not negative" diagnosis, a second veterinarian who has had first hand experience with the disease is sent to conclude on-farm investigations. If the conclusion is still "not negative", emergency measures are immediately put in place, consisting of farm quarantine, slaughter of all susceptible animals on the infected property, disease investigation on all neighbouring and high risk properties and extensive movement control of all susceptible animals and animal products within a minimum distance of 10km from the infected property. Samples are taken from infected animals and sent to diagnostic laboratories at Pirbright and Nong Sarai for confirmation. These laboratories provide confirmation of the disease, and also type the virus.

Seven immunological types of FMDV exist (A, O, C, Asia-1, SAT-1, SAT-2, and SAT-3), within which there are numerous subtypes. Diagnostic assays have been developed both for primary diagnosis on suspect materials, and for subsequent identification and typing of a particular isolate. Detection of infective-FMDV is carried out by animal inoculation (guinea pig, suckling mice, cattle) or by the isolation of virus in tissue cultures (1,2). Immunogenic assays may be used for rapid detection of FMDV but are generally less sensitive than virus isolation. This paper describes the development of a highly sensitive nucleic acid hybridization assay for the detection of FMDV-RNA.

MATERIALS AND METHODS

Virus purification

To determine differences between serotypes, FMDV types A_{12}, A_5, A_{22}, A(Sabana), A(Venceslau), O_1(Kaufbeuren), O_1(Campos), C_1(Oberbayern) C_1(Resende), SAT-1, SAT-2, SAT-3, and Asia-1 were grown in baby hamster kidney cells (BHK-21). Virus was harvested and purified on a cesium

chloride gradient and titered on primary bovine thyroid cells (2). Various porcine enteroviruses (PEV), an equine rhinovirus (ERV), and swine vesicular disease (SVD) virus, were propagated in appropriate tissue culture systems (PK-15, Vero and IBRS-2 respectively). Tongue epithelium submitted from field outbreaks was homogenised as a 10% suspension (w/v), filtered (20m) and the nucleic acids were extracted.

RNA extraction-purification

All manipulations were done on ice with the addition of a RNase inhibitor (RNAsin). Samples were solubilized and digested with proteinase K in a Tris/EDTA buffer containing 0.5% SDS, and the nucleic acids extracted with phenol/chloroform/isoamyl alcohol and precipitated with alcohol (4). Multiple samples were applied directly to pre-moistened Gene Screen Plus membranes (New England Nuclear), with the aid of a slot-blot manifold (Schleicher and Schuell).

Probe preparation

The generation of recombinant plasmids used for hybridization has been described (5,6). Plasmids were amplified by growth in E. coli and purified on a CsCl gradient. Digestion with the appropriate restriction endonuclease released the FMDV cDNA fragment of interest which was purified from a low-melting temperature agarose gel. Two cDNA fragment coding for the polymerase gene and the VP1 structural protein were further digested to produce both a general and a serotype specific probe (6).

Labelling procedure

FMDV probes were labelled with ^{32}P (NEN) or biotin (BRL); either by nick translation or random priming (7,8). Probes were purified by gel filtration and consistently had specific activities of > 10^8 cpm/ug probe (Cerenkov).

Hybridization procedure

Nylon filters containing the diagnostic samples were processed as described elsewhere (6). Hybridization was conducted overnight at 58°C which corresponds to 25 degrees less than the calculated duplex melting temperature (Tm) in solution. A high stringency (Tm-25) wash followed hybridizations and autoradiographs were taken to detect the presence of the radiolabel.

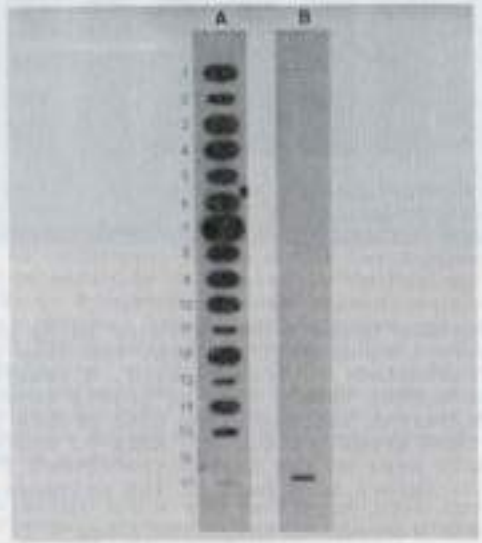
RESULTS

Sensitivity

Under high stringency conditions only 2 of the A_{12} probes exhibited no cross hybridization when 10^9 $TCID_{50}$s of PEV were present as contaminants (data not shown). One of these probes, a fragment from the genome coding for part of the virus infection associated antigen (VIAA), appeared to be highly conserved among all serotypes of FMDV and constituted a good "general" probe for FMDV detection. When this probe was used with homogenates prepared from bovine tongue epithelium clinically affected with FMD in the field a strong signal was detectable in each instance, Fig. 1. The minimal detection level under high stringency conditions using a ^{32}P label was 1 pg of genomic FMDV-RNA (6) and when PK-15 cells were mixed with known amounts of infective FMDV and total RNA was extracted and probed, 100 $TCID_{50}$ of virus was detected. The sensitivity compares favourably with direct detection tests currently used for FMDV detection. Lowest detectable levels of 7.8 ng/ml, and > 500 ng/ml of FMDV have been reported for

the trapped ELISA and CFT respectively (9). A likely method of increasing the sensitivity of the DNA hybridization assay would be to use the polymerase chain reaction (PCR) on cDNA copies of viral RNA produced by a reverse transcriptase reaction.

The sensitivity for diagnosing FMD is critically important for a country such as New Zealand since the potential damage from a false negative determination is enormous.



(Fig. 1) Hybridization between a cDNA probe from the polymerase gene region (A_{12}) and FMDV-RNA extracted from field cases.

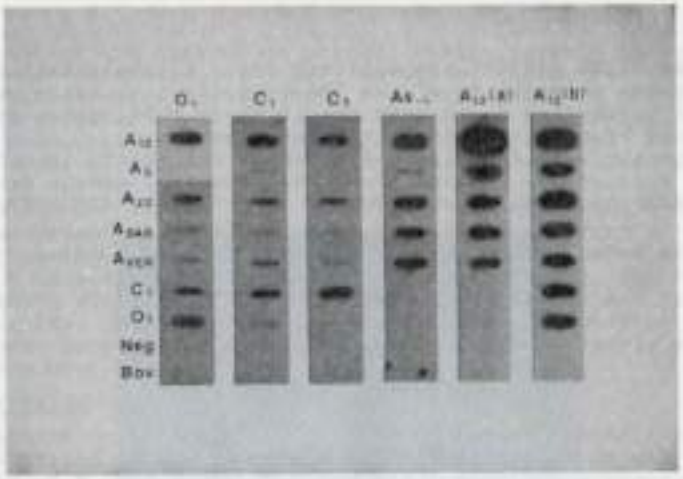
Rows 1 to 15 contain the nucleic acid from 0.1g of tongue epithelium (FMD samples) or other infected tissue (other viruses). In Panel A: C_3, Asia 1/2, SAT 3/3, SAT 1/1, A_{32}, A_{17}, O_2, A_{10}, A_{14}, A_{22}, C_1, Asia 1/1, SAT 1/7, SAT 2/1, SAT 3/1. In Panel B: Bovine herpes mammilitis (slot 1 & 2), Lumpy skin disease (slot 3 & 4), Malignar catarrhal fever (slot 5 & 6), Rinderpest (slot 7 & 8), Contagious bovine pleuropneumonia (slot 9), Vesicular exanthema (slot 10 & 11), Vesicular stomatitis (slot 12 & 13).

Rows 16 and 17 were a negative control (5ug bovine thymus DNA) and a positive control (0.1ng FMDV A_{12}-RNA) respectively.

Differentiation between virus strains

When selected cDNA probes from FMDV types A_{12}, C_1, C_3, O_1, and Asia-1 were hybridized against viral RNA from FMDV types A, O, and C at high stringency conditions, cross hybridization occurred between different serotypes in addition to homologous binding. It was possible to distinguish between FMDV strains C_1, O_1 and the A serotypes (Fig.2).

The differentiation between FMDV field isolates has major relevance for the tailoring of vaccines to a particular outbreak and epidemiological studies to determine the origin and movement of FMDV. The use of FMDV probes from different serotypes appears to be useful in differentiating between major groups of the virus.



(Fig. 2) Differentiation between FMDV strains using selected cDNA probes. Rows contain 1 ng or purified FMDV-RNA from types A_{12}, A_5, A_{22}, A(Sahana), A(Venceslau), C_1(Oberbayern), O_1(Kaufbeuren), and Asia-1, application buffer (Neg), 5 ug of bovine thyroid DNA (Bov). Columns refer to different radiolabelled cDNA probes derived from the VP1 region of FMDV types O_1, C_1, C_3, Asia-1, and A_{12}. Probe A_{12} (a) refers to a small area of the genome that codes for a structural protein, and A_{12} (b) refers to a part of the polymerase gene.

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SUMMARY

A hybridization assay was developed in order to detect the presen of foot-and-mouth disease virus (FMDV). Genetic probes were lebelled with the radioisotope ^{32}P in order to detect target RNA sequences, which had been extracted from infected bovine epithelium or tissue culture. Using a 'general' probe from the polymerase region, samples from field cases of FMD were consistently detected under the specified assay conditions. Serotypic differentiation among FMDV A, O and C was determined by the use of cDNA probes from different FMDV serotypes This appears to be a useful and sensitive test for the early detection and differentiation of viruses causing field outbreaks.

RESUMEN

Un ensayo de hibridizacion fue desarrollado con el objectivo de detectar la presencia del virus de Fiebre aftosa (foot-and-mouth disease virus (FMDV). Fragmentos de DNA fueron marcados con el radio-isotopo ^{32}P con el objectivo de detectar las sequencias del RNA blanco, el cual habia sido extraido del epitelio de bovino infectado o cultivo detejido. Usando un fragmento de DNA general derivado de la region de polimerasa del genoma, las muestras provenientes de animales infectados fueron analisadas de acuerdo a las condiciones especificadas de ensayo. La Diferenciacion Serotipica entre FMDV A, O and C se determino mediante el uso de fragmentos de cDNA provenientes de diferentes serotipos de FMDV. Este parece ser un util y sensitivo test para la temprana deteccion y diferenciacion de virus en brotes bajo condiciones de campo.

RESUME

Un test d'hybridation a ete developpe pour deceler la presence du virus de la fievre aphteuse. Des fragment d'ADN ont ete marque par le radioisotope P^{32} afin d'identifier les sequences ARN cibles qui avaient ete extraites soit d'epithelium bouin infecte ou de culture ole tissu. En utilisant um fragment de la region polymerase du genome les echantillous provenant de cas de fievre aphteuse ant ete regulier-ement identifies dans les conditions prescrites par le test. La differentiation serotypique parmi les virus A, O et C a ete etablie par l'emploi de sondes cADN provenant de differents serotypes ole fievre aphteuse. Le test semble etre utile et precis pour l'identification rapide et la differentiation des virus causant l'epidemic.

HERZKATHETERISIERUNG IN VERBINDUNG MIT DER ECHOKARDIOGRAPHIE BEIM RIND

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Einleitung

Die Echokardiographie hat die diagnostischen Möglichkeiten der buiatrischen Kardiologie in letzten Jahrzehnt am nachhaltigsten beeinflußt. Mit ihr sind neben Informationen zur Morphologie auch solche über die Funktion myokardialer, valvulärer sowie vaskulärer Strukturen zu erzielen.

Soll zukünftig das gesamte Spektrum der Echokardiographie genutzt werden, so ist zunächst eine intra vitam Objektivierung insbesondere funktioneller Veränderungen mittels Herzkatheterisierung als Referenzmethode notwendig. Darüberhinaus ist neben klinischen Fragestellungen die Herzkatheterisierung in der Untersuchung hämodynamischer Medikamenteneffekte unverzichtbarer Bestandteil.

Die bisher übliche Praxis der Herzkatheterisierung unter röntgenologischer Lagekontrolle der Katheter birgt insbesondere in der Großtiermedizin aufgrund der hohen zu durchdringenden Schichtdicken die Nachteile einer nötigen aufwendigen Apparatur sowie einer erheblichen Strahlenbelastung des untersuchenden Personals.

Aus diesem Anlaß wurde geprüft, inwieweit eine kontrollierte Herzkatheterisierung mit Hilfe der Sonographie möglich ist.

Material und Methoden

Die Untersuchungen wurden zunächst an 5 Kälbern sowie Jungbullen (60 bis 150 Kg KGW) im Alter von 2 bis 5 Monaten durchgeführt. Bei diesen Tieren lagen keine Hinweise auf krankhafte Veränderungen im Herz- sowie Lungenbereich vor. Darüber hinaus wurden 3 Kälber mit angeborenen Herzmißbildungen (1 Kalb mit Fallot'scher Tetralogie, 2 Monate alt, 85 kg KGW; 2 Kälber mit Ventrikelseptumdefekt, 4 Wochen alt, 55 kg KGW;) untersucht. Ein weiteres Kalb (2 Monate alt, 65 kg KGW) litt an einer fibrinösen Bronchopneumonie sowie Pleuritis mit sekundärem Lungenemphysem. Nach Praemedikation mit 0,03 mg Xylazin/kg KGW i.m. (Rompun®, Fa. Bayer, FRG) wurden die Tiere in linker Seitenlage abgelegt. Bei jedem Tier wurde die Katheterisierung der rechten sowie linken Herzabschnitte dreimal unmittelbar nacheinander durchgeführt.

Die sonographischen Untersuchungen wurden mittels eines Microimager 1000 der Fa. Ausonics (Sydney, Australien) vorgenommen. Verwendet wurden ein 3,5 MHz (kurze Brennweite) sowie 5 MHz (Veterinärausführung) Sektorscanner. Die verwendeten zweidimensionalen Schnittbildebenen orientierten sich an den von STADLER et al. (1988)[4] am Pferd erarbeiteten Positionen. Alle Aufnahmen wurden von rechts im 3. oder 4. Interkostalraum gemacht. Dokumentiert wurde mittels Video Graphic Printer Sony® UP-850 (Eickemeyer/Tuttlingen, FRG).

Der Blutdruck wurde mittels Gould-Statham Druckwandlers (Gould Medical, Düsseldorf, FRG) als proportionales Stromsignal erfaßt und auf einer Registrieranlage der Fa. Kontron (Watford, England; Supermon 7210, Modul 7266, Recorder 7336) aufgezeichnet.

Als Zugang für den Rechtsherzkatheter (Pulmoball, 7F; Vygon/Aachen, FRG sowie USCI-NIH-Katheter, 8F; Medimex/Hamburg, FRG) wurde die V.jugularis, für den Linksherzkatheter (USCI NIH-Katheter, 8F; Medimex/Hamburg, FRG) die A.carotis gewählt. Nach Lokalanästhesie und Freilegung wurde in jedes der beiden Gefäße gemäß der

Seldingertechnik eine Kathetereinführungsschleuse (USCI, Typ Hemaquet "Standard", 8F; Medimex/Hamburg, FRG) eingebracht. Druckdom (Microflo-Flush-Unit 2; Vygon/Aachen, FRG) und Katheter wurden über ein Mikroflo-Monitoring-Set (Vygon/Aachen, FRG) mit heparinisierter istonischer Kochsalzlösung (Ratiopharm GmbH/Blaubeuren, FRG) luftblasenfrei gefüllt. Kontrastdarstellungen wurden durch Bolusinjektion von 5 ml genannter Kochsalzlösung bei Unklarheit über die Lage der Katheterspitze vorgenommen.

Aufzeichnungen des Elektrokardiogramms wurden mittels der bereits genannten Registrieranlage der Fa. Kontron (Modul 7271) sowie des Sonographen als "Base-Apex"-Ableitungen[2] durchgeführt.

Röntgenaufnahmen wurden mit Hilfe eines mobilen Gerätes (Siemens "Mobil XR", Hannover, FRG; Rubinfolie, Siemens, Hannover, FRG) parallel zur Blutdruckregistrierung sowie Sonographie jeweils bei erkennbarem Übertritt des Katheters in einen Herzabschnitt erstellt.

Ergebnisse und Besprechung

Seit SWAN UND GANZ (1970)[3] den sogenannten Rechtsherzeinschwemmkatheter entwickelten, besteht prinzipiell die Möglichkeit einen Katheter ohne Durchleuchtungskontrolle nur unter Registrierung der typischen kardialen Blutdruckkurven über die rechten Herzabschnitte in die Pulmonalarterie zu manipulieren. Zur Vermeidung von Komlikationen, wie z.B. der intrakardialen Verschlingung des Katheters oder -bei Vorliegen von Herzmißbildungen- das Verlassen der physiologischen Blutstrombahn sowie ohnehin für die Katheterisierung des linken Herzens ist eine Einführung unter Sichtkontrolle notwendig[1,2].

Bei allen lungengesunden untersuchten Tieren waren die von STADLER et al. (1988)[4] am Pferd erarbeiteten sonographischen Schnittbilder des Herzens, aufgenommen von der rechten Thoraxseite im 3. oder 4. Interkostalraum, darstellbar. Damit konnten beide Vorhöfe und Ventrikel, die A. pulmonalis, die Aorta sowie die entsprechenden Klappenebenen mittels Echokardiographie eingesehen werden (s. Abb. 3 - 6). Eine Ausnahme bildete das Kalb mit hochgradigen entzündlichen Veränderungen im Lungen- und Pleurabereich. Bei diesem Tier war einerseits die Pleura derart "echogen" sowie andererseits die rechtsseitigen Lungenabschnitte offensichtlich soweit vergrößert, daß das für die Sonographie notwendige "Schallfenster" keine verwendbaren Aufnahmen des Herzens mehr zuließ.

Sowohl der Rechtsherz- als auch der Linksherzkatheter stellten sich mittels Sonographie ausnahmslos in den einzelnen Herzabteilungen sowie den abführenden großen Gefäßen deutlich sichtbar dar (s.a. Abb. 3 - 6). Insbesondere der an der Spitze des für die rechten Herzabschnitte verwendeten SWAN-GANZ-Katheters befindliche luftgefüllte Ballon (sowie dessen sonographischer Schallschatten) erleichterte die Auffindung des Katheters im rechten Ventrikel (s. Abb. 3 u. 4). Sofern dennoch einmal Unklarheit über die Position der Katheterspitze herrschte wurden einige Milliliter isotone Kochsalzlösung über den Katheter zur Kontrastdarstellung sooft injiziert, bis der Entstehungsort des Kontrastes und damit das Katheterende ausgemacht war.

Durch den wiederholten Wechsel von Längs- zu Querschnitten des Herzens war der Katheter stets nicht nur in seinem gesamten Verlauf innerhalb des Herzens zu verfolgen, sondern darüberhinaus auch eine gute räumliche Vorstellung von der Katheterposition zu bekommen. Dies war bei der Rechtsherzkatheterisierung mittels SWAN-GANZ-Katheters weniger bedeutend, da dieser ohnehin die Tendenz hat mit dem Blutstrom in A.pulmonalis zu schwimmen. Vorteilhaft erwies sich dies bei der retrograden Katheterisierung des linken Ventrikels, d.h. der Passage der Aortenklappen entgegen der Klappenbewegung sowie des Blutstroms. Durch den wiederholten Wechsel der Schnittebenen ließ sich der in der Aorta liegende Katheter im Gefäßlumen zentrieren, so daß bei gleichzeitiger Beobachtung der Klappenbewegungen diese im geöffneten Zustand problemlos passiert werden konnten.

Stets stimmte das sonographisch gewonnene Bild mit der erwarteten Blutdruckkurve sowie dem Roentgenbild überein. Insbesondere die Passage der Herzklappenbereiche konnte, verglichen mit der Blutdruckregistrierung, sonographisch ausnahmslos gut verfolgt werden.

Übersicht 1: Sichere Identifizierung der Katheterlage in verschiedenen Positionen bei 5 herzgesunden sowie 3 Tieren mit angeborenen Herzmißbildungen mittels verschiedener Methoden.

Katheterposition	Echokardiographie	Roentgen	Blutdruckregistrierung
V.cava cran.	-	+	-
V.cava caud.	-	+	-
Rechter Vorhof	+	+	+
Passage Trikuspidalklappe	+	+	+
Rechter Ventrikel	+	+	+
Rechtsventr.Ausflußtrakt	+	+	-
Passage der Pulmonalklappe	+	+	+
Truncus pulmonalis	+	+	+
Äste der A. pulmonalis	-	+	-
A.carotis	-	+	-
Aortenwurzel	+	+	+
Passage der Aortenklappe	+	+	+
Linker Ventrikel	+	+	+
Intrakardiale Verschlingung	+	+	-
Passage eines VSD	+	+	-
+ = sichere / - = keine Identifizierung möglich			

Herzrhythmusstörungen traten regelmäßig bei Passage der Aortenklappe für wenige Herzaktionen sowie bei Berührung des Endokards der linksventrikulären Wand mit der Katheterspitze auf. Letztgenanntes Ereignis war mittels zweidimensionaler Echokardiographie sowie im Röntgenbild stets feststell- und korrigierbar. Bei der Katheterisierung des rechten Herzens wurden keine Rhythmusstörungen beobachtet.

Bei einem Kalb mit angeborenem Ventrikelseptumdefekt (VSD) sowie einem Kalb mit FALLOT'scher Tetralogie ließ sich der Rechtsherzkatheter (hier USCI-NIH-Katheter) unter sonographischer Kontrolle über den VSD in den linken Ventrikel sowie in die Aorta manipulieren.

Unbeabsichtigt knickte bei einem Tier -nach längerer Verweildauer- der in den linken Ventrikel vorgeschobene Katheter selbständig ab. Dies war erwartungsgemäß im Röntgenbild aber auch zuverlässig im zweidimensionalen echokardiographischen Bild darstellbar (Abb.6: nach erfolgtem Rückzug des Katheters in den Klappenbereich).

Eine bei der Rechtsherzkatheterisierung wiederholt auftretende Schwierigkeit war, daß beim Vorschieben des aus der V.cava cran. kommenden Katheters dieser sofort in die V.cava caudalis überwechselte. Beide Gefäße waren röntgenologisch, nicht jedoch sonographisch darstellbar. Dies Ereignis war jedoch sonographisch insofern erfaßbar, daß der beim Vorschieben des Katheters erwartete erkennbare Eintritt der Katheterspitze in den Klappenbereich des rechten Vorhofs ausblieb. In diesem Fall wurde der Katheter zurückgezogen und erneut bis zum feststellbaren Eintritt in den rechten Vorhof vorgeschoben. Ein vergleichbares Problem trat bei der Linksherzkatheterisierung nicht auf. Der über die A. carotis eingeführte Katheter ließ sich ausnahmslos ohne Schwierigkeit bis in den sonographisch kontrollierbaren Bereich der Aortenwurzel vorschieben.

Damit ermöglicht die zweidimensionale Echokardiographie beim Rind nicht nur eine nicht-invasive kardiologische Befunderhebung, sondern darüberhinaus -im gleichen Arbeitsgang- eine gerichtete Katheterisierung der Herzkammern, des Truncus pulmonalis sowie der Aortenwurzel. Sie beinhaltet dabei gegenüber der röntgenologischen Durchleuchtung die Vorteile eines hohen Maßes an Flexibilität und Mobilität (keine stationäre Einheit notwendig) sowie keiner Strahlenbelastung für den Untersucher (damit unbegrenzte Wiederholbarkeit).

Abb.1: Durchleuchtungsaufnahme des über die V.cava cran., den rechten Vorhof sowie den rechten Ventrikel in den Pulmonalklappenbereich vorgeschobenen SWAN-GANZ-Katheters.

Abb.2: Durchleuchtungsaufnahme des über die Aorta in den linken Ventrikel vorgeschobenen Katheters.

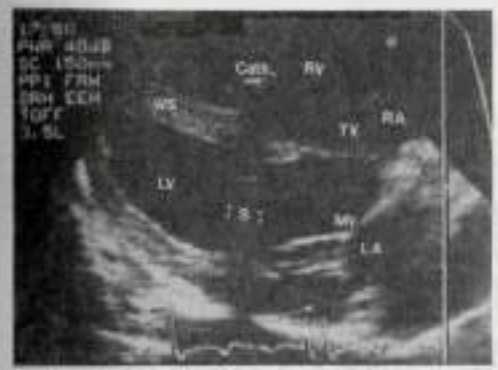

Abb.3: Zweidimensionales echokardiographisches Bild (Längsschnitt; von rechts; 4.I.C.): In RV deutlich sichtbarer Ballon (Cath., sowie dessen Schallschatten: S) des SWAN-GANZ-Katheters. Passage der Trikuspidalklappe in dieser Ebene sicher zu verfolgen.

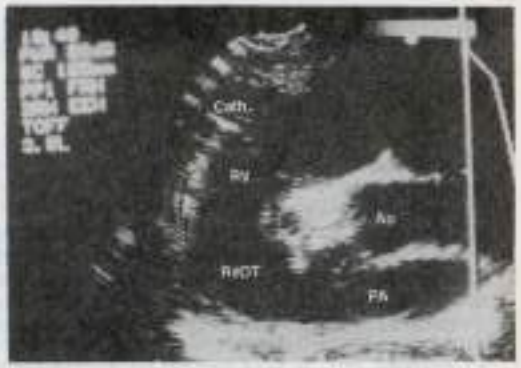

Abb.4: Zweidimensionales echokardiographisches Bild (Längsschnitt; von rechts; 3.I.C.): In dieser Schallebene läßt sich der Verlauf des Katheters in RV, über den RVOT bis in die PA identifizieren.

LE PROTOCOLE DE CONTROLE DES VACCINS I.B.R. (Rhinotrachéite infectieuse bovine) AU L.P.B.*

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1- INTRODUCTION

Le contrôle des vaccins I.B.R. est effectué dans le cadre des demandes d'A.M.M.(Autorisation de Mise sur le Marché) au L.P.B.*. Il s'agit d'un triple contrôle : innocuité, activité et efficacité effectué sur l'espèce sensible : le bovin (1).

. Innocuité : - recherche des réactions locales et générales lors de chaque vaccination,
- recherche de la non diffusibilité de la souche,
- recherche de la stérilité bactériologique et fongique

. Activité : - recherche des anticorps vaccinaux par sérologie,

. Efficacité : - une épreuve virulente sur bovins doit mettre en évidence une protection clinique et virologique.

2- PRINCIPE DE LA METHODE

Le principe consiste à comparer : 6 animaux vaccinés avec le vaccin à tester à 6 animaux recevant du sérum physiologique et, à des animaux vaccinés avec un vaccin ayant déjà son AMM, ce vaccin est l'IFFAVAX de Rhône-Mérieux.

Le vaccin en contrôle doit avoir au moins autant de qualités sur les trois plans : innocuité, activité et efficacité que le vaccin français actuellement sur le marché (3, 4).

3- CHOIX DES ANIMAUX

Pour contrôler un vaccin il faut 18 bovins âgés de 8 à 18 mois avec comme préférence 12 mois.

La race et le sexe ne sont pas des critères de choix. Mais ces animaux doivent être indemnes d'I.B.R. : il est demandé au fournisseur de vérifier l'absence d'anticorps vis-à-vis de l'I.B.R. Nous vérifions la sérologie à l'arrivée des animaux et durant une période de quarantaine de 1 mois dans notre ferme (2, 6).

Durant la quarantaine les animaux sont :

- marqués avec des n° de travail,
- tirés au sort,

. 6 témoins
. 6 vaccinés X
. 6 vaccinés IFFAVAX avec un vaccin du commerce.

- déparasités : douves, strongles, varrons,
- pose d'anneaux naseaux
- vaccinés avec un vaccin trivalent OAC contre la fièvre aphteuse,
- tondus sur l'encolure droite, un travers de main en avant de l'épaule sur une surface équivalente à celle de deux mains.

4- CONTROLE D'INNOCUITE ET D'ACTIVITE

Il se fait dans notre ferme en étable classique.

Il débute à J+0 sur animaux. Il est précédé du contrôle de stérilité bactérienne et fongique.

Les animaux vaccinés reçoivent les vaccins à la dose d'emploi et par la voie recommandées par les fabricants.

Les animaux témoins reçoivent 5 ml de sérum physiologique par voie sous cutanée sur l'emplacement tondu.

Quotidiennement de J-3 à J+7 compris sont relevées, la température rectale et de J 0 à J+7, l'épaisseur du pli de peau à l'aide d'un cutimètre (à J 0 immédiatement après vaccination).

Les personnes effectuant ces relevés n'ont pas la répartition des bovins par vaccins sous les yeux.

Tous les 8 jours à partir de J 0, il y a une prise de sang, sur tube sec sous vide de 10 ml pour sérologie IBR (2, 6) :

- sur les vaccinés il s'agit du contrôle d'activité,
- sur les témoins (qui doivent rester négatifs) cette sérologie permet de détecter le passage d'un virus sauvage.
- à J+14, rappel de vaccin anti-aphteux trivalent OAC,
- à J+28, rappel des vaccins IBR et injection de sérum physiologique aux témoins.

Ce rappel se fait dans les mêmes conditions que la primo-vaccination et avec le même suivi clinique.

5- CONTROLE D'EFFICACITE

Les bovins sont rentrés dans les étables protégées du Laboratoire de Lyon entre J+35 et J+42.

L'épreuve débute 3 semaines après le rappel (J+49). Elle se déroule en zone protégée.

5.1- La souche utilisée n° VE 85 est une souche isolée d'un cas du terrain. L'inoculum est produit par un 2ème ou 3ème passage sur cellules primaires de rein ou de testicules de veaux.

5.2- Inoculation (J+49) :

. un écouvillon de coton monté sur tige plastique de 30 cm est plongé dans la culture pure. Il sert à écouvillonner chaque narine d'un animal en remontant de façon drastique dans les cornets. L'écouvillon doit ressortir taché de sang (1 écouvillon par animal).

5.3- Surveillance des animaux :

. température :

relevé quotidiennement 3 jours avant l'inoculation,
relevé biquotidiennement du jour de l'inoculation jusqu'après le retour à la normale soit environ de J+49 à J+56,
relevé quotidien ensuite jusqu'à J+60 environ.

. symptômes :

symptômes respiratoires : il faut noter la toux, le jetage, la dyspnée.
Autres symptômes : il faut noter larmoiements ou divers autres symptômes.

. la notation est la suivante :

JETAGE :	Nul	0
	Séreux	1
	Muqueux	2
SALIVATION :	Absent	0
	Présent	1
LARMOIEMENT :	Absent	0
	Présent	1
TOUX :	Absent	0
	Présent	1
POLYPNEE :	Absent	0
	Présent	1

. prélèvements :

- Prise de sang sur tube sec 10 ml pour sérologie : tous les 8 jours.
- Ecouvillons nasaux pour titrage de virus :

2 jours	J+51
4 jours	J+53
	après inoculation
6 jours	J+55
9 jours	J+59

5.4- Abattage, autopsie :

- S'il n'y a pas de mortalité, les animaux sont abattus à J+63 et J+64.
- L'appareil respiratoire est examiné après une coupe longitudinale de la tête (cornets, sinus, larynx, trachée, poumons). On note les autres lésions s'il y a lieu.

5.5- Traitement des écouvillons

Le virus excrété est recherché sur cellules primaires de rein de veau et le titrage est effectué par effet cytopathogène (5).

Les sérologies sont effectuées par une micro technique d'hémagglutination passive avec des dilutions de sérum de raison géométrique en partant du 1/4 et allant de 2 en 2 (2).

6- EXPLOITATION DES RESULTATS

6.1- Innocuité : (primo-vaccination et rappel)

Les résultats sont traités grâce à la création d'un signe :

. pour la température :

note 0 si la température est inférieure à 39°5 ,
note 1 si la température est supérieure ou égale à 39° C.

. pour l'épaisseur du plis de peau :

note 1 s'il y a doublement de l'épaisseur du plis de peau.
note 0 s'il n'y a pas doublement.

Toutes les notes de l'épreuve sont ajoutées ce qui donne une note pour chaque groupe d'animaux. Ce sont les 3 notes qui sont comparées.

6.2- Activité :

Il s'agit d'une comparaison des moyennes des titres des animaux vaccinés lors de chaque prise de sang.

6.3- Efficacité :

. Les températures se traitent à l'aide d'un signe :

note 0 température inférieure à 40° C pour un relevé,
note 1 température supérieure ou égale à 40° C pour un relevé.

Toutes les notes de l'épreuve sont additionnées et donnent un total par groupe. Ces 3 chiffres sont comparés.

. Pour les signes et symptômes tous les chiffres quotidiens sont ajoutés dans un même groupe. Les totaux sont faits pour toute l'épreuve et chaque lot d'animaux est représenté par une note.

Pour l'autopsie :

Il y a création du signe pour chaque organe examiné :

Note 0 : pas de lésion
Note 1 : "lésion pouvant être rapportée à l'IBR ou à une sur-infection bactérienne post-IBR". (Ce qui exclu bien sûr toute lésion chronique).

Là, encore, il faut éventuellement comparer les 3 notes.

CONCLUSION

Cette épreuve à l'avantage de se dérouler sur l'animal sensible donc de donner une bonne idée de la valeur réelle des vaccins I.B.R., d'autant plus, qu'il y a toujours concordance entre la clinique, la sérologie et la virologie (4).

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RESUME

Les auteurs décrivent le contrôle que subissent les vaccins contre la Rhinotrachéite Bovine au CNEVA-Laboratoire de Pathologie Bovine (FRANCE) pour obtenir leur A.M.M.* Il s'agit d'un contrôle sur bovin par épreuve virulente avec des tests d'innocuité, d'activité et d'efficacité.

SUMMARY

The autors discribe the challenge that the I.B.R vaccines undergo at the CNEVA-Laboratoire de Pathologie Bovine* for their official licence. This challenge is a virulente one including test of innocuity, activity and efficacity.

INTERFERON STATUS IN BOVINE FOETUSES, NEONATES AND COWS

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INTRODUCTION

Neonatal animals in comparison to adults are known to have increased susceptibility to disseminated and devastating infections. Although it has been documented that the immune system of newborns is deficient in several aspects of humoral and cellular immunity, the precise mechanisms involved in this age-related sensitivity have not been well defined /1,2,3,4/. Since interferon has been shown to play an important role in antiviral resistance /5,6/, studies were carried out to determine the status of the interferon system in bovine foetuses, neonatal calves and cows after delivery.

MATERIALS AND METHODS

Experimental animals

Samples of blood were taken by jungular venipuncture from ten calves and dams immediately after delivery. The samples of blood were also taken from the same calves 2 or 3 days after birth. Foetuses were obtained by cesarian section from healthy dams and blood was taken from umbilical cord. The age of foetuses was defined on the basis of their length and anatomical features. Ten healthy, nonpregnant cows were included as controls.

Whole blood technique /7/

Heparinized blood was mixed at a ratio 1:5 with RPMI 1640 medium supplemented with penicillin 100 u/ml, and streptomycin 100 ug/ml, without additional serum.

Seperation of peripherel blood lymphocytes /PBL/

PBL were isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation. After washing with phosphate buffered saline /PBS/, cells were suspended at a concentration of 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% calf serum and antibiotics. The number of cells and their viability was determined in hemocytometer using trypan blue exclusion test. The types of white blood cells were determined by microscopic examination of cells stained with Wright-Giemsa stain.

Induction of interferon

PBL isolated from blood of foetuses, calves, dams and cows and also cultures of whole blood cells were induced with Radom strain of NDV /10 $TCID_{50}$ or 1 $TCID_{50}$/ cell /and induced with PHA and Con A/10 ug/ml or 1 ug/ml/. Cultures were incubated at 37°C for 24 hrs after induction with virus, and 72 hrs after induction with mitogens. Supernatant fluids after centrifugation were harvested and assayed for interferon activity.

Interferon assay

Since bovine IFN was found to be partially unstable at pH 2, the infectivity of residual virus, used for interferon induction, was destroyed by uv irradiation /54 000 J/m^2/. Interferon was assayed in 96 well plastic plates using bovine embryonic fibroblasts /BEF/, at a level of 3 to 20 passages with vesicular stomatitis virus /VSV/, Indiana serotype as challenge. Interferon titers were calculated as the reciprocal of the dilution causing 50% reduction of cytopathic effect and expressed as mean ± S.D. The statistical significance was assessed by the Student's t test. The laboratory standard of semi-purified bovine interferon /8/ was included in each assay.

RESULTS

Since IFN has been shown to play an important role not only in antiviral resistance but also in immunoregulation /9,10/, the magnitude of interferon production in vivo may determine, in part, the animal's ability to resist infections. The present investigation demonstrates that neonatalcalf leukocytes examined by the whole blood technique produce considerably less interferon in response to viral induction in comparison with leukocytes of adult animals /Figure 1/ and in comparison to leukocytes of foetuses /data not presented/. This deficiency correlates with the neutrophilia so it may be due to the low number of interferon producing cells /mainly monocytes, large granular lymphocytes/ or to the presence of soluble inhibitors released by other cells /3,11/.

The mononuclear cells /about 94% of mononuclear/ after separation on Ficoll-Hypaque were more effective producers of interferon, for the same number of cells, than the nonseparated population of leukocytes, but the differences in interferon yield from newborn and adult leukocytes were still present /Table 1/.

When blood leukocytes or separated mononuclear cells were induced with PHA or Con A, a very low level or no interferon was detected in cultures derived from neonatal calves /Figure 1 and Table 1/. However leukocytes of foetuses and 2-3-day-old calves responded to the virus and mitogens with interferon production much higher than those observed in leukocytes of newborns.

Since in sera of newborn calves no soluble inhibitors of interferon production were detected /data not presented/, this suggest, that if they exist, they are rather nonsteble.

It is also interesting that leukocytes of dams produced lower interferon levels in comparison with leukocytes of nonpregnant cows. This phenomenon also correlated with leukopenia and neutrophilia and no soluble serum inhibitors of interferon production were detected.

However, we cannot exclude that hormones or other mediators like prostaglandins /12,13/ can influence the leukocyte metabolism in vivo and low interferon production in vitro can be the consequence of their action.

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Table 1. Production of interferon in mononuclear blood cells of foetuses, calves, dams and nonpregnant cows after induction with NDV-R and mitogens.

Animal	Interferon titer $\log_{10}$U/ $2x10^6$ of cells induced with:					
	NDV-R /$TCID_{50}$/cell/		PHA µg/ml		Con A µg/ml	
	10	1	10	1	10	1
5-month-old foetuses	3,37±2,88 a	2.02±1.61	2.12±1.48	1.20±1.40	2.15±1.15 a	1.36±1.12
dams	3.47±1.27 b	2.38±1.33	2.40±1.20	1.52±1.52	2.38±1.33 b	1.52±1.66
neonatal calves	3.04±1.20	2.06±1.12	1.34±1.67	1.27±1.55	1.32±1.64	1.20±1.40
2-3-day-old calves	3.43±1.23	2.57±1.39	2.56±1.29	1.90±1.53	2.58±1.30	1.90±1.53
cows	3.41±1.28	2.46±1.13	2.40±1.14	2.25±1.11	2.55±1.25	2.15±1.15

a versus b $p \leq 0,05$

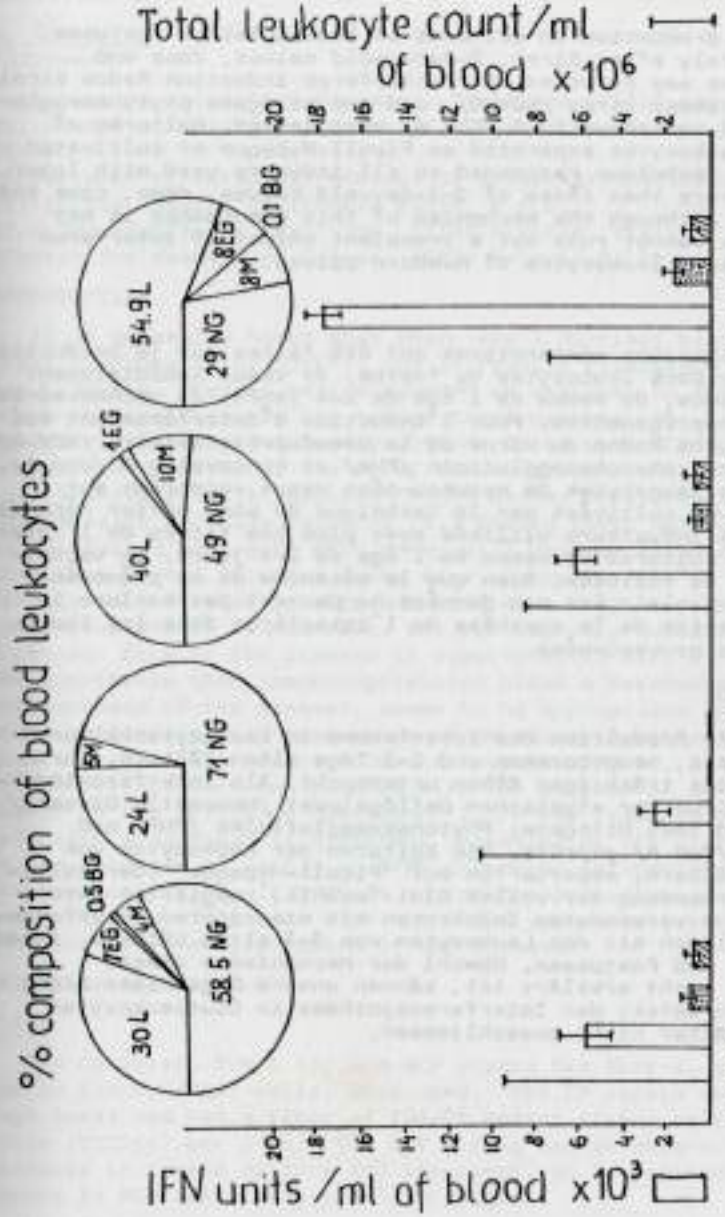

Figure 1 Production of INF in blood cells of calves, dams and cows after induction in vitro with NDV-R, PHA and con A ☐ NDV-R ▨ PHA ▨ con A
NG - neutrophils, EG - eozynophils, BG - basophils, M - monocytes, L - lymphocytes

SUMMARY

Interferon production in cultures of leukocytes of foetuses, calves immediately after birth, 2-3-day-old calves, dams and nonpregnant cows was compared. For interferon induction Radom strain of Newcastle Disease Virus /NDV-R/, and two mitogens phytohemagglutinin /PHA/ and concanavalin A /Con A/ were tested. Cultures of newborn-calf leukocytes separated on Ficoll-Hypaque or cultivated by whole blood technique responded to all inducers used with lower interferons titers than those of 2-3-day-old calves, dams, cows and even foetuses. Although the mechanism of this phenomenon is not clear, our data cannot rule out a transient defect of interferon synthesis in blood leukocytes of newborn calves.

RESUMÉ

Les investigations comparatives ont été faites sur la production de l'interféron dans leucocytes du foetus, de veaux immédiatement aprés la naissance, de veaux de l'âge de 2-4 jours, de vaches-méres et de vaches nonprégenantes. Pour l'induction d'interféron ont été examinés la souche Radom du virus de la pseudopeste aviaire /NDV-R/, et deux mitogens: phytohemagglutinin /PHA/ et concavalin A /con A/. Les cultures de leucocytes de nouveau-nées veaux,-separées sur Ficoll-Hypaque ou cultivées par la technique du sang entier répondait mieux aux touts inducteurs ulilisés avec plus bas titers de l'interférons que les cultures de veaux de l'âge de 2-4 jours, de vaches--méres et même de foetuses. Bien que le mécanism de ce phénoméne n'est pas encore clair les nos données ne peuvent pas exclure le déffaut transitoire de la synthése de l'interféron dans les leucocytes des veaux nouveau-nées.

ZUSAMMENFASSUNG

Es wurde die Produktion des Interferons in Leukozytenkulturen von den Foetussen, neugeborenen und 2-3 Tage alten Kälbern, ihren Müttern und nicht trächtigen Kühen untersucht. Als Interferoninduktoren wurden Virus der atypischen Geflügelpest /Newcastle Disease/ Radom Stamm und zwei Mitogene: Phytohaemagglutinine /PHA/ und Concavaline A /Con A/ geprüft. Die Kulturen der Leukozyten von neugeborenen Kälbern, separierten auf "Ficoll-Hypaque" oder kultivierten mit Verwendung der vollen Blut-Technik, reagierten unter dem Einfluss von verwendeten Induktoren mit niedrigeren Interferontitern im Vergleich mit den Leukozyten von 2-3 alten Kälbern, ihren Müttern, Kühen und Foetussen. Obwohl der Mechanismus dieses Prozesses noch nicht erkklärt ist, können unsere Ergebnisse einen vorübergehenden Defekt der Interferonsynthese in Blutleukozyten neugeborener Kälber nicht ausschliessen.

A STUDY OF SOME PATHOGENETIC ASPECTS OF BOVINE VIRAL DIARRHEA VIRUS INFECTION

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INTRODUCTION

It is generally known that there are 2 distinct biotypes of Bovine Viral Diarrhea Virus (BVDV): one is readily cytopathic (CP) the other is not. Several studies have suggested an explanation for the mechanism by which the two biotypes can induce fatal disease (6). It begins with an early transplacental infection of the fetus with NCP BVDV prior to the complete maturation of the fetal immune system and the animals that eventually survive fetal infection might become persistently viremic with no neutralizing antibody response (9). Should these animals be subsequently superinfected with a CP BVDV, they will undergo fatal disease. It has also been proven (3) that acute form of the disease is observed in persistently infected cattle with NCP BVDV when they are superinfected with homologous BVDV. In contrast, the persistently infected cattle develop a chronic form of the disease if superinfected with a heterologous BVDV. The hypothesis that immunosuppression plays a peramount role (11) in the pathogenesis of the disease, seems to be appropriate in view of the evidence that at least a proportion of persistently infected animals may have a permanently impaired immune response (8, 10, 12).

The present work was planned with the purpose of making an attempt to answer to the following questions: 1. Is the response of calves to the infection significantly different depending on the biotype of BVDV? 2. Is the response of cattle to BVDV infection influenced by an immunosuppressive state? 3. If BVDV is an immunosuppressive agent itself, could a simultaneous infection with the 2 biotypes of the virus result in the classically fatal expression of the disease?

MATERIALS AND METHODS

Virus

The CP strain TVM-2 (5) and NCP strain New York-1, grown in bovine embryo kidney (BEK) cells, were used. The CP strain was at its 6th passage level and had a titer of $10^{6.50}$ median tissue culture infectious doses ($TCID_{50}$) per 1 ml. The NCP strain had an undetermined number of passages in tissue culture and its titer, as determined by immunofluorescence in BEK cells, was $10^{5.50}$ $TCID_{50}$/ml.

Experimental design: inoculation of calves

Twenty-two 30-40 days old Friesian calves, devoid of neutralizing antibody to BVDV, were subdivided into 5 groups of 6, 6, 4, 4 and 2 respectively. The calves of Group 1 and 2 were inoculated with CP or NCP strains of BVDV, respectively. In Groups 3 and 4, 2 calves in each group were inoculated as above, i.e. with CP or NCP BVDV, whereas the remaining 2 calves in each group were given dexamethasone (DMS) in addition to BVDV. Finally, calves of Group 5, received simultaneously both, the CP and the NCP BVDV. The CP and NCP BVDV were inoculated intravenously (i.v.), each calf receiving 5 ml of virus. The DMS was given i.v. and each calf was injected daily for 4 consecutive days with 0.1 mg/kg of body weight per day, with the first injection being made a few hours before virus inoculation.

Viral isolation

Attempts to isolate virus in BEK cells were carried out on pharyngeal swabbings and leukocytes obtained from each calf at pre-determined intervals after infection. Samples were considered negative for virus if cytopathic effects (CPE) failed to appear or immunofluorescence (fluorescein-isothiocianate conjugated with USDA anti-pestivirus 445 BVD serum of porcine origin) was negative after 2 serial subpassages of the inoculum had been made. The virus recovered from the samples was identified as BVDV by serum neutralization tests.

RESULTS

Response of calves to experimental infection with BVDV

- Group 1: CP BVDV

As shown in Table 1 all 6 animals inoculated with CP strain of BVDV

TABLE 1. The response of calves exposed to experimental infection with cytopathic TVM-2 strain of Bovine Viral Diarrhea Virus.

Calf No.	Clinical signs: Fever ≥40°C onset/duration[a]	Nasal discharge onset/duration	Diarrhea onset/duration	Leukopenia onset/duration	Oral lesions on PID	Virus recovery in BEK cells on PID from: PS	LE
141	3/4	3/7	4/1	2/6	None	2,11	2[b]
132	2/2	2/7	4/1	2/5	None	2,11	2,6,17
133	3/7	2/4	2/5	2/7	None	2,11	2,17
134	3/5	2/10	6/1	2/6	None	2,11	11,17
135	5/6	3/7	3/5	2/10	None	2,11	4,11,17
136	2/7	2/6	2/6	3/10	4	2,11	N.I.

a = Post infection day (PID)/days; b = Samples were collected on PID 2, 4, 6, 7, 11, 17; PS = Pharyngeal swabbings; LE = Buffy coat; BEK = Bovine embryo kidney; N.I. = Virus was not isolated.

had a clinical response very similar to that observed previously (1)

with this viral strain. The calves developed a febrile reaction, leukopenia, respiratory symptoms and diarrhea and, in one case, mouth lesions. All animal recovered in about 2 weeks. Virus was consistently recovered from the pharyngeal swabbings of all calves from PID 2 through PID 11, whereas virus recovery from the buffy coat was less regular. At PID 30 neutralizing antibody were found at titers ranging 1:32-1:128.

- Group 2: NCP BVDV

The 6 calves inoculated with NCP strain New York-1 of BVDV did not show any clinical sign of the disease. The body temperatures, as well as the leukocyte counts, remained normal. However, the virus was isolated from pharyngeal swabbings and from buffy coat of all calves. The immunologic response of these calves was similar to that described for calves exposed to CP BVDV.

Response to BVDV infection of calves treated or not treated with DMS

- Group 3: CP BVDV

As depicted in Table 2, the clinical response of calves to CP strain of BVDV was generally more severe in the case of those animals which were treated with DMS. The two calves (Nos. 193, 194) which were given

TABLE 2. The response of calves treated or not treated with dexamethasone (DMS) and exposed to experimental infection with cytopathic TVM-2 strain of Bovine Viral Diarrhea Virus.

Calf No.	DMS treatment	Clinical signs: Fever ≥40°C onset/duration[a]	Nasal discharge onset/duration	Diarrhea onset/duration	Leukopenia onset/duration	Virus recovery in BEK cells, on PID from: PS	LE
191	T	5/10	6/9	5/13	10/4	2	2,6[b]
192*	T	4/4*	6/1*	5/3*	6/2*	2,6	2,6§
193	NT	3/6	9/2	7/5	3/6	2,6	2
194	NT	5/3	7/1	7/7	3/5	6	2

a = Post infection day (PID)/days; b = Samples (PS pharyngeal swabs; LE buffy coat) were collected on PID 2 and 6; BEK = bovine embryo kidney; T = treated; NT = not treated; * Calf 192 died on PID 7; § Virus was also isolated from lung and spleen of this calf.

the virus only, had a clinical response similar to that described above for the CP BVDV infection of Group 1 calves in that they reacted with fever, nasal discharge, diarrhea and leukopenia and recovered in about two weeks. The 2 calves (Nos. 191, 192) which, beside the virus, were also given DMS for 4 consecutive days, developed symptoms later than the former animals, but they were much more severe and lasted longer; moreover, one calf (No. 192) died 3 days following the last injection of DMS, i.e. on PID 7. At necropsy the mucosa of the small intestine was slight congested and the mesenteric lymph nodes appeared enlarged.

Virus was isolated from both groups of calves from both pharyngeal swabbings and from buffy coat. Virus was also isolated from the spleen and from the lung of the dead calf. Neutralizing antibody titers were found in the serums of all calves which survived, and at PID 30 they were in the same range (1:32 - 1:128) as those observed for the other groups of calves.

- Group 4: NCP BVDV

In the case of those calves exposed to NCP BVDV, the simultaneous treatment with DMS induced a severe clinical disease (Table 3). The 2 DMS treated calves had high fever, nasal discharge, leukopenia and diarrhea. One calf died on PID 11 and the other on PID 17. At necropsy congestion was observed in the upper digestive tract and superficial erosions were found in the mucosa of small intestine. Mesenteric lymph nodes were hemorrhagic and enlarged. In contrast, the 2 calves which

TABLE 3. The response of calves treated or not treated with dexamethasone (DMS) and exposed to experimental infection with non-cytopathic New York-1 strain of Bovine Viral Diarrhea Virus.

Calf No.	DMS treatment	Clinical signs: Fever ≥40°C onset/duration[a]	Nasal discharge onset/duration	Diarrhea onset/duration	Leukopenia onset/duration	Virus recovery in BEK cells*, on PID, from: PS	LE
149§	T	5/10§	6/8§	6/10§	6/8§	2,4,11[b]	2,6,8,11
150°	T	6/5°	6/2°	6/5°	6/5°	2,4,8	2,6,8°
151	NT	None	4/2	None	None	2,11	2,6,8,11
152	NT	None	10/1	None	None	4,11	2,6,8,11

a = Post infection day (PID)/days; b = Samples (PS pharyngeal swabs; LE buffy coat) were collected on PIDs 2, 4, 6, 8, 11; * Virus detection was made by immunofluorescence; Died on PID 17 (§) or PID 11 (*); ° Virus was also detected in the spleen and lung of this calf; T = treated; NT = not treated.

were only injected with the NCP BVDV showed no clinical signs of disease, with the exception of a slight nasal discharge. Virus was consistently recovered from pharyngeal swabbings and buffy coat samples without any difference being observed between the two groups of calves. Virus was also isolated from spleen and lung of the calf that died on PID 11.

Group 5: Response of calves to simultaneous infection with CP and NCP BVDV

The clinical response of the 2 calves was analogous to that observed in the calves of Group 1 which were used to test the pathogenicity of the CP BVDV. It seems, therefore, that the mixed infection did not result in any particular unexpected pathological situation.

DISCUSSION

In this study it was demonstrated that CP TVM-2 strain of BVDV induced in calves a severe disease, whereas the calves inoculated with the NCP New York-1 strain remained clinically normal. The data are still insufficient to decide whether the key factor for a different pathogenic role of the virus can be related to its ability to induce cytopathology or not. However, it becomes increasingly obvious that among BVDV isolates there can be a potential diversity in their pathogenic pathway toward the host. This study confirms that the clinical response of cattle to BVDV infection is significantly affected by an immunosuppressive state of the host. Evidently, as already suggested (11), the immunosuppression represents a further "key factor" in the pathogenesis of bovine viral diarrhea. To conclude: The BVDV infection should be regarded as a multifactorial syndrome (7) where several factors might be involved in altering the pathogenesis, such as: 1. An immunotollerant state induced in persistently infected cattle with NCP (and/or CP also?) BVDV. These cattle when superinfected with an homologous BVDV (3) undergo an acute and, eventually, fatal expression of the disease. 2. The biotype of BVDV, seems to influence significantly the development of the disease. 3. The immunosuppressive state of the animals is responsible for a severe form of bovine viral diarrhea. In this case the biotype does not seen to play any particular role in the development of the disease which, whatever biotype is involved, is almost always fatal. 4. The immunosuppressive activity of BVDV could be a property peculiar to certain strains of the virus. On the other hand, there is evidence that under natural conditions BVDV might be responsible of immunologic dysfunctions in cattle (11).

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SUMMARY

The cytopathic (CP) strain TVM-2 of bovine virus diarrhea virus (BVDV) induced in calves a severe disease, whereas the calves inoculated with the non-cytopathic (NCP) New York-1 strain, remained clinically normal. When calves were immunosuppressed with dexamethasone (DMS) they underwent an overt, generally fatal disease. This result was obtained with either the CP and the NCP strain of BVDV. It was speculated that the immunosuppressive activity of BVDV could be a property peculiar to certain isolates of the virus.

RESUME

La souche cytopathique (CP) TVM-2 du virus de la diarrhée bovine virale (BVDV) a provoqué chez les veaux une grave maladie tandis que les veaux inoculés avec la souche non cytopathique (NCP) New York-1 sont restés cliniquement normaux. Quand les veaux ont été soumis à une immunosuppression par dexaméthasone (DMS), ils ont été frappés d'une maladie manifeste, généralement mortelle. Ce résultat a été obtenu tant avec la souche CP qu'avec la souche NCP de BVDV. L'hypothèse a été émise que l'activité immunosuppressive du BVDV pourrait être une propriété particulière à certains isolats du virus.

ZUSAMMENFASSUNG

Der zytopathologische (CP) Stamm TVM-2 des BVD Virus (BVDV) führte bei Kälbern zu einer schweren Erkrankung, dagegen blieben die mit nicht zytopathologischem (NCP) Stamm New York-1 geimpfter Kälber klinisch normal.

Wenn das Immunsystem der Kalber mit Dexamethason (DMS) unterdrückt wurde, kam es normaler Weise zum offenen Ausbruch einer tödlichen Erkrankung. Dieses Risultat wurde sowohl mit dem CP-Stamm als auch dem NCP-Stamm erzielt.

Es wird spekuliert, dass die das Immunsystem unterdrückende Eigenschaft des BVDV eine spezifische Eigenheit einiger isolierter Viren darstellt.

CLINICAL, IMMUNOLOGICAL AND HAEMATOLOGICAL REACTION TO VARIOUS ANTIGENS IN CALVES PESISTENTLY INFECTED WITH BOVINE VIRUS DIARRHOEA VIRUS (BVDV).

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INTRODUCTION

Chronic bovine virus diarrhoea (BVD) seems to be a disease of increasing importance in the Norwegian cattle population (1). During the last years several outbreaks have been reported. The diagnosis is difficult to make on the basis of clinical and pathological findings. Serological and virological examinations must form the basis for the diagnosis.

Non-cytopathogenic strains of BVDV are found in the majority of cases with clinical symptoms (2). In clinical outbreaks, persistently infected calves are often detected by virological examination of blood samples.

The present work was undertaken to study possible differences in haematological and immunological parameters between calves persistently infected with BVDV and non-infected calves, and so study the reaction to various antigens in these two groups.

MATERIALS AND METHODS

Animals

Nine calves, 6-10 months old, persistently infected with non-cytopathogenic BVDV, were detected in a herd with outbreaks of chronic BVD. They were transported to the Medical Clinic, Norwegian College of Veterinary Medicine (NCVM), where they were kept until slaughter.

Ten animals of the same age from the experimental farm of NCVM were used as control group. These animals were negative for both BVD virus and BVD antibodies.

Experimental design

On experimental day 0 and 21, all the calves were inoculated subcutaneously with the following antigens:

1. Prevacun FT: Influenza A equi 1,2 and 2F, and tetanus toxoid, 2 ml.
2. Pneumabort K: Equin herpesvirus serotype 1, 2ml.
3. Parvopig: Porcine parvovirus, 2 ml.
4. Pastacidin: Vaccine consisting of Pasteurella multocida, Pasteurella haemolytica, Staphylococcus aureus, Actinomyces pyogenes, 2 ml.
5. Ovalbumin (200 ug/ml) 0.25 ml + 0.25 ml alhydrogel.
6. Corynebacterium diphteriae toxoid (50 lf/ml) 0.25 ml + 0.25 ml alhydrogel.

Blood examination

Blood samples from all animals were taken on experimental day (ED) 0, ED 21 and ED 42. They were examined for various blood parameters using routine methods. The blood samples were also tested in a lymfocyte transformation test (LTT) for mitogene respons.

Virological and serological examination

Serum samples were inoculated on a calf kidney cell culture which was examined for non-cytopathogenic strains of BVDV by an indirect peroxydase staining technique. Serum samples were also examined for neutralizing antibodies against BVDV, and for antibodies against the various antigens inoculated on the calves.

Statistical methods

The Kruskal Wallis test was used to test for significant differences between blood parameter levels at start and end of the experiment. The test was also used to compare start level and end level with midperiod level.

Kruskal Wallis test was used to test the effect of time on the blood parameters by use of the NPAR1WAY procedure in SAS (SAS Institute Inc. SAS/STAT Guide for Personal Computers, Version 6. Cary, NC:SAS Institute Inc., 1987. 1028 pp.)

Analysis of covariance by use of proc GLM in SAS was also performed. However, due to deviation from normality, the statistical analysis are mainly based on the Kruskal Wallis test.

RESULTS

None of the animals showed any symptoms of disease during the experiment. Fig. 1 show the average values of haemoglobin, packed cell volume, leukocytes, total serum protein and serum gamma globulins in both groups on the 3 sampling dates.

On the first sampling day (ED 0), no significant difference was found in haemoglobin content, packed cell volume, number of leukocytes and total serum protein between the two groups, while serum gamma globulin was significantly lower ($p < 0.01$) in the infected group than in the control group.

On the last testing day (ED 42) there was a significant difference between the groups with regard to haemoglobin content ($p < 0.002$), packed cell volume ($p < 0.005$), leukocyte number ($p < 0.01$) and serum total protein ($p < 0.002$), but no significant difference was found in serum gamma globulin levels.

If we compare the differences between the groups from the first to the last sampling day, there was a significant difference in haemoglobin content ($p < 0.002$), packed cell volume ($p < 0.005$), leukocyte number ($p < 0.01$) and serum total protein ($p < 0.002$), but not for serum gamma globulin.

Fig. 2 shows the mitogen response in the two groups on the first sampling day (ED 42). The infected group had a significantly stronger response to PHA ($p < 0.0025$) and PWM ($p < 0.05$) than the control group, while no significant difference was found for ConA.

Fig. 2 also shows the antibody titres to tetanus toxoid, diphteriae toxoid, and to P. haemolytica on the 3 sampling dates. The titres in the two groups on the first sampling date (ED 0) was significantly different for tetanus toxoid ($p < 0.01$) and P. haemolytica ($p < 0.05$), but not for diphteriae toxoid. On the second sampling date (ED 21) they were significantly different for tetanus toxoid ($p < 0.05$) and for diphteriae toxoid ($p < 0.01$), but not for P. haemolytica. No significant differences were found on the third sampling day (ED 42). A similar pattern was found for the other antigens tested.

DISCUSSION

None of the animals showed symptoms of illness. All the animals in the infected group were persistently infected with non-cytopathogenic BVDV. At slaughter the carcasses were accepted by the meat control without comments.

On the first sampling day, before the animals were exposed to the experimental antigens, no significant differences were found in the examined blood parametres, except for serum gamma globulin. The difference in gamma globulin values is in accordance with earlier findings in animals with clinical chronic BVD, which were found to have substantially reduced serum gamma globulins, even if they had a longstanding secondary infection with pyogenic bacteria (1).

The inoculation with several antigens affected the two groups differently. Haemoglobin content, packed cell volume, number og leukocytes and serum total protein all increased in the uninfected calves, while these values fell in the infected group. The mean values were significantly different between the two groups. The serum gamma globulin level increased in the infected group and fell slightly in the control group, and on day 42 no significant difference could be found between the groups.

These findings may indicate that the infected group reacted slowly to various antigens, judged by certain blood parameters, but were stimulated by the antigens to produce more gamma globulins.

The mitogen response, shown in fig. 6, was somewhat unexpected. The mean lymphocyte responses to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were significantly higher in the infected group than in the control group, while no significant difference was found for concanavalin A (ConA). This is in accordance with findings of Løken et al in virus inoculated kids (3), but in contrast to decreased response to mitogens by mononuclear blood cells derived from pestivirus-infected cattle or sheep, found by other workers (4).

On the two first sampling days (ED 0 and ED 21), the antibody titres to all antigens were higher in the control group than in the infected group, and for some of the antigens the difference was significant. However, both groups had a similar response to the antigen challenge, and no significant difference could be found in titre values in ED 42. The pattern was the same for all the antigens tested, and ties well in with the increasing gamma globulin levels in the infected group.

The experiment shows that persistently infected animals react differently from non-infected animals to various antigenic challenges. Further research is needed to show what happens when the defence mechanisms in infected cattle breaks down after challenge by secondary microorganisms.

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SUMMARY

An experiment was carried out, where a group of calves persistently infected with bovine virus diarrhoea virus (BVDV) was compared with a similar group of non-infected calves. Both groups were inoculated with various antigens, and clinical condition and changes in haematological values were examined. Blood samples were also examined for antibody titres against the various antigens, and a mitogen induced lymphocyte transformation test was performed.

When the experiment started, no differences in the blood values examined were found between the two groups, except for serum gamma globulin values, which were significantly lower in the infected group. After antigenic challenge, haemoglobin values, packed cell volume, number of leukocytes and total serum protein values all decreased in the infected group , and were significantly different from those of the control group when the experiment ended. For gamma globulin, the pattern was different, and no significant difference was found in the end of the experiment.

The infected animals showed an increased lymphocyte response to PHA and PWM, while no difference was found for Con A. The infected group also had a lower starting point in antibody titres than the control group, but the increase in titres after challenge was similar in the two groups.

RÉSUMÉ

Une expérience a été réalisée, au cours de laquelle un groupe de veaux contaminés du virus bovin diarrhétique persistant (BVDV) a été comparé à un groupe analogue de veaux non contaminés. Différents antigènes ont été inoculés aux deux groupes, et les conditions cliniques et les changements dans les valeurs hématologiques ont été examinés. Les prélèvements de sangs ont également fait l'objet

de recherches de titres d'anticorps contre les différents antigènes, et un test de transformation des lymphocytes d'origine mitogène a été réalisé.

Lorsque l'expérience a débuté, aucune différence des valeurs du sang examiné n'a été enregistrée entre des deux groupes, sauf pour les valeurs du serum de gamma-globuline, qui étaient considérablement plus faibles que pour le groupe contaminé. Après une provocation antigénique, l'ensemble des valeurs de l'hémoglobine, du volume de cellules tassées, le nombre de leucocytes et le total des valeurs des protéines du sérum ont diminué dans le groupe contaminé et elles étaient considérablement différentes de celles du groupe de contrôle à la fin de l'èxpérience. En ce qui concerne le gamma-globuline, la situation était différente, et aucune différence sensible n'a été relevée à la fin de l'experience.

Chez les animaux contaminés on a pu relever une réaction accrue des lymphocytes à PHA et PWA, alors qu'aucune différence n'a été constantée pour Con A. Le groupe contaminé avait également un niveau inférieur au départ de titres d'anticorps que le groupe de contrôle, mais l'augmentation des titres après provocation était identique pour les deux groupes.

ZUSAMMENFASSUNG

In einer Versuchsreihe wurde eine persistent mit dem BVD-Virus ("bovine virus diarrhoea virus") infizierte Kälbergruppe einer nichtinfizierten, gleichartigen Kälbergruppe vergleichweise gegenübergestellt. Bei beiden Gruppen erfolgte eine Impfung mit Antigenen, worauf der klinische Befund and Änderungen in den hämatologischen Werten untersucht wurden. Es wurden auch Blutproben auf Antikörpertiter gegenüber den eingeimpften Antigenen analysiert sowie ein mitogenisch-induzierter Lymphozytentransformationstest durchgeführt.

Bei Beginn der Versuchsreihe wurden keinerlei Differenzen in den untersuchten Blutwerten bei den zwei Versuchgruppen festgestellt, mit Ausnahme der Serumgammaglobulinwerte, die bei der infizierten Gruppe beträchtlich niedriger ausfielen. Nach erfolgter Antigen-Anregung ergab sich bei der infizierten Gruppe ein Abfallen in den Hämoglobinwerten, im Haftzellvolumen, in der Anzahl der Leukozyten und den Gesamt-Serumproteinwerten, d.h. es ergaben sich deutliche Abweichungen von den Werten der Kontrolgruppe bei Abschluss der Versuchsreihe. Bei Gammaglobulin war der Ablauf verschieden, da sich bei Versuchende keine bemerkenswerte Differenz ergab.

Die infizierte Tiergruppe wies eine erhöhte Lymphozytenreaktion gegenüber PHA und PWM auf, während jedoch bei Con A keine Abweichung feststellbar war. Bei den infizierten Tieren ergab sich auch ein niedrigerer Startpunkt in Antikörpertitern als bei der Kontrollgruppe, doch war der Titeranstieg nach Antigen-Anregung dann bei beide Gruppen ähnlich.

RESUMEN

Se llevó a cabo un experimento, en el que un grupo de becerros con infección persistente del virus de la diarrea bovina (BVDV, bovine virus diarrhoea virus) fue comparado con un grupo similar de becerros no infectados. Ambos grupos fueron inoculados con varios antígenos, y se examinaron las condiciones clínicas y los cambios de los valores hematológicos. Se examinaron también pruebas de sangre para comparar los valores de anticuerpos con los diferentes antígenos, y se efectuó una prueba de transformación linfocítica inducida por mitógeno.

Cuando se inició el experimento no se encontró ninguna diferencia para los valores examinados en la sangre entre le dos grupos, excepto los valores de suero de gamma globulina, que eran considerablemente más bajos en el grupo infectado. Tras desafió antigénico, los valores de hemoglobina, el volumen comprimido de células, el numero de leucocitos y los valores del total de proteínas séricas, todos disminuyeron en el grupo infectado, y fueron considerablemente diferentes de aquellos del grupo de control cuando terminó el experimento. Para la gamma globulina el esquema fue diferente, y no se encontró ninguna diferencia significativa cuando terminó el experimento.

Los animales infectados mostraron un crecimiento en la respuesta de linfocitos ante PHA y PWM, mientras que no se encontró ninguna diferencia con respecto a Con A. El grupo infectado también tenía un punto de partida más bajo en los valores de anticuerpos que el grupo de control. No obstante, el incremento de los valores tras el desafío fue similar en los dos grupos.

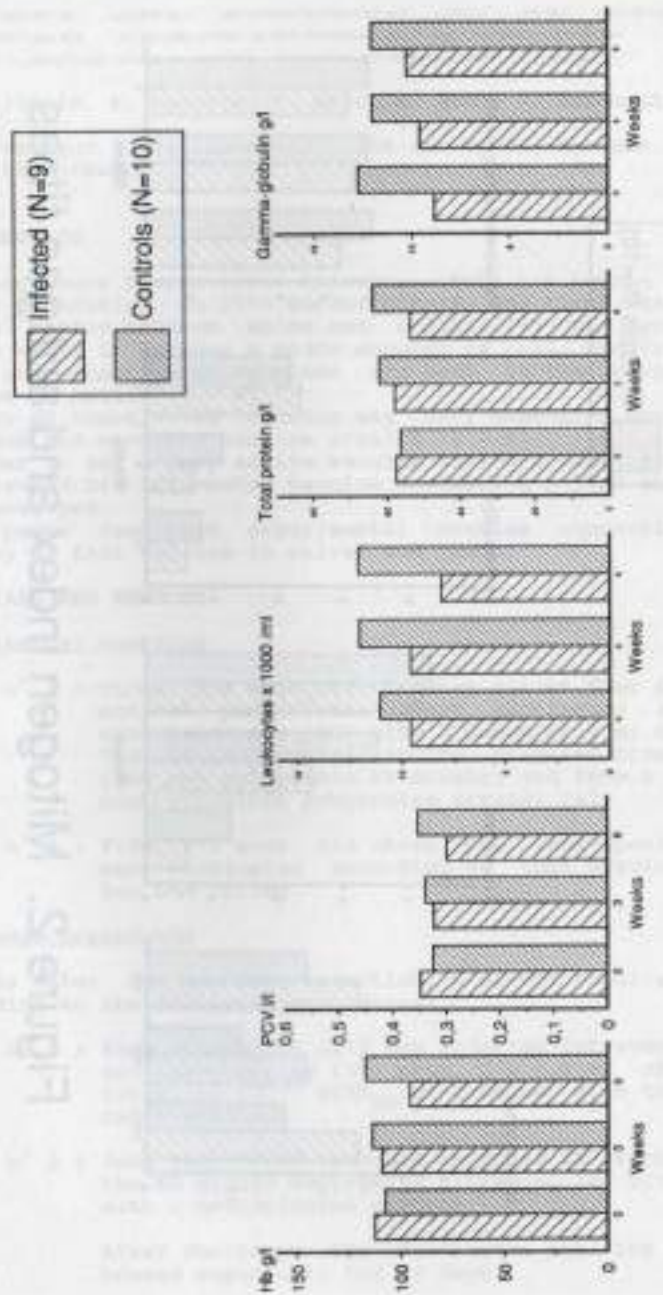

Figure 1. Blood parameters

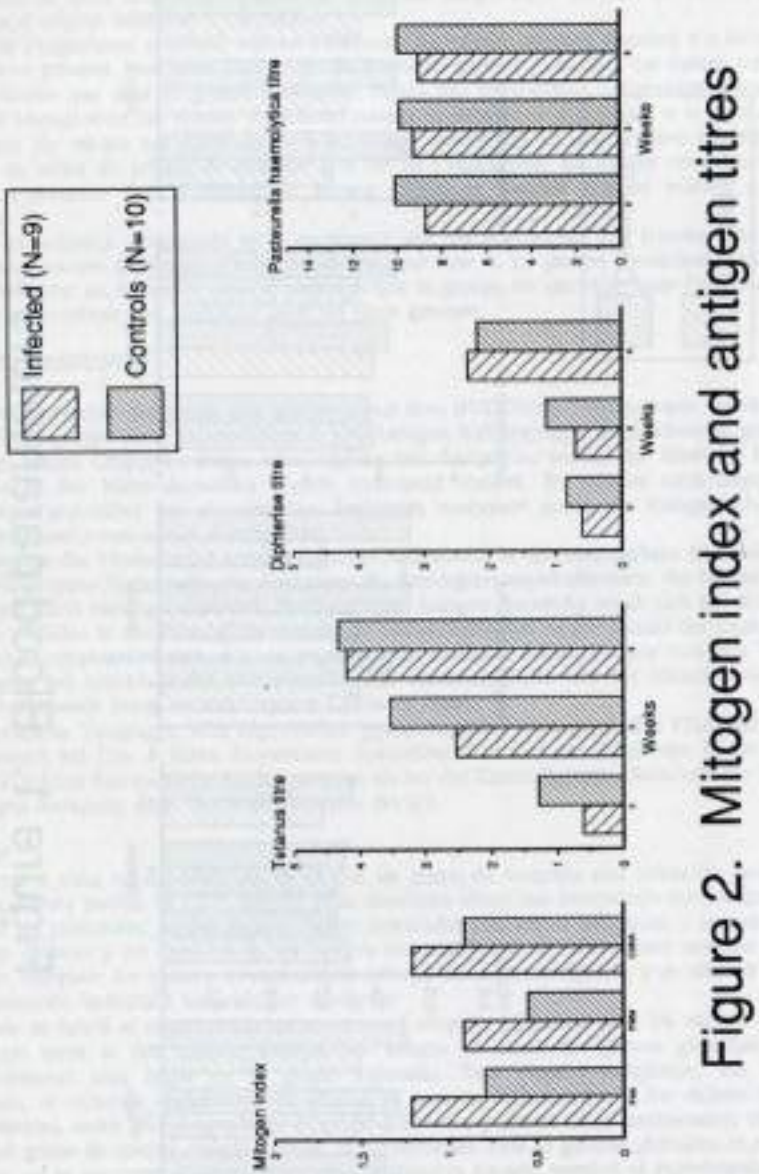

Figure 2. Mitogen index and antigen titres

SAFETY AND POTENCY OF AN OIL ADJUVANTED INACTIVATED VACCINE AGAINST THE BOVINE PESTIVIRUSES

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INTRODUCTION

For many years Bovine Viral Diarrhea (BVD) has been a problem in the cattle population. In 1959 Border Disease was first described on sheep at the border between Wales and England (7) and spread throughout Europe where it because a major concern (4 - 8). Modified Live Vaccine (MLV) and inactivated vaccines are used in the prophylaxis of the disease in cattle (3 - 6)
Potency of inactivated vaccine may vary depending upon the type of adjuvant and upon the vaccine straine (5 - 6).
In order to get a very active vaccine giving a wide range of cover, an Inactivated Oil Adjuvanted vaccine containing a BVDV and a BDV strain was developed.
This paper describes experimental studies concerning safety and potency of that vaccine in calves and lambs.

MATERIALS AND METHODS

Experimental overview

Trial n° 1 : Three, 3-6 week old Friesan calves free from antibodies against pestiviruses were vaccinated 3 weeks apart subcutaneously (SC) with 2 doses of 2 ml of an inactivated oil adjuvanted vaccine prepared from a BVD strain (the non cytopathic NY strain) and from a BD strain (the non cytopathic Aveyronite strain) (4).

Trial n° 2 : Five, 4-8 week old sheep free from specific antibodies ware vaccinated according to that previously described for the calves.

Challenge procedures

3 weeks after the booster injection, a direct challenge took place according to the following procedures.

Trial n° 1 : Each vaccinated calf was injected intravenously with 2 ml and intranasally (IN) with 2 x 0,5 ml of the NY strain titrating $10^{7,5}$ $TCID_{50}$/ml together with three unvaccinated controls.

Trial n° 2 : Each vaccinated lamb was injected IN with 2 x 2,5 ml of the BD strain Aveyronite titraging 10^{5} $TCID_{50}$/ml together with 5 unvaccinated control .

After challenge the vaccinates ant the controls were housed separately for 14 days.

Clinical procedures (trials n° 1 & n° 2)

After the first injection and after the booster injection local and general (temperature) reactions were monitored for 2 weeks.

After challenge, the animals were observed daily in the morning for evidence of clinical signs and rectal temperatures were recorded.
For each group of vaccinates and controls, the daily mean temperatures were calculated and it was considered as fever when the mean temperature was 40° C or above for the lamb or 39.5 or above for the calves.

Sampling and laboratory procedures (trials n° 1 and n° 2)

- Blood samples were taken for serumneutralization tests (SNT) on the following days : day of vaccination, day of booster, day of challenge and 2 weeks after challenge.

- Nasal swabs and blood samples on anticoagulant were taken daily from day 0 to day 14 after challenge.
The samples collected for White Blood Cell (WBC) counts were kept at + 4° C untill they were processed.
The samples collected for virus isolation were frozen and kept at - 20° C untill they were processed in the laboratory. A bovine cell line was used with BVD virus and a sheep cell line with BD virus.

- Virus isolation from nasal swabs and blood
After thawing samples were put onto cells. After 1 hour at 37° C, the monolayer were rinced and fresh medium was added. After 48 hours the cell culture was frozen at - 20° C. Final reading on the 2nd passage on cells was done by IF after 3 days.

- Serum neutralization
Three fold dilutions of decomplemented sera (20 mn/56 C) were incubated at 37° C under a volume of 0. 1 ml with 0.1 ml of either BVDV or BDV virus (320 $TCID_{50}$ per ml). After 1 hour 30 000 cells were added to each microwell. Final reading was done by IF after three days.

RESULTS

Clinical signs

Fig. 1 / CLINICAL SIGNS / CALVES

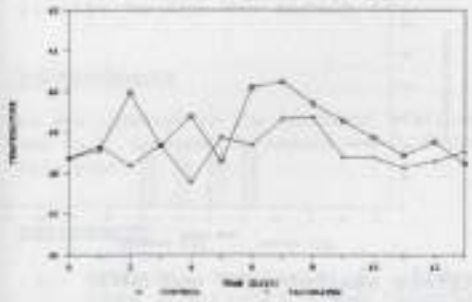
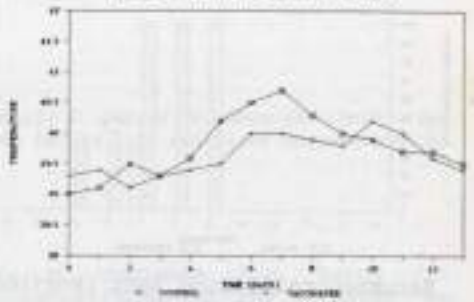


Fig. 2 / CLINICAL SIGNS / LAMBS



Leucopenia

Fig. 3 / LEUCOPENIA / CALVES

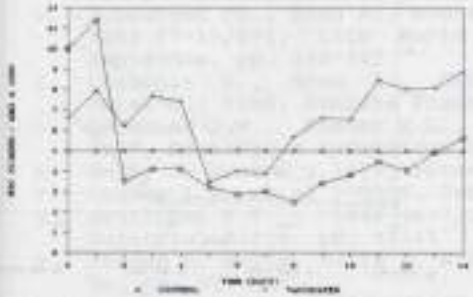
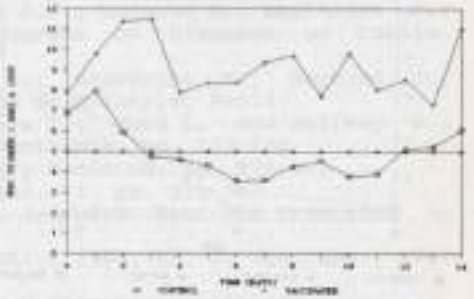


Fig. 4 / LEUCOPENIA / LAMBS



Virus isolation : % of positive animals

- *Blood samples*

Fig. 5/VIRUS ISOL/BLOOD SAMPLE/CALVES

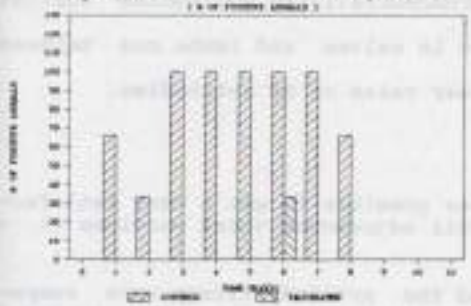
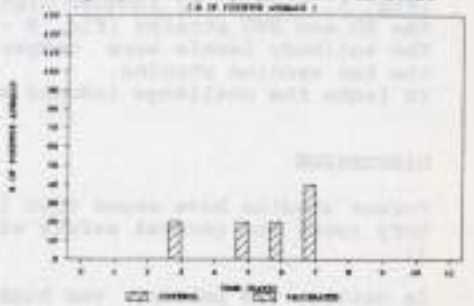
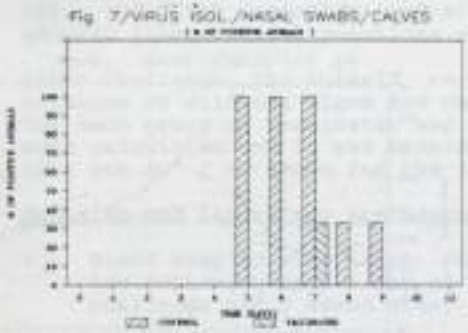
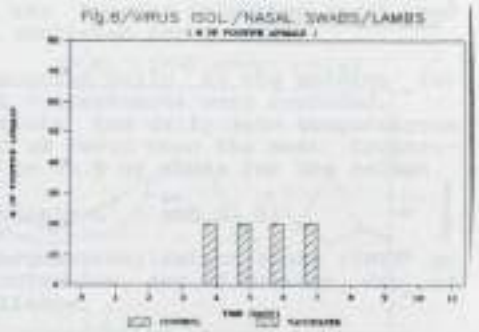


Fig. 6/VIRUS ISOL/BLOOD SAMPLE/LAMBS

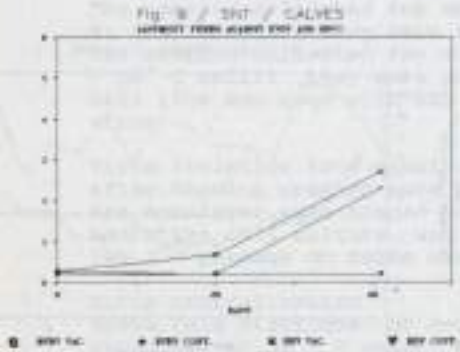


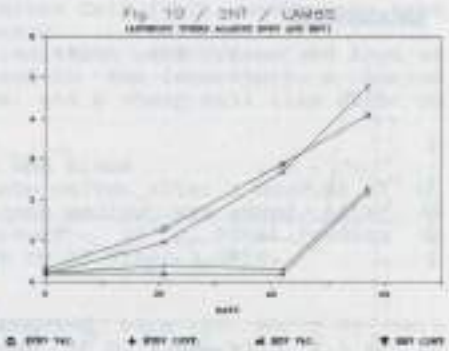
Nasal swabs





Serumneutralization test (antibody titers against BVDV and BDV)





The effects of the vaccination are comparable in calves and in lambs. The vaccination prevented from hyperthermia after challenge (Fig. 1 & Fig. 2) and did not induce reduction in the number of WBC while the leucopenia was dramatic in the control groups (Fig. 3 & Fig. 4). The vaccination suppressed the viremia and nasal excretion after challenge (Fig. 5, 6, 7 & 8) induced high serumneutralizing antibodies against the BD and BVD strains (Fig. 9 - 10).
The antibody levels were comparable in calves and lambs and between the two vaccine strains.
In lambs the challenge induced a clear raise of SN antibodies.

DISCUSSION

Former studies have shown that it was possible to get a very satisfactory local and general safety with oil adjuvanted viral vaccines (1 - 2).

In cattle, the potency was high and the antibody titers were comparable to those obtained by Dubourget et al. (3) with a MLV produced with the Oregon C24V and to those observed by S. R. Bolin et al. (9) 3 weeks after a single dose injection of two different MLV.

The vaccine was able to induce an almost total protection of the vaccinated lambs. This confirmed that effective vaccines against BD should contain at least one strain similar to the Moredun reference strain (i.e the Aveyronite strain) and one strain antigenically similar to the BVD strain (7).

Acknolefgements

We are indebted to the technical staff especially C. Devaud, G. Roux and their team and to F. Limoson, D. Lapalu and P. Cooper who helped with the final preparation of this paper.

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SUMMARY

An oil adjuvanted inactivated vaccine was manufactured using a BVDV and a BDV strains. Calves and lambs were vaccinated twice at an interval of 3 weeks. 3 weeks later the vaccinates were challenged together with controls : the calves with a BVD strain, the sheep with a BD strain.
In both species the vaccination reduced the hyperthermia, suppressed Leucopenia, reduced dramatically or suppressed the viremia and nasal viral excretion. The antibody titers against the two virus strains were very high and comparable to those generally observed with modified live vaccines.

RESUME

Un vaccin huileux inactivé a été produit à partir du virus de la maladie des muqueuses et du virus de la pestivirose ovine. Des veaux et des agneaux ont reçu deux injections de vaccins espacées de 3 semaines. 3 semaines plus tard les animaux vaccinés ainsi que des animaux témoins ont été éprouvés : les veaux avec le virus de la maladie des muqueuses, les agneaux avec une souche Border.
Dans les deux espèces la vaccination a limité l'hyperthermie, a supprimé la leucopénie, a réduit considérablement ou supprimé la virémie et la ré-excrétion nasale du virus. Les titres en anticorps ont été très élevés et sont comparables à ceux généralement observés avec des vaccins atténués.

SUMÁRIO

Uma vacina inativada oleosa foi produzida com uma cepa de pestivirus dos bovinos (BVDV) e uma cepa de pestivirus dos ovinos (BDV). Os vitelos e os carneiros foram vacinados com duas injeçoes com um intervalo de três semanas. Apos três semanas os vacinados foram desafiados com os controles : os vitelos com a cepa BVD, os carneiros com a cepa BD.
Nas duas especias, a inocuidade foi satisfactória, e a vaccinação reduziu a temperatura, suprimiu a leucopenia, e reduziu muito ou suprimiu a viremia e a re-excreção nasal. Os títulos em anticorpos contra as duas cepas virais foram muito altos e comparaveis a aqueles que sao geralmente obtidos com as vaccinas vivas.

PREVALÊNCIA DA LEUCOSE ENZOÓTICA DOS BOVINOS ADULTOS, EM ANIMAIS DA RAÇA JERSEY, CRIADOS NO ESTADO DE SÃO PAULO, BRASIL.

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INTRODUÇÃO

A Leucose Enzoótica dos bovinos adultos é uma doença infectocontagiosa caracterizada por proliferação e neoplasias do sistema linforeticular, determinada por um vírus: Retroviridae - Oncornavirinae - Oncornavirus C de mamíferos - Vírus da Leucose Bovina (VLB) (7). Atualmente é considerada cosmopolita, tendo sido descrita como entidade nosológica característica desde o século passado e a partir da década de 50 como uma enzootia (8). No Brasil a Leucose Bovina é, cientificamente, reconhecida a partir de 1969 (6, 11) e descrita como ocorrendo em bovinos leiteiros importados e crioios. Após a detecção do vírus e seu primeiro isolamento internacional (13) e no Brasil (2, 3) bem como após a utilização de seus antígenos capsulares-gp 51, (15) com fim diagnóstico através a prova de imunodifusão radial dupla de Outcherlony em ágar gel, realizaram-se inúmeros trabalhos sobre epidemiologia e controle desta insidiosa moléstia (7).

No Brasil a determinação imunosorológica de bovinos leiteiros reagentes ao VLB iniciou-se em 1978 (1), quando determinou-se, em São Paulo, ocorrência de aproximadamente 25% de animais sororeagentes ao antígeno capsular gp-51 do VLB. A seguir realizam - se levantamentos epidemiológicos em Minas Gerais (12), Paraná (10), São Paulo (4, 5) e Rio Grande do Sul (9).

A maioria dos trabalhos publicados no Brasil referem-se a bovinos leiteiros da raça holandesa ou seus mestiços e não fazem referência às faixas etárias ou sexo dos animais reagentes. Estes fatos torna necessária a avaliação da prevalência da infecção pelo VLB em animais leiteiros da raça Jersey, dando-se destaque à distribuição etária e ao sexo dos bovinos sororeagentes.

MATERIAL E MÉTODOS

A fim de estabelecer-se a prevalência da infecção pelo VLB em bovinos da raça Jersey, examinaram-se amostras de soro sanguíneo de 868 animais, sendo 764 fêmeas e 52 machos. A população examinada foi estratificada em 7 faixas etárias,constituindo-se os seguintes grupos experimentais: bezerros com até 3 meses de idade e bezerros com idades variando entre 3 e 6 meses e 6 e 12 meses; garrotes e novilhas com idade entre 12 e 24 meses e bovinos adultos com idades variando entre 24 e 48; 48 e 72 meses e idade maior do que 72 meses.

As amostras provieram de animais criados em fazendas localizadas em 11 municípios do Estado de São paulo: Buri; Bragança Paulista; Cotia; Igaratá; Itatiba; Itú; Itupeva; Jacareí; Jaguariúna; São Carlos e Tatuí.

A determinação dos animais sororeagentes ao Vírus da Leucose Bovina (VLB) foi feita através a técnica da imunodifusão radial dupla de Outcherlony em gel de ágar, utilizando-se como antígeno a glicoproteína (gp-51) da cápsula do VLB (3).

RESULTADOS

Os resultados são apresentados em 3 tabelas e 1 figura. A tabela 1 demonstra a ocorrência da infecção em bovinos da raça Jersey criados nos onze municípios paulistas incluídos nesta pesquisa, havendo rebanhos com diferentes índices de infecção (16,7% a 78,4%). Os focos de Leucose Enzootica dos Bovinos Adultos, no Estado de São Paulo, são distribuídos em um mapa e apresentados na figura 1.

Tabela 1 - Bovinos da raça Jersey sororreagentes ao antígeno gp-51 do Vírus da Leucose Bovina, criados no Estado de São Paulo. Resultados distribuídos segundo o município de origem. São Paulo, 1990.

Município	Animais soro reagentes nº	%	Examinados não-reagentes nº	%	Total de animais nos municípios nº	%
Itupeva	29	78,4	8	21,6	37	4,30
Tatuí	29	67,4	14	32,6	43	5,00
Itú	59	58,4	42	41,6	101	11,60
Itatiba	71	50,7	69	49,3	140	16,10
Jaguariúna	59	49,6	60	50,4	119	13,70
Igaratá	11	45,8	13	54,2	24	2,80
Buri	44	41,1	63	58,9	107	12,30
São Carlos	15	33,3	30	66,7	45	5,20
Jacareí	63	32,8	129	67,2	192	22,10
Cotia	8	26,7	22	73,3	30	3,45
Bragança Paulista	5	16,7	25	83,3	30	3,45
Total	393	45,3	475	54,7	868	100,10

Figura 1 - Representação dos focos de infecção pelo VLB, em bovinos da raça Jersey criados no Estado de São Paulo. São Paulo, 1990.

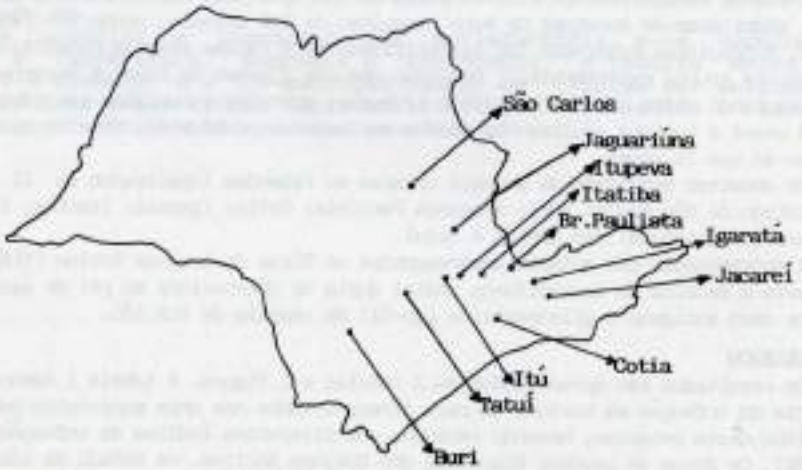

Tabela 2 - Bovinos da raça Jersey sororreagentes ao antígeno gp-51 do Vírus da Leucose Bovina, criados no Estado de São Paulo. Resultados distribuídos segundo as faixas etárias da população estudada. São Paulo, 1990.

Faixa etária idade em meses	Animais examinados soro reagentes nº	%	não-reagentes nº	%	Total de animais nos grupos etários nº	%
⟵ 3	35	38,9	55	61,1	90	11,0
3 ⟵ 6	8	11,8	60	88,2	68	8,3
6 ⟵ 12	13	15,5	71	84,5	84	10,3
12 ⟵ 24	31	22,8	105	77,1	136	16,7
24 ⟵ 48	81	46,0	95	54,0	176	21,6
48 ⟵ 72	89	65,0	48	35,0	137	16,8
72 ⟵	108	86,4	17	13,6	125	15,3
Total	365	44,7	451	55,3	816	100,0

Tabela 3 - Bovinos da raça Jersey sororreagentes ao antígeno gp-51 do Vírus da Leucose Bovina, criados no Estado de São Paulo. Resultados distribuídos segundo o sexo e faixas etárias da população estudada. São Paulo, 1990.

Sexo dos animais	FÊMEAS			MACHOS		
Faixa etária idade em meses	Número de amostras	Soro reagentes nº	%	Número de amostras	Soro reagentes nº	%
⟵ 3	77	35	45,5	13	-	-
3 ⟵ 6	61	6	9,8	7	2	28,6
6 ⟵ 12	76	13	17,1	8	-	-
12 ⟵ 24	122	30	24,6	14	1	7,1
24 ⟵ 48	170	81	47,6	6	-	-
48 ⟵ 72	135	89	65,9	2	-	-
72 ⟵	123	106	86,2	2	2	100,0
Total	764	360	47,1	52	5	9,6

A tabela 2 apresenta a prevalência da infecção pelo VLB em bovinos da raça Jersey criados em São Paulo, distribuindo os resultados segundo a faixa etária dos animais utilizados neste levantamento clínico-epidemiológico, destacando que o índice de infecção aumenta significativamente com o evoluir da idade. A tabela 3 apresenta o índice de infecção pelo VLB em bovinos fêmeas e machos, da raça Jersey, demonstrando que o mesmo é significativamente menor nos machos.

DISCUSSÃO

O índice geral de infecção pelo VLB em bovinos da raça Jersey, criados em São Paulo (44,7%) foi semelhante ao obtido em animais da raça holandesa, em 1988(44,9%) e superior ao observado em 1979(35,6% em 16 raças bovinas) (1, 4). A infecção foi detectada nos 11 municípios de São Paulo estudados, correspondendo a 6 focos novos no Estado, em relação aos trabalhos anteriores (1, 4). A prevalência foi maior do que as referidas no Paraná, Rio Grande do Sul e Minas Gerais (9, 10, 12, 14).

O índice de infecção foi maior nos animais mais idosos, atingindo valores máximos nos grupos etários, com idade variando entre 48 e 72 meses e superiores a 72 meses; respectivamente, 65,0 e 86,4%. Este fato é similar ao encontrado em bovinos da raça holandesa (5). O número relativo de animais sororeagentes no grupo etário de até 3 meses de idade(38,9% - imunidade passiva colostral, associada às infecções congenitas), diminuiu nos bezerros desmamados(11,8% - bezerros entre 3 e 6 meses de idade) por degradação dos anticorpos de origem colostral. A seguir os índices crescem gradativamente em consequência a infecções ativas. Variações idênticas já tinham sido observadas em bovinos da raça holandesa(5).

O índice de infecção pelo VLB foi significativamente maior nos bovinos Jersey fêmeas (47,1%) do que nos machos(5,0%), não existindo referência sobre o assunto, na bibliografia brasileira compulsada, que elucidem o fato, a diferença encontrada pode ser atribuida à forma de manejo e ao maior isolamento dos machos, nos rebanhos leiteiros que mantêm touros ou recriam bezerros machos para a venda de reprodutores.

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RESUMO

Em 868 bovinos da raça Jersey, criados no Estado de São Paulo, determinou-se a prevalência da infecção pelo Vírus da Leucose Bovina (VLB), utilizando a prova de imunodifusão radial dupla em gel de ágar utilizando-se o antígeno capsular glicoproteíco (gp-51). A estratificação da população em faixas etárias demonstrou ser a prevalência maior nos animais mais idosos. Os resultados obtidos foram os seguintes: bezerros até 3 meses de idade - 38,9%; de 3 a 6 meses - 11,8%; de 6 a 12 meses - 15,5%; nas novilhas e garrotes com idade entre 12 e 24 meses - 22,8% e nos bovinos adultos de 24 a 48 meses-46,0%; de 48 a 72 meses - 65,0% e com idade maior do que 72 meses - 86,4%. Os resultados demonstraram a existência de focos de infecção do VLB nos 11 municípios paulistas examinados. A prevalência em fêmeas bovinas da raça Jersey foi significativamente maior do que a observada nos machos.

RÉSUMÉ

Dans 868 bovins de la race Jersey on a déterminé la fréquence de l'infection pour le virus de la Leucose Bovine utilisant l'immunodiffusion en gélose avec l'antigène glycoprothéique (gp 51). On a observé 393 animaux réactifs (45,3%). La stratification selon les différentes ages a demonstré qu'est plus grand la fréquence dans les animaux plus agés. Voici les résultats trouvés: jusqu'à 3 mois = 38,9%; de 3 à 6 mois = 11,8%; de 6 à 12 mois = 15,9%; de 12 à 24 mois = 22,8%; de 24 à 48 mois = 46,0%; de 48 à 72 mois = 65,0% et plus de 72 mois = 86,4%. Les résultats ont démontré qu'il y a des foyers d'infection pour le virus de la Leucose Bovine dans 11 villes étudiés. Dans les femelles le numéro rélatif des animaux réactifs a été significativement plus grand que ce qu'on a trouvé dans les mâles.

ABSTRACT

In 868 bovines of the Jersey bread, it was determined through immunodiffusion in agar-gel, with the use of glycoprotein (gp 51) antigen, that there is a predominance of the infection by the bovine leucosis virus; 393 animals reacting to the serum were observed (45,3%). The samples arranged according to various age groups, showed a higher predominance in older animals. The values obtained were the following: up to 3 months-38,9%, 3-6 months - 11,8%, 6-12 months - 15,5%, 13-24 months - 22,8%, 24-48 months - 46,0%, 48-72 months - 65,0%. The results showed that there exist foci of the infection by the Bovine Leucose Virus within the 11 studied municipalities. The relative number of male animals reacting to the serum was significantly lower than that found in females.

ZUSAMMENFASSUNG

Bei 868 Jerseyrindern wurde durch Immundiffusions-test in Agar-gel unter Verwendung von Glykoseprotein (gp 51) als Antigen das Vorherrschen einer Infektion durch den Rinderleukosevirus festgestellt, und 393 Tiere, die auf das Serum reagierten(45,3%). Die Versuchstiere, nach verschiedenen Altersstufen eingeteilt, zeigten, dass der Befall stärker bei älteren Tieren ist. Die festgestellten Werte waren folgende: bis zu 3 Monaten - 38,9%, 3-6 Monaten - 11,81%, 6-12 Monaten - 15,5%, 12-24 Monaten - 22,8%, 24-48 Monaten - 46,0%, 48-72 Monaten - 65,0% und älter als 72 Monaten - 86,4%. Die Resultate zeigen, dass Infektionsherde durch Rinderleukosevirus in den 11 untersuchten Bezirken bestehen. Unter den männlichen Rindern ist die Zahl der auf das Serum reagierenden Tiere wesentlich geringer als bei den weiblichen Rindern.

IMPFSTOFFE GEGEN VIRUSKRANKHEITEN BEIM RIND

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Die ersten viralen Impfstoffe wurden zum Schutz vor Maul- und Klauenseuche entwickelt. Heutzutage werden sie noch in zahlreichen europäischen und außereuropäischen Ländern eingesetzt. Frei von Maul- und Klauenseuche sind Ozeanien (Australien, Neuseeland, und verschiedene Inselstaaten) sowie Amerika von Mexiko bis Kanada. In den Ländern der Europäischen Gemeinschaft wird derzeit darüber diskutiert, ob die noch impfenden Länder sich der Strategie der nichtimpfenden Länder anschließen sollen oder nicht bzw. unter welchen Konditionen. Bei den MKS-Impfungen werden im Normalfall trivalente, inaktivierte Vakzinen verwendet, die in den reinen Impf- oder Nachbarländern hergestellt werden. Die reinen MKS-Impfstoffe bleiben bei den weiteren Ausführungen unberücksichtigt. Außerdem bleiben die Tollwutimpfstoffe, die monovalent oder bivalent (mit MKS-Impfstoffen) unter bestimmten Seuchenbedingungen eingesetzt werden, unerwähnt.

Nach dem 2. Weltkrieg kam es zu wesentlichen Strukturveränderungen in der landwirtschaftlichen Haltungsform von Rindern und damit verbunden nahm die Zahl der Rinder pro Betrieb, ausgehend von den USA, auch in Europa zu. Die hygienischen Bedingungen wurden nicht in gleichem Maße verbessert, so daß es im Zusammenwirken von schnellen Passagen im idealen Wirt zu Virulenzsteigerungen kam, und durch ungünstige äußere Faktoren zu den sogenannten Faktorenkrankheiten. Faktorenkrankheit in diesem Zusammenhang bedeutet, daß die Infektion mit einem schwach oder gar apathogenen Erreger dann zu einer klinischen Erkrankung führt, wenn äußere Umstände synergistisch wirken.

Da sowohl beim Auftreten der monokausal wie auch der multikausal bedingten Krankheiten zum Teil beträchtliche wirtschaftliche Schäden entstehen können, wurden, beginnend in den 50iger Jahren, Impfstoffe gegen diese Krankheiten entwickelt, und zwar zunächst wieder in den USA, später dann auch in Europa. Nachstehend wird eine Übersicht über die in den Ländern der EG und der USA zugelassenen Impfstoffe gegeben.

Monovalente Impfstoffe

Entsprechend der Bedeutung der Erreger gibt es gegen die wirtschaftlich bedeutendsten Krankheiten monovalente Vakzinen und zwar gegen die BHV1-Infektionen, Bovine Virusdiarrhoe (BVD), die BRSV-Infektion und Parainfluenza 3. Welche Arten und wieviele Lizenzen es den USA und den EG-Ländern gibt, ist Tabellen 1 und 3 zu entnehmen.

TABELLE 1. Monovalente BHV1-Vakzinen

Impfstoffarten	Anzahl der Lizenzen in den USA	einzelnen EG-Ländern[1]			
		B	D	F	GB
inaktiviert	2		5[3]	2[3]	
lebend[2]	9	1	1		1

1) in Dänemark und Griechenland gibt es keine Lizenz; in Luxemburg sind lizenzierte Impfstoffe der Nachbarländer zugelassen. Von den nicht erwähnten Ländern der EG wurde keine Information zur Verfügung gestellt.
2) darunter sind auch die ts-Mutantenimpfstoffe
3) darunter ist auch eine Subunit-Vakzine

Kombinationsvakzinen

Bei den Kombinationsvakzinen werden nicht nur die Erreger der Faktorenkrankheiten berücksichtigt, sondern auch alle erdenklichen Kombinationen von den in Tabellen 1 und 3 zusammengestellten monovalenten Impfstoffen. Die Mehrzahl der Kombinationen hängt mit den BHV1-Impfstoffen zusammen, worüber Tab. 2 Auskunft erteilt. Die anderen Kombinationen - also ohne BHV1-Erreger oder -Antigen - sind in Tab. 4 zusammengefaßt. Impfstoffe zur Mutterschutzimpfung und Neugeborenenprophylaxe zum Schutz vor Diarrhoe sind in Tab. 5 aufgeführt.

Adjuvanzien

Einem Teil der parenteral applizierbaren Impfstoffe sind Adjuvanzien beigemischt; zugelassen sind bisher Aluminiumhydroxid und Aluminiumphosphat sowie Mineralöle.

TABELLE 2. BHV1-Kombinationsvakzinen

Zusammensetzung (jeweils (BHV1 plus)	Impfstoffarten	Anzahl der Lizenzen in den USA	einzelnen EG-Ländern[1] B	D	F	GB
+ PI3	inaktiviert	1	-	-	-	-
	lebend	12	2	1	-	1
+ PI3 + BRSV	inaktiviert	-	-	-	-	-
	lebend	2	-	-	-	-
+ BRSV	lebend	1	-	-	-	-
+ BVDV	inaktiviert	6	-	-	-	-
	lebend	8	-	-	-	-
+ BVDV + PI3 (+ BRSV)	inaktiviert	6(3)	-	-	-	-
	lebend	11(2)	-	-	-	-
	inaktiviert lebend[2]	2(2)	-	-	-	-
+ BVDV + BRSV	lebend	1	-	-	-	-
+ PI3 + Adeno3	lebend	-	1	-	-	-
+ Adeno3 + BVDV + REO + PI3	inaktiviert	-	-	-	-	1
+ PI3 + REO 1+3 + Adeno 1+3	inaktiviert	-	-	1	-	-

1) siehe Tabelle 1
2) inaktiviert ist die BVDV-Komponente

TABELLE 3. Andere monovalente Impfstoffe

Virus bzw. Antigen	Impfstoffarten	Anzahl der Lizenzen in den USA	einzelnen EG-Ländern[1] B	D	F	GB
BVDV	inaktiviert	8	-	-	-	-
	lebend[2]	7	1	3	2	-
BRSV	inaktiviert	3	-	-	-	-
	lebend	3	1	1	1	1
PI3	lebend[2]	-	-	3	1	1

1) und 2) - siehe Tabelle 1

TABELLE 4. Andere Kombinationsvakzinen gegen Krankheiten im Bereich der Luftwege

Virus bzw. Antigen	Impfstoffarten	Anzahl der Lizenzen in den USA	einzelnen EG-Ländern[1] B	D	F	GB
+ PI3 + REO + Adeno	inaktiviert	-	-	1	1	-
+ PI3	lebend	1	-	-	-	-
+ BVDV + BRSV	lebend	-	-	1	1	-

TABELLE 5. Impfstoffe zur Mutterschutzimpfung und Neugeborenenbehandlung gegen Diarrhöen

Virus bzw. Antigen	Impfstoffarten	Anzahl der Lizenzen in den USA	einzelnen EG-Ländern[1] B	D	F	GB
+ Rota	lebend	1	2	2	1	-
+ Corona[2]	inaktiviert	-	1	-	1	-
+ Rota + Corona + Parvo[2]	inaktiviert	-	-	1	-	-
+ Rota[2]	lebend	-	-	-	-	1

1) - siehe Tabelle 1
2) - meinst mit E.coli-Antigen

DISKUSSION

Unter den monovalenten Vakzinen sind jene gegen BHV1 am zahlreichsten, gleiches trifft für Kombinationsvakzinen zu, die BHV1 oder BHV1-Antigen enthalten. Dabei fällt auf, daß es sich bei der Mehrzahl um Lebendimpfstoffe handelt, insbesondere wenn das Verhältnis lebend/inaktiviert in den USA betrachtet wird. Es verhält sich wie 9:2 bei den monovalenten Vakzinen und bei den inaktivierten Vakzinen wie 39:16. Außerdem fällt auf, daß es in einigen EG-Ländern keine BHV1-Lebendvakzinen gibt. Dies hängt einmal von der Bekämpfungsstrategie der einzelnen Länder, zum anderen von der Auffassung der verschiedenen Länderexperten ab.

Was die BVD-Impfstoffe, monovalent und bi- bzw. polyvalent betrifft, so halten sich inaktivierte und Lebendimpfstoffe in etwa die Waage. Auffällig ist, daß in den EG-Ländern, mit einer Ausnahme (GB), keine inaktivierten Mono- oder Mehrfachimpfstoffe zur Verfügung stehen, obwohl bekanntermaßen die Anwendung von Lebendvakzinen ein gewisses Risiko in sich birgt (2).

Der prophylaktischen Impfung von Muttertieren oder Neugeborenen gegen Durchfallerkrankungen beim Kalb wird in den USA offensichtlich wenig

Beachtung geschenkt, und in den EG-Ländern sind die Erfahrungen und Ansichten offensichtlich recht unterschiedlich, denn Dänemark verwendet beispielsweise überhaupt kein derartiges Produkt.

Gleiches trifft für die "Faktoren"-Prophylaxe zu. In den meisten Ländern stehen Impfstoffe, die Adeno- und oder Reoviren enthalten, überhaupt nicht zur Verfügung. Ob daraus auf die Bedeutungslosigkeit dieser Erreger im Krankheitsgeschehen geschlossen werden darf oder ob dies ein echter Mangel ist, kann nicht zuverlässig abgeklärt werden.

Bezüglich der Schutzimpfung gegen BRSV ist anzumerken, daß eine Vakzinierung unter Umständen sogar die Krankheit verschlimmern kann, da es durch den Erregerkontakt zur Allergisierung kommen kann. Es wurde auch deutlich herausgearbeitet, daß hierbei Antikörpern der Klasse IgE eine wesentliche Bedeutung zukommt (1; 3).

Schließlich ist auch noch nicht eindeutig geklärt, wie sinnvoll es ist, BVD-Virus in derart vielen Kombinationen anzubieten, zumal bekannt ist, daß dieses Virus immunsuppressiv wirkt (2). Nach eigenen Erfahrungen kann diese Wirkung sogar durch inaktivierte BVD-Virusstämme erzeugt werden. Ganz offensichtlich setzen sich in den USA sowohl Produzenten als auch Anwender über diese Imponderabilien hinweg.

Die Zahl der Lizenzen ist in den USA ungleich größer als jene in den EG-Ländern, z.T. sind die Lizenzträger sogar identisch. Daraus resultiert auch eine gewisse Marktbeherrschung.

Gentechnologische Vakzinen sind bisher nicht auf dem Markt. Ob sie zur Bekämpfung der Krankheit erforderlich sind oder ob hinter der Entwicklung nur Wettbewerbsinteressen stehen, wird die Zukunft klären.

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Für die Zusammenstellung wurden die Angaben der Zulassungsbehörden der Länder verwendet (Stand 1990), für deren Überlassung Dank gesagt sei.

ZUSAMMENFASSUNG

Es wird ein Überblick über die verschiedenen mono-, bi- und polyvalenten Impfstoffe gegeben, die zur Applikation beim Rind bei den verschiedenen Problemen zugelassen sind. Dabei fällt auf, daß in den USA der Schwerpunkt bei Lebend- und in den EG-Ländern - soweit die Daten erhältlich waren - bei inaktivierten Vakzinen liegt. Außerdem werden prophylaktische Impfungen gegen Faktorenkrankheiten und Diarrhöen der Neugeborenen in den USA offensichtlich kaum durchgeführt.

SUMMARY

A brief review is given concerning the different mono-, bi and trivalent vaccines licensed for prophylactic measures in the USA and EC countries. It is noteworthy to point out that in contrast to Europe more attenuated vaccines are used in the USA. Furthermore vaccinations against neonatal diarrhoeas and diseases of the respiratory tract influenced by environmental factors do not seem to play a role in the USA.

RESUME

Un bref exposé sommaire est donné sur les vaccins mono-, bi- et polyvalents qui sont admis pour la prophylaxie des maladies des boeufs en CE et EU. Il est remarquable que dans les Etats-Unis on préfère les vaccins vivants. Parcontre, en CE on préfère les vaccins inactivés. De plus, les vaccinations contre les diarrhées néonatales et contre les infections réspiratoires qui se manifestent dans de conditions défavorables sont peu utilisées apparemment dans les EU.

SAFETY AND POTENCY OF AN OIL-ADJUVANTED SUBUNIT BHV-1 VACCINE

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INTRODUCTION

Live as well as inactivated vaccines are used in the prophylaxis of diseases caused by BHV-1.
Live vaccines may be used intranasally or parenterally (7,9,11). The fact that they may establish latency (7) can probably be considered as a disadvantage for their use in eradication programs. Indeed, by corticoid treatment, reexcretion of vaccine virus has been reported.

Inactivated vaccines do not pose these problems. Their potency is mostly to be enhanced by the use of oil adjuvants. Whole virus oil-adjuvanted vaccines have proven their efficacy (8,9) but postvaccinal reactions were noticed (4,5).

Both live and inactivated vaccines proved to be efficacious for reducing the excretion of virulent virus from experimentally challenge exposed animals (8,9,10,11).

Results obtained by Chappuis et al. (2) using cat herpesvirus as an experimental model, showed the importance of the viral capsid proteins in eliciting postvaccinal reactions. Therefore, in our laboratories, herpesvaccines solely composed of the viral envelope were developed and tested in various species such as feline, swine, bovine and equine.

This paper describes further experimental results concerning safety and efficacy obtained with a subunit glycoprotein oil-adjuvanted BHV-1 vaccine* with particular emphasis on short term protection.

MATERIALS AND METHODS

Experimental design

Group 1: out of 12 calves, one month old, 5 were vaccinated subcutaneously with one dose (2 ml) of vaccine. Four days after the vaccination, the 7 remaining calves were treated as follows: two were intranasally inoculated with 10^{6} $TCID_{50}$, in 5 ml Pbs, of a virulent IBR-strain and the other five were not treated and served as contact controls. The challenge strain represented the second passage on primary calf kidney (CK) cells.

Group 2: five one-month-old calves were vaccinated twice at a 3 week interval. Two weeks later, they were, together with 5 non-vaccinated control calves, intranasally inoculated as described for the two inoculated animals in group 1.
Starting on the day of inoculation the following clinical parameters were monitored daily :

1 : hyperthermia
2 : nasal discharge
3 : red nose or epiphora
4 : membranous lesion

From these parameters a total daily clinical score was established. Results are expressed (figures 1 and 2) as a clinical score for constant number of 5 animals per group.

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For proof of virusexcretion, nasal swabs were taken from the day of inoculation until day 18 after challenge in group 1 and day 14 in group 2.
Prior to vaccination, blood samples were taken for the determination of seroneutralizing antibody titers as well as on the day of inoculation in group 2.

Fig.1: Clinical score - group 1 animals

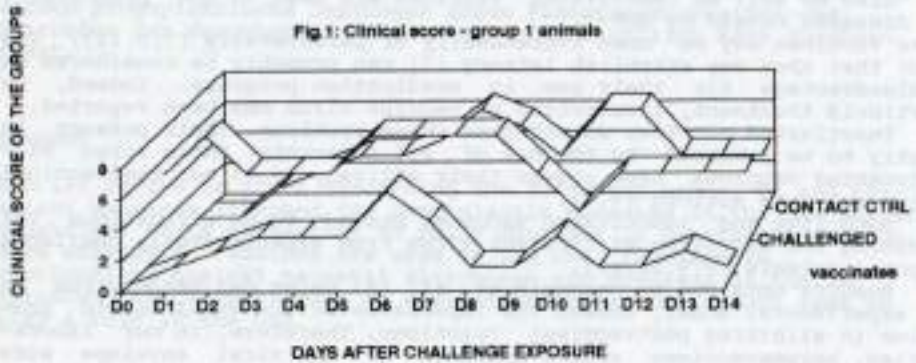
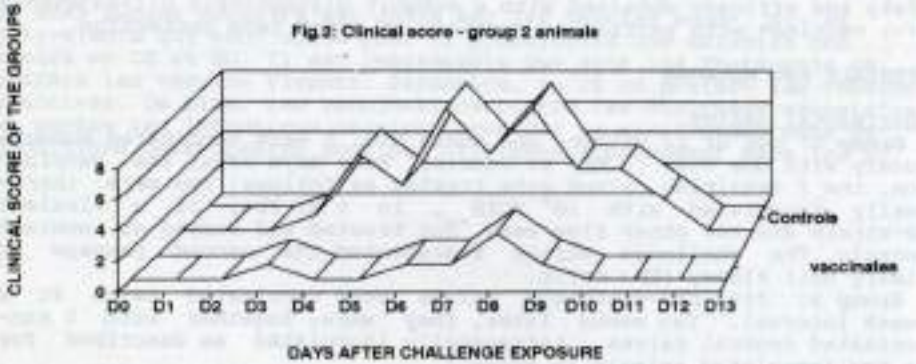


Fig.2: Clinical score - group 2 animals



Virusisolation
Swabs were stored at - 20° C. After thawing and centrifugation, microtiter plates containing CK-cell suspension were inoculated with tenfold dilutions. Final reading was done after seven days.Titer was calcutated according to Kärber.
Seroneutralisation test (SNT)
0.1 ml of three fold dilutions of inactivated sera (20 mn 56 C) were incubated at 37°C with 0.1 ml containing 30 TCID50 IBR virus. After 24 hours 30.000 CK cells were added in each microwell. Final reading was done after seven days.

RESULTS

Clinical results of challenge
Group 1: inoculated animals developed typical IBR-symptoms from day 1 after inoculaton and the contact controls from day 2 p.i.. One inoculated animal died on day 18 p.i. and one contact control on day 20 p.i.. Vaccinated animals showed only mild IBR-signs.

Group 2: just like in group 1, control animals developed typical IBR- symptoms whereas the vaccinates were only very slihgtly affected.

Clinical scores (figures 1 and 2) confirm the clinical protection provided by the subunit vaccine.

Neither in group 1 nor group 2, clear postvaccinal reactions could be observed on the inoculation site.

Virusisolation

In both groups, virusexcetion was much lower in the vaccinated animals (figures 3 and 4). The differences between vaccinated and control animals was highly significant (variance analysis $P < 0.01$).

Fig.3: Viral excretion after challenge in group 1 animals.

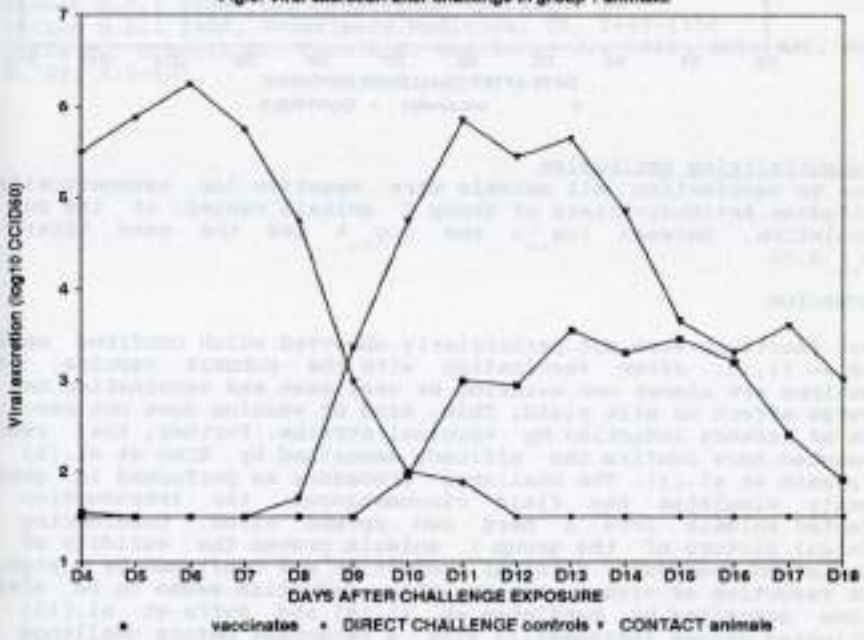
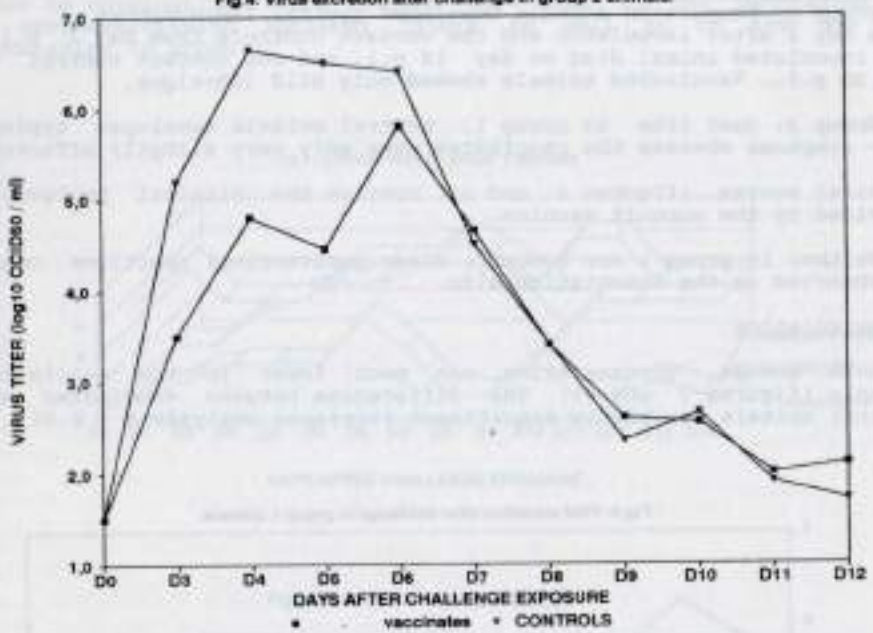


Fig 4: Virus excretion after challenge in group 2 animals.



Seroneutralizing antibodies

Prior to vaccination all animals were negative for seroneutralizing antibodies.Antibody-titers of group 2 animals ranged, at the day of inoculation, between $\log_{10}2$ and $\log_{10}4$ and the mean titer was $\log_{10}$ 2.70.

DISCUSSION

Local reactions were not particularly observed which confirms earlier studies (1,3): after vaccination with the subunit vaccine, local reactions are almost not existing or very weak and vaccination has no adverse effect on milk yield. This kind of vaccine does not bear the risk of latency induction by vaccinal strains. Further, the results presented here confirm the efficacy described by Brun et al.(1) and Guillemin et al.(3). The challenge procedure as performed in group 1 closely simulates the field circumstances: the introduction of infected animals into a herd can spread virus. Considering the clinical picture of the group 1 animals proves the validity of the inoculation procedure. Clinical protection was confirmed by a significant reduction of virusexcretion. This reduction seems to be similar to one described by Nettleton et al.(6) and Zuffa et al.(11) who vaccinated calves intranasally with a ts-mutant before challenge two months to nine weeks later. The present results indicate that the subunit vaccine induces a rapid onset of protection in cattle

comparable to one observed by Sutton (10) using a modified-live-virus by intramuscular injection and similar contact challenge conditions. It may be concluded the subunit BHV-1 vaccine is safe and efficient and due to its high degree of safety, it seems to be an interesting vaccine for use under different management conditions.

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SUMMARY

Envelope glycoproteins of virulent IBR-virus were used as an oil-adjuvanted vaccine in calves. Calves were vaccinated once or twice with three weeks interval. Once vaccinated calves were, 4 days after vaccination, brought in contact with intranasally infected calves together with non-vaccinated control calves. Non-vaccinated calves experienced severe IBR-disease not seen in the vaccinated once. Excretion of challenge virus was significantly reduced in the vaccinated calves compared to the controls. The twice vaccinated animals were also well protected against a severe challenge when considering virus excretion and clinical protection.

RESUME

Les glycoprotéines de l'enveloppe d'un virus IBR virulent ont été utilisées en vaccin huileux sur des veaux. Les veaux ont été vaccinés une ou deux fois. Dans le cas de la vaccination unique, les veaux vaccinés ont été mis en contact avec des veaux éprouvés par voie intranasale, seulement 4 jours après la vaccination, ainsi que des veaux témoins non vaccinés-non éprouvés. Alors que les veaux témoins ont été atteints de la maladie IBR typique, les vaccinés n'ont guère présenté de symptômes et l'excrétion virale était réduite de façon drastique. En considérant également l'excrétion virale et la protection clinique, les veaux vaccinés deux fois ont été également bien protégés.

ZUSAMMENFASSUNG

Hüllglykoproteine eines virulenten BHV -Virusstammes wurden als Impfstoff mit öligem Adjuvans bei Kälbern eingesetzt. Eine Gruppe wurde einmal, eine zweite zweimal vakziniert. Die Gruppe der einmal vakzinierten Tieren wurde am 4. Tag p. vacc. in Kontakt mit BHV -infizierten Kälbern gebracht. Während die infizierten Tiere unter den typischen Symptomen der IBR erkrankten, zeigten die Impflinge nur geringgradige klinishce Symptome. Sie schieden auch im Vergleich zu den nicht geschützten Tieren wesentlich weniger Virus aus. Entsprechend geschützt, erwiesen sich auch die Kälber der zweimal geimpften Gruppe.

NACHWEIS DES BOVINEN HERPESVIRUS TYP 1 (BHV1) MITTELS INTRAKUTANTEST

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EINLEITUNG

Für den Nachweis des bovinen Herpesvirus Typ 1 (BHV1) werden heute routinemäßig der direkte Virusnachweis (Virusisolierung oder Immunfluoreszenz) und der indirekte Virusnachweis (Blut- oder Milchserologie mittels Neutralisationstest oder ELISA) angewandt.
Mit der Anwendung dieser Methoden bleiben zwei für ein BHV1-Bekämpfungsprogramm wichtige Probleme ungelöst :
- die Differenzierung von passiv und aktiv erworbenen Antikörpern, und
- die Erfassung latent infizierter, serologisch negativ oder fraglich reagierender Tiere.

1970 wurde erstmals über allergische Reaktionen bei BHV1-immunen Rindern berichtet (6), 1972 erschien der erste Bericht über einen BHV1-Intrakutantest (5). Ein derartiger Test scheint geeignet, die beiden Probleme zu lösen. Basis des Intrakutantestes ist der Nachweis einer durch BHV1-Antigen induzierten zellulären Immunität, wobei eine lokale Oberempfindlichkeitsreaktion vom verzögerten Typ (delayed hypersensitivity) diagnostisch verwertet wird.
Eine Reihe weiterer Untersuchungen (1,2,3,4,7,8,9,10) waren Anlaß zu vorliegend beschriebenem Feldversuch.

MATERIAL UND METHODEN

Tiere

Der Versuch wurde durchgeführt mit insgesamt 722 Tieren in 15 Betrieben, von denen 3 BHV1-frei und 12 BHV1-infiziert waren:
345 Tiere älter als 6 Monate, BHV1-frei,
270 Tiere älter als 6 Monate, BHV1-infiziert,
107 Tiere jünger als 6 Monate, davon 77 serologisch negativ und 30 Tiere mit passiver Immunität gegenüber BHV1.

Testantigen

Für die Versuche wurde ein BHV1-Antigen der *Behringwerke AG, Marburg, Bundesrepublik Deutschland* verwendet (Vermehrung des Virus in Zellkulturen, Reinigung des Antigens durch Zentrifugation und Filtration, Konzentrierung durch Ultrafiltration, Präzipitation mit Polyethylenglycol). Das rückgelöste Antigenkonzentrat hatte einen Virusgehalt von $10^{6,8}$ GKID$_{50}$/0,1 ml.

Durchführung des Intrakutantestes

Vor dem Versuch wurde an der Injektionsstelle am Schulterblatt die Hautfaltendicke mit einem Federkutimeter gemessen. Danach injizierte man 0,1 ml des BHV1-Antigens intrakutan. Am 3. Tag (ca. 72 Std.) nach der Injektion wurde die Hautfaltendicke an der Injektionsstelle nachgemessen und die Differenz gegenüber dem Ausgangswert bestimmt.
Parallel zum Intrakutantest ist von jedem Tier eine Blutprobe entnommen und im ELISA (TRACHITEST, Firma Bommeli AG, Bern/Schweiz) untersucht worden.

ERGEBNISSE DER VERSUCHE

BHV1-freie Tiere im Alter von über 6 Monaten

Von den 345 serologisch negativen Tieren reagierten im Intrakutantest 344 Tiere (99,7%) mit einer Hautdickenzunahme von nicht mehr als 1,0 mm. 1 Tier (0,3%) hatte eine Hautdickenzunahme von 1,9 mm. Der Mittelwert der Hautdickenzunahme bei den 345 serologisch negativen Tieren betrug 0,2 mm (Abb. 1).

BHV1-infizierte Tiere im Alter von über 6 Monaten

Bei den 270 BHV1-infizierten , serologisch positiven Tieren erbrachte der Intrakutantest folgende Ergebnisse: 3 Tiere (1,1%) zeigten eine Hautdickenzunahme bis zu 1,0 mm, 1 Tier (0,4%) eine Zunahme von 2,0 mm und 266 Tiere (98,5%) reagierten mit Hautdickenzunahmen zwischen 2,1 mm und 16,6 mm. Der Mittelwert der Hautdickenzunahme bei den 270 BHV1-infizierten Tieren betrug 6,3 mm. In den einzelnen Betrieben reagierten die Tiere unterschiedlich stark. Die Mittelwerte variierten zwischen 5,2 mm und 10,7 mm (Abb. 1).

Abb. 1: Ergebnisse des Intrakutantestes bei über 6 Monate alten Tieren

Serologischer Befund	Tierzahl	Hautdickenzunahme (mm) bis 1,0	1,1-2,0	2,1-4,0	4,1-6,0	6,1-8,0	8,1-10,0	10,1-12,0	12,1-14,0	14,1-16,0	16,1-18,0	Mittelwert
Negativ	345	344 (99,7%)	1 (0,3%)									0,2
Positiv	270	3 (1,1%)	1 (0,4%)	50 (18,5%)	75 (27,0%)	89 (33,0%)	39 (14,4%)	9 (3,3%)	4 (1,5%)	1 (0,4%)	1 (0,4%)	6,3

Tiere im Alter bis zu 6 Monaten

Von den 107 getesteten Jungtieren waren 77 Tiere serologisch negativ und 30 Tiere waren auf Grund einer passiven Immunität serologisch positiv. Sowohl die serologisch negativen als auch die passiv immunen Tiere reagierten ausnahmslos mit Hautdickenzunahmen von weniger als 1,0 mm. Die maximale Zunahme betrug 0,8 mm, die durchschnittliche Hautdickenzunahme betrug nur 0,2 mm.

SCHLUSSFOLGERUNGEN

Auf Grund der in vorliegenden Untersuchungen sowie bei ergänzenden Untersuchungen (7,11) erzielten Ergebnisse bietet sich für die praktische Anwendung des BHV1-Intrakutantestes folgendes Verfahren an. Das Antigen wird intrakutan am Schulterblatt in einer Menge von 0,1 ml pro Tier appliziert. Es empfiehlt sich die Kennzeichnung der Injektionsstelle und die palpatorische Kontrolle des Injektionserfolges. Als Bewertungsmaßstab gilt die Hautdickenzunahme, die sich aus der Differenz

der Meßwerte vor der Antigeninjektion und 3 Tage (72 Std.) nach der Injektion ergibt. Hautdickenzunahmen bis zu 1,0 mm werden als negativ, solche von mehr als 2,0 mm als positiv bewertet. Hautdickenzunahmen von 1,1 mm bis 2,0 mm gelten als fraglich.
Gegenüber anderen Labormethoden bietet der Intrakutantest vor allem folgende Vorteile:
- Möglichkeit der Differenzierung von passiver und aktiver Immunität,
- schnelle Diagnose innerhalb 3 Tagen,
- Unabhängigkeit vom Diagnostik-Laboratorium,
- Möglichkeit der Entdeckung latent mit BHV1 infizierter, serologisch negativer Tiere,
- Kostenersparnis.

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ZUSAMMENFASSUNG

Ein Intrakutantest zur Diagnose der BHV1-Infektion ist unter Praxisbedingungen an insgesamt 722 Rindern in 15 Betrieben erprobt worden.Als Referenz galten die Ergebnisse der serologischen Blutuntersuchung im ELISA (TRACHITEST, Fa. Bommeli AG, Bern/Schweiz).
Das Testantigen (konzentriertes, gereinigtes, inaktiviertes BHV1, Behringwerke AG, Marburg, Bundesrepublik Deutschland) wurde intrakutan appliziert (0,1 ml). Die Hautdickenzunahme 3 Tage nach der Antigeninjektion diente zur Beurteilung des Testergebnisses.
Von 345 serologisch BHV1-negativen, über 6 Monate alten Tieren hatten 344 (99,7%) eine Hautdickenzunahme bis zu 1,0 mm und 1 Tier (0,3%) reagierte mit einer Zunahme von 1,9 mm. Die durchschnittliche Hautdickenzunahme betrug 0,2 mm.
Von 270 BHV1-infizierten Tieren reagierten 266 (98,5%) mit einer Hautdickenzunahme von mehr als 2,0 mm. Bei 3 Tieren (1,1%) betrug die Zunahme bis zu 1,0 mm und 1 Tier (0,4%) zeigte eine Reaktion von 2,0 mm. Die durchschnittliche Hautdickenzunahme betrug 6,3 mm.
Von den 107 Tieren im Alter von bis zu 6 Monaten waren 77 serologisch BHV1-negativ und 30 Tiere waren passiv immun. Die Hautdickenzunahmen aller Tiere betrug ausnahmslos weniger als 1,0 mm; der Mittelwert betrug 0,2 mm.

SUMMARY

An intradermal test (delayed hypersensitivity test) for the diagnosis of BHV1 infection was evaluated in 722 cattle of 15 dairy farms. The skin reactions were compared with the results of serological examinations using a comercial BHV1 ELISA kit (TRACHITEST, Bommeli AG,Bern-Switzerland).
As antigen concentrated, purified and inactivated BHV1 was used (Behringwerke AG, Marburg, Fed.Rep.Germany). The skin reaction (increase of the skin fold thickness) on the third day after injection of the antigen was used for the interpretation of test results.
From 345 serologically BHV1 negative cattle with an age of more than 6 months 344 (99,7%) had a skin reaction up to 1,0 mm and 1 animal (0,3%) had a reaction of 1,9 mm respectively. The mean increase of skin fold thick-ness was 0,2 mm.
Out of 270 BHV1-infected cattle 266 animals (98,5%)showed a skin reaction of more than 2,0 mm, in 3 animals (1,1%) a skin reaction up to 1,0 mm was observed and 1 animal (0,4%) had a reaction of 2,0 mm. The mean increase of the skin fold thickness was 6,3 mm.
Among 107 animals with an age of up to 6 months 77 were BHV1 negative and 30 were serologically positive by passive immunity. In all animals the skin reaction was less than 1,0 mm, the mean was 0,2 mm.

RESUMO

Um teste intradérmico (teste de hipersensibilidade de reação tardia) foi provado em 722 bovinos de 15 rebanhos. Como método de referência foi usado o ELISA (TRACHITEST, Bommeli AG, Berna/Suica).
O antígeno utilizado (herpesvirus dos bovinos, tipo 1 , HVB1), fornecido pelo Behringwerke AG, Marburg, Alemanha Ocidental) foi inoculado intradérmicamente (0,1 ml). A leitura foi feita 3 dias depois da inoculação do antigeno. A interpretação do teste baseou-se no aumento da grossura da pele no lugar da inoculação.
Dos 345 animais adultos, sorològicamente HVB1-negativos, 344 animais (99,7%) mostraram uma reação até 1,0 mm e 1 animal mostrou uma reação de 1,9 mm. A reação média neste grupo foi 0,2 mm.
Dos 270 animais infectados por HVB1 266 (98,5%) mostraram reações entre 2,1 e 16,8 mm, 3 animais mostraram reações até 1,0 mm e 1 animal (0,4%) mostrou uma reação de 2,0 mm. A reação media neste grupo foi 6,3 mm.
Dos 107 terneiros (77 animais sorològicamente negativos e 30 animais com imunidade passiva) todos reagiram com menos de 1,0 mm. A reação media neste grupo foi 0,2 mm.

POSTER

AFFICHE

ABORTO A VIRUS DE LA DIARREA VIRAL BOVINA EN UN RODEO DE CRIA DE LA PROVINCIA DE BUENOS AIRES, ARGENTINA.

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INTRODUCCION

En los rodeos de cría de la provincia de Buenos Aires, las enfermedades venéreas (tricomoniasis y campylobacteriosis) son los principales agentes causales de problemas reproductivos y pérdidas económicas. En numerosos rodeos, el control de las mismas ha mejorado sensiblemente su eficiencia productiva. Sin embargo, las etiologías virales no deben ser subestimadas como causas de fallas reproductivas, pese a su incompleta caracterización. El virus de la diarrea viral bovina (DVB) fue reconocido como causal de fallas reproductivas y acción fetopatogénica en numerosas epizootias. (1, 2, 3). En Argentina si bien existen descripciones sobre brotes de enfermedad clínica y relevamientos serológicos el aislamiento viral a partir de fetos abortados no ha sido reportado. En el presente trabajo se presentan los datos referidos al aislamiento del virus de DVB de fetos y neonatos en un rodeo de cría con problemas reproductivos.

MATERIALES Y METODOS

Historia del rodeo original

En un establecimiento de cría bovina del sudeste de la provincia de Buenos Aires se observaron bajos porcentajes de preñez y abortos en vaquillonas. Un rodeo de 170 vaquillonas Aberdeen Angus (Rodeo A) vacunadas cuando terneras (3-6 meses de vida) contra brucelosis, en buen estado nutricional fueron servidas durante 3 1/2 meses (invierno) con toros Aberdeen Angus libres de tricomoniasis y campylobacteriosis. El porcentaje de preñez al mes de retirados los toros fue del 68,2%. Se detectaron 18 vaquillonas abortadas (10,5%) con fetos de aproximadamente 4 meses de edad. Se examinaron 7 de las vaquillonas las que fueron serológicamente negativas para brucelosis y rinotraqueitis infecciosa bovina (IBR). Los cultivos de moco cervical de dichas vacas resultaron bacteriológicamente negativas a Tritrichomonas foetus, Brucella abortus y Campylobacter fetus. Los cultivos bacteriológicos de un feto de 4 meses también fueron negativos a dichos patógenos, el cultivo virológico para IBR fue negativo. No se realizaron siembras para aislamiento de virus DVB. Histológicamente, en el hígado se observaron focos necróticos , en los espacios porta áreas con infiltrado mononuclear y macrófagos y miocarditis multifocal con vasculitis. Excepto los problemas reproductivos mencionados, no se observaron síntomas clínicos de DVB en las vacas.

Rodeo problema

Del lote de vaquillonas mencionadas anteriormente (Rodeo A), se

vendan 107 hembras preñadas (Rodeo B) las que son transferidas a otro establecimiento. La mayoría de ellas tenían una preñez de aproximadamente 7 meses. A los 15 días de arribar al establecimiento se observaron abortos y mortalidad neonatal. Algunos terneros mostraron signos de ataxia, escaso desarrollo corporal y ceguera. No se observaron signos clínicos, excepto los mencionados en ninguna de las vacas.

Examen de los fetos y neonatos

Se realizó la necropsia de 4 fetos y neonatos. Se recolectaron estérilmente para cultivos bacteriológicos y virológicos los siguientes órganos y fluidos: cerebro, cerebelo, líquido céfalo-raquídeo, líquido abomasal, bazo, linfonódulos abdominales, timo, hígado, riñón, pulmón e intestino y la placenta de un feto abortado. Los órganos mencionados fueron muestreados para histología de rutina, se fijaron en formol al 10% y teñidos con hematoxilina y eosina.

Examen bacteriológico

Los órganos fetales de neonatos y placenta fueron cultivados en agar sangre y en medios selectivos para aislamiento de T.foetus, Campylobacter fetus, B.abortus, microorganismos aerobios y microaerófilos.

Examen virológico

Los órganos muestreados fueron molidos en un mortero con arena estéril y suspendidos al 10% en medio esencial mínimo Eagle (MEM) suplementado con suero bovino al 10% libre de anticuerpos. Posteriormente fue clarificado por centrifugación y el sobrenadante inoculado por duplicado en volúmenes de 0,2 ml. en tubos de cultivo Leighton con células de testículo fetal bovino (TFB) en MEM con 80% de confluencia. Luego de 45 minutos de adsorción, los tubos se incubaron a 37°C durante 7 días . Los cultivos donde no se observaba efecto citopático (ECP) fueron pasados a un cultivo de TFB de forma similar a la expuesta. Posteriormente, las laminillas fueron fijadas y coloreadas mediante la técnica de inmunofluorescencia indirecta (IFI) con anticuerpo conjugado de referencia para DVB (NVSL, Ames, Iowa, USA) (4, 5).

RESULTADOS

Fallas reproductivas

Finalizada la parición de las 107 vaquillonas originalmente preñadas se obtuvieron 75 terneros viables (70,1%).

Se observaron abortos (de 7 a 8 meses de gestación) y mortalidad perinatal en 9 (8,4%) animales. Los principales hallazgos clínicos, virológicos y patológicos se describen en la Tabla 1.

También se observó el nacimiento de 10 terneros (9,3%) con menor desarrollo corporal, algún grado de ataxia y dismetría, y ceguera en algunos de ellos. Al realizar el destete, de los 75 terneros se observaron animales con escaso desarrollo corporal y dos ciegos.

Bacteriología

Los cultivos bacteriológicos de los fluidos y órganos de fetos y neonatos procesados resultaron negativos.

Virologia

Se aisló virus DVB de ECP de todos los materiales sembrados de un feto y un ternero con ataxia, mientras que virus sin ECP se aisló de dos terneros a término (Tabla 1). En todos los casos los aislamientos virales fueron positivos a IFI. No se aisló virus de IBR en ninguna de las muestras procesadas.

DISCUSION

El aislamiento de virus de DVB con y sin ECP en fetos y neonatos provenientes de un rodeo de vaquillonas preñadas (rodeo B) en el presente caso confirma las severas pérdidas económicas ocasionadas por el virus. El presente brote evidencia la facilidad con que el virus puede atravesar la placenta después de los 50 días de preñez en vacas no inmunizadas provocando fetopatología o nacimiento de terneros infectados, como ya fue mencionado (6). En Gran Bretaña, se estima que el 39% de las vacas se infectan con DVB durante las primeras tres preñeces (3) y uno de cada 16 terneros en dichos rodeos estarían en riesgo de presentar anomalías en el desarrollo o muerte intrauterina como resultado de la exposición al virus de DVB (6). La carencia de información con respecto a la situación de DVB en nuetro país nos obliga a insistir en la necesidad de recabar más información. Las evidencias epizootiológicas del Rodeo A hacen presumir que el virus de la DVB habría sido el responsable de un severo efecto sobre la performance reproductiva. La falta de signos clínicos, excepto los problemas reproductivos en ambos rodeos (A y B) sugiere que la mayoría de las infecciones de DVB en adultos son inaparentes coincidiendo este hallazgo con lo mencionado por otros autores (3, 6). Las pérdidas reproductivas encontradas en el presente brote (31,8% en el rodeo A y 29,9% en el rodeo B) están dentro del rango mencionado por otros autores (3). Las pérdidas embrionarias son difíciles de evaluar y si bien el virus no inhibiría la concepción en vacas seropositivas, al ser inoculado intrauterinamente en forma experimental en el momento del servicio en vacas seronegativas ocasionó bajos porcentajes de preñez (7). En el presente caso se observó toda la gama de pérdidas reproductivas desde repeticiones de celo, mortalidad fetal, neonatos y nacimiento de terneros débiles permitiendo suponer que el período de infección ocurrió antes de los 180 días de preñez momento en el que el feto es inmunocompetente y el virus no tendría acción deletérea (2, 8). Por otro lado, los cultivos bacteriológicos de fetos y placenta y la serología de vacas, permitiría descartar organismos bacterianos, protozoarios, y el virus de IBR como agentes involucrados en el presente brote.

El aislamiento del virus DVB sin efecto citopático a partir de neonatos confirmaría la infección transplacentaria cuando el feto es inmunológicamente inmaduro (< 125 días) (9). Dichos terneros podrían nacer persistentemente infectados (2) localizándose el virus en la neuronas de la corteza cerebral e hipocampo (10). Estos animales infectados intrauterinamente podrían desencadenar un brote de enfermedad de las mucosas al sobreinfectarse con virus citopático.

La presencia de abortos y pérdidas neonatales en el presente caso permite suponer que la infección viral se produjo debido a la carencia de inmunidad en las madres. Por otro lado, la presencia de inmunidad materna minimizaría tales pérdidas reproductivas (9). Las infecciones de vaquillonas entre los 100 y 150 días de preñez serían las responsables de malformaciones congenitas con ceguera y ataxia como los observados en algunos terneros en el presente caso de los cuales fueron aislados virus DVB con ECP. Las lesiones macroscópicas e histopatológicas aquí descriptas coinciden con las mencionadas por otros autores (2, 8). Si bien el virus de DVB puede introducirse por diferentes vías en un rodeo (contacto con un animal persistentemente infectado, toro eliminador de virus por semen, etc) la verdadera vía de infección en este caso no pudo determinarse. El 60,7% de las vaquillonas (65/107) tuvieron seroconversion al ser sangradas una vez finalizada la parición (Daguerre y col, datos sin publicar). Títulos séricos > de 32 protegerían al feto cuando la hembra fue experimentalmente expuesta en cualquier estadio de lo gestación. Sería de esperar que la inmunidad activa adquirida en dichas madres minimizara los riesgos de pérdidas reproductivas en un nuevo brote de DVB (9).

La vacunación contra DVB podría ser una opción viable para el control de la enfermedad. El riesgo del empleo de vacunas vivas lo hacen desaconsejable por la posibilidad de difundir el virus o producir efecto fetopatogénico en vaca preñada no inmunizada. El empleo de vacunas a virus inactivados produciría respuestas de anticuerpos y protegería contra la infección de DVB (12).

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RESUMEN

Un brote de abortos, pérdidas reproductivas y nacimientos de terneros atáxicos fue incriminado al virus de la DVB en un rodeo de cria de la provincia de Buenos Aires, Argentina.

Sobre 107 vaquillonas Aberdeen Angus preñadas se destetaron 75 terneros (70.1%). Las pérdidas ocasionadas por abortos y mortalidad neonatal fue del 8,4%. Se observó el nacimiento de 9,3% terneros con menor desarrollo corporal, ataxia y ceguera. Se aisló virus de DVB de efecto citopático en un feto de 7 1/2 meses y en un neonato, mientras que virus de DVB sin efecto citopatico se aisló de dos terneros neonatos. Se observaron lesiones macro y microscópicas de hipoplasia cerebelosa y retinopatia. No se detectaron signos clinicos excepto los reproductivos, en las vaquillonas donde se registró el brote. Se describen las severas pérdidas económicas ocasionadas por el presente brote.

SUMMARY

An outbreak of abortion reproductive losses and the born of weak ataxic calves due to bovine virus diarrhoea (BVD) virus was detoted in a beef herd in Buenos Aires province, Argentina. 107 pregnant Aberdeen Angus heifers weaned 75 calves (70.1%). Abortion and stillbirths were 8.4% weak, ataxic and blindness were observed in 9.3% of calves. Both cytopathic and non-cytopathic BVD virus were isolated from a foetus, stillbirth and 2 stillbirths, respectively. Post mortem and histological findings were the typical for BVD. No clinical signs, except reproductive losses, was observed in the pregnant heifers. Economical losses due to this outbreak are discussed.

RÉSUMÉ

Le virus de la DVB a été soupçonné d'etre le responsable des avortements, des pertes reproductives et de la naissance de veaux ataxiques dans un troupeau de bovins à viande de la province de Buenos Aires, Argentine. A partir de 107 genisses detectes gestantes, on a eu seul 75 veaux sevres (70.1%). Les pertes du à des avortements et des morts autour de la mise bas ont été del ordre de 8.4%. Les veaux avec un certain degree de'ataxie et des problèmes de la vision ont atteint le 9.3%. Le virus de la diarrhée viral bovine (DVB) avec un effet citopatique a été isole d'un fetus de 7 1/2 mois et d'un nouveau né, tandis que le même virus sans effet citopatique a été isole a partir de deux veaux noveaunes. Des lesions macro et microscopiques d'hypoplasie cerebelleuse et de la retinopatie ont été observées. On n'a pas observes d'autres signes cliniques que les reproductives déjà decrits chez les genisses du lot problème. On discute les sevères pertes economiques produites par la maladie.

TABLA I: Hallazgos clínicos, patológicos y virológicos en fetos y neonatos afectados por el virus de la Diarrea Viral Bovina.

Ternero N	Edad	Clínica	Aislamiento virus DVB	Patología
1	7 1/2 meses de gestación	Abortado	Positivo, ECP	Tamaño normal, edema subcutáneo, petequias generalizadas en cavidad torácica y abdominal, hígado ??, aspecto marmóreo. Necrosis hepática centrolobulillar, vasculitis, proliferación endotelial media en S.N.C. Infiltración macrofágica perivascular.
2	Nace vivo, necropsiado a los 3 días	Ataxia, disenteria, ceguera, escaso desarrollo corporal	Positivo, ECP	Opacidad corneal bilateral. Hipoplasia cerebelosa, severa degeneración cística de lóbulos dorsales. Cerebelo con agenesia parcial, atrofia y edema de folia,necrosis de capa granulosa externa y molecular con desaparición de células de Purkinje. Vasculitis y meningitis no supurativa. Hígado consistente, marmóreo. Hiperplasia periportal en hígado con infiltrado mononuclear y necrófagos
3	A término no	Aparente distocia, 32 kg. de peso	Positivo, sin ECP	Congestión en hígado y bazo
4	A término no	Aparente distocia, 32 kg. de peso	Positivo, sin ECP	Congestión en timo, edema en cabeza y cuello. Proliferación periportal en hígado. Meningitis no supurativa, hiperplasia endotelial en cerebelo.

OPTIMIZATION OF MEMBRANE INTEGRITY EVALUATION OF BOVINE SPERMATOZOA BY SWELLING TEST

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INTRODUCTION

The Hypo-osmotic Swelling Test (HOS) has been used as assay for investigating the functional integrity of human sperm membrane (3). This test assesses functional integrity of sperm membrane by count of swollen tail spermatozoa, after a hypo-osmotic treatment.

There are important correlations between HOS and zona free hamster egg penetration assay with human semen samples (1,4). Therefore Chan et al. (2) have found that specific sperm tail patterns of swollen, % normal sperm morphology and kinetic parameters can discriminate samples exhibiting sperm in vitro fertilizing capacity.

The objective of this study is the optimization of Hipo-osmotic Swelling Test for bovine semen.

MATERIALS AND METHODS

20 samples of frozen bovine seminal material, diluited with milk or Tris-egg yolk based extender were used. The samples were thawed in water-bath at 37 C for two minutes.

Seminal material concentration (millions/ml) (CON), total motility (%) (TM), progressive motility (%) (PM), mean velocity (um/sec) (MV), acrosome anomalies (%) (AA), tail anomalies (%) (TA), total sperm anomalies (%) (SA) and proximal cytoplasmatic drops (%) (PD) were calculated. Concentration was calculated with a Coulter Counter (Coulter mod. ZM). Motility was assessed with a computerized videomicrography using an automatic image analyzer (SM system, Mini Tub) with sixteen image digitalizations a second per spermatozoon. Average curvilinear velocity (V) as sum of vectors between single digitalized points per spermatozoon and linear velocity (S) as distance between each spermatozoon's first and last image were calculated for all those spermatozoa with at least 10 images. Those spermatozoa with V > 25 um/sec were considered as mobile while those with V > 25 um/sec and S/V > 0.8 were considered progressively mobile. Cytomorphology was assessed by means of contrast interference microscopy (x1250) after fixation in suspension of the seminal material with 0.2% glutaraldehyde solution in PBS without calcium and magnesium (1:2 v/v). The HOS was carried out by hypo-osmotic solution (150 mOsm) (3) for 30 or 60 minutes of incubation, with or without previous centrifugal washing with PBS (2 x 1200 rpm for 10 minutes). At least 200 spermatozoa were analysed by contrast interference microscopy (DIC) and phase contrast microscopy (PH) and classified according to Chan et al., (2) (figure 1). The variance analysis was assessed according to the following linear model:

$$Y_{ijkl} = m + S_i + W_j + I_k + bX_{ijkl} + e_{ijkl}$$

where Y = dependent variable (HOS); m = general average; S = i-th microscopic system effect (i=1,2); W = j-th washing effect (j=1,2); I = k-mo incubation effect (k=1,2); X = covariate (seminal concentration); e = common random error effect.

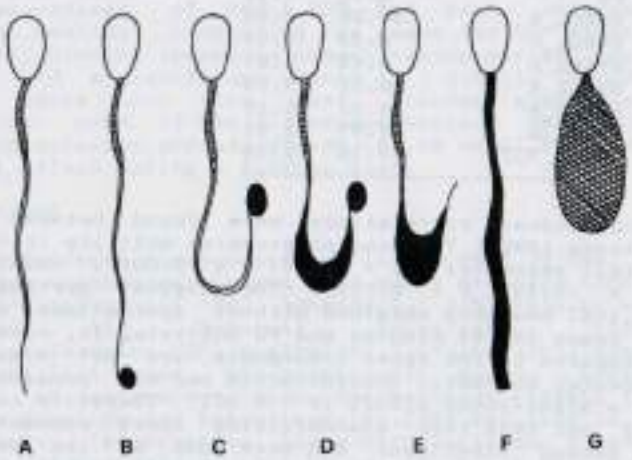

Figure 1 HOS classification according to Chan et al., (2) (A = normal spermatozoon; B = SWELL_B; C = SWELL_C; D = SWELL_D; E = SWELL_E; F = SWELL_F; G = SWELL_G).

RESULTS AND DISCUSSION

The our semen analysis results are displaied in table 1. The mean percentage of total swollen spermatozoa was 35.30%. The main type of swelling (24.20 %) was that of the terminal part of the tail (SWELL_B).

TABLE 1. Mean (+/- SD) semen quality results (N=160).

PARAMETERS	Mean	SD
CON	47.05	27.70
TM	50.80	17.88
PM	34.93	17.00
MV	64.30	8.32
AA	19.73	7.39
TA	10.95	7.91
SA	32.30	12.23
PD	2.80	1.89
SWELL_B	24.20	6.99
SWELL_C	4.33	4.78
SWELL_D	5.49	5.87
SWELL_E	0.31	0.89
SWELL_F	0.03	0.18
SWELL_G	0.97	2.07
SWELL_TOT	35.30	13.19

Interesting linear correlations were found between total swollen spermatozoa (SWELL_TOT) and progressive motility (r = 0.36; P < 0.001), tail anomalies (r = -0.45; P < 0.001), total sperm anomalies (r = -0.39; P < 0.001). The highest percentage of SWELL_TOT (40.15%) has been obtained without spermatozoa washing, incubating the semen for 60 minutes and PM analysing it, even if the differences compared to the other treatments were not significant (P > 0.05). However spermatic concentration per ml, considered as covariate, had a significant effect (P < 0.001). Therefore could be best to carry out this test standardizing sperm concentration. Therefore a second experiment has been made by the best HOS procedural method defined in the first experiment and utilizing standard concentration samples (samples = 10; N = 20). There was not significant difference (P > 0.05) between the SWELL_TOT with or without concentration pre-adjustment (49.5% vs 47.1%).

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SUMMARY

20 samples of frozen bovine seminal material were treated with hypo-osmotic solution (150 mOsm) for 30 or 60 minutes, with or without previous centrifugal washing with PBS. At least 200 spermatozoa were analysed by contrast interference microscopy (DIC) and phase contrast microscopy (PM). The Coulter-Counter concentration, computer motility and DIC morphology were evaluated. The mean percentage of total swollen spermatozoa (SWELL_TOT) was 35.30%. The main type of swelling (24.20 %) was that of the terminal part of the tail. Interesting linear correlations were found between SWELL_TOT and progressive motility (r = 0.36; P < 0.001), tail anomalies (r = -0.45; P < 0.001), total sperm anomalies (r = -0.39; P < 0.001). The highest percentage of SWELL_TOT has been obtained without spermatozoa washing, incubating the semen for 60 minutes and PM analysing it. However spermatic concentration per ml, considered as covariate, had a significant effect (P < 0.001). Therefore it is best to carry out this test standardizing nemaspermatic concentration, even if the difference between SWELL_TOT with or without concentration pre-adjustment (49.5% vs 47.1%) did not have a significant effect during a further test.

ZUSAMMENFASSUNG

20 Proben gefrorenen Rindersamenmaterials wurden 30 bzw. 60 Minuten lang mit hypo-osmotischer Lösung (150 mOsm) mit oder ohne vorherigem Zentrifugal-Waschen mit PBS behandelt. Schließlich wurden 200 Spermatozoen mit Nomarski Interferenz (DIC) und Phasekontrastmikroskop (PM) analysiert. Coulter-Zahl-Konzentration, Computer-Mobilität und DIC-Morphologie wurden bewertet. Der Hauptanteil an aufgeschwollenen Spermatozoen (SWELL_TOT) betrug 35,30%. Der Haupttyp der Schwellung (24,20%) war jene im Endbereich des Schwanzes. Interessante Linearkorrelationen wurden zwischen SWELL_TOT und Vorwärts-Beweglichkeit (r = 0,36; P < 0,001), zwischen SWELL_TOT und Schwanzanomalien (r = -0,45; P < =,001), zwischen SWELL_TOT und Spermaanomalien insgesamt (r = -0,39; P < 0,001) festgestellt. Die Höchstrate an Membranintegrität wurde ohne Waschen der Spermatozoen erreicht, bei einer Inkubation des Samens von 60 Minuten und mit PM-Analyse. Jedenfalls ist die Spermakonzentration pro Milliliter, die als Kovariante angesehen wird, von entscheidender Bedeutung (P<0,001). Daher ist es am besten, diesen Test mit einer standardisierten Nemasperma-Konzentration durchzuführen, auch wenn der Unterschied zwischen SWELL_TOT mit oder ohne vorbestimmter Konzentration (49,5% gegenüber 47,1%) bei einem weiteren Test keine wesentlichen Auswirkungen hatte.

RESUMEN

Se trataron 20 muestras de material seminal de bovino congelado con una solución hipo-osmótica (150 mOsm) durante 30 o 60 minutos, con o sin previo lavado centrífugo con PBS. Se analizaron al menos 200

espermatozoides mediante interferencia Nomarski (DIC) y microscopia de contraste de fase (PH). La concentración del coulter-contador, movilidad de procesador y la morfología DIC se evaluarón. El promedio de porcentaje de los espermatozoides hinchados (SWELL_TOT) era del 35.30%. El mayor tipo de hinchazón fué del (24,20%) que era en la parte final de la cola. Se encontraron correlaciones lineales interesantes entre el SWELL_TOT y movilidad progresiva ($r = 0,36$; $P<0,001$), entre el SWELL_TOT y anomalies de cola ($r = -0,45$; $P < 0,001$) y entre el SWELL_TOT y anomalias espermáticas totales ($r = -0,39$; $P<0,001$). El mayor porcentaje de integridad de la membrana se ha obtenido sin el lavado de los espermatozoides incubado el semen durante 60 minutos y analizando el PH. No obstante, la concentración espermática por ml, considerada como covariante, tenía un efecto significativo ($P < 0,001$). Por tanto, es mejor llevar a cabo este test estandarizando la concentración nema-espermática incluso si la diferencia entre el SWELL_TOT con y sin la pre-regulación de la concentración (49,5% vs 47,1%) no ha tenido un efecto significante durante un ulterior test.

A MURINE MODEL FOR THE STUDY OF MYCOTIC PLACENTITIS AND ABORTION

Why bovine mycotic abortion occurs late in pregnancy - A new hypothesis

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INTRODUCTION

In most countries loss due to mycotic placentitis and abortion is of great importance in cattle breeding. A number of different fungi have been isolated from spontaneous cases of bovine mycotic abortions. In most cases members of the genera Aspergillus, Candida, Absidia, Rhizopus, Mucor, and Mortierella are recovered from aborted mycotic material, with Aspergillus fumigatus being the main causative fungus. The main characteristics of the bovine mycotic placentitis/abortion are: 1) It occurs predominantely in the terminal state of gestation. 2) The fungi are spread haematogenously to the placenta. 3) The portal of entry is most probably the lung and/or gastro-intestinal tract. 4) The development of initial mycotic lesions is in placentomes whereas the chorioallantoic membrane is infected secondarily. 5) An acute necrotizing inflammation develops in the placentas. 6) Foetal mycotic skin plaques may occurs in the most severely affected cases.

The pathogenesis of mycotic abortion is still unclear. However, from experimental studies Hill et al. (1971) has introduced a theory for the spread of aspergillosis (A.fumigatus) in the bovine placenta. In brief, they considered mycotic lesions to originate from a limited number of placentomes (primary mycotic lesions), from which the mycosis extends to the chorioallantoic membrane and previously uninfected placentomes, by lateral spread between the endometrium and the chorioallantoic membrane.

In order to study the pathogenesis of mycotic placentitis and abortion we recently developed a new murine model for the study of experimental mycotic placentitis and abortion [5,6], and the aim of the present paper is to propose a new hypothesis for the development of mycotic placentitis, based on results obtained by the murine model, and apply it to other mammalian species in general, including the pregnant cow.

MATERIALS AND METHODS

In two experiments (A & B) prinipregnant female BALB/cABom mice were inoculated intravenously with conidia from A. fumigatus (A.T.C.C. 42202) on day 10 of pregnancy. A complete necropsy was performed on each animal, and the uterine contents and histopathological findings were recorded individually. A detailed description of the materials and methods employed is being published elsewhere [5,6].

In experiment A [5] 8 groups of mice were challenged intravenously with $1x10^1$ to $1x10^8$ conidia. Except for mice that either succumbed or were killed severely ill, the mice were euthanized on day 19 of pregnancy. A complete necropsy was performed on each animal, and the uterine contents and histopathological findings were recorded individually. The post-mortem findings are given in Table 1 and 2.

In experiment B [6] 3 groups of mice were inoculated intravenously with $1x10^5$ conidia. Consecutively all mice in each group were euthanized corresponding to day 14, day 16, and day 18 of pregnancy. The post-mortem findings are given in Table 3.

RESULTS

The histopathological findings are described in detail elsewhere [5]. From experiment A it appeared that most mice aborted or had placental lesions when inoculated with $1x10^3$ or more conidia (Table 2). Furthermore, polymorphonuclear (PMN) cell infiltrations were found in the uterus of 75% of the mice which aborted following the inoculation of $1x10^3$ conidia or more. Extrauterine lesions were closely related to the dose of conidia inoculated, and organs showed inflammatory change with decreasing frequency as follows: liver > lung > kidney > brain > heart. Concerning the growth of hyphae the following pattern was found: kidney > brain > lung.

In experiment B hyphal growth outside the foeto-placental units was not found. A detailed description of the results is being published elsewhere [6]. The distribution of foeto-placental units with hyphal growth is given in Table 3. Characteristically the growth of hyphae started in and was consistently found in the periphery of the placental disc, which is the area in which the natural necrosis initiates in the terminal state of pregnancy [1]. Further progression of hyphal growth was found around and along the more or less degenerated Reicher's membrane (Fig 1). Progressively, fungi spread along the extrafoetal membranes, which frequently were penetrated by the hyphae giving fungal access to the amnionic cavity and the foetus (Fig. 1). Foetal infection was first established in the skin, from which it sometimes penetrated to underlying tissues. In experiment A the mice with foetuses were euthanized on day 12 and day 19 of pregnancy, and in these only necrosis was produced due to the growth of hyphae. In infected foetuses from mice in experiment B, which were euthanized on day 14 to day 18 of pregnancy, the growth of hyphae was further accompanied by haemorrhage and infiltration by PMN and mononuclear cells.

Table 1. Findings in extrauterine organs from female BALB/c mice inoculated intravenously with different concentrations of A. fumigatus conidia on day 10 of pregnancy.

	Number of mice challenged									
	4	4	4	4	4	3	4	2	2	3
Dose	C	$1x10^1$	$1x10^2$	$1x10^3$	$1x10^4$	$1x10^5$	$1x10^6$	$1x10^7$	$.5x10^8$	$1x10^8$
Liver	0/0	1/0	1/0	3/0	2/0	1/0	4/0	2/0	2/0	3/0
Lung	0/0	0/0	0/0	1/0	1/0	*2/0	2/0	2/0	2/0	3/0
Kidney	0/0	0/0	0/0	0/0	0/0	3/0	1/0	3/3	2/2	3/2
Brain	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/0	*1/1	2/1
Heart	0/0	0/0	0/0	0/0	0/0	*0/0	0/0	0/0	0/0	2/0

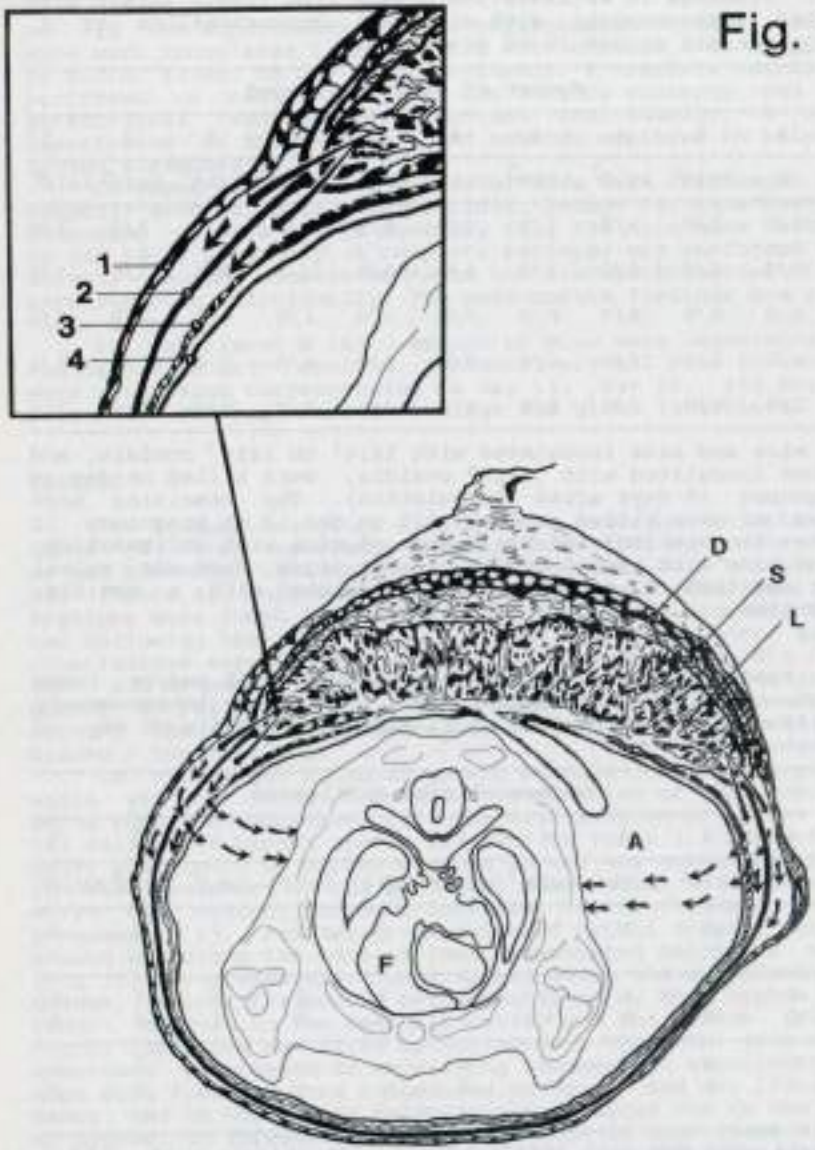
Control mice and mice inoculated with $1x10^1$ to $1x10^7$ conidia, and one mouse inoculated with $.5x10^8$ conidia, were killed on day 19 of pregnancy (9 days after inoculation). The remaining mice succumbed or were killed severely ill on day 12 of pregnancy (2 days after inoculation). No/No: number of mice with inflammation/number of mice with hyphal growth. *) The organ from one animal was not examined. C: control group, inoculated with a conidia-free solution.

Table 2. Findings in placentas and the uterine wall and/or lumen (when aborted) of female BALB/c mice inoculated intravenously with different concentrations of A. fumigatus conidia on day 10 of pregnancy.

		Number of mice challenged									
		4	4	4	4	4	3	4	2	2	3
Dose		C	$1x10^1$	$1x10^2$	$1x10^3$	$1x10^4$	$1x10^5$	$1x10^6$	$1x10^7$	$.5x10^8$	$1x10^8$
Aborted		1	0	1	4	1	3	4	2	0	0
Uterus	I)	0	0	1	3	2	3	2	2	1	1
	H)	0	0	0	0	0	0	0	0	0	0
Placentae	I)	0	0	0	-	0	-	-	-	2	3
	H)	0	0	0	-	0	-	-	-	2	3

I) Animals with PMN cell infiltrations. H) Animals with hyphal growth. -) Had aborted when necropsied. C) Control group, inoculated with a conidia free solution. See also legend to Table 1.

Fig. 1.



Arrows indicate the initiation of hyphal growth in the periphery of the placental disc (L+S)(inset) and lateral spread in the foeto-placental unit of mice challenged with A. fumigatus conidia (i.v.) on day 10 of pregnancy. A: Amniotic cavity. D: Decidua. F: Foetus. L: Labyrinth. S: Spongiotrophoblasts. 1: Uterine wall. 2: Reicher's membrane. 3: Splanchnopleura. 4: Amnion.

Table 3. Findings in the uterus of mice euthanized on day 14 (Group A), day 16 (Group B), and day 18 (Group C) of pregnancy after intravenous inoculation of $1x10^5$ A. fumigatus conidia on day 10 of pregnancy.

Groups	Number of mice	Foeto-placental units	
		Total number	Number with hyphal growth
A	4 inoculated	35	4 in 1 mouse
	1 control	6	0
B	4 inoculated	37	12 in 4 mice
	1 control	8	0
C	4 inoculated	23	13 in 2 mice, 1 aborted
	1 control	7	0

DISCUSSION

The present murine model supports the opinion that fungi are spread haematogenously to the placenta, a route generally accepted for mycotic infections in bovine placentas. Furthermore, it was found that subsequent spread along and penetration of the extrafoetal membranes might give hyphae access to the foetus, in which the infection started in the skin. These events are similar to what is believed to take place in the development and progression of the bovine mycotic placentitis. In mice inoculated intravenously with A. fumigatus conidia the kidney has hitherto been considered the target organ for hyphal growth [8,9]. However, the present study demonstrates that A. fumigatus more readily infected the placenta than any other organ when mice were inoculated on day 10 of pregnancy. A comparable propensity for hyphal growth in placental tissue has also been found in pregnant ewes and cows when inoculated intravenously with A. fumigatus conidia [2,4]. In the murine placentas hyphal growth originated in the periphery of the placental discs, the area in which natural necrosis appears in the terminal stages of pregnancy [1]. From these findings, and the fact that trophoblasts are capable of phagocytosis of particles [7], including yeasts [3], we infer that the growth of fungi might originate from dorminant fungal elements contained in trophoblasts. The conidia are believed to be released for germination during the process of naturally occurring necrosis. Applied to cattle the hypothesis implies that bovine trophoblasts, during early pregnancy, accumulate fungal elements. Germination and spread laterally between the endometrium and the chorioallantoic membrane in the late state of pregnancy could explain why bovine mycotic placentitis and abortion is predominantly a phenomenon of this period. An alternative hypothesis is that trophoblasts are only capable of containing fungi during early pregnancy, whereas later on this ability is lowered or lost due to the advanced aging of the trophoblasts.

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SUMMARY

Based on a newly developed murine model for the study of fungal-induced placentitis the following theory is proposed: During pregnancy fungal elements are engulfed by phagocytosis in the trophoblastic cells. In the intact cell the fungal material is dormant. However, as pregnancy progresses the ability of the aging trophoblastic cells to maintain the fungal elements dormant decreases. This hypothesis explains why bovine mycotic abortion is predominantly a phenomenon of late pregnancy.

ZUSAMMENFASSUNG

Begründet in einem kürzlich entwickelten Maus-Modell zum Studium mykotisch-induzierter Plazentitis folgende Theorie ist vorgelegt: Während der Trächtigkeit werden mykotische Elemente durch Phagozytose von den Trophoblast-Zellen aufgenommen. In der intakten Zelle ist das mykotische Material im Ruhezustand. Bei fortgeschritteners Trächtigkeit, die Fähigkeit der alternden Trophoblast-Zellen die mykotischen Elemente in Ruhe zu halten, ist indessen abgenommen. Diese Theorie erklärt weshalb bovine mykotische Aborte hauptsächlich ein Phänomen in der späten Trächtigkeit sind.

RÉSUMÉ

Basé sur l'expérience réalisée avec une modèle de souris pour etudier la formation du placentite mycosique - on propose la théorie suivante: Pendant la gestation, les organismes mycosiques sont engloutis par phagocytose dans les cellules trophoblastiques. Dans la cellule intacte la matière mycosique est en repos. Cependant, avec l'évolution de la gestation les cellules trophoblastiques mûres reduirent leur capacité à soutenir les organismes mycosiques en repos. Cette hypothese explique pourquoi lávortement dû aux mycoses chez les bovines sont essentiellement un phénomène a la fin de gestation.

UTILIZACION DEL LICOR FOLICULAR EN EL MEDIO DE MADURACION DE OVOCITOS BOVINOS PARA FERTILIZACION IN VITRO (F.I.V.)

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INTRODUCCION

Actualmente, es de gran importancia disponer de una fuente económica de embriones, tanto para aplicar estrategias de mejora ganadera como para investigaciones en nuevas tecnologías genéticas. Hoy es posible obtener en el laboratorio, mediante F.I.V., embriones en todas las etapas de desarrollo, partiendo de ovocitos inmaduros, los cuales han sido madurados utilizando diversos medios de cultivo, con o sin adición de hormonas, en presencia o ausencia de células de ayuda (células de la granulosa, células de tapiz de oviducto)(2,6).También se ha efectuado la maduración en el interior de folículos disecados (8,7). De acuerdo con los resultados de varios estudios el porcentaje de formas desarrolladas obtenidas a partir de ovocitos madurados in vitro, es considerablemente menor (menos del 50%), en comparación con las obtenidas a partir de ovocitos madurados in vivo (5). Es necesario concentrar esfuerzos en investigaciones sobre los sistemas de cultivo de maduración, buscando a la vez, alternativas de menor costo,aspecto fundamental para la viabilidad de la técnica de F.V.I. particularmente en los países del tercer mundo. En este trabajo se evalúa el licor folicular (LF) como componente del medio de maduración de ovocitos bovinos.

MATERIALES Y METODO

Colección de ovocitos inmaduros a partir de folículos

Los ovocitos se obtuvieron de ovarios extraídos en el frigorífico inmediatamente después del sacrificio de la vaca, los cuales fueron transportados al laboratorio en una solución de ClNa 0,9%, a 37°C. Los ovocitos se aspiraron de folículos cuyo diámetro se encontraba entre 2 a 5 mm, mediante jeringa de 5ml y aguja 20G. Previamente la jeringa se cargó con 2ml de PBS a la que se adicionó el 1% de suero de ternero (ST) y penicilina y estreptomicina (100.000U.I. 100mg/l). El contenido de los folículos se virtió en tubos de ensayo mantenidos a 37°C.

Selección, maduración

El contenido de los tubos fue transferido a cajas de Petri grandes (90mm). Una vez identificados, con el microscopio estereoscópico, los ovocitos se pasaron con pipeta Pasteur de punta afinada a cajas de Petri chicas (35mm) conteniendo PBS + 1% ST + penicilina, estreptomicina procediéndose a sucesivos lavados. Posteriormente se clasificaron de acuerdo con la calidad de los cúmulus, descartándose los que se encontraban desnudos, y se formaron dos grupos con dos diferentes medios de maduración. En el grupo N1, compuesto por 72 ovocitos, se utilizó como medio de maduración el Medio 199,(95%), ST(5%) y penicilina, estreptomicina (100.000U.I.,100mg/l). En el grupo N2 fueron madurados 66 ovocitos en Medio 199(65%), LF(30%), ST(5%) y penicilina, estreptomicina. El LF fue obtenido de folículos preovulatorios, centrifugado y el sobrenadante inactivado a 56°C por 30 minutos. El cultivo se efectuó en gotas de 50 microlitros cubiertas por aceite mineral, durante 20 horas, en incubadora a 38,5°C, 5% de CO_2 en aire y 99% de humedad. Finalizado el tiempo de maduración (20 horas), ésta fue evaluada por la expansión de los cúmulos y los ovocitos fueron posteriormente lavados en el medio de inseminación.

Capacitación del semen

A efectos de capacitar el semen y facilitar la reacción acrosómica,se utilizó el medio de Brackett y Oliphant (BO)(1), al cual se adicionó heparina (10 microgramos/ml), cafeína benzoato de sodio 5mM, penicilina y estreptomicina (100.000 U.I., 100mg/l). Se descongelaron pastillas de semen de toro a 37°C, se lavó el semen por dos veces en medio BO mediante centrifugado a 500 g, se retiró el sobrenadante y se incubó en tubo de ensayo con 5 ml del medio descrito, durante 2 horas a 38,5°C en 5% de CO_2 en aire. Al fin de las dos horas se transfirió el estrato superior a otro tubo y se calculó la concentración de espermatozoides, ajustándola a 1 x 10^6espermatozoides/ml con medio BO con una concentración de albúmina de suero bovino de 10 mg/ml.

Fertilización

Con el semen se formaron gotas de 100 microlitros cubiertas por aceite mineral y en ellas se incluyeron los ovocitos a razón de 10 por gota. Se cultivaron juntos durante 5 horas, al final de las cuales se extrajeron los ovocitos, se lavaron en medio 199 con 5% de ST y se incluyeron con sus cúmulos en las gotas en las cuales pasaron el período de maduración. La primera evaluación de la división se efectuó a las 48 horas de la inseminación clasificándose según el número de células, se prosiguió evaluando cada 48 horas cambiando el medio cada vez, hasta el día 13.

RESULTADOS

El cultivo para el desarrollo fue controlado hasta el día 13 de comenzado el experimento (12 post-inseminación). La maduración fue evaluada por la expansión de los cúmulus, se pudo apreciar claramente una mayor expansión de los cúmulus de los ovocitos que fueron madurados en el medio que contenía LF (grupo 2). Para los resultados en la división y desarrollo, se consideraron los blastocistos (BL) y los blastocistos expandidos (BE). En el grupo 1, se obtuvieron 12 embriones desarrollados, 5BL(6,94%) y 7BE(9,72%). En el grupo 2 se obtuvieron en total 25 embriones en estado avanzado de desarrollo, 10BL(15,15%) y 15 BE(22,73%). Los resultados fueron significativamente favorables ($p<0,05$) para el grupo que contenía el LF en el medio de maduración. (Ver tabla 1).

TABLA 1. Rangos de desarrollo de embriones bovinos MIV-FIV en dos diferentes medios de maduración.

Grupo	Medio IVM	N°	BL	BE	Total
1	M-199+5%ST	72	5(6,94%)	7(9,72%)	12(16,67%)
2	M-199(65%)+	66	10(15,15%)	15(22,73%)	25(37,88%)

Channingycol.(3), postularon la presencia en el LF de un inhibidor de la maduración del ovocito, en cerdos y hamster, que fue aislado, el cual dejaría de actuar a partir del pico de LH. Este factor no ha sido aislado en el bovino. Liebfried y col.(5) reportan buenos resultados en FIV cuando mantienen los ovocitos 20 minutos en LF previo al cultivo de

maduración. Recientemente Romero y col. (9) reportaron altos índices de maduración IV (85,3% en Metafase II), de ovocitos bovinos, empleando M-199 más 40% de LF extraído de folículos preovulatorios 20 horas después del pico de LH. Nuestros resultados al utilizar el LF en el medio de maduración y el co-cultivo con células del cúmulus en los cultivos de FIV y desarrollo son semejantes a los reportados recientemente por Kajihara y col.(4), quienes efectuando el co-cultivo con células del cúmulus y con células del cúmulus más monocapas de células endometriales obtuvieron un 25,9% y 39,4% de blastocistos, alientan a continuar las investigaciones.

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RESUMEN

El objetivo de este trabajo es evaluar el comportamiento del licor folicular (LF), obtenido de folículos preovulatorios, como componente del medio de maduración de ovocitos posteriormente fertilizados in vitro(FIV). Se aspiraron 138 ovocitos de folículos de ovarios de vacas provenientes del frigorífico y se formaron dos grupos. En el grupo 1, 72 ovocitos fueron madurados en medio 199(95%) más suero de ternero (ST) 5% con adición de penicilina y estreptomicina (100.000U.I. 100mg/l). En el grupo 2, se maduraron 66 ovocitos en medio 199(65%), ST(5%), LF(30%), penicilina y estreptomicina. El cultivo se efectuó en gotas cubiertas por aceite mineral durante 20 horas en incubadora a 38,5% de CO_2 en aire y 99% de humedad. A efectos de la fertilización se utilizó semen de toro congelado en pastillas, el cual luego de descongelado fue capacitado durante 2 horas mediante swim-up en medio BO al cual se adicionó heparina (10 microgramos/ml) y cafeína (5mM). Se procedió al co-cultivo de los ovocitos con el semen durante 5 horas. La concentración fue de $1x10^6$ esperma/ml. La primera evaluación de la división se realizó a las 48 horas de la inseminación, cambiándose el medio de cultivo y luego sucesivas evaluaciones cada 48 horas hasta el día 13. En el grupo 1 se obtuvieron 12 blastocistos (16,67%) y en el grupo 2 se obtuvieron 25 blastocistos (37,88%). Los resultados favorables al segundo grupo fueron significativos a un nivel de significación de 0.05.

SUMMARY

The aim of this paper is to evaluate Follicular Fluid(FF) from pre - ovulatory follicles as a component of oocite maturation medium for In Vitro Fertilization (IVF).138 oocites were aspirated from follicles of ovaries obtained from cows at the slaughter house. These oocites were divided into two groups.
Group 1: 72 oocites were maturated in TCM 199(95%) + Calf Serum (CS) (5%) with penicillin and streptomycin (100.000 IU and 100 mg/l).
Group 2: 66 oocites were maturated in TCM 199(65%) + CS(5%) + FF(30%) with penicillin and streptomycin.
The oocites were cultured in drops under mineral oil for 20 hours in an incubator, at 38.5°C of temperature, 5% of CO_2 in air and 99% of humidity. Frozen semen (pellets) was used for fertilization procedure. The semen was thawed and capacitated for 2 hours Throug swim-up treatment with BO medium plus heparin (10 ug/ml) and caffeine (5mM). Oocites and semen were cultured together for 5 hours. Semen concentration was $1x10^6$ cells/ml. 48 hours after insemination cleavage ratio was checked and culture medium changed. After that evaluations were performed every 48 hours until day thirteen. 12 blastocytes were obtained from Group 1, and 25 from Group 2. The results showed favorable to group 2 ($P<0.05$).

RESUME

L'objectif de ce travail est d'évaluer les propriétés du liquide folliculaire(LF), obtenu de follicules préovulatoires, comme composant du milieu de maturation pour les ovocites destinés à la fécondation in vitro (FIV). Cent trente huit ovocites ont été aspirés des follicules d'ovaires de vaches d'abattoir, et ils ont été distribués en deux groupes. Dans le groupe 1 la maturation de 72 ovocites a été réalisée sur milieu TCM 199 (95%) plus sérum de veau (ST,5%) additionné de penicilline (100.000,UI) et de streptomicine (100 mg/l) Dans le groupe 2 (66 ovocites) la maturation a été obtenue sur milieu TCM 199 (65%), ST (5%), penicilline et streptomicine, avec adjonction de LF (30%). La culture a été faite dans des gouttes sous huile minérale pendant 20 heures dans une couveuse à 38,5°C, sous atmosphère d'air avec 5% de Co_2 et 99% d'humidité. Pour la fécondation nous avons utilisé de la semence congélée (pellets) de taureau. La semence a été décongélée et capacitée, pendant deux heures par swim-up en milieu BO additionné d'héparine (10 ug/ml) et de caféine (5mM). Les ovocites et les spermatozoïdes ($1x10^6$/ml) on été mis en culture ensemble pendant 5 heures. La première évaluation de la division a été faite 48 h après l'insemination, avec renouvellement

du milieu de culture. Par la suite, des évaluations ont été faites toutes les 48 heures jusqu'au treizième jour. Dans le groupe 1 nous avons obtenu 12 blastocytes (16,67%), contre 25 (37,88%) du groupe 2 ($p<0,05$).

PRESENÇA DE AGLUTININAS ANTI-BRUCELA EM HEMO-SÔRO DE BÚFALAS (Bubalus bubalis) NO ESTADO DA BAHIA.

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INTRODUÇÃO

O incremento da criação de búfalos no país e no Estado da Bahia, passou a exigir o exame sanitário dos rebanhos existentes, procurando-se através do diagnóstico das enfermidades, evitar a propagação dessa zoonose, lançando-se mão de medidas profiláticas. Esta zoonose em bubalinos tem sido descrito em vários países, porém os animais que vivem em contato com bovinos são mais susceptíveis a contrair a infecção.

A brucelose, segundo (17) é considerada problema mundial por dois aspectos: o da repercussão na saúde do homem, e de interesses econômicos, como zoonose caracterizada por acarretar emagrecimento, infertilidade, abortos, diminuição da secreção láctea dos animais, crias fracas e conseqüentemente uma redução na produção de bezerros. Segundo ainda o referido autor, os animais zootécnicos originalmente afetados são os bovinos, caprinos e suínos.

No Continente Europeu (8), excluindo os países escandinavos, de 15 a 30% do gado leiteiro estão infectados. No Brasil (17) a produção é de 10 a 20%, abrangendo gado leiteiro e de corte.

A brucelose em búfalos, em condições naturais e similares, ocorre da mesma forma que nos bovinos (9), sendo seu agente etiológico, sob o ponto de vista bioquímico, biológico e sorológico, o mesmo para as duas espécies (7).

De acordo (2, 18), já se preocupavam com os índices de infecção brucélica no rebanho bubalino de seus países, que apresentavam, segundo eles, incidências de 23% e 8% na Itália e Egito, respectivamente.

Conforme (1), na Índia, país detentor do maior rebanho de búfalos do mundo, a prevalência de brucelose, nesta espécie animal, permanecia entre 6,4% a 10,2%. Porém (13), citam que em fazendas do governo onde os animais dispõem de controle adequado, através de vacinações e testes, o índice é de cerca de 4,8%.

Recentemente, (6), também na Índia, relataram que nestas fazendas a prevalência da brucelose encontra-se em torno de 0,2% e atribuem este baixo índice ao alto nível de imunidade conferido pela vacina, nesta espécie.

Já no Brasil, estudos sobre a incidência e prevalência desta enfermidade na esfera reprodutiva em búfalos são escassos. (16) já se preocupava com o elevado grau de infecção brucélica do rebanho bovino e bubalino da Ilha de Marajó, e recomendava a vacinação dos animais, pouco utilizada nesta região.

Investigando a brucelose em 66 amostras de soros destes animais (14), no Vale da Paraíba, em São Paulo, encontrou 40,9% de positividade. Doze anos depois, (4) no Estado de Goiás, constataram 17,3% de positividade.

Segundo (5), mesmo os búfalos que apresentam títulos altamente significativos na prova rápida em placa e que reagem positivamente no Card Test não demonstram problemas causados pela brucelose, tais como aborto, retenção de placenta e morte de neonatos. Enquanto (15) encontraram ao examinarem 992 amostras séricas de búfalos empregando a prova de soro aglutinação rápida em placa e o Card Test, constataram positividade em cada método de 4,3% e 5,3% respectivamente.

Em búfalas brucélicas, os principais sintomas clínicos observados são

descritos como sendo higroma articular (11), endometrite (10) e aborto (12). Ainda afirma (12) que a incidência de aborto em vacas búfalos brucélicas é baixa, atingindo cerca de 3,7% dos reagentes.

Tendo em vista, portanto, a carência de informações nacionais sobre o assunto, especialmente no Estado da Bahia, a presente investigação objetiva-se determinar a ocorrência de brucelose, nesta espécie em apreço.

MATERIAL E MÉTODOS

O material constou de hemo-sôro de 2.670 fêmeas da espécie bubalino, coletado aleatoriamente em 141 propriedades de 28 municípios do Estado da Bahia, nos anos de 1987, 1988 e 1989.

O sangue foi coletado diretamente da jugular dos animais em frascos de vidro, tipo penicilina, devidamente identificados com o número do animal correspondente.

Após a retração do coágulo, as amostras séricas foram submetidas à prova de sôro aglutinação rápida em placa de Huddleson para verificação de aglutininas antibrucélicas, de acordo com as recomendações da OPAS (1968), utilizando-se antígenos **B. abortos**, amostra 1119/3.

Para cada amostra utilizou-se as diluições de 1:25, 1:50, 1:100 e 1:200. Como positividade, foi adotado o título de 1:100 na forma indicada pela Portaria nº 23/76 do Ministério da Agricultura.

Das 2.670 amostras examinadas, eram procedentes de animais não vacinados variando a idade entre dois a dez anos.

RESULTADOS

Foram investigadas aglutininas antibrucela em sôro sangüíneo de 2.670 fêmeas da espécie bubalino, nos anos de 1987, 1988 e 1989, encontrando-se, no total, 5,46% de positividade, conforme se vê na Tabela 1.

Tabela 1 - Investigação sorológica para Brucelose bubalino, distribuída segundo número de propriedade, sôros testados e percentuais de positividade.

ANO	NÚMERO DE MUNICÍPIOS	Nº DE PROPRIEDADES	Nº DE SOROS TESTADOS	% DE REAGENTES	
				POSITIVO	SUSPEITO
1987	9	12	377	8,7	8,2
1988	18	34	1.666	4,1	5,9
1989	1	95	627	7,0	5,6
TOTAL	28	141	2.670	5,5	6,1

Tabela 2 - Investigação sorológica para brucelose bubalino, distribuída segundo propriedades e municípios trabalhados e com animais reagentes.

ANO	NÚMERO DE MUNICÍPIOS	Nº DE PROPRIEDADES	Nº DE SOROS TESTADOS	(1)		(2)	
				POSITIVO	SUSPEITO	POSITIVO	SUSPEITO
1987 ...	9	12	377	75,0	16,6	88,8	-
1988 ...	18	34	1.666	58,8	26,5	55,5	27,7
1989 ...	1	95	627	27,4	16,8	(3)	-
TOTAL	28	141	2.670	39,0	19,1	67,8	17,8

(1) % propriedades com animais reagentes
(2) % municípios com animais reagentes
(3) não foi calculado, por ter apenas trabalhado em um município.

DISCUSSÃO E CONCLUSÃO

Considerando que as matrizes representam a continuidade do rebanho, poder-se-á considerar como elevado o percentual encontrado, no total, de 5,5% (Tabela 1), ressaltando-se para o ano de 1987, foram mais elevados os percentuais de positivos (8,7%) e suspeitos (8,2%), conforme Tabela 3.

Tabela 3 - Investigação sorológica para brucelose bubalino distribuída segundo municípios e número de propriedades trabalhadas - 1987

MUNICÍPIOS	NÚMERO DE PROPRIEDADES	NÚMERO DE SOROS TESTADOS	% DE ANIMAIS POSITIVOS	REAGENTES SUSPEITOS
Feira de Santana	3	43	2,3	16,3
Ipiaú	1	11	45,4	18,2
Eunápolis	1	15	13,3	13,3
Itapetinga	2	104	14,4	4,8
Itabuna	1	72	1,4	12,5
Itororó	1	40	5,0	-
Floresta Azul	1	30	6,7	20,0
Caatiba	1	40	12,5	-
Macaraní	1	22	-	-
TOTAL	12	377	8,7	8,2

Os achados na presente investigação, são inferiores aos 40,9% encontrados por (14), em São Paulo, e aos 17,3% encontrados por (4) em Goiás, usando todas as investigações aludidas, a mesma metodologia. (15) com outra metodologia, o Card Test, em São Paulo, encontraram resultados semelhantes 5,6% e 4,3% para sôro aglutinação rápida.

Percentual inferior ainda, ao encontrado para o rebanho bovino de corte e leite no país (17) no Continente Europeu (2) em bubalino.

Dos 28 municípios trabalhados nos três anos que abrangeram a investigação (Tabelas 3, 4 e 5) em cinco deles não foram constatados animais reagentes; este dado, entretanto, não permite inferência, face ao pequeno número de amostras coletadas.

Tabela 4 - Investigação sorológica para brucelose bubalino distribuída segundo municípios e número de propriedades trabalhadas - 1988

MUNICÍPIOS	NÚMERO DE PROPRIEDADES	NÚMERO DE SOROS TESTADOS	% DE ANIMAIS REAGENTES	
			POSITIVOS	SUSPEITOS
Canavieiras	1	83	-	-
Recôncavo Baiano	6	449	3,3	5,3
Prado	1	79	-	3,8
Ibicuí	1	102	14,7	6,9
Ubaíra	2	107	0,9	-
Itambé	1	30	3,3	3,3
Alcobaça	1	16	-	-
Ipiaú	2	26	-	26,9
Itagí	2	74	-	-
Gongogí	3	124	6,4	2,4
Feira de Santana	1	45	-	-
Eunápolis	1	29	3,4	-
Maiquinique	2	107	6,5	-
Itapetinga	4	195	6,7	9,2
Macaraní	3	64	1,6	1,6
Encruzilhada	1	38	5,3	5,3
Itarantim	1	61	1,6	6,5

Itororó	1	37	-	-
TOTAL	34	1.666	4,1	5,9

Pode-se observar ainda que alguns animais considerados negativos (não vacinados) reagiram na diluição de 1:25, evidenciando, assim, que nos bubalinos também pode ocorrer com freqüência este tipo de reação, sem o auxílio da vacina.

Tabela 5 - Investigação para brucelose bubalino distribuída segundo municípios e número de propriedades trabalhadas - 1989

MUNICÍPIOS	NÚMERO DE PROPRIEDADES	NÚMERO DE SOROS TESTADOS	% DE ANIMAIS REAGENTES POSITIVOS	% DE ANIMAIS REAGENTES SUSPEITOS
Cravieiras	-	-	-	-
Recôncavo Baiano	-	-	-	-
Prado	-	-	-	-
Ibirapuã	-	-	-	-
Ubaíra	-	-	-	-
Itambé	-	-	-	-
Alcobaça	-	-	-	-
Ipiaú	-	-	-	-
Itagí	-	-	-	-
Gongogí	95	627	7,0	5,6
Feira de Santana	-	-	-	-
Eunápolis	-	-	-	-
Maiquinique	-	-	-	-
Itapetinga	-	-	-	-
Macaraní	-	-	-	-
Encruzilhada	-	-	-	-
Itarantim	-	-	-	-
Itororó	-	-	-	-
TOTAL	95	627	7,0	5,6

Os sintomas clínicos observados nos animais que reagiram positivamente ao teste de sôro aglutinação rápida para brucelose estão descritas na Tabela 6. Nota-se que entre os bubalinos brucélicos, a ocorrência de aborto (5,5%) e de retenção de placenta (4,1%) não são freqüentes, coincidindo com as afirmações de (12, 5) respectivamente. Diagnosticou-se ainda nestes animais quatro casos de endometrite mucopurulenta. Conforme descrição de (10) é um caso de higroma articular, igualmente citado por (11).

Tabela 6 - Número e percentagem de casos clínicos apresentados pelos 145 animais que reagiram positivamente ao teste de sôro aglutinação rápida para brucelose

CASOS CLÍNICOS NÚMERO	CASOS CLÍNICOS %	SINTOMAS APRESENTADOS
8	5,5	- Aborto
6	4,1	- Retenção de placenta
4	2,7	- Endometrite mucopurulenta
1	0,6	- Higroma articular
2	1,3	- Morte neonatos

As investigações realizadas na Bahia, a prevalência de brucelose em búfalos foi num total de 5,5% permitem aos autores a sugestão que sejan adotadas medidas mais enérgicas de contrôle para essa antropozoonose, em búfalos, o que certamente reduzirá a taxa de infecção em humanos e perda econômica.

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RESUMO

Objetivando-se estudar a epizootiologia da Brucelose em fêmeas da espécie bubalina, foram analisadas, através da prova de soro aglutinação rápida em placa de Huddleson, para detectar aglutininas antibrucélicas, 2.670 amostras séricas de búfalas, de diversas faixas etárias, em 141 propriedades de 28 municípios que compõe diversas regiões da Bahia, nos anos de 1987, 1988 e 1989. A prevalência de animais positivos encontrado, foi no total de 5,36% de positividade. Para cada amostra utilizou-se as diluições de 1:25, 1:50, 1:100 e 1:200. Como positividade, foi adotado o título de 1:100, as amostras examinadas, eram procedentes de animais não vacinados. Os sintomas clínicos observados nos animais brucélicos foram aborto (8 casos), retenção de placenta (6 casos), endometrite muco purulenta (4 casos), higroma articular (1 caso) e mortalidade neonatal (2 casos).

SUMMARY

Study objective in epizootiology from the brucellosis in water buffalos female, agglutination teste. Antibrucella aglutinins wese faund in 5,46% of 2.670 cows investigated at 141 farms in 28 diferent countris of Bahia dusing the years 1987, 1988 and 1989. For each sample from animals without vaccine, hod been used the dilution of 1:25, 1:50, 1:100 e 1:200. Inthe samples, was adapted the title of 1:100 for positivenes. The clinical symptoms diagnosed in buffaloes cows with brucellosis in cluded abortion (8 cases), retained placenta (6 cases), mucupurulent endometritis (4 cases), articular higroma (1 case) and neonate mortality (2 cases).

RESUMÊN

El objetivo de estudiar la epizotiologia de la Brucelôsis en hembras de la espêcie Bubalina fueron analizadas, a través de la prova de suero-aglutinación rápida en placa de Huddleson, para detectar aglutininas anti-brucélicas, 2670 muestras de suero de búfalos de diversas edades, en 141 propiedades de 28 municipios que componen diversas regiones de la Bahia, en los anos de 1987, 1988 e 1989. La predominancia de animales positivos encontrados fue de 5,36% de positividad. En cada muestra fue utilizada las diluciones de 1:25, 1:50, 1:100 y 1:200. Como positividad fue adptado el titulo de 1:100, las muestras examinadas fueron procedentes de animales no vacunados. Los síntomas clínicos observados en los animales brucélicos fueron aborto (ocho casos) retención de la placenta (seis casos) endometritis muco purulenta (quatro casos) higroma articular (un caso) y muerte neonatal (dos casos).

A STUDY ON MAJOR ORGAN DEVELOPMENT WITH RESPECT TO FETAL CROWN-RUMP LENGTH OF COWS

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INTRODUCTION

Growth curves plotted on body weight and crown-rump length (CRL) against pregnancy progress in bovine fetuses indicate their growth rates to peak around pregnancy day (PD) 230. Moreover, withers height (WH) and CRL of bovine fetuses show a highly enhanced growth rate at pregnancy month (PM) 5 and 7. Further, WH has been indicated to grow at a higher rate than that of CRL initially, but the reverse occurs as the fetal stage terminates.

Our present investigation attempted to measure fetal body parameters such as body weight, CRL, WH, chest-girth, head-length against time, and the changes were plotted and expressed in a growth curve by using a polynomial regression equation. The above parmeters in relation to strains, sexes, daily increase rates of body weight and correlation coefficients were compared.

MATERIALS and METHODS

A total of 286 fetuses from pregnant cows (136 Hostein-Freizein and 150 Japanese Black cows) were isolated at Shibura Abattoir, Tokyo. Fetal parameters such as body-weight, CRL, WH, chest-girth and head-length were measured. CRL was defined as the length from the tip of the head to the tip of the rump, WH as the linear distance to the tip of hoof, chest-girth as the circumferential distance of the the narrowest region of the breast (immediately posterior to scaple) and head-length was the distance from tip of the head to tip of the nare.

Growth pattern of external morphological parameters such as body weight, CRL, WH, chest-girth and head-length were plotted according to analysis on the above data with a polynomial regression equation derived from an application statistical verification program package (BMD/SPSS) of a supercomputer (M-680H) installed at Tokyo University. If the polynomial regression model was expressed as $Y = \alpha + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 + \beta_k X^r + \varepsilon$, where Y is the dependent variable or the vector of changes in proportional values of the dependent variable (population) between times t and t+r; X is the vector of changes in proportional values of the independent (symptomatic) variables; the β's are the regression coefficient; α is a constant; and ε is a vector of stochastic errors (A). In this case, the maximum regression variate was 4 in this polynomial regression model: by performing regression on the observed parameters with the use of the Table of Residual, the plot was predicted with the observed values and sum of square. Regression distribution was confirmed with the contribution rate F value.

On the correlation of CRL against fetal age (in pregnancy days based on the day of insemination with reference to previously published results (1) in Fig 1), CRL and FA were taken as independent and dependent variables, respectively. Based on the polynomial regression model, a bivariate regression equation where $Y = 31.0099 + 3.72606X - 0.01333X^2$ was derived. Using this binomial regression equation, the estimated FA was derived from the CRL. This estimated FA was taken as the index of gestation changes. For comparative studies on the in-

the index of gestation changes. For comparative studies on the influencing factors such as strains and sexes on fetal growth, variance analysis was employed, where FA was expressed in months (this was achieved by dividing the FA in days by 30). Further, to verify any daily increases in body weight gain and CRL, PD was substituted in the defined binomial regression equation.

RESULTS

1) Relationship between FA and fetal pararmeters

Crown-rump length (CRL): The relationship between CRL and estimated FA shown in Table 1 for fetuses of 150 Japanese Black and 136 dairy cows was expressed as a binomial regression equation: $Y = -3.31774 + 0.13953X + 0.00083X^2$, where the constant was -3.31774, the respective regression coefficients for β_1 and β_2 were 0.13953 and 0.00083. Standard errors (B) derived from the regression coefficients B1 and B2 of the equation were 0.00434 and 0.00001, respectively. Variance analysis that accompanied the regression analysis indicated the F value as 9509.6250. Residual, the sum of square, was 1.00279. The contribution rate of the regression analysis was appropriated as R^2=99.94. This finding was distinctly clear even when viewed from the derived binomial regression equation. CRL increases were observed even at the early period of FA.

Body weight: This parameter of 37 Japanese Black and 61 dairy cows was measured. Relationships between body weight and FA for the Japanese Black and dairy cows were expressed by the following binomial regression equations: $Y=12.92632-0.23564X+0.00198X^2$ (where R^2=92.40) and $Y = 16.74565 - 0.31815X + 0.00142X^2$ (where R^2=95.28), respectively. From the regression thus derived, the daily body weight increase during the early fetal stage was low, made gains eventually during the mid-fetal stage to peak suddenly around fetal age day (FAD) 180.

Withers height (WH): This parameter in 133 Japanese Black and 76 dairy cows was measured. Based on these measured values, relationships of WH with FA for the Japanese Black and dairy cows were derived from the polynomial regression model, and expressed as $Y = 2.89500 - 0.01173X + 0.00103X^2$ (R^2=96.10) and $Y= - 0.61913 + 0.02078X + 0.00089X^2$ (R^2=99.04), respectively.

In our comparartive studies on WH-CRL relationships in 133 Japanese Black and 76 dairy cows, WH increased proportionally with respect to CRL gains in a linear manner with WH values concentrated along the linear regresssion plot closely. WH-CRL relationships for the Japanese Black and dairy cows were expressed as $Y = -2.40763 + 0.98837X - 0.01997X^2 + 0.00044X^3$ and $Y = -0.16528 + 0.59719X - 0.00162X^2 + 0.00010X^3$, respectively.

Chest-girth: This parameter in 147 Japanese Black and 106 dairy cows was measured. By substituting these values in the polynomial regression model, relationships of the chest-girth parameter with FA for the Japanese Black and dairy cows were derived as $Y = 0.31616 + 0.07945X + 0.00066X$ (R^2=96.02) and $Y = - 0.51351+0.07287X + 0.00073X^2$ (R^2=0.986), respectively.

In our comparative studies on girth-CRL relationships between 147 Japanese Black and 106 dairy cows, the chest-girth varied with respect to CRL changes in a similar linear manner as illustrated for WH-CRL relationship, depicted above with WH values concentrated along the linear regression equation. Girth-CRL relationships for the Japanese Black and dairy cows were expressed as $Y = - 1.11961 + 0.92483X - 0.00842X^2 + 0.00015X^3$ and $Y = 1.20239 + 0.64322X + 0.00067X^2 + 0.00004X^3$, respectively.

Head-length: This parameter in 123 Japanese Black and 103 dairy

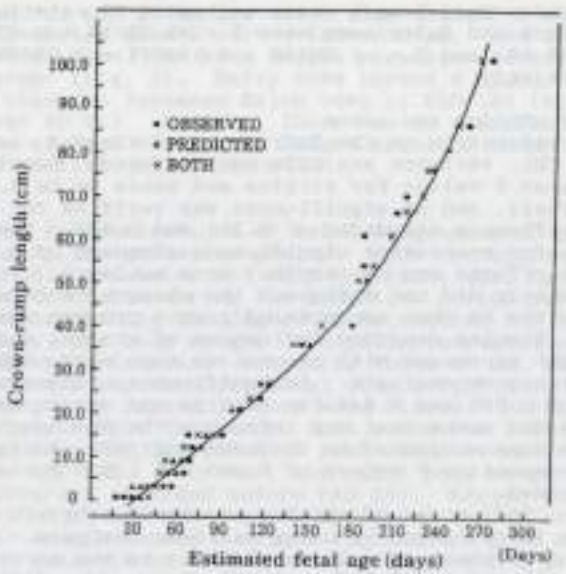

Fig. 1 Standard plot of fetal crown-rump length against track-recorded fetal age (in days), and the relationship between these 2 parameters was expressed as $Y=31.0089+3.7260x-0.0133x^2$. Estimated fetal age was therefore derived from this multiple regression equation.

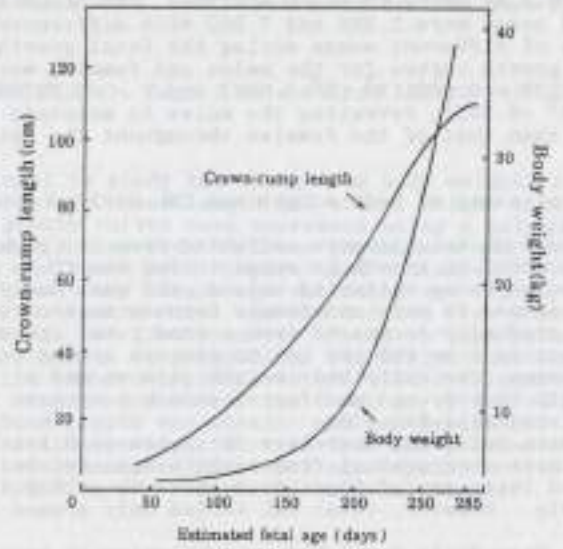

Fig. 2 Changes the body weight and Crown-rump length against estimated fetal age in combined Holstein with Japanese breeds.

cows was measured. Head-length vesus estimated FA relationships for the Japagese Black and dairy cows were $Y = -2.25015 + 0.077381X + 0.00006X^2$ (R^2=88.88) and $Y = -1.36108 + 0.03908X + 0.00009X^2$ (R^2 =94.22), respectively.

2) Influence of strains and sexes

CRL: For verification on the influences of strains, sexes and FA (in months) on CRL, variance analysis was employed. Results of analysis indicated F values for strains and sexes to be 1.570 and 0.232, respectively, and no significance was verified for their respective significance standadrd of 0.211 and 0.630. However, significant values for respective significance standard of 0.001 and 0.001 for major effects and FA (months) were achieved. Thus, strains and sexes definitely did not influence the changes on fetal growth rate so long as the FA that accompanied growth process was concerned.

Body weight: Results depicted influences of strains and sexes on daily body weight increases with respect to time were obtained by employing the variance analysis. Insignificance standards for respective F values of 3.595 and 0.844 for strains and sexes, were obtained.

WH: Strains and sexes that may influence the WH changes with respect to time were subjected to variance analysis. Insignificance standards for respective F values of 0.007 and 1.223 for strains and sexes were obtained.

Chest-girth: Influences of strains and sexes on girth changes against FA were investigated by using variance analysis. The respective significant F values ($p<0.05$) applicable in the strain and sex factors were 4.469 and 1.233, respectively. As such, differences in chest-girth that appeared during the process of fetal growth were subjected to influences of strains and sexes. The growth curves for male and female were $Y = -0.72325 + 0.09079X + 0.00063X^2$ and $Y = 0.73577 + 0.05987X + 0.00076X^2$ where differences in the constant were depicted.

Head-length: On investigating the influences of strains and sexes on the head-length by using variance analysis, the respective F values for strains and sexes were 1.386 and 7.000 with differences appeared in head-lengths of different sexes during the fetal growth process. The respective growth curves for the males and females were $Y = -0.96762 + 0.0552X + 0.00011X^2$ (R^2=0.904) and $Y = -2.73783 + 0.08242X + 0.00003X^2$ (R^2 =0.893), revealing the males to maintain a head-length greater than that of the females throughout the fetal growth period.

3) Mean daily increases of body weight and CRL derived from the regression model

Fetal CRL and body weights were estimated from the growth polynomial regression model on growth by substituting the PD in the equation. Based on these estimated values, the mean daily CRL and body weight increases in male and female fetuses were derived. The mean daily CRL gradually increased from around fetal age day (FAD) 50 to suddenly elevated from FAD 150 but to plateau around FAD 250. Against CRL changes, the daily body weight gain showed slight increases until FAD 50-100, and manifested sudden increases (after FAD 150) that persisted till FAD 250.

CRL: On the mean daily CRL increases for Japanese Black and dairy cows, the increases were gradual from early pregnancy period. This parameter showed increases of about 2, 3 and 4 mm at FAD 150, 200 and 250, respectively. However, fetal CRL varied only around 4 mm after FAD 250.

Body weight: Mean daily fetal body weight gains in both Japanese Black and dairy cows showed rapid increases until parturition. However, this parameter showed gains that were consistently lower than

CRL increases for any given gestation time until FAD 250, a point where both parameter growth curves intersected. And from this intersection point of time, the body weight parameter reversed to a higher increase thereof (Fig. 2). Dairy cows showed a higher daily body weight gain than the Japanese Balck cows at FAD 160 (where CRL measured about 40 cm). However, there was no significant difference when the data of mean daily body weight gains of these 2 strains were subjected to statistical verification.

DISCUSSION

It is vital for evaluations of fetal developmental growth to be performed during the gestation period. From conception to parturition, this parameter not only expresses well the developmental conditions of the fetus during the gestation period but also serves as an index in interpreting the degree of maturation for the fetus. In our experiments, the body weight especially increased suddenly from FAD 150 to 220 with increases exceeding those of CRL around FAD 250 when growth curves representing fetal body weight and CRL changes during the pregnancy period in cows were viewed. Particularly on the daily fetal body weight increases, this parameter indicated a growth rate increase from FAD 220-285 with peak value exceeding 300g/day registered on FAD 285. Moreover, the daily body weight increase were low, below 100g/day recorded before FAD 250. Beside this parameter, growth curves of WH and chest-girth indicated a similar trend. However, head-length showed a slight tendency to grow at a lower rate. From variance analysis on such findings, there was a difference in the growth pattern manifested by dairy and Japanese Black cows. Further, a difference in chest-girth and head-length growth rate was displayed between the sexes.

From these findings, our results on fetal CRL increases coincide well with previous documentation, but as for the body weight parameter where prominent increases were recorded even after FAD 250 in dairy and Japanese Black cows, our results differ from past findings in Jersey cows.

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SUMMARY

In an attempt to study the changes in body weight, crown-rump length, chest-girth, head-length and wither height with respect to time, their growth curves were expressed using a polynomial regression model where daily increases of these parameters and their correlations with strains and sexes were compared and evaluated. From previous findings on crown-rump length against fetal age (in days) relationship, estimated fetal age was derived from a polynomial regression model, and growth curves of the various parameters were expressed as binomial or polynominal regression equations. On comparison made in the growth patterns of fetal development with reference to strains and sexes, the chest-girth was strain-dependent whereas the head-length was strain- and sex-dependent. Daily increases in fetal body weight derived from the polynomial regression model indicated increases from fetal age day 220 to 285 with the peak value exceeding 300g/day registered on fetal day 285.

ESTROUS SYNCHRONIZATION IN BOS INDICUS CATTLE WITH A PROSTAGLANDIN ANALOG (LUPROSTIOL) USING A REDUCED DOSE VIA INTRAVULVOSUBMUCOSE.

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INTRODUCTION

In Costa Rica 90% of the beef cattle have a zebu breed component of 50 to 75%. Nation wide the pregnancy rate in Bos Indicus cattle is estimated to be 50% and the interval between parturitions around 24 months. (4) In order to improve these indexes, better management systems have to be applied. One of the basic requirements to obtain good beef production is a continuous effort to inprove the genetics. The shortest way to obtain this is having a greater number of pregnancies through artificial insemination, using sires that have been proved to be genetically superior. Estrous synchronisation is a great tool that could be used to establish an intensive artificial insemination progran. This would allow the insemination of more cows in a relative short period of time and therefore reduce the management and personnel cost. Prostaglandins have been used as a heat synchronization drug, in different doses an routes of administration: intramuscular (2,3) intrauterine (9) subcutaneous (8) and intravulvosubmucous (1,7). In field trails the administration of reduced doses of PGF2 alpha then salt into uterine horn ipsilateral to the ovary bearing a mature Corpus Luteum was very effective even when the dosage as reduced to 1/25 of the luteolytic dose (9). Local ipsilateral transport of prostaglandins from the uterus to the corresponding ovary has been proposed as the pathway of natural luteolysis during late diestrous in sheep. A venoarterial pathway has been proposed by Ginther (5) while Heap et al (6) demostrated a combination of vascular and lymphatic pathway. In both cases a close anatomical relationship between uterine drainage and arterial supply of the ovary have been demonstrated. In 1982 Ono et al (7) used the intravulvosubmucous route in Bos taurus cows with good results. More recently, Chauhan et al (1) using the IVSM reported that dose reduction was effective down to 1/4 of the luteolytic intramuscular dose.
The present experiment was designed to study the luteolytic effect of prostaglandins in zebu cows using the intravulvosubmucous route.

MATERIALS AND METHODS

Animals were located in a farm at the dry Pacific area of Costa Rica, between 0 and 20 ms above sea level, ambient temperature range of 23-33° C and annual rain fall of 309 m.m.
From the artificial insemination beeding herd of 307 cows, 50 fenales bearing a mature corpus luteum (CL) on either ovary, selected by rectal examination were assigned at random to one of the following treatments:
GROUP A (N:16) Injected with complete luteolytic dose (15 mg) of a prostaglandin analog, Luprostiol ("Prosolvin", Intervet International BV, Boxmeer, Holland), via intramuscular.
GROUP B (N:17) Injected with 1/2 dose (7,5 mg) of Luprostiol via intravulvosubmucousus (IVSM) ipsilateral to the ovary bearing the CL.
GROUP C (N:17) Injected with 1/4 dose (3,75 mg) of Luprostiol via IVSM ipsilateral to the ovary with the CL.
Heat detection was performed continuesly using teaser bulls and direct observation particularly during early morning and late evening periods.

Cows were artificially inseminated (AI) at the end of first estrous post treatment.
Blood samples were colleted at time of prostaglandin injection, at time of artificial insemination (AI) or day 5 after injection if estrous was not detected. Plasma was inmediatly separated, trasported at 5° and stored at -20°C for posterior progesterone (P4) radioinmunoassay.
Pregnancy diagnosis was done by rectal examination around 40 days post-service using the methodology described by Zemjanis (11).

RESULTS

Table 1 compiles the results for P4 values before and after treatment, estrous and fertility data. Mean P4 levels (ng/ml) at time of prostaglandin injection were 4.1 (±2.73), 4.6 (±3.23) and 3.5 (±2.24) for groups A,B and C respectively. All but 5 cows had progesterone levels greater than 1 ng/ml which gave a correlation between clinical diagnosis of a mature CL and progesterone levels above 1 ng/ml of 90%.
Diagnosis of-palpable luteal structures in the ovaries of zebu cows is less precise compared with Bos taurus. In our laboratory we have adapted the criteria for CL diagnosis recomended by Zemjanis (11) and we put more emphasys in the increase of size and change of consistency of the ovary bearing a corpus luteum as compared with the opposite ovary (10). At time of AI or day 5 post treatment progesterone levels for same group were: 0.22 (±0.20), 0.17 (±0.18) and 0.37 (±0.54). The analysis of individual data showed that luteolysis occurred in 96% of cows treated. These results clearly show that prostaglandin injected via IVSM caused regression of the corpus luteum present in the ovary ipsilateral to site of injection. The way prostaglandin reaches the ovarian circulation when injected into the vulva has not been demostrated.

In light of recent studies (6) showing lymphatic pathways from the uterus to the ovary, the possibility of lymphatic transport from the vulva to the ovary should be considered.

Heat detection rate (%) was 75, 94 and 84 for groups A, B and C.respectively. A total of 42 out of 50 cows were detected in heat as they showed good mounting behavior and were monitored by an experienced technician and reliable personnel.

Interval from treatment to initiation of heat behaviour was: Group A: 64,50 (±16,48), group B:68,30 (±15.84) and group C: 77,73 (±17,64). Frequency distribution of cows in heat is shown in figure 1. Duration of heat manifestations for groups A, B and C respectively was (hrs): 13, 17, 11.20 and 11,56 and first service conception rate (%) for same groups was: 50,62 and 85. Cost/cow injected with Luprostiol was ($)5.50 2.75 and 1.37 for complete, half and one fourth dose repectively.

CONCLUSIONS

We concluded that the intravulvocubmucous route of injecting prostaglandins in reduced doses was effective in causing luteolysis with normal fertility in zebu cows under the conditions of present study.

ACKNOWLEDGEMENTS:

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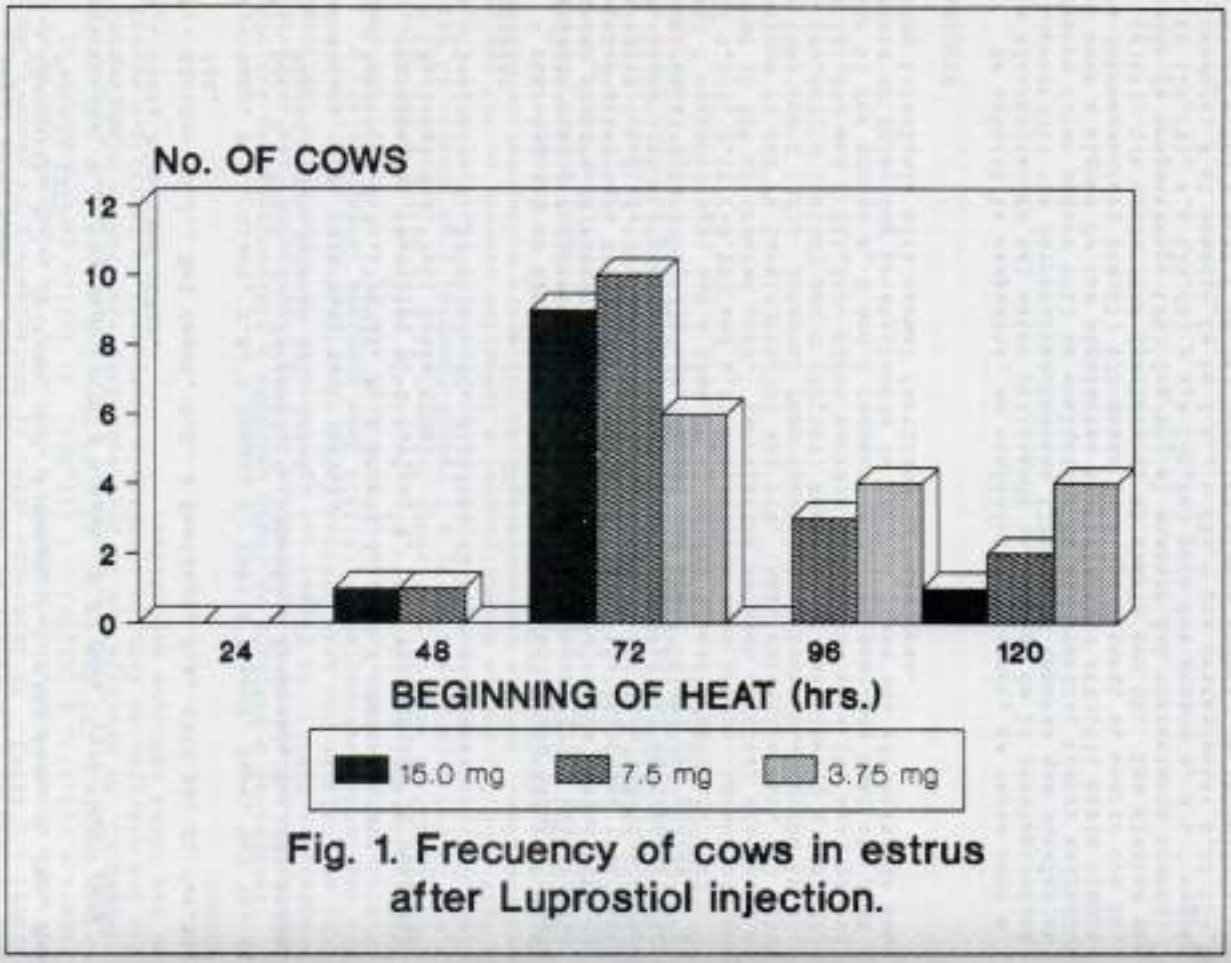

Fig. 1. Frecuency of cows in estrus after Luprostiol injection.

TABLE 1. Plasma progesterone levels, Heat detection rate, Interval from treatment to heat observation and first service conception rate for the 3 experimental groups.

Groups	Mean Progesterone Levels (ng/ml) (SD)		Heat detection rate	Interval from treatment to initiation of heat	First Service Conception Rate
	Time of Treatment	Time of A.I. or day 5 post-treatment	N (%)	(hrs) (SD)	N (%)
A N:16	4,1(± 2,73)	0,22(± 0,20)	12/16 (75)	64,58(± 16,48)	6/16 (50)
B N:17	4,6(± 2,73)	0,22(± 0,20)	16/17 (94)	68,30(± 15,84)	10/17 (62)
C N:17	3,6(± 2,24)	0,37(± 0,54)	14/17 (84)	77,73(± 17,64)	12/14 (85)

Group A: Complete dose (15 mg) of Luprostiol i.m.
Group B: 1/2 dose (7.5 mg) of Luprostiol IVSM
Group C: 1/4 dose (3.75 mg) of Luprostiol IVSM

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SUMMARY

The response of zebu cows to estrous synchronization using a prostaglandin analog (Luprostiol) via intravulvosubmucous (IVSM) was studied under field conditions. 50 females with a mature corpus luteum (CL) on either ovary were assigned at random to one of the following treatments: A (N:16) complete intramuscular dose; B (N:17) 1/2 dose IVSM)ipsilateral to ovary with CL. Mean progesterone levels (P4)(ng/ml) at time of treatment were: 4,10 (±2,73), 4,6 (±3,23) and 3,6 (±2,24) for groups A, B, and C respectively; and a time of AI or day 5 post treatment: 0,22 (± 0,20), 0,17 (±0,18) and a,37 (±0,54) for same groups. Luteolysis occurred in 96% of treated cows. Correlation between clinical diagnosis of mature CL and P4 levels >1ng/ml was 90%. Heat detection rate (%) A(75%) B (94) and C (82). Interval from treatment to heat observation (hrs): A (64,58), B (68,30) and C (77,73). Duration of heat (hrs): A (13,17), B (11,20) and C (11,56). First servive conception rate (%) was: 50, 62 and 85 for groups A, B and C respectively. We conclude that the IVSM route of injecting prostaglandin in reduced doses was effective in causing luteolysis with normal fertility in zebu cows.

RESUMEN

Se estudió la respuesta, en condiciones de campo, de vacas cebú a la sincronización del estro utilizando un análogo de la prostaglandina (Luprostiol) vía intravulvosubmucosa (IVSM). 50 hembras que poseían un cuerpo luteo maduro (CL) en cualquiera de sus ovarios, fueron asignados al azar a alguno de los siguientes tratamientos: A(N:16): dosis completa intramuscular; B(N:17) 1/2 dosis IVSM ipsilateral al ovario con CL; C (N:17): 1/4 dosis IVSM ipsilateral al ovario con CL. Los niveles promedio de progesterona (P4) (ng/ml) al momento del tratamiento fueron: 4,10 (±2,73), 4,6 (±3,23) y 3,6 (±2,24) para los grupos A, B y C respectivamente; y al momento de la I.A., o día 5 pos tratamiento: 0,22 (±0,20 0,17 (± 0,18) y 0,37 (±0,54) para los mismos grupos. La luteolisis ocurrió en el 96% de las vacas tratadas.

Existió una correlación del 90% entre el diagnóstico clínico de CL y los niveles de P4 >1ng/ml. La tasa de detección de celo fue: A(75%), B(94%) y C (82%). El intérvalo entre el tratamiento y la observación del celo (hrs): A(64,58), B(68,30) y C(77,73). La duración del estro (hrs): A (13,17), B (11,20) y C (11,56). El índice de concepción al primer servicio (%) fue de 50, 62 y 85 para los grupos A, B y C respectivamente. Se concluye que la prostaglandina vía IVSM en dosis reducidas fue efectiva en inducir luteólisis acompañada de fertilidad normal, en las vacas cebuinas estudiadas.

RESUMO

Estudou-se a resposta em vacas zebuínas a sincronização do cio utilizando um análogo da prostaglandina F2 Alfa (Luprostiol) via intravulvosubmucosa (IVSM). 50 fêmeas que apresentavan um corpo luteo funcional (CL) em qualquer dos seus ovários foram distribuidos ao acaso em algun dos seguintes tratamentos: A (N:16): dose completa intramuscular; B (N:17): 1/2 dose IVSM ipsilateral ao ovário com CL e C (N:17): 1/4 dose IVSM ipsilateral ao ovário com CL. Os niveis medios de progesterona (P4) (pg/ml) na hora do tratamento foram: 4,10 (±2,73), 4,6 (±3,23) e 3,4 (±2,24) para grupos A, B, e C respetivamente; e no momento da inseminação artificial ou no dia 5 pos tratamento: 0,22 (±0,20), 0,17 (±0,18 e 0,37 (±0,54) pra os mesmos grupos. A luteolise ocorreu no 96% das vacas tratadas. Existiu uma correlação do 90% entre o diagnóstico clínico do CL e os niveis de P4 >1ng/ml. A taxa de detegão do cio foi (%): A (75), B (94) e C (82). O intervalo entre o tratamento e a observação do cio (hrs): A (64,68), B (68,30) e C (73,73). A duração do periodo de estro (hrs): A (13,17), B (11,20) e C (11,56). O índice de concepção ao primeiro serviço (%) foi de 50, 62, e 85 pra os grupos A, B e C respectivamente. Conclui-se que a prostaglandina vía IVSM en dose reduzida é efectiva em induzir luteolise acompanhada de fertilidade normal nas vacas zebuínas estudadas.

THE INFLUENCE OF LOW BLOOD GLUCOSE VALUES ON THE FERTILITY OF DAIRY COWS

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INTRODUCTION

The high producing cow reaches her maximal feed consumption 6-8 weeks after calving. In this situation a negative energy balance is initiated with considerable weight loss as a consequence (1). The condition seems to interfere with the cow's ability to get pregnant in reasonable time (2).

There is, however, limited knowledge on how the metabolic state at the time of first insemination influences the pregnancy rate.

The aim with the present study, was to increase our knowledge about the relationships between blood glucose values at first insemination and fertility of the cow.

MATERIAL AND METHODS

352 cows from 18 farms participated in the investigation. The Swedish Red and White Breed constituted 51 per cent of the material, the remaining cows (49 per cent) represented the Swedish Friesian Breed. The cows yielded on an average 6814 kg fat corrected milk.

Milk and jugular blood samples were collected four times, 21-28, 35 and 42-29 days post partum and finally at first insemination. The samples were collected during daytime from about 9.00 am to 15.00 pm, and centrifuged 4-8 hours later. Blood plasma was stored at -20°C until the analyses were performed.

Blood plasma was analysed for glucose and the milk for acetone and progesterone. A number of other variables describing the metabolic state were also determined but excluded from this presentation due to limited value.

The statistical analysis was made with general linear models of the Statistical Analysis System (SAS). The pregnancy rate at first insemination was evaluated with a model including herd, calving season, breed, lactation number class, plasma glucose at first insemination and interval calving to first insemination. Glucose at first insemination was evaluated with a model including herd, breed, calving season, lactation number class, milk acetone and plasma glucose in the fourth lactation week, milk acetone in week five and interval calving to first insemination.

RESULTS AND DISCUSSION

The total pregnancy rate at first insemination was 0.54, which is in level with Swedish official figures. It was significantly influenced by calving season ($p \leq 0.05$), lactation number class ($p \leq 0.05$) and plasma glucose at first insemination ($p \leq 0.05$). Least-square means for the four calving seasons February - April, May - July, August - October and November - January were 0.51, 0.62, 0.46 and 0.39, respectively. This means that the pregnancy rate was considerably higher after calving in the summer compared with the darker seasons. Least-square means for the three lactation number classes (lactation 1, lactations 2-3 and lactations 4 or higher) were 0.43, 0.43 and 0.60, respectively. The higher frequency in older cows is in accordance with for example Janson (3).

The coefficient of regression of plasma glucose at first insemination on pregnancy at first insemination was 0.14. This means that an increase of plasma glucose by 1 mmol/l increased the pregnancy rate by 14 per cent. This result can be compared with studies by King (4), who reported decreased fertility in cows with energy under-nourishment and/or ketosis.

Plasma glucose at first insemination was significantly influenced by herd ($p \leq 0.05$) and milk acetone in the fifth week of lactation ($p \leq 0.01$; coeff. of regr. = -0.18). The effect of plasma glucose at the fourth week of lactation was nearly significant ($p = 0.06$; coeff. of regr. = 0.11). This means that cows with low plasma glucose at first insemination often had hyperketonaemia and/or hypoglucaemia earlier in the lactation. Accordingly, it ought to be possible to increase the fertility of hyperketonaemic cows by giving them hyperglucaemic substances before first insemination.

The progesterone values were classified in three classes according to estrus, intermediate phase and luteal phase. Corresponding values were 0-4.9, 5.0-9.9 and ≥ 10.0 nmol/l, respectively.

The distribution of 325 progesterone values and the pregnancy rate are presented in Table 1.

TABLE 1. Distribution of progesterone values and pregnancy rates at first insemination.

	Progesterone nmol/l		
	0-4.9	5.0-9.9	≥10.0
Number of cows	294	21	10
Frequency, %	90.4	6.5	3.1
Pregnancy rate, %	52.4	71.4	70.0

The table also shows that 9.6 per cent of the cows were inseminated despite of progesterone values above 5.0 nmol/l. The pregnancy rate with cows between progesterone 5.0-9.9 was 71.4 per cent. Corresponding figures for progesterone above 10.0 was 70.0 per cent. It might be possible, that a combination of milk sampling and insemination in early proestrus and an exceptional sperm survival can explain these confusing results.

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SUMMARY

In a study of 352 Swedish cows, it was shown that low plasma glucose at first insemination is one reason for low pregnancy rate. It was also shown that milk acetone or plasma glucose determination in early lactation can predict plasma glucose at first insemination. Despite of high progesterone levels pregnancy rate was unexpectedly high in some cows. Possible reasons will be discussed.

RÉSUMÉ

Une recherche comprenant 352 vaches suédoises a montré qu'une plasma glucose basse à la première insémination est une raison d'un mauvais niveau de fertilité. On a aussi montré qu'une détermination de l'acétone de lait ou plasma glucose au début de la lactation peut prédire la plasma glucose à la première insémination. De dépit des niveaux de progesterone élevés, la gestation de quelques vaches était inopinément bonne. Des raisons possible sont discutées.

ZUSAMMENFASSUNG

Es wurde in einer Untersuchung von 352 schwedischen Kühen gezeigt, dass niedrige Plasma-Glukose bei erster Besamung einen Anlass zu schlechter Fruchtbarkeit ist. Es wurde auch gezeight, dass Bestimmung von Milchazeton oder Plasma-Glukose in früher Laktation die Plasma-Glukose bei erster Besamung voraussagen kann. Trotz hohen Progesteronstufen war die Fertilität einiger Kühe überraschend hoch. Mögliche Ursachen werden diskutiert.

RELACION ENTRE PALPACION RECTAL DE CUERPO LUTEO Y NIVELES DE PROGESTERONA EN VAQUILLONAS HOLANDO.

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INTRODUCCION

La condición reproductiva de la hembra bovina puede ser evaluada fundamentalmente en base a antecedentes de comportamiento sexual y mediante examen clínico-ginecológico (11). La palpación rectal de los ovarios es una técnica de enorme utilidad, que permite obtener muy valiosa información respecto a la presencia de estructuras relacionadas con la ciclicidad, gestación o procesos patológicos de la mas diversa etiología (1,5, 8). La detección de cuerpos lúteos, por ejemplo es de fundamental interés en los programas de sincronización de celos basados en la administración de compuestos de acción luteolítica (3,4,6).

En condiciones prácticas la palpación rectal de las estructuras ováricas posee limitaciones que determinan errores en el diagnóstico o en la interpretación del momento del ciclo reproductivo en que se encuentra la vaca (7,8,12).

La presente comunicación tiene por objetivo analizar la precisión de la palpación rectal de cuerpos lúteos en los ovarios en relación a su función endócrina, tomando como referencia las concentraciones de progesterona presentes en la leche al momento de efectuado el examen.

MATERIAL Y METODOS

Evaluación Ginecológica

El ensayo fue realizado en vaquillonas Holando de primera cría, no gestantes, que se encontraban entre 35 y 75 días postparto. Mediante palpación rectal minuciosa se incluyeron en la experiencia 110 vaquillonas que evidenciaron la presencia de al menos un cuerpo lúteo en sus ovarios.

Los cuerpos lúteos detectados fueron clasificados clínicamente en dos categorías: funcionales y no funcionales. Los primeros se caracterizaban por un buen desarrollo, consistencia firme y clara protrusión sobre la superficie ovárica. Se consideraron como cuerpos lúteos no funcionales a aquellos pequeños en tamaño y muy consistentes al tacto.

Cuando en un mismo animal se detectaban ambos tipos de cuerpos lúteos, se lo incluía en la categoría de los endócrinamente activos.

Dosificación de Progesterona

Al momento de efectuarse el examen ginecológico se extrajeron 10 ml de leche que era depositada en frascos herméticos y transportada al laboratorio sin conservadores. Las muestras fueron centrifugadas en refrigeración (6°C) a 250 g durante 20' y, luego de remover la materia grasa, congeladas a -24°C hasta su procesamiento.

La dosificación de progesterona se efectuó mediante RIA en fase sólida, utilizando como marcador I^{125}. Los kits fueron suministrados por el Laboratorio Seibersdorf, disponiéndose de una curva estándar de 0,79 a 25,2 ng/ml. Todas las muestras fueron procesadas por duplicado, y el promedio considerado como el valor real de la hormona. La concentración crítica para establecer la presencia de un cuerpo lúteo endócrinamente activo fue de 1 ng/ml, considerando niveles inferiores como no indicativos de actividad luteal.

Procesamiento de los Datos

La precisión en la estimación rectal o valor predictivo, así como la sensibilidad y especificidad de la técnica ginecológica, fueron establecidas según la fórmula descrita por Sprecher y col (1988). Las diferencias entre tipo de cuerpo lúteo en relación a niveles de progesterona se analizaron mediante la prueba de Chi-Cuadrado con una significación de $p < 0,05$.

RESULTADOS

En los 110 exámenes ginecológicos incluidos en el ensayo se diagnosticó la presencia de cuerpo lúteo funcional en 76 de las vaquillonas, y no funcional en las 34 restantes.

El valor predictivo global entre el diagnóstico ginecológico y el perfil hormonal fue del 77% (85/110). La estimación de la funcionalidad endócrina presentó importantes variaciones según el tipo de cuerpo, tal como se observa en la Tabla 1.

Tabla 1. Valor predicitvo de la palpación rectal de los cuerpos lúteos en relación al perfil de progesterona en leche.

Diagnóstico Rectal	n°	Progesterona		Valor Predictivo
		ing/ml	ing/ml	
C.L. Funcional	76	23	53	70 %
C.L. no Funcional	34	32	2	94 %
Total	110	55	55	77 %

En los cuerpos lúteos considerados funcionales existió un 30% de discordancia respecto a las concentraciones de progesterona, situación que se presentó tan solo en el 6% de los no funcionales. Estas diferencias fueron estadísticamente significativas a $p < 0,005$.

En razón de lo expuesto la especificidad de la palpación rectal fue baja para los cuerpos lúteos funcionales (58%), a pesar de que existió una elevada sensibilidad (96%). Una situación totalmente inversa se observó en el caso de cuerpos lúteos no funcionales, con alta especificidad y escasa sensibilidad.

DISCUSION

La precisión de la exploración rectal de las estructuras ováricas y su relación con la función endócrina, constituyen aspectos de enorme interés clínico. Los resultados obtenidos en el presente estudio concuerdan con la mayoría de los estudios sobre el tema. En tal sentido debe destacarse que el valor predictivo de la palpación rectal ha oscilado, según diferentes investigadores, entre el 71 y 79% (1,5,7,8,10,12).

Por tal motivo es de importancia enfatizar que si bien en algunas oportunidades se ha informado de aciertos superiores al 80% (6,11), en condiciones prácticas parecería difícil superar dicho umbral (7).

Las posibles causas de error que motivan los porcentajes observados pueden relacionarse con varios factores. Lo primero a analizar es la experiencia del técnico actuante, que evidentemente tiene una gran influencia sobre los resultados (4,12), pero que no puede ser considerada como una limitante intrínseca de la técnica. Ensayos en matadero han demostrado que las diferencias que se observan entre profesionales bien entrenados son de pequeña magnitud y carentes de significación (2).

Una segunda causa de error, no imputable a la perica del clínico, radica en la inexistencia de una relación directa entre presencia anatómica de un cuerpo lúteo y su funcionalidad endócrina (4,7). En tal sentido deberá tenerse presente que el cuerpo lúteo deja de segregar progesterona tres días antes de su regresión anatómica, situación imposible de identificar mediante exploración rectal (7). Ello implica que en el trascurso del ciclo estral existe un período de desfasaje entre anatomía y función de casi el 20%. Los errores que se presentan en estas circunstancias deberán ser atribuídos a la técnica y no a la falta de entrenamiento.

En el presente ensayo las divergencias de importancia derivaron de considerar como funcionales a cuerpos lúteos cuyo perfil hormonal evidenció como no funcionales. Este error se presentó en porcentajes superiores a los constatados en otras investigaciones, aunque en algunas de ellas también fue relativamente elevado: 24%(10) y 20%(1).

Respecto al valor predictivo en cuerpos lúteos considerados no funcionales se obtuvieron coincidencias muy elevadas, que en general superaron a las descritas por otros autores (1,7,8,12). Ello significaría que en la evaluación ginecológica serían muy escasos los casos de vacas en anestro erróneamente diagnosticadas como cíclicas.

Las observaciones precedentes permiten concluir que si bien la palpación rectal de los ovarios constituye una práctica de enorme interés ginecológico, pueden existir limitaciones de importancia al estimar la función endócrina. Estos errores podrán ser minimizados con entrenamiento, disponibilidad de registros reproductivos y, eventualmente, perfiles hormonales.

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RESUMEN

La precisión de la palpación rectal de los ovarios fue analizada en un ensayo efectuado en vaquillonas Holando de primera cría, no gestantes, que se encontraban entre 35 y 75 días postparto. Se incluyeron en

el estudio 110 vaquillonas que en el examen ginecológico evidenciaron al menos un cuerpo lúteo en sus ovarios. Los cuerpos lúteos fueron clasificados en dos grupos: funcionales y no funcionales. La precisión del diagnóstico clínico fue determinada por intermedio de la concentración de progesterona en leche, utilizando la técnica de RIA en fase sólida. El valor predictivo global de la palpación rectal fue de 77%(85/110). En el caso de cuerpos lúteos considerados no funcionales la precisión fue muy elevada (94%), mientras que en los funcionales disminuyó significativamente (70%). Los resultados obtenidos coinciden con los observados por la mayoría de los investigadores, estimándose que en condiciones prácticas es difícil obtener un valor predictivo global superior a 80%. Se concluye que es posible minimizar los errores en base a entrenamiento, historias reproductivas individuales, repetición de exámenes y, eventualmente, perfiles de progesterona.

RESUMO

A exatidão da palpação retal dos ovários foi pesquisada em novilhas de raça Holandesa de primeira cria, não gestantes, aos 35-75 dias após o parto. Foram incluidas no trabalho 110 novilhas as quais evidenciaram, ao menos, um corpo lúteo no seus ovários. Os corpos lúteos foram classificados em funcionais e não funcionais. A exatidão do diagnóstico clínico foi determinada através dos níveis de progesterona no leite segundo a técnica de RIA em fase sólida. O valor predictivo geral da palpacão retal foi 77%(85/110). Tratando-se de corpos lúteos não funcionais a exatidão foi de (94%), porem nos corpos lúteos funcionais houve uma diminuçao significativa (70%). Os resultados concordaram con aqueles obtidos pela maioria dos pesquisadores, considerando-se que sob condições da prática no campo é difícil atingir porcentagens de predição maiores que 80%. Concluíu-se que é possível reduzir erros através do treinamento, registros reprodutivos individuais, repetição dos exames ginecológicos e, eventualmente, dosagem da progesterona.

RESUME

La précision de la palpation rectal des ovaries fut analysé dans un essai effectué en génisses Holstein de premier élevage, non pleine, qui se trouvaient entre 35 et 75 jours pos-vêlage. On a inclu dans l'étude 110 génisses qui ont montré, lors de l'examen ginecologique, au moins un corps jaune dans leurs ovaries. Les corps jaunes furent classifiés cliniquement en deux categories: fonctionnels et non-fonctionnels. La précision du diagnostic fût determiné à travers la dosification de progesterone en lait par la technique de RIA en phase solide. La valeur de prediction de la palpation rectal fût de 77% (85/110). Dans le cas des corps jaunes considerés non fonctionnels la précision fut tres elevé (94%), tandis que dans les fonctionnels elle a diminué significativement (70%). Les résultats obtenues coincident avec aux observés par la plus grande partie des investigateurs, estimant que dans les conditions pratiques, il est difficile d'obtain une value predictive superieure a 80%. En conclusion il est possible minimiser les erreurs en base a entraînement, histories reproductives complètes, repetition d'examen et, eventuelment, profils de progesterone.

SUMMARY

A field trial was conducted to evaluate the accuracy of rectal palpation of ovaries in first calf Holstein heifers. One-hundred and ten lactating heifers with at least one corpus luteum in their ovaries were selected by rectal palpation. All the heifers were non pregnant and between 35 and 75 days post partum. Corpora lutea were clinically considered by size and consistency in functional and non-functional. Diagnostic accuracy was determined by progesterone levels in mil by RIA in solid phase. Predictive value of rectal palpation were positive in 77%(85/110). The accuracy for non-functional corpora lutea was very high (94%), whereas it was significantly lower for functional corpora lutea (70%). As it has been reported by must workers, these results confirm that under field conditions it is very difficult to attain predictive values higher than 80%. As a conclusion, procedures like training, reproductive records, multiple examinations and, eventually progesterone profiles can decrease mistakes.

BOVINE FOETAL AND MATERNAL PLACENTAL AROMATASE ACTIVITY AND OESTROGEN LEVELS DURING PREGNANCY AND PARTURITION

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INTRODUCTION

A surge in oestrogen level in bovine maternal blood (8,10,15,17) and placenta (11,24) before parturition has been known such as an important phenomenon for parturition to ensure. Oestrogens are derived from thier precursors and this derivation process is limited by the enzymatic activity of aromatase (19). The foetal placenta is the major production site for oestrogens (9). Though aromatase activity (AA) in bovine fetal placenta has been reported to increase by a few folds immediately after parturition (1), negative findings are documented as well (4). Further, AA is increased by a few folds after corticosteroid administration (1). However, it is believed that AA is not affected as an increase in estrogen production after corticosteroid treatment is due to the activation on enzymes (17-OH lase, $C_{17,20}$-lyase) responsible for converting pregnenolone to dehydroepiandrosterone (6). On administering oestrogens to pregnant cows, pregnancies are somehow terminated during early or mid pregnancy (7). Oestrogens seem to play an important role at mid-term (18), but attention on their increases at this juncture and on parturition has not been focussed. Our present investigation attempted to examine the AA changes and oestrogen levels in the bovine foetal placenta and compared with those present in the maternal placenta from PM 4 to immediately after parturition. The role and the possible regulatory mechanism of oestrogens on pregnancy progress was studied and discussed.

MATERIALS and METHODS

Materials

Placentae from a total of 38 Holstein cows (31 pregnancies and 7 with normal parturition) were isolated, the foetal-maternal placentae were then separated manualy within 30 min either after slaughter or parturition prior to freezing with dry-ice. Samples isolated were stored at -80 ℃ before assay. Pregnancy terms of foetuses were estimated from foetal crown-rump length (CRL) according to previous findings (22).

Determination of aromatase activity (AA)

Enzymatic activity was determined according to Bellino and Osawa (2) method. Placental samples were homogenized with 0.25 M sucrose in a teflon-glass homogenizor (B.Braun Melsungen Corpn.). Homogenate was centrifuged at 10,000xg for 15 min at 4 ℃, and the supernatant was taken as the enzyme source. To enzyme source sample (expressed as an approximate value of 0.3 mg protein), 20 mM phsphate buffer, 0.2 μ M EDTA-2Na, 1 μ M NADPH and 5 nM of androstenedione {[1β -^{3}H(N)] as substrate; specific activity: 27.4 Ci/mM, New England Nuclear Chemical Co.} were added and allowed to react by incubating the reaction mixture at 37 ℃ for 10 min, agitating at a frequency of 120/min. Reaction was terminated on cooling with ice-water, chloroform extraction and charcoal absorption were performed to remove non-metabolized substances, and AA in the supernatant was assayed with the protein concentration determined according to Lowry et al.(12) method.

Inhibitor test

To investigate whether androstenedione was aromatized to estrone, aromatase inhibitor test was carried out. Samples containing 1, 2, 4, 8 nM of 4-hydroxy-androstenedione (Sigma Chemical Co.) as the inhibitor were compared with that (100% AA) devoid of inhibitor.

Aromatase reaction (aromatization) velocity test

Maximum aromatase reaction velocity (Vmax) and Michaelis constant (Km) were determined by measuring the AA using 80 pM~ 280 nM ^{3}H-androstenedione as the substrate.

Determination of E_1 and E_2 levels

E_1 and E_2 levels in placental homogenates were determined by a radioimmunoassy method as described previously (13). Antisera against E_1-6-CMO-BSA and E_2-6-CMO-BSA were purchased from Teikoku hormone Mfg. Co.(Tokyo, Japan). Cross-reactivities of the E_1 antiserum for E_2 and estriol were 2.0% and 1.6%, whereas those of E_2 antiserum for E_1 and estriol were 3.0% and 2.0 %, respectively. Variation coefficients of intra- and inter-assays for E_1 were 11.2% and 17.4%, whereas those for E_2 were 15.7% and 19.6%, respectively.

Statistical analyses

Statistical significance was verified with the Student's t test, and the correlation coefficients were evaluated.

RESULTS

Profile of AA method

When AA of supernatants of fetal placentae immediately after parturition was determined using 0.1~ 0.5 mg protein weight, a linear plot was obtained (Fig.1). Approximately 0.3 mg samples (wet weight≈ appro. 10 mg) were used in our experiments. The Vmax and Km values obtained were 0.27 pM/mg protein/min and 6.9 nM, respectively (Fig.2). For the inhibition test, AA at respective pregnancy months was proportionally supppressed by the inhibitor concentration increases and a rather straight linear plot was phtained; 50% of the control value was suppressed with 1~ 2 nM inhibitor concentration (Fig.3).

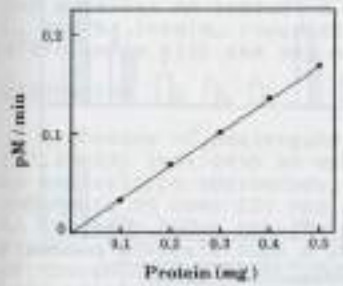

Fig.1 Aromatase activity using various protein weight in the bovine foetal placenta of parturition.

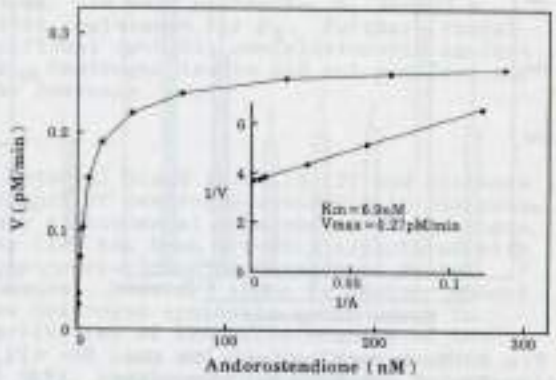

Fig.2 Velocity of aromatization with various substrate concentration (androstenedione).

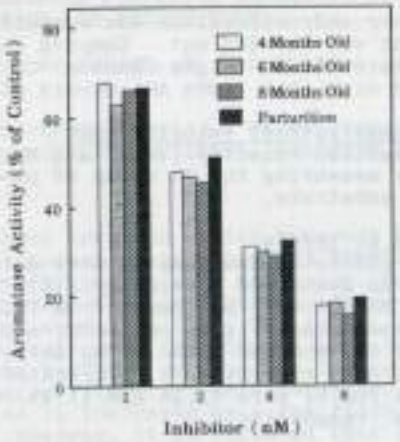

Fig.3 Inhibition ratio of aromatase activity by inhibitor(4hydroxy-androstenedion).

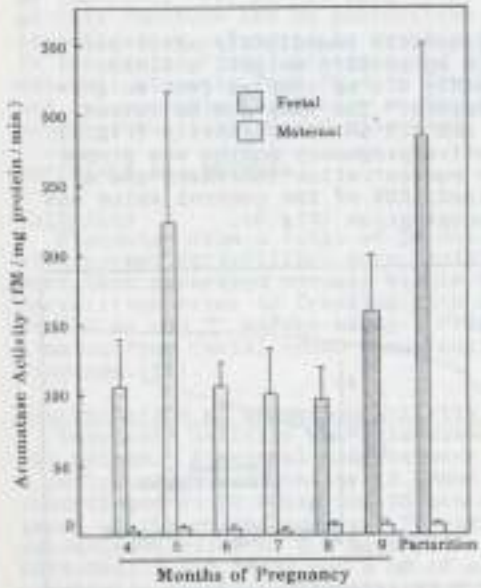

Fig.4 Aromatase activity in the bovine foetal and maternal placentae at various pregnant periods. Each bar represents the mean± SEM.

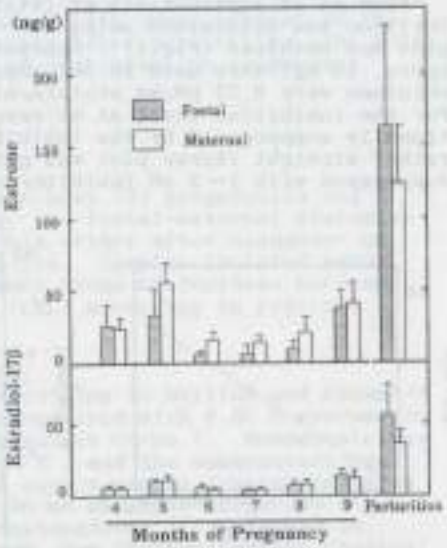

Fig.5 Estrone and estradiol-17β levels in bovine foetal and maternal placentae at various pregnant periods. Each bar represents the mean± SEM.

AA in foetal and maternal placentae

The relationship of mean ± standard error values of AA against pregnancy term in months is shown in Fig.4. AA at PM 4 registered 106.6± 35.2, significantly ($p<0.05$) elevated to an initial peak of 268.5± 92.5 at PM 5, suppressed thereafter to 107.5± 17.6 at PM 6 suppressed thereafter to 107.5± 17.6 at PM 6 and cascaded further to 99.5± 34.9 fM/mg protein/min at PM 7 in foetal placenta tissue. The AA decline was reversed at PM 9 and thereafter to register a significantly ($p<0.05$) subsequent second peak value of 284.3± 67.2 fM/mg protein/min immediately after parturition. A biphasic pattern of AA in foetal placenta was illustrated. On the other hand, in the maternal placenta tissue, AA registered 3.8± 1.2 at PM 4 and indicated similar low readings of 4.6± 0.6 fM/mg protein/min at PM 5, indicating extremely meagre values which were significantly ($p<0.001$) lower than those recorded in the foetal placenta.

E_1 and E_2 levels in foetal and maternal placentae

The respective E_1 levels of foetal and maternal placentae increased from 26.1± 16.0 and 24.7± 7.5 at PM 4 to 34.5± 18.4 and 55.8± 25.6 ng/g at PM 5. At PM 6~ 7, both placentae showed extremely low levels, indicating significant ($p<0.05$) decreases against the values at PM 5 (Fig.5). These low values reversed to substantial increases at PM 8, and both foetal and maternal E_1 levels respectively peaked at 158.9± 61.2 and 123.9± 36.1 ng/g immediately after parturition with significant ($p<0.05$) increases recorded against those measured at PM 6~ 8. E_1 levels in both foetal and maternal placentae showed a parallel change against pregnancy term with significant ($p<0.01$) correlationship.

Foetal and maternal E_2 levels showed slight increases from 6.0± 3.6 and 5.2± 1.6 at PM 4 to 10.4± 3.6 and 10.9± 3.7 ng/g at PM 5, respectively (Fig.5). On reaching PM 6~ 7, both levels returned to the PM 4 level. Their values started to increase gradually from PM 8 to indicate peak foetal and maternal levels of 57.4± 23.2 and 37.6± 8.2 ng/g immediately after parturition, respectively. Significant foetal ($p<0.05$) and maternal ($p<0.01$) E_2 increases were verified between the values recorded immediately after parturition against those registered at PM 4~ 8. E_2 levels of foetal and maternal placentae showed approximately similar values throughout the pregnancy term with significant ($p<0.01$) correlationship. In both placentae, E_1 showed a value 2~ 3 folds higher than that registered for E_2. Further, foetal and maternal AA indicated significant ($p<0.01$) correlationship against E_1 and E_2 levels, respectively. Oestrogen levels did not manifest any differences with the sex of the foetuses.

DISCUSSION

Increases of oestrogens in maternal blood (8,10,15,17) and placenta (11,23,24) implicate an enhancement of oestrogen synthesis in placenta as parturition approaches. Such a phenomenal enhancement in oestrogen synthesis in cows (1) and sheep (14) has been directly associated with AA increase. Our results on post-parturition AA changes in foetal placenta advocate such a phenomenon. However, there is another school of thoughts (4,6) that proposes oestrogen synthesis enhancement to have attributed to increased activities of synthetic enzymes of the oestrogen precursors, such as 17α -OH lase and $C_{17,20}$-lyase, rather than aromatase. In addition to this, corticosteroid hormonal increases on parturition in cows (6) and sheep (14,20) have also been associated to enhanced activation of such enzymes. All in all, however, reports on AA in foetal placenta with respect to pregnancy progress have not been documented. Our present experiments revealed AA to exhibit a biphasic pattern, where an initial peak activity appeared at PM 5 and

a subsequent peak value ensued after immediately parturition. In spite of the fact that peak AA values appeared at PM 5 and immediately after parturition, E_1 level in foetal placenta registered a concentration of approximately 4.6 folds higher than that recorded at PM 5. Coupling past findings with our present data, enhancement of oestrogen synthesis in placenta is not associated with only increased activities of 17α -OH lase and $C_{17,20}$-lyase but elevated activity of AA is also implicated.

The most stimulating findings in our present experiments are the increases in E_1 and E_2 levels that accompanied the enhanced AA in bovine foetal placenta observed at PM 5. With regards to such a data, a parallel finding of the presence of a maximal concentration of E_1 -sulfate in urine solution at PM 4.5 has also been reported (18). Moreover, a recent document (11) has also reported on the extremely high E_1 synthetic production in foetal placenta at PM 6.5. Though past findings (11,18) indicate oestrogen levels to peak at pregnancy term slightly different from our present data, the marked oestrogen synthetic production increase at mid-pregnancy converges well with our present data. Nevertheless, it is still unclear as to why such a physiologically phenomenal increase in oestrogen production occurred at mid-pregnancy. Maximal increases in monthly rate on weights of bovine foetal membrane and uterine capacity have been reported to occur at PM 5 (21). During early pregnancy, changes in foetal membrane weight are closely correlated to placental oestrogen levels in cows (3). Further, increases in oestrogen level have also been associated to uterine enlargement, a phenomenon that has been believed to be due to influences of actomyosin, collagen and enlargement of uetrine muscle cells (25). From the above and our present findings, the abrupt increase in placental oestrogen synthetic production at mid-pregnancy may implicate the necessary preparations to essentially accomodate the extremely fast growth appropriates by the foetus during late pregnancy.

With regards to regulatory factors influencing the sudden increase in AA at mid-pregnancy and on parturition, it is still unknown. Corticosteriods do not exert any direct influences on the AA (6), and adrenocortical functions have been known to decline at mid-pregnancy when compared with those observed during early pregnancy (5). Such findings reveal straight forwardly that 'unknown factors' are regulating the AA; oestrogen synthetic production at term is regulated by corticosteroids plus the unknown factors, whereas that at mid-pregnancy is believed to have been merely influenced by those unknown factors per se.

When oestrogen synthetic productions of maternal and foetal placentae are compared in tissue cultures, the respective quantities of E_1 produced in the free and conjugated forms are 2 (9,16) and a few 10 (9) folds higher in the foetal than maternal placenta. The latter findings coincide well with our present data of the very much higher AA found in foetal than maternal placenta at mid-pregnancy. Not only low values in AA were registered in the maternal placenta, the activity recorded did not behave in a biphasic pattern exhibited by AA in the foetal placenta. Such findings reconfirm the oestrogen production site in bovine placenta to locate mainly in the foetal compartment. However, when oestrogen levels in foetal and maternal placentae were compared, significant differences were not evaluated between the two; AA changes were somewhat parallel in foetal site. As such, the site of production for and existence of oestrogen was distinctly located, and our findings advocate further that oestrogens were transferred from foetal to maternal placenta.

From the above findings, we conclude that 1)maximal oestrogen synthetic production in bovine placenta occurred twice in the pregnancy-parturition process; an initial peak appeared at mid-pregnancy ensued

by a subsequent peak level registered at term, 2)some unknown factors were involved in regulating oestrogen production in foetal placenta, and 3)those synthetically produced oestrogens have been confirmed to play a vital role in appropriating an ideal environment for promoting development/growth and eventual parturition of the foetus in achieving a successful birth, enabling life forms to continue existence in the environment so bestowed upon their beings.

Acknowledgments

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Summary

Aromatase activities, oestrone and oestradiol-17β levels from bovine foetal and maternal placentae, isolated separately from cows of pregnancy month 4 to 9 and immediately after parturition, were determined by substrate-metabolism and radioimmunoassay methods, respectively. Aromatase activities in foetal placenta registered an initial high value of 266.54± 92.53 fM/mg protein/min on pregnancy month 5, declined thereafter to 107.5± 17.8 fM/mg protein/min at PM 6, peaked again at 284.31± 67.21 fM/mg protein/min immediately after parturition, indicating a biphasic response. However, aromatase activity recorded in maternal placenta revealed a low value of 4.63± 0.60 fM/mg protein/min on pregnancy month 5 compared to that observed in the foetal placental tissue. Oestrone and oestradiol-17β values in foetal placenta were 34.5± 18.4 and 10.4± 3.6 ng/g whereas those measured in maternal placenta registered 55.8± 25.6 and 10.9± 3.7 ng/g, respectively. Comparison between both oestrogens indicated no significant difference in both tissues, and these values varied in a paralel manner to changes in aromatase activities of foetal placenta. We conclude therefore; 1) oestrogen synthetic activity was enhanced to a maximum during the pregnancy-parturition process for at least twice; during mid-term pregnancy and immediately before parturition, 2) some factors were involved in the regulation of estrogen synthesis in foetal placenta, and 3) the synthesized estrogen was confirmed to play an important role by appropriating an ideal environment in promoting the proper development of foetal growth followed by parturition.

EFECTO DEL DESTETE TEMPORAL EN EL PORCENTAJE DE PREÑEZ EN UN HATO BRAHMAN

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INTRODUCCION

La ganadería en Centro América se ha convertido en los últimos años en el punto focal de un gran esfuerzo productivo. En Guatemala aproximadamente el 35% de las tierras aprovechables está constituido por pasturas, de las cuales la mayor parte corresponde a explotaciones de ganado bovino de carne. Debido a las condiciones tropicales de nuestro país, uno de los principales limitantes con las que se encuentra el productor de ganado bovino, es una baja eficiencia reproductiva, como consecuencia de una alta incidencia en la presentación de anestro postparto y al reducido número de vacas gestantes al final de la época de monta. En nuestro medio uno de los métodos tradicionales en el manejo de las ganaderías de carne consiste en mantener a la vaca con el ternero al pié amamantando continuamente durante un período de 7 a 9 meses, ocasionando la presentación de anestros prolongados con intervalos entre partos más largos. Actualmente en las explotaciones ganaderas de carne se han venido utilizando diversos métodos para mejorar la eficiencia reproductiva. Dentro de este tipo de manejo se encuentran tres modalidades diferentes que son: el destete precoz, el amamantamiento restringido y el destete temporal. Siendo éste último poco conocido en nuestro país, por lo que se hacia necesario su evaluación, que fue el objetivo primordial de este trabajo.

MATERIALES Y METODOS

Localización

La presente investigación se realizó en la finca "Magdalena" ubicada en el municipio de Retalhuleu, departamento de Retalhuleu, en la República de Guatemala. Descrito según De la Cruz (3) en una zona de vida clasificada como bosque subtropical húmedo (cálido), con una altura de 156 pies sobre el nivel del mar, una precipitación pluvial que varía de 1500 a 3200 milímetros, distribuidos de mayo a octubre, con una temperatura promedio de 30 grados centígrados, con la latitud Norte de 14°18' y una longitud Oeste de 19°55' y con un tipo de suelo clasificado como Ixtan arcilloso.

Procedimiento

Se procedió a seleccionar dentro del hato comercial, 45 vacas, con las siguientes características: 1. Vacas vacías con períodos postparto, entre 60 y 90 días con ternero al pié y que a la palpación rectal presentaran ovarios funcionales. 2. Edad de la vaca entre 7 a 10 años. 3. Número de partos de la vaca entre 3 a 6. Los tratamientos se organizaron al azar, en grupos de 15 vacas cada uno, de la siguiente manera: Tratamiento I: (testigo) manejo normal de la finca, que consite en mantener a la cría al pié de la madre hasta el destete (7 meses). Tratamiento II: separación del ternero por un período de 48 horas, una vez cada mes durante la estación de monta. Tratamiento III: separación del ternero por 72 horas una vez cada mes durante la estación de monta. Todos los tratamientos pertenecieron en un mismo lote. Los terneros de los tratamientos II y III se les proporcionó una suplementación a base de un concentrado elaborado en la finca cuando estos estuvieron separados de la madre. De manera complementaria se realizó antes del inicio del trabajo, pruebas de fertilidad a los toros con el propósito de evitar cualquier efecto del toro. La relación toro vaca que se utilizó fue de 1:22. Dos meses después de terminada la época de monta se realizó a las vacas el diagnóstico de gestación, a través de palpación rectal. La duración de la estación de monta fue de tres meses.

Análisis estadístico

Para el análisis estadístico de los datos se utilizó la prueba de comparación de dos proporciones binomiales de la siguiente forma: se comparó los grupos de dos en dos con el objeto de determinar si existen diferencias entre las proporciones de ambos grupos y de esta forma se presentan los resultados.

$$Zo = \frac{\hat{P}\,1 - \hat{P}\,2}{\sqrt{\frac{\hat{P}(1-\hat{P})}{n} + \frac{\hat{P}(1-\hat{P})}{m}}}$$

En donde:

- P 1 = proporción de preñez del tratamiento I.
- P 2 = proporción de preñez del tratamiento II.
- P = proporción de preñez de ambos tratamientos.
- n = número de vacas del tratamiento I.
- m = número de vacas del tratamiento II.

RESULTADOS

Efecto del destete temporal del ternero en las tasas de preñez.

El cuadro No.1 muestra el número de vacas gestantes en relación a las no gestantes en los diferentes tratamientos expresados en porcentaje. Se estableció un porcentaje de preñez del 93.33%, 80.00% y 93.33% para los tratamientos I, II, III respectivamente. Al hacer la comparación de dos proporciones binomiales utilizando la prueba Z, no se encuentran diferencias significativas (p 0.05). Resultados similares fueron observados por Rodríguez, A. et al. (8), quienes realizaron un estudio con vacas Brangus, utilizando destete temporal por 96 horas, encontrando un aumento del 4% en el porcentaje de preñez; asumiendo que el manejo de la lactación no tuvo ningún efecto significativo para incrementar la fertilidad y que el retiro de la cría como única práctica de manejo no provoca un estímulo suficiente para restablecer la actividad ovárica. De igual forma Menéndez y Wiltbank (7), usaron 106 vacas en las que considerando la condición corporal, midieron la respuesta estral y el porcentaje de gestación en hembras 60 días postparto con o sin retiro de la cría por un período de 48 horas. Concluyendo que es más importante el efecto de la condición corporal al parto, que los cambios de peso postparto o el retiro de la cría.

Rodríguez, A. et al. (8) y Rodríguez, A. et al. (9), atribuyeron que las diferencias encontradas en cuanto a beneficios de esta práctica se deben en parte a condiciones como manejo, nutrición, condición corporal al parto, intensidad de selección y raza. Probablemente estos factores fueron los causantes de no encontrar ningún efecto en esta técnica en el presente estudio, ya que en la finca en la que se realizó, el manejo a que son sometidos los animales es bastante adecuado para mantener índices altos de natalidad, los cuales están alrededor del 75% (registros de 1989), comparado con los índices nacionales que se encuentran en un 52% de natalidad. Sin embargo Wettemann (10) y Horta (5) encontraron aumentos en la presentación celos y en los porcentajes de gestación utilizando la separación del ternero por 48 horas.

Es importante mencionar que en la presente investigación a pesar de que no se encontraron diferencias estadísticas significativas (p 0.05) entre los tratamientos, biológicamente el tratamiento II fue inferior a los otros, ya que se presentó un 13.33% más bajo en la tasa de preñez, que es una diferencia de dos vacas gestantes menos. Esta diferencia tiene una repercusión negativa en el aspecto económico, ya que se perdería, debido a una menor cosecha de terneros, así como en un aumento de los gastos de mantenimiento de las vacas, ya que presenta el mayor número de vacas no gestantes.

Es muy importante observar que el destete temporal sí puede contribuir a acortar el intervalo parto concepción, como se puede apreciar en el cuadro No.2. El mismo permite establecer que en la primera separación el tratamiento I presentó un total de 5 vacas gestantes (33.33%), el tratamiento II, 6 vacas (40.00%) y el tratamiento III, 3 vacas gestantes (20.00%). En la segunda separación el tratamiento I presentó 3 vacas gestantes (20.00%), el tratamiento II, 6 vacas gestantes (40.00%) y el tratamiento III, 3 vacas gestantes (20.00%). En la tercera separación, el tratamiento I presentó 6 vacas

Cuadro No.1. PORCENTAJE DE PREÑEZ, VACAS GESTANTES Y NO GESTANTES Y ESTADO DE PREÑEZ DE LOS DIFERENTES TRATAMIENTOS EN UNA ESTACION DE MONTA DE 120 DIAS.

TRATAMIENTO	NUMERO DE VACAS	VACAS GESTANTES	VACAS NO GESTANTES	PORCENTAJE DE PREÑEZ	ESTADO DE PREÑEZ X
I	15	14	1	93.33 % a	2 meses 27 días
II	15	12	2	80.00 % a	3 meses 17 días
III	15	14	1	93.33 % a	2 meses 2 días

Valores en una misma columna, seguidos de letras iguales no difieren significativamente (p 0.05) según la prueba de distribución binomial.

CUADRO No.2. NUMERO Y PORCENTAJE DE VACAS GESTANTES EN CADA SEPARACION DEL TERNERO EN LOS DIFERENTES TRATAMIENTOS

TRATAMIENTO	1a. SEPARACION DEL TERNERO		2a. SEPARACION DEL TERNERO		3a. SEPARACION DEL TERNERO	
	No. DE VACAS	% DE PREÑEZ	No. DE VACAS	% DE PREÑEZ	No. DE VACAS	% DE PREÑEZ
I	5	33.33 b	3	20.00 a	6	40.0
II	6	40.00 b	6	40.00 a	0	----
III	11	73.33 a	3	20.00 a	0	----

* Valores en una misma columna, seguidos de letras iguales no difieren significativamente (p. 0>) según la prueba de distribución binomial.

gestantes (40.00%), mientras que en los tratamientos II y III no hubo presentación de vacas gestantes. Al realizar el análisis estadístico de los datos con la prueba de Z; durante la primera separación los tratamientos I y II no presentaron diferencias estadísticas significativas (p 0.05), siendo el tratamiento III diferente a estos.

En la segunda y tercera separaciones no hubo diferencias estadísticas significativas (p 0.05) entre ninguno de los tratamientos. Como se puede observar, en la primera separación el tratamiento III (destete por 72 horas), presentó el mayor porcentaje de concepciones, teniendo por consiguiente que el mayor número de vacas quedaron preñadas en los primeros 30 días de iniciada la época de monta. Al hacer la comparación entre tratamientos, el tratamiento III supera a un 33.33% al tratamiento II y en un 40.00% al tratamiento I. De igual manera Menéndez y Wiltbank (7) encontraron que el retiro de la cría por 48 horas reduce el tiempo de aparición del estro y aumenta los porcentajes de ovulación y preñes en los primeros 24 días siguientes a la separación, ya que hubo un 25% más de vacas gestantes en comparación a vacas que mantienen el ternero al pié. Rodríguez, A. et al. (8) concluyeron que los mejores porcentajes de gestación al inicio de la monta en los animales tratados tal vez se deban a que el destete temporal aumenta la liberación de gonadotropinas.

Bugner, M. (1) concluyó que la separación del ternero acelera el restablecimiento de la actividad reproductiva postparto, debido a que elimina el efecto de la liberación de gonadotropinas por la pituitaria, causado por el amamantamiento, lo que conlleva un rápido desarrollo folicular. Castañeda, N. et al. (2), Laster, D. (6) y Gregg, D. (4) encontraron que el amamantamiento retrasa la actividad cíclica postparto al causar una inhibición de la secreción de factores liberadores de gonadotropinas, así como la reducción de las concentraciones de LH.

Es importante señalar en la presente investigación que en la segunda separación los porcentajes de preñez fueron similares (p 0.05) para los tres tratamientos; no obstante, biológicamente se puede observar un mayor número de vacas gestantes en el tratamiento II comparado con el tratamiento I. En la tercera separación solamente el tratamiento I (manejo normal de la finca) presentó un alto número de vacas gestantes, lo que nos indica que con este manejo se necesita un mayor número de días para la concepción, lo cual puede tener implicaciones en el intervalo entre partos.

En el cuadro No.1 se presenta el número de vacas y porcentaje de preñez según el estado de gestación. Se encontró que el tratamiento I tiene un promedio de gestación de 3 meses con 27 días, el tratamiento II, de 3 meses con 17 días y el tratamiento III de 4 meses con 2 días.

Estos resultados indican que los tratamientos II y III presentan 20 y 35 días más de gestación que el tratamiento I. Siendo de esta forma que el destete temporal acorta el intervalo parto concepción, lo que favorece a que las vacas lleguen a la nueva época de monta con mayor número de días postparto y con terneros de mayor edad.

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RESUMEN

Este trabajo tuvo como objetivo estudiar el efecto de la interrupción temporal del amamantamiento del ternero por diferentes períodos sobre la tasa de preñez en vacas Brahman. Fueron utilizadas 45 vacas con edades de 6 a 10 años, con 4 a 6 partos y con 80 a 90 días postparto. Los animales fueron distribuidos al azar en tres grupos: Tratamiento I: las crías permanecen con sus madres durante todo el período experimental. Tratamiento II: Las crías fueron separadas de sus madres por un período de 48 horas al inicio del experimento y cada 30 días durante el transcurso del mismo. Tratamiento III: semejante al tratamiento II, con separación por período de 72 horas. Los resultados demostraron no haber diferencia estadística en el porcentaje de preñez entre los diferentes tratamientos. Sin embargo biológicamente el tratamiento II fue inferior. Se observó también que los tratamientos II y III presentaron un estado de gestación más avanzado que el tratamiento I. Se concluye en este estudio que a pesar que el destete temporal no mejoro la tasa de preñez en vacas Brahman, esta práctica de manejo permite disminuir el intervalo parto-concepción.

RÉSUMÉ

Ce travail eut comme but l'étude de l'effet de l'interuption temporelle del'allaitement des veaux, dans des périodes differentes, sur le taux de gestation desvaches Brahman. Quarantecing (45) vaches furent employees entre les ages de six ot dix ans, avec quatre (4) ou 6 (six) parturitions et entre quatre vingt (80) et quatrevingt dix (90) jours d'apres-parturition. Les animaux furent partages en trois groupes. Traitement I: Les nourrisons demeureut pendant toute pa période experimentale avec ses méres. Traitement II: les nourrisons furent separes pendant quarante nuit hewres, au debut de l'experiment, et puis, chaque trente (30) jours. Traitement III: Le merne traitment que II, maisdes des périodes de soixante douze (72) heures. Les resultatsne montrerent aucune difference statistique dans le pourcentage de gestation entre les diferentes groupes. Tout de meme, Biologiquement, le traitement II fut inferiewr. Et les observations montrerent aussi que les groupes II et III presenterent des gestations plus avancées que le groupe I. L'etude coóclut que, mene si le sevrage temporel n'a pas amelioré la taux de gestación des vaches Brahman, celle pratique fermet la diminution de l'intervalle parturition-conception.

SUMMARY

Pregnancy rates of Brahaman cows were evaluated when calf nursing was temporarily interrupted. Forty five cows ranging from six to ten years of age, four to six parturitions, and 80 to 90 days postpartum were randomly alloted to three treatment groups. I) Calves remained the whole experimental time with the mother. II) Calves were removed 48 hours from calf nursing at hte beginning of the experimental. Every 30 days the procedure was repeated. III) Same as treatment two, but with a 72 hours calf removal. No significant difference (P 0,05) was observed in pregnancy rates although cows with a 48 hours removal had numerically a lower rate. The 48 and 72 hours removal cows had more advanced pregancies than the non-removed calf cows. Although temporarily removing calves from nursing did not improve pregnancy rate of Brahman cows. This practice allowed a shorter partum-conception period.

LA OVARIOTOMIA EN VACAS DE DESCARTE

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INTRODUCCION

Esta es una operación quirúrgica, típica de campo, de muy fácil realización, a pesar de lo cual está muy poco difundida.El conocimiento de sus ventajas tanto a nivel de productores como de veterinarios, estimulará a éstos a especializarse en dicha operación.
Sus objetivos concretos son:
1o) Asegurar la infertilidad definitiva de las vacas viejas,después del último parto y también de las vaquillas de descarte.
2o) Incrementar en menor tiempo el engorde, comparado con vacas testigo NO castradas.
3o) Obtener con esta operación, la ganancia de mayor peso, comparada con vacas testigo, engordadas en campo nativo.
4o) Aumentar la calidad y aspecto de la carcasa y su mayor rendimiento que alcanza del 4 al 8 % en más, semejándose al novillo gordo.
5o) Facilitar el manejo y ubicación de vacas de invernada, incorporándolas en potreros con novillos y toros.
6o)Obtención de terneros "baby beef" de 300 a 400 kilos de peso,sin seleccionar sin ningún suplemento, sólo con la leche materna.
7o)Evitar que el país pierda por fetos y envolturas en frigoríficos,un promedio de 15 kilos en el 60 % de vacas de faena, pués sólo en Río Grande del Sur,se perdieron por esa causa en 5 años -1978/82-14.500 toneladas, o sea 2.900 toneladas por cada año.Estos son datos del Dr.Oscar Silveira Collares de Bagé. **(1)**

HISTORIA DE LA OVARIOTOMIA

Año 1830 - El veterinario francés Dr. Charlier descubre la incisión dorso-longitudinal de la vagina, que permite el acceso directo a los ovarios;a continuación efectuaba su extirpación cruenta, motivando abundantes hemorragias, causa principalísima de las peritonitis.
Año 1890 - El veterinario belga Dr. Degive inventa la ligadura elástica del cordón ovárico, impidiendo las inevitables y temidas hemorragias
Año 1908 - El veterinario italiano Dr. Marco Dutto inventa el ovariótomo o instrumento que permite aplicar la ligadura elástica en ambos cordones ováricos, en sólo 1 a 2 minutos por vaca, según la práctica del veterinario.El rendimiento es de 30 a 50 vacas por hora.NO hay hemorragias y NO hay muertes, ya que NO se producen peritonitis.

INSTALACIONES Y PERSONAL NECESARIO

La operación se hace con la vaca en pié,en el tubo, inmovilizada con el cepo o mucho mejor sin él.Se inpide su retroceso con 1 caderatravesaño de madera, un poco por encima de los garrones.La cantidad ideal de personal son 5 operarios para el ganado y 1 instrumentista.Con 2 instrumentistas el trabajo es mucho más rápido.-

EL PORCENTAJE DE MUERTES EN OVARIOTOMIA

Es el 0 % y debe ser siempre el 0 %.No podemos imaginar el éxito de otro modo.Unicamente con ese éxito, la profesión veterinaria logrará extender la difusión en gran escala de esta operación tan ventajosa. El suscripto tiene clientes que sobrepasaron los 40 años consecutivos de castración de vacas.Ellos lo consideran un simple trabajo de rutina, el castrar todas las vacas viejas después de parir el último ternero.

EL OVARIOTOMO DEL DR. DUTTO - SU DESCRIPCION

Este instrumento está formado por un tubo de bronce de 12 mm x 45 cms. de longitud.Su parte anterior presenta un soporte para las 2 ligaduras

de goma (borrachas) que en forma de lazos, permiten pasar los ovarios para su extrangulación.Por el interior del ovariótomo se deslizan 2 varillas planas, flexibles,de acero inoxidable, que en su extremo anterior enganchan las ligaduras elásticas.En su parte posterior tiene dos cómodas manivelas para la tracción.

ACCESORIOS: extensor vaginal - bisturí - anillos de goma (borrachas)

Extensor vaginal: su finalidad es estirar la vagina y colocarla en la correcta posición para su incisión.Es un vástago de 50 cms. de largo, con un apéndice de 3 cms. que se introduce en el cuello del útero y concentra allí el esfuerzo para la extensión vaginal.
Bisturí: la forma aguda, cóncava y penetrante de su hoja de acero es absolutamente original y su eficiencia para el corte es extraordinaria: la punta se incrusta casi sin esfuerzo en el techo de la vagina;después se hace el corte.La hoja del bisturí es recambiable.
Anillos de goma(borrachas) y anillos plásticos: estos materiales son los elementos esenciales para efectuar la extrangulación de los cordones ováricos.-

OVARIOTOMIA CON LIGADURA ELASTICA VERSUS EXTRACCION CRUENTA OVARIOS

La extracción cruenta de los ovarios es la causa de las fatales hemorragias de las arterias ováricas: se instala peritonitis aguda y muertes. Por ningún motivo es aplicable este sistema totalmente obsoleto, anticientífico y anti-económico(por las vacas que mueren) y por el bajo rendimiento de operaciones por hora del profesional.

TRATAMIENTO DE UNA PERITONITIS YA INSTALADA Si se produce esta contrariedad, se deben tratar con urgencia las vacas enfermas.Estas lo demuestran con hinchazón del vientre (pseudo-meteorismo) desplazamiento con dolor y encorvaniento.Hay fiebre.Medicación: debe ser por inoculación intra-peritoneal únicamente, y NO por otra vía.Por esa vía se frena la mortalidad con total éxito siendo suficiente 1 sola inyección de 100 cc.de solución de tretraciclina.

LA OVARIOTOMIA AL SERVICIO DE LA PRODUCCION

Obtención de terneros "baby-beef". La vaca castrada prolonga su lactación un mínimo de 2 años, siempre que no existan fuertes crisis forrajeras.Esta propiedad se utiliza para provocar un gran desarrollo a los terneros machos del último parto y que permanecen al lado de sus madres durante 15/16 meses.En buenos campos del Uruguay, consumiendo casi constantemente praderas artificiales, alcanzan y sobrepasan los 400 kilos a esa edad.Nunca consumieron heno, ni silos, ni raciones: sólo excelentes pasturas y la leche de sus madres en cantidad constante.Por supuesto que es en regimen de pastoreo libre, sin estabulación. Es sabido que en Europa se logran esos mismos pesos, pero en estabulación más el gran costo de raciones, heno, mano de obra y estabulación en invierno.Allí se acorta el tiempo a un año de preparación pero el costo es enorme. En nuestros campos, ayuda al éxito, que los terneros nazcan muy temprano en julio/agosto o sea 2 meses antes de la primavera, para aprovechar la abundancia de las pasturas lactógenas de esa estación.

PESO DE LAS MADRES CASTRADAS DE ESOS NOVILLITOS "BABY BEEP"

Vacas Hereford que alimentaron 15 meses esos novillitos en excelentes campos y praderas artificiales alcanzaron a pesar 532 kilos con casi el 58 % de rendimiento con la riñonada.La planilla del frigorífico ilustra esos datos, de la faena.

PESO DE VACAS CASTRADAS, CRIANDO 1 AÑO ALIMENTADAS SOLAMENTE CON CAMPO

Vacas Hereford que crían sus hijos todo 1 año, alimentados ambos en

buenos campos nativos, pero sin forrajeras artificiales, pesan en general unos 100 kilos menos, o sea 430/440kilos, como se puede apreciar en la planilla de faena del frigorífico.

EL MAYOR RENDIMIENTO DE LA RES DE LA VACA CASTRADA

Los rendimientos de estas vacas oscilan del 54 al 58 % en razas inglesas, Hereford,Aberdeen A.,Shorthon y sus cruzas.Son porcentajes que muchas veces no alcanzan los buenos novillos.Su explicación científica, es que la ovariotomía modifica la tendencia de ubicación de la grasa: ésta tiende a infiltrarse íntimamente en el músculo en lugar de hacerlo en forma subcutánea. Tanto frigoríficos como carniceros,aprecian mucho esta gran cualidad y siempre tienen preferencia para la adquisición de vacas castradas, aún en meses de intensa oferta de ganado gordo, facilitando su venta.

POSIBILIDAD DE MEZCLAR EN UN MISMO POTRERO DE INVERNADA, TOROS, NOVILLOS Y VACAS CASTRADAS. Sus excepciones por falsos celos. Las dificultades corrientes de tener separadas las vacas de engorde, con los novillos, se soluciona de raíz con la castración de las hembras.Todas las categorías conviven sin inconvenientes SIEMPRE QUE NO sobrevengan falsos celos.Hay aparición de olor sui-generis, secreción de mucus, presencia de células epiteliales descamadas,etc.Con estas vacas en falso celo, los toros copulan constantemente,hasta que cesan las causas.

CUANDO SE PRODUCEN LOS FALSOS CELOS. SUEXPLICACION CIENTIFICA (2)

La causa de dicho fenómeno se debe a la alta concentración de hormonas vegetales de estructura química semejante a la foliculina, que se encuentran en las hojas verdes, de las exhuberantes pasturas en primavera y también en los buenos otoños.Cuando los pastos sazonan y endurecen, cambia la composición química y los celos desaparecen.El autor no tiene experiencia de lo que puede acontecer con referencia a falsos celos, en los climas tropicales.Es una experiencia que deberá ser escrita por los estimados colegas que trabajan en esas zonas.

LA CASTRACION DE VACAS LECHERAS DE DESCARTE

Si las vacas viejas que acusan excesivo desgaste de dientes, NO se castran, la última gestación suele terminar con sus vidas, por falta de vitalidad,hipocalcemia, anemia, y desnutrición.La revisación anual de dientes antes del parto, decide cuántas serán eliminadas por ovariotomía, a los 30 días de parir. Después de producir sin pausas durante 2 a 3 años, pueden engordarse y las vacas Holando y cruza dan facilmente 600 kilos.

INTERRUPCION DE LA PREÑEZ EN VACAS COMPRADAS PARA INVERNAR

Los invernadores que adquieren vacas para el engorde, se llevan con ellas el problema de la preñez.Durante su castración el veterinario las encontrará servidas en toda la escala de meses.Todas las que presenten gestación hasta los 3 y 1/2 meses, pueden ser operadas sin riesgos de sus vidas. El aborto se produce entre los 3 a 4 días, coincidiendo con el fin de su convalescencia y quedarán en buenas condiciones para engordar.El veterinario que actúe en estos casos, deberá tener ya adquirida una buena práctica para operar las vacas gestantes, ya que absorben el doble de tiempo por la dificultad del peso del útero y captación de ovarios.-

LAS INSTALACIONES MINIMAS PARA UNA CORRECTA OVARIOTOMIA

No puede haber, no se pueden admitir fracasos en esta operación, a causa de malas instalaciones, que también hacen perder mucho tiempo en cada vaca.Se necesita un simple tubo criollo con 1 puerta lateral (mucho mejor si hay 2).Puede haber cepo o no existir; afortunadamente, NO es indispensable;

Se trabaja mejor sin "cepos" y se impide que la vaca retroceda colocando una cadena o barra de hierro por encima y detrás de los garrones.
Un detalle indispensable: UN BUEN PISO, firme, liso, libre de grietas o pozos; de cemento sería lo ideal. Si el piso es de tierra, después de operar 15 vacas se forma mucho barro: en este caso hay que interrumpir el trabajo y construír 1 "parrilla" de varillas de madera, que se pueda colocar y sacar, una vez terminado el trabajo. Esta debe tener 3 metros de largo para que tanto la vaca como el veterinario la usen.

CON CEPOS "INEFICIENTES" NO SE PUEDE TRABAJAR BIEN

Las consecuencias de un mal "cepo" son varias a saber: 1o) dejan escapar la cabeza de las vacas (en Uruguay son mochas), ocasionando mucha pérdida de tiempo. 2o) Cuando aprietan demasiado, producen desvanecimiento por isquemia cerebral, con otra importante pérdida de tiempo. 3o) Cuando son defectuosos, producen una contusión nerviosa que produce en la vaca que sale operada una parálisis de 1 o los 2 miembros delanteros. Si la parálisis es definitiva, la vaca se entrega y debe ser sacrificada.

UNA SIMPLE SOLUCION PARA LOS "CEPOS" DEFECTUOSOS

Todos esos inconvenientes se solucionan forrando toda la cavidad del cepo, con un faja de neumático de automóvil, a la que previamente se le suprimen los cables de acero del "talón". Esa faja de neumático se debe fijar con finos bulones, pero nunca con clavos que hieren los animales.

LAS ESTACIONES Y LA TEMPERATURA CON RELACION A LA TEMPERATURA.

La primavera, el otoño y el invierno, son las mejores épocas para efectuar esta operación, porque el tiempo fresco y frío es un silencioso colaborador del éxito. Si las vacas han parido en primavera y se castran 1 mes después, todavía NO se han recuperado físicamente, y no están pletóricas de sangre, estado que produce cierta hemorragia del corte vaginal. Ella carece de importancia pero es molesta, especialmente para el novel cirujano. El único factor de importancia, si se opera en verano, es la necesidad de ofrecer sombra al ganado operado, abundante y muy cerca, en pequeños potreros. Lo ideal es el monte natural con aguada. NO hay inconveniente en que el ganado operado coma y tome agua de inmediato a la intervención.

INSTRUCCIONES GENERALES PARA EFECTUAR LA OVARIOTOMIA

1) Con el ganado a operar: Es conveniente traerlo el día anterior a los corrales, a última hora, al caer la tarde, así estará descansado y con el rumen disminuído de volumen. Si el calor es muy intenso, se trae sólo la cantidad necesaria para 1 mañana de trabajo, y de mañana temprano, se trae otro lote para operar de tarde.

2) AGUA POTABLE O AGUA HERVIDA

El personal de la estancia NO tiene la obligación de conocer lo que es la asepsia, por lo tanto el veterinario debe supervisarlo todo: el origen del agua que servirá para la higiene vaginal, la limpieza de los envases para el traslado de esa agua. El agua para ese fin, debe ser simplenente potable, que se pueda beber: debe ser de origen de pozos semi-surgentes, NUNCA de brocal, pues están contaminados. La cantidad de agua: para uso desinfectante vaginal, 3/4 litro por animal. Para la higiene exterior de los genitales 1 litro por vaca, pudiendo ser esta última, NO potable, de río, etc.

3) EL HERVIDO DEL AGUA

Cuando el agua es de río, arroyo, etc., NO es potable, por lo tanto debe hervirse y siempre el día anterior. Es muy práctico hacerlo cerca del tubo, a unos 10 metros de distancia, y en bidones de 200 litros es lo ideal; si contenía combustibles, mejor, se en-

juaga y queda pronto. El recipiente debe tener tapa y sólo el veterinario y su ayudante pueden acceder a él. El resto del personal debe ser advertido de ese importante detalle.

4) TEMPERATURA DEL AGUA

Puede ser fría, tibia o bien caliente en invierno, para estimular las manos del cirujano, contra el frío.

5) MESA PARA EL INSTRUMENTAL DE CIRUGIA

Es suficiente de 1 mt. x 0,60 y si es un poco más amplia mejor. Sobre ella se colocan los recipientes con la solución antiséptica, el instrumental, frascos, etc.

6) RECIPIENTES

Se necesitan 4 de 8 litros aproximdos. Dos de ellos, deben ser perfectamente limpios, para uso del cirujano. Otro para el cepillado e higiene externa de genitales y el cuarto debe contener agua limpia simplemente, para el enjuague final de la vulva, antes de la operación.

7) TABLA-ASIENTO PARA EL OPERARIO QUE LEVANTA LA COLA

De 25 cms. de ancho y 1,50 de largo, se coloca atravesada en el tubo, sobre la vaca, a esos efectos, mientras dura la operación.

8) HERRAMIENTAS Y UTILES DE LA ESTANCIA

Siempre es indispensable tener a mano los implementos de uso corriente en los trabajos de campo: lazos tizas, serrucho, pala, escoba, mocheta, cepillo y tijera de esquilar, estos dos últimos esenciales para la higiene externa de genitales.

PREPARACION DE LOS ANILLOS DE GOMA (BORRACHINHAS)

Estas se pueden preparar el día anterior o también varias semanas antes del trabajo. Para su preparación con los anillos plásticos, consultar el capítulo correspondiente del libro.

ELECCION DEL ANTISEPTICO PARA LA OVARIOTOMIA

He trabajado con los principales antisépticos ofrecidos en el comercio veterinario: yodóforos, amarillo de acridina, cloruro de benzalconio, etc., pero he vuelto al ESPADOL a base de dicloro-xilenol, ricinoleato sódico, aceites esenciales vegetales, y un pequeño porcentaje de solución jabonosa. La causa principalísima de su elección es su instantánea acción hemostática, especialmente importante en la operación de vaquillas al efectuarse la "episiotomía", ésta consiste en el corte y agrandamiento del anillo vulvar, en su borde superior: se produce una fuerte hemorragia que es inmediatamente detenida con sólo 5 cc. de dicho antiséptico puro.

SIMULACRO DE OVARIOTOMIA SOBRE UNA MESA (PARA LOS APRENDICES)

Practicar la operación a la vista, ahorra a los noveles cirujanos, muchas situaciones confusas, a veces irrealizables, si intentan hacerla por vez primera en una vaca. Con un ovario ficto (que puede ser el simple nudo de un pañuelo) y leyendo pausadamente las instrucciones del Capítulo IX, inciso g) respecto a la localización de los ovarios y su extrangulación el aprendiz hará su práctica en forma previa, y desde allí a la realidad, sólo hay un paso, para su éxito.

EL DIA DE LA OPERACION Todos los detalles y preparativos importantísimos para iniciar este trabajo, están muy bien detallados en el

libro del autor sobre este tema.

LA CASTRACION DE VAQUILLONAS O VAQUILLAS.EL PORQUE DE SU INTERES

Pueden ser 3 las causas que la provocan: 1) Desinterés a nivel nacional en la cría de ganado, cuando sus precios son excesivamente bajos. 2) Vaquillas cruzas: es el destino lógico que muchas veces se da a las hembras nacidas como cruzas industriales entre razas. 3) Consumo propio: obedece al placer de faenar reses jóvenes, tiernas y con una gran calidad, para el consumo del establecimiento.-
Existen dos técnicas distintas para realizar la ovariotomía en vaquillonas: 1) por vía vaginal (pero siempre con episiotomía) y 2) por laparatomía lateral (o por el flanco), previo afeite del campo operatorio. El primer método rinde el doble de animales operados por hora de trabajo. Por ser muy extenso el tema, se explica en detalle en el libro del autor.-

LA INDIVIDUALIZACION ECONOMICA, PRACTICA Y PERMANENTE DE LA VACA

El corte de 1/3 de la oreja es el método más seguro y definitivo, para detectar en el campo una vaca castrada, porque se distingue a distancia y nunca hay confusiones.No se debe permitir que esta señal se realice antes de la operación, pues si la vaca NO se opera ya no tiene arreglo. Nunca se producen miasis por corte de orejas, nunca.

LA SANIDAD PARASITARIA INTERNA Y EXTERNA DE LA VACA PARA INVERNAR

Es indiscutible que la máxima conversión del alimento se obtiene con el ideal de sanidad parasitaria.En Uruguay, la parasitosis que más preocupa es el "saguaypé" o fasciola h.Muchísimas veces se sub estima, por su acción sub clínica. Garrapata y piojo succionando desconocidos volúmenes de sangre atrasan la invernada, y producción láctea: la medicación con ALBENDAZOLE en v.lecheras logró que la alimentación se incrementara en 52 lts.por vaca en 90 días de producción, eliminando la lombricosis,según experiencias de colegas brasileños en el Estado de Río de Janeiro(3)

EL PRIMER TRABAJO DE OVARIOTOMIA DE UN PROFESIONAL

Es absolutamente indispensable tener la práctica suficiente antes de lanzarse a efectuar el primer trabajo.Si el Veterinario tiene éxito en sus primeras vacas, conquistará para siempre la confianza en sí mismo y en su técnica y tendrá entusiasmo para difundir esta operación.Pero un fracaso creará en su mente un pesimismo al respecto y habrá cerrado para siempre, una importante especialización como profesional.Una buena práctica,previa al primer trabajo, se adquiere trabajando al lado de un profesional amigo o también realizando repetidas prácticas en frigoríficos o mataderos (que siempre colaboran) en vacas a faenarse ese mismo día o al siguiente.El aprendiz debe elegir animales magros, pués la vaca gorda dificulta el encuentro y manipulación de los ovarios.Las vacas lecheras, por su mansedumbre son las ideales para aprender.
En un trabajo particular NO SE DEBE insistir muchos minutos en operar un mismo animal, porque es peligroso para su vida: es preferible decir simplemente NO SE PUEDE OPERAR y adquirir MAS PRACTICA.

La ovariotomía es una verdadera transferencia de tecnología aplicada en el medio ganadero.Efectuarla con SEGURIDAD y sean cuales sean las dificultades que se presenten al profesional, será la mejor garantía de su progresiva difusión en el ámbito ganadero y un paso importante en la aplicación de técnicas de producción, de la ganadería de América.

RESUMEN El autor propone la alta difusión de esta sencilla operación de campo, la cual, técnicamente bien efectuada, no involucra riesgos de mortalidad.
Utiliza el instrumento "ovariótomo", ideado en 1908 por su padre, Dr. Marco Dutto, que aplica una pequeña ligadura elástica en cada cordón ovárico.
Con las sencillas instalaciones corrientes, cinco operarios y un instrumentista, el profesional especializado puede castrar de 200 a 300 vacas por día.
Las ventajas de esta operación son extraordinarias a saber:

PARA EL PAIS: Evita la inútil gestación en vacas de abasto, con pérdidas por nonato, envolturas,etc. de 15 kilos promedio.En Río Grande del Sur, en 5 años -1978/1982- constatóse el 60 % de preñez en vacas de abasto.Esa pérdida representó la cifra de 14.587 toneladas, o sea 2.587 toneladas anuales.Son cifras proporcionadas por el distinguido colega brasileño Oscar Silveira Gálares - Bagé 1986.-

PARA EL INVERNADOR:
1) Garantía para terminar el engorde sin preñez.
2) Aumentar el rendimiento de la res -similar al novillo- alcanzando del 54 al 58 % (carne con riñonada).
3) Acelerar el engorde: estimada entre 30 a 45 días la menor duración del mismo.
4) Crianza excepcional del último ternero macho: con sólo 16 meses alcanzan 380/400 kilos, sin raciones, sólo con excelentes forrajeras de invierno y verano y la permanente lactancia de la madre castrada, compartiendo las mismas forrajeras.(Hereford).
5) Mezclar vacas castradas con novillos en potreros de engorde.
6) Provocar el aborto sin riesgos en gestaciones hasta 3 y 1/2 meses, en vacas compradas para engorde.
7) Eliminar vaquillas descarte, cruzas, etc.

INSTALACIONES
Tubo y puerta lateral para cirujano.NO es indispensable el cepo.
Individualización: el mejor sistema permanente es despuntar un tercio de oreja.Nunca hay miasis.

PARA EL VETERINARIO
La castración de vacas representa una nueva, constante y vigorosa corriente de ingresos, que se complementa con otros trabajos de campo.-

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NOVO MÉTODO DE DESCORNA EM BOVINO ADULTO

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INTRODUÇÃO

QUIN (1945), relata um método de descorna muito cruento, indolor, com emprego de anestesia local. A contenção foi feita por cabresto, com animal em pé e aplicação de formigão. A hemostasia foi feita com o pinçamento do tronco da artéria cornual e o arrancamento brusco.

O'CONNOR (1950), indica a descorna para evitar traumatismo no momento da alimentação e para aumentar o valor comercial do animal. A anestesia é local. Podem aparecer complicações como sinusites.

WALKER (1960), recomenda anestesia do nervo cornual, antissepsia e tricotomia no local da cirurgia. A incisão da pele foi feita a uma polegada da frente do chifre, indo da borda dorsal à base. A segunda incisão era praticada na base ventral do chifre, estendo-se ventralmente. A incisão da pele foi feita circundando o chifre e dissecando a pele da sua borda, do osso frontal e da área entre o chifre e a base do olho. Os grandes vasos foram pinçados durante esse processo. A pele dissecada forma duas dobras que foram afastadas no sentido antero-posterior. O chifre podia ser serrado por uma serra no sentido crânio-caudal. Os vasos foram ligados e a pele saturada. Em seguida, retira-se o 2º chifre. Aos 10 dias foram retirados os pontos.

KAMAN & CERVENY (1962), informaram que a artéria cornual medial e lateral eram ramos terminais da artéria temporal superficial, dispostos em torno da base do chifre. O ramo das veias temporais superficiais não correspondiam às artérias.

GRIGORESCU et alii (1965), compararam os seguintes métodos de descorna: sutura de pele, amputação comum e compressão elástica. As principais vantagens foram para o método por sutura pericornual.

ROZTOCIL (1965), enfoca a influência da descorna na produtividade de vacas leiteiras. O descornamento sempre reduz temporariamente a produção de leite.

BUTLER (1967), informa que dois nervos são envolvidos na descorna de bovinos, os ramos infra-troclear e nervo zigomático-temporal. Um 3º nervo, que pode estar também envolvido, é o ramo dorsal do C_2. Seu ramo final inerva a pele, entre a base do chifre e a orelha. O nervo infra-troclear é um ramo do nervo Trigênio que inerva a região frontal, o osso frontal e um de seus ramos inerva a base dos cornos em animais chifrudos. O nervo zigomático-temporal passa através da fossa temporal sob a camada muscular que é aderida à pele. O ramo principal inerva o chifre e a pele ao seu redor, principalmente na parte caudal. A anestesia do nervo zigomático-temporal foi feita no ramo cornual. O resultado é a completa dessensibilização da área do chifre. O bloqueio dos ramos cornuais dos nervos infra-troclear e zigomático-temporal, na vaca, é simples e efetivo.

A remoção de cornos, para VILLAGRAN & MATAMOROS (1969), é prática comum no gado devido a estes atributos se constituirem em riscos permanentes para quem os manejam e para outros animais, tornando-os ainda mais estéticos e uniformes.

GODINHO E GETTY (1971), descrevem que o interesse anatômico nessa área foi provavelmente o motivado pela aplicação clínica, assim como a deter

minação dos sítios e áreas de infiltração anestésica. O nervo oftálmico origina-se no foramen órbito "rotundum" em um tronco comum com o nervo maxilar. Após a origem o nervo oftálmico dá as seguintes raizes: zigomático-temporal, lacrimal, frontal, ramo seio fraontal, muscular e nasociliar. O nervo zigomático-temporal emite vários ramos cornuais, que se distribuem na superfície lateral e caudal da base do chifre. O nervo infra-troclear (ramo do nasociliar) emite ramos para a pela da região frontal e os ramos cornuais, que estão distribuidos na superfície rostro lateral da base dos cornos.

Segundo MEYER (1973), o chifre, nos Alpes, é considerado atributo de beleza e nobreza da vaca. As condições para descornar bovinos devem ser: ausência de dor e complicações para o animal, uso de material adequado, tranquilidade no ambiente, pouca hemorragia, sem sequela e com resultados positivos, rapidez no método e economia.

LEHR (1974), descreve que a técnica deve ter a vantagem de exigir pequeno esforço físico, custo e tempo mínimos, controle perfeito do instrumento e fácil hemostasia. Não se observar complicações.

MEISCHKE; RAMSAY; SHAN (1974), estudaram o efeito da presença de cornos, em bovinos, na contusão de carcaças. Os animais do lote chifrudo apresentaram duas vezes mais contusão de carcaças.

LAZZERI et alii (1975), descreveram uma técnica de fácil execução e destituída de complicações pós-operatórias. Após a incisão cutânea fusiforme em torno da base do chifre, amputavam o corno por meio de serra de lâmina com hemostasia. A pele foi descolada e suturada por pontos separados em "U" em pé (sutura de Matress), com fio de algodão.

Para HICKMAN & WALKER (1978), o gado adulto deve ser descornado em pé, com anestesia local e contenção do animal em tronco.

Somente a partir de 1967, começou aparecer, o interesse dos fazendeiros pela descorna dos bovinos adultos. O método de descorna mais comum era a amputação dos cornos, empregando-se vários instrumentos, como fio serra, guilhotina, descornador de Barnes e outros. Não se fazia uso de hemostasia por pinçamento e nem a síntese da ferida. Mais tarde, introduziu-se a descorna cirúrgica plástica, com a publicação do primeiro trabalho nacional por LAZZERI et alii (1975); hoje a técnica é apresentada nos livros textos.

Um método eficiente de descorna deve ser indolor, livre de complicações, com material adequado, pouca hemorragia, sem sequela, resultados positivos, rapidez e economia.

O objetivo desto trabalho é apresentar modificações nas técnicas usuais, basicamente constituídas por cirurgia indolor, menos cruenta, mais estética e redução do tempo cirúrgico, permitindo ao animal deambular e alimentar-se após a intervenção cirúrgica. Considerando os resultados obtidos, julgou-se conveniente a publicação da presente pesquisa.

MATERIAL E MÉTODOS

O material empregado foi uma bandeja, um cabo de bisturi nº 4 e lâminas, um arco de serra de serralheiro com lâmina, duas agulhas de Uttrech médias, uma tesoura curva de ponta romba, uma pinça de dissecção com dente, uma pinça de Kelly de 18 cm, seringas descartáveis de 10 ml agulhadas, lidocaína com vaso constritor, fio de algodão, corda para contenção, penicilina associada a streptomicina, cicatrizante e repelente, antisséptico, sabão, depilador e lâmina de gillete.

Foram usados 50 bovinos, com idade acima de 24 meses, fêmeas, mestiços Zebu x Holandês. Os animais não foram submetidos a jejum prévio. A contenção dos animais foi feita segundo método de LAZZERI (1983). Após antissepsia da região frontal os nervos zigomático-temporal (nervo cornual) e o infra-troclear foram anestesiados por 10 e 5 ml de xilocaína a 2% com adrenalina, próximo a base do processo cornual, respectivamente. A tricotomia foi feita de modo que servio para demarcar o local exato onde os cornos seriam amputados. A seguir, fez-se uma incisão semi-circular iniciando na crista do occiptal passando pela base frontal do processo córneo, terminando na crista frontal próximo ao arco zigomático-temporal. A pele foi dissecada no sentido do osso frontal, a apófise córnea serrada até 3/4 de sua circunferência no sentido crânio-caudal e, em seguida, fraturada. O deslocamento da pele, da região posterior da base do processo córneo, conseguiu-se por movimento de alavanca em sentido caudo-cranial, tendo como ponto de apoio a base serrada. Pinçou-se a artéria temporal superficial e fez-se a ablação final do chifre. Coágulos e esquírulas ósseas, que poderiam penetrar nos seios frontais, foram removidos. Colocou-se antibiótico no seio frontal. Realizou-se a sutura com fio inabsorvível 4-0, iniciando o primeiro ponto no ângulo ventral da ferida, tendo a agulha penetrado na pele, tecido subcutâneo e sob a artéria temporal superficial, onde a pinça hemostática foi previamente aplicada, concluiu-se o primeiro ponto sem cortar o fio. Realizou-se o restante da sutura, fazendo-se uma sutura contínua simples em toda a extensão da ferida, mantendo-se cada passada de linha presa à mão. Em seguida procedeu-se da seguinte forma: a linha originária do primeiro ponto foi cortada e o cabo livre foi usado para dar o segundo ponto com a passada seguinte; novamente, cortada a linha, procedeu-se à idêntica manobra, obtendo-se o terceiro ponto e assim, sucessivamente, até a conclusão da sutura. Sobre a ferida suturada foi aplicado, por aspersão, um medicamento cicatrizante, repelente e larvicida (Lepecid) e, decorridos 12 dias, retiraram-se os pontos de 43 animais, sendo que, em sete, os pontos foram retirados aos 20 dias. Os animais foram mantidos em observação durante 4 meses.

RESULTADOS

Das 50 vacas descornadas 43 foram retirados os pontos aos 10 dias e 07 aos 20 dias. Observou-se em 18 vacas corrimento nasal sanguinolento nas primeiras 24 horas após a descorna. Nas sete vacas que os pontos foram retirados aos 20 dias observou-se em 05 vacas corrimento nasal sanguinolento nas primeiras 24 horas e pontos inflamados e em 02 vacas apenas pontos inflamados.

RESUMO

Foram descornados 50 bovinos, fêmeas, com idade acima de 24 meses, mestiços Holandês x Zebu, sem jejum prévio. A contenção dos animais foi feita segundo LAZZERI (1983). Após a antissepsia da região frontal, próxima à base do processo cornual, os nervos zigomático-temporal e infra-troclear, foram anestesiados com respectivamente 10 e 5 ml de lidocaína a 2% com adrenalina. A tricotomia foi adotada para determinar a simetria das amputações dos cornos. A seguir, fez-se uma incisão semi-circular, próximo à base frontal do processo córneo. A pele foi dissecada no sentido do osso frontal, a apófise corneal serrada até 3/4 de sua circunferência no sentido crânio-caudal e em seguida fraturada. O deslocamento da pele da região posterior da base dos cornos, conseguiu-se por movimento de alavanca em sentido caudo-cranial, tendo, como apoio, a base serrada. Pinçou-se a artéria temporal superficial e fez-se a ablação do chifre. Coágulos e esquírulas ósseas foram removidos. Colocou-se antibiótico no seio frontal. Realizou-se a sutura com fio de algodão 4-0, iniciando-se o primeiro ponto no ângulo ventral da referida, tendo a agulha penetrado na pele, tecido subcutâneo e sob a artéria temporal superficial, onde a pinça hemostática estava aplicada. Concluiu-se o primeiro ponto sem cortar o fio. Realizou-se o restante da síntese com sutura contínua simples, mantendo-se cada

passada da linha presa à mão. A linha originária do primeiro ponto foi cortada e o cabo livre foi usado para dar o segundo ponto (nó) com a passada seguinte: novamente cortada a linha, procedeu-se a idêntica manobra, obtendo-se o terceiro ponto e assim sucessivamente até a conclusão da sutura. Os pontos foram retirados a partir do 12º dia.

RESUMÉN

Fuerón descornados 50 bovinos, hembras, con edad superior a 24 meses, mestisos Holandes x Zebu, sín _ juno prêvio. La contensión de los animales fue hecha segun LAZZERI (1983). Después de una antisepsia en lã región rrontal, próximo a la base del proceso cornual, los nervios zigomático-temporal y infra-troclear fuerón anestesiados con respectiva cantidade de 10 y 5 ml de lidocaína a 2% asociada a adrelina. La tricotomia fue hecha para determinar la simetria de las amputaciones de los cuernos. Continuando se hizo una insición semi-circular, próximo a la base frontal del proceso córneo. La piel que disecada en el sentido del hueso frontal, la apófise córnea serrada hasta 3/4 de su circunferencia en el sentido crânio-caudal y en seguida fracturada. La disección de la piel en la región posterior de la base de los cuernos, se consiguió por movimiento de palanca en sentido caudo-cranial, teniendo como apojo, la base serrada. Se pinzó la artéria temporal superficial e se hizo la ablación de los cuernos. Cuágulos e esquírulas oseas fueron removidos. Se colocó antibiótico en el seno frontal. Se realizó la sutura con hilo de algodón 4-0, se inició el primer punto en el ángulo ventral de la herida, haviendo penetrado la aguja en la piel, tejido subcutâneo y por debajo de la artéria temporal superficial, donde la pinza hemostática estaba aplicada. Se completó el primer punto sín cortar el hilo. Se realizó el restante de la síntese con sutura contínua simples, manteniéndose cada pasada del hilo sujeta a la mano. El hilo originário del primer punto fue cortada y la extremidad libre fue usada para dar el segundo punto (nudo) con la pasada seguiente; nuevamente cortado el hilo, se procede a una idéntica manobra, obteniéndose el tercer punto e asî sucesivamente hasta la conclusión de la sutura. Los puntos fuerón retirados a partir del 12º dia.

SUMMARY

Fifty female bovines, half-breed Holandes x Zebu, aged alone 24 months, without previons fasting, were dehorning. Animals contention was performed according to LAZZERI (1983). After antisepsis os the frontal region, near the horny process base, the zygomatic-temporal and infra-trochlear nerves were anesthetized with 10 and 5 ml lidocaine 2% with adrenalin, respectively. The trichotomy was adopted to determine the symmetry of the horns amputation. After that, a semicircular incision was performed near the frontal base of the horny process. The skin was dissected toward the frontal bone, the corneous apophyasis sawed until 3/4 of its circunference in the cranio-caudal direction and then it was fractured. The skin displacement of the posterior region of the horn's base was obtained by lever movement, in the caudo-cranial direction, supported by the sawed base. The superficial temporal artery was pinched and the horn ablation was done. The coagulum and bone small were removed. Antibiotic was put on the frontal sinus. The suture was performed with 4-0 cotton cord, initiating the first knot in the ventral wound angle, having the needle penetrating the skin, subcutaneous tissue and over the superficial temporal artery, where the hemostatic tweezers was. The first knot was concluded without cutting the cord. The remainder synthesis was performed with countinous simple suture, Keeping each cord transpierce on the hand. The cord that comes from the first knot was cut and the free string was used to do the second knot with the following transpierce; the cord was cut again, the procedure was repeated again in the same way so the third knot was obtained and so on until the suture finished. The knot were removed from 12 days on.

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ERFAHRUNGEN MIT DER MARSUPIALISATION UND SPÜLUNG VON UMBILIKALVENENABSZESSEN MIT LEBERBETEILIGUNG BEIM KALB

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EINLEITUNG

Nabelentzündungen beim Kalb sind meist die Folge einer aszendierenden bakteriellen Infektion im Verlaufe der ersten Lebenstage (6). Die Technik der Nabeloperation wurde schon verschiedentlich beschrieben (1,2,3,5) und ist zu einem Standardeingriff geworden. Beim Vorliegen einer kompletten, d.h. bis in die Leber reichenden, abszedierenden Omphalophlebitis gestaltet sich die vollständige chirurgische Entfernung des Krankheitsherdes jedoch schwierig. Die Technik der paramedianen Marsupialisation wurde in der Literatur als mögliche Therapie solcher Fälle erwähnt (2).
Der Sinn der vorliegenden Arbeit ist es, die Erfahrungen mit unserer klinikiblichen Marsupialisationstechnik zu beschreiben, sowie Nachbehandlung und Langzeitresultate von 8 Kälbern mit Umbilikalvenenabszess zu diskutieren.

TIERE, MATERIAL UND METHODE

Kriterien für die Selektion der Fälle:
Zur Auswertung herangezogen wurden alle Kälber, welche die folgenden Kriterien erfüllten:
a) Zeitraum der Einweisung in die Veterinär-Chirurgische Klinik zwischen November 1987 und Oktober 1989,
b) Ultrasonographische und chirurgische Diagnose Omphalophlebitis mit Leberbeteiligung,
c) ungenügende Drainage des Nabelinhaltes,
d) kein Hinweis für eine Streuung des Infektes in andere Organsysteme.
Nicht zur Auswertung herangezogen wurden alle Kälber, welche
a) eine unvollständige, d.h. nicht bis zur Leber reichende Omphalophlebitis aufwiesen und
b) nach ultrasonographischer Diagnosesicherung aus wirtschaftlichen Gründen sofort verwertet wurden.

Patientengut:
Acht Kälber erfüllten die oben aufgeführten Kriterien. Es handelte sich dabei um 1 männliches und 7 weibliche Tiere verschiedener Rassen. Das Alter der Kälber betrug bei der Einweisung zwischen 1 und 9 Wochen, im Durchschnitt 5 Wochen (Tab. 1).

Untersuchung:
Bei allen Kälbern wurde am Eintrittstag eine klinische Allgemeinuntersuchung durchgeführt. Das Hauptaugenmerk galt jedoch der eingehenden Untersuchung des Nabels. Diese bestand aus Adspektion und Palpation des ausseren Nabels, sowie beidhändiger Palpation und sonographischer Untersuchung des inneren Nabels. Bei der Sonographie wurde so vorgegangen wie früher beschrieben (12,13).

Erste Operation:
Die Kälber wurden mit 0,2 mg/kg Körpergewicht (KGW) Xylazin intramuskulär sediert. Die Narkoseeinleitung erfolgte mit 2 mg/kg KGW Ketalar intravenös. Anschliessend wurden die Patienten endotracheal intubiert und in Rückenlage auf den Operationstisch gebettet. Die Narkose wurde mit einem Sauerstoff-Lachgas-Halothan-Gemisch weitergeführt und das Operationsfeld nach sterilen Kautelen vorbereitet.
Der äussere Nabel wurde mit dem Skalpell spindelförmig umschnitten. Nach Eröffnung der Bauchhöhle wurde auch der innere Nabel durch Lösen der Adhäsionen vom umliegenden Gewebe befreit. Anschliessend wurde die Operationswunde nach kranial entlang der linea alba bis ca. 3 cm kaudal des Xyphoids verlängert. Der Nabelstrang wurde an den kranialen Wundwinkel verlegt und dort mit Einzelknopfheften in zwei Lagen an der Bauchdecke befestigt. Als Nahtmaterial kam ein Glycolsäurecopolymerfaden der Stärke 2-0 zur Anwendung. Die Bauchhöhle wurde zur Prävention von Verklebungen mit 0,5 Liter Polyvinylpyrrolidon und Heparin (50 I.E./kg KGW), zur Verhütung von Infektionen mit einer Neomycin-Penicillin-Suspension (10 mg Neomycin/kg KGW und 20'000 I.E. Procain-Penicillin/kg KGW) versorgt. Die Bauchdecke wurde in der üblichen Weise (12,13) fortlaufend in drei Etagen verschlossen. Die Adaptation der Haut erfolgte mit einem Hautklammergerät.

Nachbehandlung:
Die antibiotische Versorgung mit Penicillin (60'000 I.E./kg KGW), welche spätestens 2 Std. vor der Operation einsetzte, wurde noch bis zum 3. Tag post operationem weitergeführt. Der nach extraabdominal verlagerte Anteil des Nabelstranges wurde, je nach Patient, 2 bis 120 Std. post operationem ca. 1cm von der Bauchdecke entfernt abgesetzt. Dies erlaubte das Abfliessen von angestautem Eiter. Nach der Marsupialisation wurde ein Schlauch in das Lumen der Umbilikalvene eingeführt, möglichst weit Richtung Leber vorgeschoben und an der Haut fixiert. Ueber diesen Schlauch wurde die Nabelvene täglich mit 1 Liter einer 10%igen Polyvinyl-Jod-Lösung unter Druck gespült, bis die Spüllösung über mehrere Tage klar zurückfloss. Anschliessend wurde der Spülschlauch entfernt und das Tier nach Hause entlassen.

Zweite Operation:
Die zweite Operation erfolgte 1 bis 2 Monate nach dem ersten Eingriff. Narkose und Lagerung des Patienten wurden genau gleich durchgeführt wie bei der Erstoperation. Der verbliebene Nabelvenenstrang wurde umschnitten, freipräpariert und möglichst nahe an der Leber abgesetzt. Die Bauchdecke wurde in der üblichen Weise verschlossen.

Nachkontrolle:
Diese erfolgte telefonisch beim Besitzer je nach Patient 6 1/2 bis 20 Monate nach dem ersten Eingriff.

RESULTATE

Anamnese, Signalement und Eintrittsbefunde:
Anamnese, sowie Signalement und die wichtigsten Eintrittsbefunde der 8 Kälber sind in Tabelle 1 aufgeführt.

Befunde bei der Erstoperation:
Die präoperativ ultrasonographisch erhobenen Befunde an der V. umbilicalis, sowie die Mitbeteiligung der Leber am Krankheitsgeschehen konnten ausnahmslos bestätigt werden.
Bei allen 8 Patienten lagen grossflächige Verklebungen zwischen Netzanteilen, parietalem Bauchfell und der infizierten Nabelvene vor.

Postoperative Phase: Die Resektion des nach extraabdominal verlagerten Nabelanteiles erfolgte bei Kalb Nr.5 nach 2 Std., bei allen anderen

Kälbern frühestens 48 Std. nach der Operation. Es dauerte 8 - 17 Tage bis kein Eiter mehr aus der Abszesshöhle herausgepült werden konnte. Von 6 Patienten wurde die tägliche Nabelspülung mit verdünnter Polyvinyl-Jod-Lösung gut ertragen. Die übrigen 2 Kälber (Nr.6 und 8) reagierten jedoch mehrmals mit einer perakut auftretenden Verschlechterung des Allgemeinbefindens, welche jeweils noch während oder unmittelbar nach der Spülung auftrat. Folgende Symptome konnten festgestellt werden: Anstieg der Pulsfrequenz, der Atemfrequenz, der Körpertemperatur, Saugunlust, Streckkrämpfe und Festliegen. Die Gesamtleukozytenzahl stieg von 14'800 Z/ul beim Eintritt auf 26'000 Z/ul nach dem Anfall bei Kalb Nr.6, resp. von 12'500 Z/ul auf 17'100 Z/ul bei Kalb Nr.8. Innerhalb von 12 bis 24 Std. trat jeweils bei beiden Kälbern wieder eine Normalisierung des Allgemeinbefindens ein. Während Kalb Nr.6 diese Episoden überlebte, musste Kalb Nr.8 während eines solchen Anfalls in extremis euthanasiert werden. Die Sektion von Kalb Nr.8 ergab folgende pathologisch-anatomischen Diagnosen: Nabelvenenabszess mit Durchbruch in die Portalvene und die hintere Hohlvene, multiple Leberabszesse, hochgradige, akute, interstitielle Pneumonie.

Wundheilung:
Die Operationswunde heilte bei 7 Kälbern gut ab. Bei Kalb Nr.5 kam es zur Bildung mehrerer kleiner Abszesse im Operationsgebiet. Die Dauer zwischen dem Ersteingriff und dem Absetzen des extraabdominalen Nabelanteiles betrug bei diesen Tier lediglich 2 Stunden.

Befunde bei der Zweitoperation:
Bei 4 von 8 Kälbern (Nr.1 bis 4) wurde die Restnabelvene in einem Zweiteingriff entfernt. Die Zeitspanne zwischen den beiden Operationen betrug zwischen 29 Tagen bei Kalb Nr.2 und 58 Tagen bei Kalb Nr.4. Bei 3 Kälbern (Nr.1, 2 und 4) bildete sich als Folge der Erstoperation eine Bauchdeckenhernie im Gebiet der Marsupialisation. Der Maximaldurchmesser der Umbilikalvene war bei allen Tieren gegenüber dem Ersteingriff deutlich kleiner geworden (Tab.1 und 2) und ein Inhalt fehlte. Bei zwei Kälbern (Nr.1 und 4) war das Venenlumen am Eingang zur Leber obliteriert (Tab. 2).

Langzeitresultate:
Die Kälber Nr.1 bis 4 erholten sich gut. Sie wurden zur Aufzucht verwendet. Gewichtszunahme und Allgemeinbefinden wurden von den Besitzern als gut beurteilt. Kalb Nr.5 erholte sich ebenfalls gut. Es wurde gemästet und im Alter von 12 Monaten geschlachtet. Kalb Nr.6 wurde schon 3 Mte. nach der Erstoperation geschlachtet, da seine Gewichtszunahme weit unter den Erwartungen zurückblieb. Ein Schlachtbefund fehlt, der Schlachtkörper konnte jedoch nicht für den menschlichen Verzehr freigegeben werden. Die Kälber Nr.7 und 8 mussten noch während der Behandlung euthanasiert werden. Ein direkter Zusammenhang zur Omphalophlebitis bestand nur bei Kalb Nr.8, während Kalb Nr.7 aufgrund eines Dünndarmileus getötet werden musste.

DISKUSSION

Beim Vorliegen einer abszedierenden Omphalophlebitis mit Leberbeteiligung ist die Forderung nach einer chirurgischen Totalsanierung des Infektionsherdes gleichbedeutend mit einer partiellen Leberresektion. Da es sich dabei um einen risikoreichen Eingriff handelt, empfehlen verschiedene Autoren die Marsupialisation des Abszesses (1,2). Auch in der Humanpädiatrie wird bei Vorliegen eines Leberabszesses die chirurgische Drainage als Therapie der Wahl angesehen (7,9,15).

Unsere Operationstechnik unterscheidet sich in mehreren Punkten von der in der Literatur beschriebenen (2). Die Nabelvene wurde lediglich nach kranial verlegt. Auf die gleichzeitige Verschiebung nach paramedian rechts wurde verzichtet. Die Kranialverlagerung war durch eine Verlängerung der medianen Inzision leicht realisierbar. Die Gefahr einer Kontamination der Bauchhöhle konnte mit dieser Technik gering gehalten werden, da der extraabdominale Nabel nie mit der Bauchhöhle in Kontakt kam. Die zusätzliche Verschiebung nach rechts erschien uns unnötig, da der Sekretabfluss auch mit unserer Technik gewährleistet war.
Die Resektion des extraabdominalen Anteiles erfolgte in der Regel nicht unmittelbar nach der Operation, sondern erst frühestens nach 48 Std. Mit dieser Massnahme sollte das Infektionsrisiko im frischen Operationsgebiet reduziert werden. Die Inzision heilte bei 7 Kälbern komplikationslos ab. Die Entstehung multipler Abszesse bei Kalb Nr.5 führen wir darauf zurück, dass dessen Nabelstumpf ausnahmsweise schon 2 Std. post operationem entfernt wurde.
Die bei allen Patienten wiederholt durchgeführte Spülung des marsupialisierten Abszesses mit verdünnter Polyvinyl-Jod-Lösung unter Druck erwies sich in einem Fall (Nr.8) mit Sicherheit und in einem weiteren Fall (Nr.6) wahrscheinlich als folgenschwerer Fehler. Physiologischerweise obliteriert nach der Geburt der Ductus venosus arantii aufgrund der veränderten Strömungsverhältnisse des Blutes. Gleichzeitig kontrahiert sich die V. umbilicalis, was zum Verschluss dieses Gefässes führt. Der beschriebene Prozess ist etwa im Alter von 3 Wochen abgeschlossen (11). Wird jedoch vor dessen Beendigung eine Flüssigkeit unter Druck in die Vena umblilicalis injiziert, so kann diese den Weg des sauerstoffreichen Blutes im fötalen Kreislauf nehmen und in den linken Hauptstamm der V. portae, möglicherweise sogar via Ductus venosus in die V. cava caudalis gelangen (8). Bakterien und Polyvinyl-Jod-Lösung erreichen so den grossen Kreislauf. Dies führt zu einer anaphylaktischen Reaktion und zu einer fatalen Streuung und Ansiedlung von Keimen im Organismus, wie es bei den Kälbern Nr.6 und 8 geschah.
Diese Problematik ist in der Humanpädiatrie bestens bekannt. Beim Kind entstehen Leberabszesse vorwiegend als Folge des Nabelvenenkatheterismus (10). Bei der Behandlung wird deshalb nur die Drainage, nicht jedoch die Spülung empfohlen (7,9). Bei älteren Kälbern (> 2 Monate) mit Omphalophlebitis darf mit einiger Sicherheit erwartet werden, dass die Involution des fötalen Kreislaufs abgeschlossen ist und sich gleichzeitig der Entzündungsprozess abgekapselt hat. Ab diesem Alter ist die Gefahr eines Durchbruches der Spülflüssigkeit ins Gefässystem minimal. Eine Analyse der Altersverteilung unserer Patienten erhärtet diese Annahme. Die Kälber, welche mit einer Komplikation auf die Spülung reagierten, waren 2 1/2 resp. 3 Wochen alt, während bei allen 3 Tieren, welche 9 Wochen alt waren die Spülung problemlos verlief (siehe Tab. 2). Daraus kann geschlossen werden, dass die Spülung eines marsupialisierten Leberabszesses unter Druck bei Kälbern die jünger sind als 2 Monate nicht durchgeführt werden sollte.
Mit der Zweitoperation konnte in allen 4 Fällen der Infektionsherd vollständig entfernt werden. Gleichzeitig konnte die als Folge der Erstoperation entstandene Hernie beseitigt werden. Andere Autoren erachten eine Zweitoperation als nicht notwendig (2,14). Obwohl Kalb Nr.5 auch ohne Zweitoperation eine normale Rekonvaleszenz aufwies, erscheint uns bei Aufzuchtkälbern der zusätzliche Aufwand dieses Eingriffes gerechtfertigt, da die Rezidivgefahr reduziert werden kann.
Die Ultrasonographie erwies sich, wie früher schon beschrieben (4,12,13), auch bei diesen Patientengut als sehr wertvolle Zusatzuntersuchung des inneren Nabels. Sie ermöglichte die Diagnosestellung bei allen 8 Patienten mit grosser Sicherheit.

Tabelle 1

Signalement, Anamnese und Eintrittsbefunde bei 8 Kälbern mit Umbilikalvenenabszess

Kalb Nr.	1	2	3	4	5	6	7	8
Geschlecht	w	w	w	w	w	m	w	w
Alter (Wo)	9	9	9	1	2	3	3	2,5
Rasse	BV	BV	BSH	BV	HF	BSH/BV	BV	HF
Ansteckende Verhaltensstörung	ja	ja	ja	ja	ja	ja	ja	ja
Innere Körpertemperatur (°C)	39,2	39,8	38,8	39,8	40,2	39,5	39,5	39,6
Herzfrequenz (pro Min.)	84	128	96	132	136	90	80	92
Atemfrequenz (pro Min.)	32	60	56	60	42	40	32	32
Singultus	n	n	mtl	mtl	mtl	mtl	n	mtl
Drainage durch Nabelfistel	fehlt	fehlt	eingeklappt	fehlt	fehlt	eingeklappt		
Maximaldurchmesser der Nabelvene (mm) vertikal bei der Erstoperation	50	20	40	30	40	50	35	15
Maximaldurchmesser der Nabelvene (mm) horizontal bei der Erstoperation	60	25	40	30	45	30	35	30

w = weiblich, m = männlich, BV = Braunvieh, BSH = Red-Holstein, HF = Holstein-Friesian, n = normal, mtl. = mühsam

Tabelle 2

Therapeutisches Vorgehen und Behandlungsresultate bei 8 Kälbern mit Umbilikalvenenabszess

Kalb No.	1	2	3	4	5	6	7	8
Zeitspanne zwischen 1. Op und Kenntnis des entnekrotisierten Nabels (in Std.)	48	48	120	72	2	48	48	72
Dauer der Nabelspülung (in Tagen)	14	37	9	14	51	8	-	13
Resultat	ob	ob	ob	ob	ob	2 tgl. nach Kalbes Abszess	ob	ob
Reaktion auf Druck-Spülung des Nabels	stark	stark	stark	stark	stark	nicht	ja	stark	ja
Hernienbildung	ja	ja	stark	ja	verschwind.	stark	stark	
Zeitspanne zwischen 1. und 2. Operation (in Tg)	30	29	34	38	-	-	-	-
Maximaldurchmesser der Nabelnarbe bei der Zweitoperation (mm) vertikal	15	8	20	13	-	-	-	-
Maximaldurchmesser der Nabelvene bei der Zweitoperation (mm) horizontal	15	3	28	15	-	-	-	-
Durchgängigkeit der Umbilikalvene	stark	ja	ja	stark	ja	-	-	-
Zeitspanne zwischen 1. Op und Nachfrage resp. Schlachtung (Monate)	11	20	14,5	6,5	12	3	0,3	2,5
Behandlungsresultate	gut	gut	gut	gut	gut	ungen.	Kachexie	

ob = ohne Besonderheiten, schwk. = schwankend, ungen. = ungenügend

ZUSAMMENFASSUNG:

Die Krankheitsverläufe von 8 Kälbern mit Omphalophlebitis mit Leberbeteiligung werden beschrieben und diskutiert. Die Diagnose wurde mit Hilfe der Ultraschalluntersuchung des inneren Nabels und der Leber gestellt. Die Therapie erfolgte in mehreren Schritten: Marsupialisation des Umbilikalvenenabszesses, Spülung desselben und Resektion der kollabierten Umbilikalvene. Bei 5 Kälbern war das Behandlungsresultat gut, 1 Kalb musste wegen eines Dünndarmileus euthanasiert werden, bei den restlichen 2 Kälbern trat eine folgenschwere Komplikation ein. Ein Teil der unter Druck injizierten Spüllösung gelangte bei diesen 2 Patienten in den systemischen Kreislauf und führte zu einer anaphylaktischen Reaktion und zu einer Keimstreuung im Organismus. Die Spülung des Nabelvenenabszesses darf deshalb bei Kälbern die jünger sind als 2 Monate nicht unter Druck durchgeführt werden.

SUMMARY:

Eight cases of calves with omphalophlebitis involving the liver are recorded and discussed. The inf - tion of the umbilical vein and the liver was diagnosed ultrasonographically, with enlargement and ec - genic material imaged within the vein. Treatment consisted of several steps: Marsupialisation of the a- fected umbilical vein, daily flushing of the drained abscess until healing occured, and excision o' be contracted umbilical vein. Five calves were clinically healthy after treatment, one calf had to be e - natized because of an intestinal incarceration and two calves suffered from a fatal complication: a part of the lavage fluid which was applied under pressure was flushed into the blood circulation of the two patients. Both calves showed signs of an anaphylactic and septic shock. One had to be euthanized immediately, the other had to be slaughtered 3 months later. With calves younger than two months the umbilical vein abscess should not be flushed under pressure.

RESUME:

On décrit l'évolution de 8 veaux presentants une omphalophlébite avec atteinte du foie, diagnostiquée par ultrasons. La thérapie est effectué en plusieurs étapes: marsupialisation de l'abscès de la veine ombilicale, rinçage puis résection de la veine ombilicale collabée.
On a obtenu un bon résultat chez 5 veaux, 1 veau a été euthanasié suite à un iléus de l'intestin grêle. 2 veaux ont présenté de graves complications: une partie de la solution de rinçage, injectée sous pression, a atteint chez ces 2 patients la circulation systémique. Elle a ainsi provoqué une réaction anaphylactique de même qu'une dispersion du germe dans l'organisme. Pour cette raison, le rinçage des abscès de la veine ombilicale ne doit pas se faire sous pression chez les veaux âgés de mois de 2 mois.

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MEASUREMENTS OF CALF SIZE IN BELGIAN BLUE CALVES AS A MEANS OF PREDICTING THE INCIDENCE OF DYSTOCIA

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INTRODUCTION

Dystocia (prolonged or difficult parturition) is a major cause of mortality of calves (1,3,7,12). Dystocia is affected by a complex of variables, some of which have a more pronounced effect at first calving, e.g. foeto-pelvic incompatibility and others affecting all parities equally, e.g. malpresentation (7). Calf weight as a measure of calf size is the factor most highly correlated with calving difficulties (8,10). Beef cattle seem to be more prone than dairy and dual-purpose cattle to dystocia asociated with foetal disporportion (10) particularly double-muscled cattle (2,5,8). This reported higher incidence of dystocia in cattle with double-muscling may be attributable to the dam or the calf (5) . There was a higher incidence of dystocia in double-muscled cows mated to double-muscled bulls (2) attributable to the smaller area of pelvic opening in the dam.

Hindson (6) found a good correlation between interischial distance and both the horizontal and vertical pelvic diameters whereas other workers (4) have found a low correlation between external measurements and dystocia. Others (2) have used pelvimetry to predict the likely incidence of dystocia.

The Belgian Blue calf has increased in popularity in the UK in the last few years because of its double-muscling characteristics and hence higher than normal amount of lean meat. This breed is associated with a high incidence of dystocia. There are clearly several characteristics of the sire, dam and calf that could be responsible. The purpose of the present study was to compare various measurements of the newborn calf with its birthweight. The study was on calves born by elective Caesarean so measurements could be compared with pelvic anatomy of the dam as a means of predicting the necessity for Caesarean section.

MATERIALS AND METHODS

Animals Used

The recipient dams studied were Friesian or Hereford-Friesian heifers and cows kept in a commercial herd of 160 milking cows. They had been implanted with pure Belgian Blue calf embryos. They were housed indoors in yards during the winter housing period and kept at grass in summer. A month before the expected calving data they were brought into a yard and kept together with other periparturient cattle. Regular observations of the dams were carried out at intervals thoroughout the day and night starting two weeks before the calving date.

Elective Caesarean section was performed as the dam went from first to second stage labour. In the Friesian crosses the udder development was less pronounced than in the pure Friesian dams. The onset of second stage labour was usually confirmed when the dam stood with her tail raised for over half an hour.

The dams were brought to the University Veterinary Hospital for elective Caesarean section under paravertebral anaesthesia. Following delivery the cow and calf were kept together in loose-boxes for six months.

Dimensions of cow and calf

The recipient dam and its pure Belgian Blue calf were measured immediately following delivery.

Calf: The average digital diameter taken at the fetlock level of the calf and the average diameters of the carpii and hocks were recorded using calipers. The distance between the supraorbital ridges of the eyes and the circumference of the dome of the head and the chest circumference at the level of the point of the elbow and in front of the hips were also measured. The distance between the tuber coxae, the interischial distance and the distance between the lateral wing of the ilium and the lateral tuber ischii were also measured in the calf. Finally the calf was weighed.

Cow: The distance between the tuber coxae (see 11), the interischial distance (see 6) and the distance between the lateral wing of the ilium and the lateral tuber ischii were measured in the recipient dam.

RESULTS

There was good correlation between calf weight and digital diameter ($P<0.001$) (Table 1, Fig. 1). The calf weight and calf digital diameter were both correlated with the carpus ($P<0.05$) and hock ($P<0.01$) diameters, the distance between the supraorbital ridges ($P<0.05$), the circumference of the dome of the head ($P<0.001$) and of the chest ($P<0.001$) (Fig. 2). The digital diameter was also correlated with the distance between the wing of the ilium and the ischium ($P<0.05$) (Table 1). The digital diameters were very similar in each fetlock joint of the calves studied.

The mean calf weight 48.4 $\pm$ 1.40 (SEM) kg was significantly correlated with the sex of the calf ($P<0.01$).

Pelvic dimensions of the foetus showed no correlation (Table 2) whereas in the recipient dam the distance across the tuber coxae and distance between the wing of the ilium and the ischium were related ($P<0.05$)(Table 3).

The calf's weight was significantly correlated with the cow's tuber coxae measurement (Table 4, Fig. 3) but not with the interischial distance. The digital diameter was not related to any of these measurements.

TABLE 1. Correlation between various measurements of the neonatal calf n=22

	Digital diameter	Carpus diameter	Hock diameter	Supra-orbital ridges	Dome of head circumference	Chest circumference	Hip circumference	Inter-ischial distance	Tuber coxae	Ilium to ischium
Calf weight	P<0.001	P<0.05	P<0.01	P<0.05	P<0.001	P<0.001	NS	NS	NS	NS
Calf digital diameter		P<0.05	P<0.01	P<0.05	P<0.001	P<0.001	NS	NS	NS	P<0.05

NS = not significant

In addition
- hock and carpus diameter — P<0.05
- chest and hips circumference — P<0.01
- supraorbital distance and circumference of the dome of the head — NS

FIG 1. RELATIONSHIP OF CALF BODY WEIGHT AND DIGITAL DIAMETER

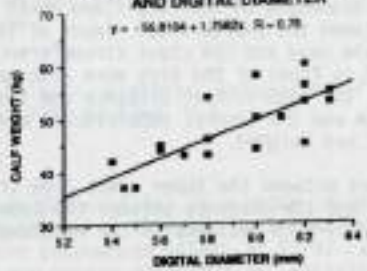

FIG 2 RELATIONSHIP OF CALF BODY WEIGHT AND CHEST CIRCUMFERENCE

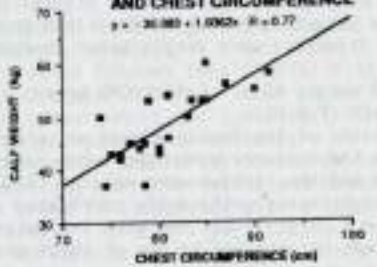

FIG 3 RELATIONSHIP OF CALF WEIGHT AND DAM TUBER COXAE DISTANCE

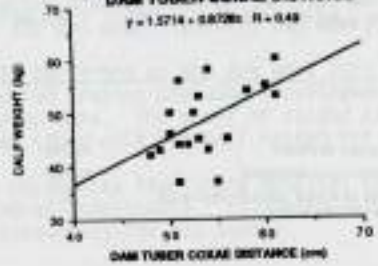

TABLE 2. Correlation between the pelvic measurements of the foetus.

	Tuber coxae distance	Ilium to ischium	Hip circumference
Interischial distance	NS	NS	NS
Tuber coxae	-	NS	-
Hip circumference	NS	NS	-

TABLE 3. Correlation between the pelvic measurements taken on the recipient dam n=22

	Tuber coxae distance	Ilium to ischium
Interischial distance	NS	NS
Tuber coxae	-	P<0.05

TABLE 4. Correlation between the neonatal calf and the dam pelvic measurements n=22

Dam	Interischial	Tuber coxae	Ilium to ischium
Calf			
Weight	NS	P<0.05	NS
Digital diameter	NS	NS	NS
Hip circumference	NS	NS	NS
Chest "			

DISCUSSION

The calf's birthweight in pure Belgian Blue calves can be estimated from its digital diameter as has been shown previously in other breeds (6,11). The preliminary results indicate that the digital diameter can also give an accurate indication of the size of the calf's head and chest circumference but not of the size of the pelvis which is important in double-muscled calves (5). This is useful as this measurement can be readily obtained prior to calving the recipient cow.

Birth weight, however, is not clearly associated with ease of calving in various breeds, (8,11), i.e. large calves were not closely associated with difficult calvings. The dam:calf weight ratio has not always been associated with ease of calving (11), although useful in some surveys (4).

The calf's weight was correlated with the sex of the calf in the present study. Previous studies (3) indicate a higher preponderance of male calves being involved in dystocia. Skeletal measurements are more highly heritable than birth weight (9) but their correlation with dystocia was much lower. This suggests that in a borderline case calf anatomy would be important.

The Friesian dam has the highest calving problems especially at first calving and the highest relative birth weights (4). The dam pelvic opening explains 10% of the variation in calving difficulty (8). The pelvis is late maturing and its growth keeps pace with or exceeds increases in body weight between 2 and 6 years which is why foeto-pelvic disproportion is less important in multiparous cows than in heifers. Pelvic area is positively correlated with body size, however larger cows with larger pelvises tend to have correspondingly larger calves. The interaction of these factors and variations with breed or age is important in predicting the necessity for Caesarean section. The fact that in the present study the recipients varied in age and some were crosses was compensated by using foetal-maternal comparative measurements (Table 4).

A good correlation has previously been shown between interischial distance and both horizontal and vertical pelvic diameters (6). However in the present study the interischial distance had no positive predictive value for foeto-maternal incompatibility (Table 4). This measurement has shown a high degree of random variation in various breeds (11). The high degree of correlation between birth weight and tuber coxae measurement (Fig. 3) which has been recorded in various breeds (11) will have a positive predictive value. This can be easily measured in the recipient dam and the calf's birth weight assessed from digital diameter measurements before calving. It is surprising however that the calf's digital diameter and tuber coxae measurements were not correlated. Differences in condition score important in measuring the interischial distance (4) could be less subject to error in measuring the tuber coxae distance or the dam weight using weigh bands. Evidence suggests that double-muscled cows have a smaller pelvic opening (2).

Hindson (6) showed that if the traction ratio fell below 2.3 surgery may be indicated. In view of the present findings this formula may not be valid for Belgian Blue calves in recipient dams.

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SUMMARY

Various measurements of calf size were made on Belgian Blue calves delivered by elective Caesarean section from recipient Friesian or Hereford-Friesian dams. The digital diameter was correlated with calf weight (P<0.001), distance between the supraorbital ridges (P<0.05), chest circumference (P<0.001) and diameter of the carpus (P<0.05) and hock (P<0.01). The calf weight was correlated (P<0.05) with the distance across the tuber coxae in the dam.

RESUMEN

Varias mediciones de terneno fueron hectues en feneros de la seza Azul Belge, los cueles hebión merido por medio de intervenciozies reserces electivas de madres Frisonas o cruzes de Hereford/Frison. El diametro digital estuvo correlocionedo con el peso del sernero (P<0,001), con le distoncie en tu los ar cos supraorbitales (P<0.05), le cir cumferencie torotica (P<0,001), y el diazuetio del corpo (P<0,05) y del tarso (P<0.01). El peso del ter nero estuvo correlocioueda (P<0,05) con le distoncie entre les tuberosidodes coxalesde le modu.

RÉSUMÉ

L'mesurage différent de dimension des veaus bleu de Belgique sont delivrer par opération césarienne des recevant mère Friesian ou Hereford-Friesian. Le dianètre digital se montrer la corrélation avec les poids du veau (P<0.001), la distance entre de l'elévation de l'orbite (P<0.05), la circonférence poitrail (P<0.001) et le diamètre des carpes (P<0.05) et des jarrets (P<0.01). Les poids du veau se montrer la corrélation avec la distance en travers des tubérosités coxae des mères.

ESTUDO SOMATOMÉTRICO EM BÚBALOS. III. CORRELAÇÕES ENTRE AS CARACTERÍSTICAS DO TIPO

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INTRODUÇÃO

A descrição mortométrica de cada raça, nos concede a oportunidade de avaliar a proporcionalidade entre as áreas ou regiões do corpo dos animais. Além disso, podem revelar as interrelações existentes entre essas regiões, resultando assim no equilíbrio exigido para descrever os animais de determinada raça e a caracterização do seu tipo econômico - leite ou corte.

No caso das raças bubalinas (Jafarabadi, Murrah e Mediterrânea) não há no Brasil o conhecimento das medidas zoométricas, que podem vir auxiliar na caracterização de cada uma delas, bem como, contribuir na definição da forma com a função de raças.

Entre os objetivos do presente projeto incluiu-se a descrição dos diferentes grupos genéticos de bubalinos, através da mensuração das características do tipo.

MATERIAL E MÉTODOS

Para a realização do presente estudo, contou-se com a mensuração de 150 fêmeas adultas registradas, com idades superiores a 40 meses, presentes nas exposições de São José do Rio Preto, Tiete, Araçatuba, Itapetininga e Criatórios de Búfalos do Estado de São Paulo. Os tres grupos genéticos contou com o número igual de indivíduos. As medidas tomadas foram: peso corpóreo; altura no sacro (ASAC); altura no garrote (AGAR); comprimento do corpo (CCOR); circunferência torácica (CTOR); circunferência abdominal (CABD); comprimento da cabeça (CCAB); largura da cabeça (LCAB); largura da anca; circunferência da canela (CCAN); distância de rótula a rótula (ROTU); comprimento da garupa (CGAR) e largura torácica (LTOR). Os dados foram ajustados pelo método dos quadrados mínimos, tendo como causa de variação os grupos genéticos, e a regressão linear da idade sobre as características mensuradas, além disso, determinou-se as correlações simples e parciais entre as variáveis dependentes.

RESULTADOS

As correlações do peso e medidas corporais são mostradas na Tabela 1, sendo representadas acima da diagonal, as correlações simples e abaixo desta as correlações parciais, ambas ajustadas para as variáveis raça e idade.

As correlações simples e parciais do peso com as demais medidas, mostraram-se significativas ($P \leq 0,01$), sendo que os maiores valores foram encontrados em relação a ASAC, CCOR, LTOR, LANC e CGAR; r=0,60; 0,62; 0,64; 0,65 e 0,68 respectivamente. Estes resultados acham-se em concordância com os encontrados por Peeva & Vanikov (7) para bubalinos da raça Murrah. As medidas de ASAC, LANC e CGAR, representam as regiões onde se localizam os músculos classificados como sendo superiores. O animal que apresenta equilíbrio ou porporcionalidade entre a altura no sacro, comprimento de garupa e largura mais pesado. o LTOR, indica a capacidade respiratória do animal e a possibilidade

Como pode-se observar, houve efeito significativo ($P<0,01$), para Peso, alturas e distância de rótula a rótula, enquanto a idade, mostrou exercer efeito significativo apenas para o peso corporal (b=0,849). Como foram amostradas búfalas com mais de 40 meses, estas já tinham completado a fase de crescimento do esqueleto, que segundo dados encontrados no Relatório Técnico de Melhoramento de Bubalinos no Trópico - FINEP - FMVZ - Botucatu (4), é completada entre os 24 a 36 meses. A partir dessa idade há uma tendência do acúmulo de gordura na carcaça, refletindo no peso corpóreo, que é grandemente influenciado, dentre outros, pelo estado fisiológico, alimentação, a raça e sua aptidão.

A Tabela 2, apresenta as médias ajustadas das mensurações e pesos.

Tabela 2 - Médias ajustadas das mensurações e pesos

Medidas	Raças		
	Jafarabadi	Murrah	Mediterrâneo
Peso (kg)*	716,20a	669,00b	618,80c
Altura no sacro (cm) **	144,30a	139,00b	141,50c
Altura no garrote (cm) **	142,00a	137,30b	139,00b
Distância rótola a rótula (cm) **	114,95a	115,24a	109,77b

- Médias comparadas pelo Teste Tukey
- Letras iguais na mesma linha não diferiram estatisticamente
- ** $P<0,01$

Os resultados encontrados para a raça Jafarabadi, diferem dos citados por RanjHan & Pathak (3) onde o peso médio encontrado para as fêmeas adultas em rebanhos indianos foi de 454 kg, contra 716,20 ±13,6 kg do presente estudo. Esta grande variação, pode estar influenciada pelo efeito ambiental (principalmente nutricional) sobre as raças Indianas (Murrah e Jafarabadi) exploradas no Brasil. No caso dos animais Murrah, Sampaio (5) e Cockrill (2), citam que os rebanhos indianos desta raça são de porte médio para pequeno, com peso médio de 360 a 400 kg, sendo que em nossas condições são comuns animais de maior estrutura e peso, podendo ser atribuído dentre outros fatores, a utilização de cruzamentos absorventes e utilização de linhagens portadoras destes atributos. Os valores encontrados neste estudo apresentou média de 669,00 ±13,73 kg, para raça Murrah, confirmando as observações dos autores acima citados.

Torres (6) et alii, citam os pesos e altura no garrote das três raças mais exploradas no Brasil, sendo para a Murrah 552 kg e 132 cm, Jafarabadi 650 kg e 135 cm e Mediterrânea 550 kg e 140 cm. Apesar destes resultados se apresentarem mais próximos dos encontrados, ainda os valores de altura (sacro e garrote), estão em média 3 a 4 cm menores para a raça Jafarabadi e Murrah e apenas 1 cm para a Mediterrânea. O valor encontrado de 140 cm para a altura da última raça é muito próximo do citado por Torres (6) et alii, porém chama a atenção o fato de que, dentro destas três raças, é a Jafarabadi que apresentou a maior estatura no presente estudo (143 cm) e não a Mediterrânea como encontrado pelos autores. Quanto aos pesos citados por estes autores, os encontrados neste estudo diferem em aproximadamente 60 kg para a Jafarabadi e 100 kg para a Mediterrânea e Murrah.

Na descrição das raças, Cabrera (1) apresenta valores médios de peso dos rebanhos Indianos Jafarabadi e Murrah, dentro do intervalo de 450-900 kg e 450-600 kg, respectivamente. Levando em consideração que estes intervalos apresentam uma amplitude muito grande e que os resultados encontrados se enquadram nestes intervalos, podemos considerar que as fêmeas amostradas apresentaram-se acima dos pesos médios dos rebanhos indianos. Este autor ainda cita que a raça Jafarabadi apresenta duas variedades Palitana e Gir, sendo que a primeira apresenta porte e osssatura mais desenvolvida. Estas duas variedades apresentam definições muito controvertidas e também a nível de criatório nacional esta raça é bastante heterogênea. Através das mensurações feitas neste estudo, encontramos maior número de animais com as características Gir, principalmente fêmeas, e outro fato importante é que os touros Palitanas acasalam búfalas Gir, originando indivíduos com proporções intermediárias e também um confundimento das caracterizações raciais.

Para a raça Mediterrânea, Cabrera (1) (1986) encontrou peso para fêmeas adultas entre 450 a 600 kg em rebanhos italianos, diferindo muito pouco do valor dos animais amostrados (618,80 ± 13,50). Este autor comenta que bubalinos desta raça apresentam a altura tomada no garrote maior do que no sacro, sem entrar em detalhes do valor especificamente. No valor encontrado para a Mediterrânea a altura no garrote foi de 139,00 ±0,96 cm e no sacro de 141,50 ±0,89, sendo em média 2,5 cm mais alta no sacro. Devemos ressaltar ainda que em termos de comparação com as raças Indianas (Jafarabadi e Murrah), a Mediterrânea, apresenta quase a mesma diferença entre altura no sacro e garrote que as outras duas 144,30± 0,89 e 142,00 ±0,96; 139,00± 0,90 e 137,30 ± 0,97, respectivamente. A diferença entre as proporções corporais da raça Mediterrânea em rebanhos italianos e no nosso meio, pode ter ocorrido devido aos acasalamentos entre Jafarabadi e Murrah, pois a posição intermediária do chifre resultantes destes acasalamentos, muitas vezes enquadram-se na caracterização da raça Mediterrânea, sendo que a sua origem é indiscutivelmente diferente e atualmente são enquadrados como animais mestiços.

Portanto, como se pode observar pelo teste Tukey, na Tabela 2 as alturas no sacro e garrote, diferiram significativamente entre as raças, excluindo-se desta afirmação somente a altura no garrote das raças Mediterrânea e Murrah, porém com tendência desta última apresentar menor estatura.

De uma maneira geral a altura também poderá auxiliar na caracterização das raças, sendo ainda uma informação de fácil conhecimento.

Quanto à medida distância de rótula e rótula, que tem por objetivo estimar o potencial de produção de carne, somente a raça Mediterrânea presentou valor significativamente ($P < 0,05$) inferior quando comparado com as outras duas raças. Porém este parâmetro seria mais relevante nos machos e sempre com a informação adicional do peso.

CONCLUSÕES

1) Os grupos raciais diferem quanto ao peso vivo, mantendo entre si uma diferença quase constante, sendo mais pesados os indivíduos do grupo Jafarabadi, seguidos dos Murrah e essa, do Mediterrânea.
2) Embora, tenha-se utilizado apenas animais adultos, verificou-se, independente de grupo racial que o peso vivo aumentou com a idade.
3) A idade revelou não exercer efeito significativo sobre a altura no sacro e no garrote e na distância rótula a rótula.

4) A altura no sacro e no garrote mostram-se diferentes quanto aos grupos raciais.
5) A distância rótula a rótula foi maior nos indivíduos do grupo Murrah e Jafarabadi.

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RESUMO

O objetivo do presente estudo foi de avaliar as características de tipo em búfalos, a saber: Peso corpóreo (PCOR), altura do garrote (AGAR) e sacro (ASAC) e distância de rótula a rótula (ROTU), dos três principais grupos genéticos existentes no Estado de São Paulo. Foram mensurados 50 fêmeas registradas de cada raça (Jafarabadi, Murrah e Mediterrânea), todas com idades superiores a 40 meses. Os animais Jafarabadi superaram em 7,05% o peso médio dos animais Murrah e de 15,74% aos da raça Mediterrânea. Já os Murrah foram em média 8,11% superiores a Mediterrânea. Quanto a AGAR, os animais Jafarabadi apresentaram a média de 142,00cm significativamente P<0,01 superior à média dos animais Murrah (137,30cm) e Mediterrâneos 139,00cm. Para ASAC os animais Jafarabadi apresentaram-se mais altos (144,30cm), seguidos dos Mediterrâneos (141,50cm) e Murrah (139,00cm), sendo a diferença de média dos grupos significativa, P<0,05. Os resultados de ROTU diferiram P<0,05, somente para a raça Mediterrânea, apresentando valores inferiores as outras duas raças. A idade mostrou exercer efeito linear significativo, P<0,01 apenas para PCOR (b=0,849).

SUMMARY

The purpose of this study was to avaluate some traits of the body of buffaloes, i.e., (BW), wither and hip height (WH and HH) and rotula and rotula lenght (RRL) of three main genetic groups in São Paulo State. Were recorded 50 females of the Jaferabadi, 50 Murrah and 50 Mediterranean, with age over 40 months old. Jafarabadi animals showed greater body weight (7.05%) than Murrah and Mediterraneans (15.74%), and Murrah was 8.15% greater, on average Mediterraneans.

The wither leight of Jafarabadi animals average 142.00 cm, and was greater (P>.01) than Murrah (141.50 cm) and Mediterranean (139.00 cm). For hip leight, the animals Jafarabadi to presented more leighters (144.30 cm), the Mediterranean (141.50 cm) and Murrah (139.00 cm), its being, the differents of mean of groups significants (P<.05). All that rotula to rotula leight, the Jafarabadi animals and Murrah was similar berd, both to difference, (P<.05), of the Mediterranean. The age showed linear effect (P<.01), only to the weight body (b=.849).

SOMMAIRE

L'objectif de c'etude a été d'evaluer les caracteristiques du type chez bufles, a savoir: Poids corporel, hauteur a l'epaule et du sacrum et la distance entre les rotules, des trois groupes genétiques existentes au Etât de São Paulo. On ont été mesurées 50 femelles registrées de chaque race (Jafarabadi, Murrahm et Mediterranea), toutes aux l'ages superieures a 40 mois. Les animaux Jafarabadi ont surpassé en 7,05% le poids moyenne des animaux Murrah et en 15,74% les animaux de race Mediterranea. Les Murrah ont été en moyenne 8,11% sucerieures aux Mediterranea. En rapport a l'hauteur a l'epaule, les animaux Jafarabadi montraient la moyenne de 142,00 cm significativament superieures (P<0,01) à la moyenne des animaux Murrah (137,30 cm) et Mediterranea (139,00 cm). Pour l'hauteur du sacrum les animaux Jafarabadi ont été plus hautes (144,30 cm), suivent para les Mediterranea (141,50 cm) et les Murrah (139,00 cm), et la difference entre les moyennes significative (P<0,05). Pour la distance entre les rotules, les animaux Jafarabadi et Murrah ont été egales, mais différentes des Mediterranea (P<0,05). L'age a montré un'effect linéaire significative, (P< 0,01) a paine pour le poide corporel (b=.849)

CLINICO-BIOCHEMICAL AND MICROBIOLOGICAL ALTERATIONS IN RUMEN LIQUOR IN PADDY STRAW INDUCED ALKALINE INDIGESTION IN BUFFALO CALVES

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INTRODUCTION

In Asia, large quantities of agrobyproducts and crop residues are available for livestock feeding. Cereal straws viz. wheat and rice constitute major feed resources in this region. The total availability from these crops works out to be 976 million tonnes in the world, of which 603 million tonnes are available in Asia (1). In India, about 66 million tonnes of rice straw is available every year (12). The advent of agricultural diversification, intensive cropping schedule and intensification of dairying in the country is making the situation grim on availability front of green fodder. Therefore, farmers are constrained to depend upon and utilize the available straw. Poor quality roughages viz. wheat and paddy are considered to be not only responsible for a number of deficieneies but may also be associated with forestomach disorders. Henee, the present study was undertaken to investigate the effect of exclusive feeding of paddy straw on clinico-biochemical and microbial alterations in rumen liquor of buffalo calves.

MATERIALS AND METHODS

Experimental animals and their management

Eight local bred, 1-2 year old buffalo calves were procured from local market on the basis of good body condition. All the calves were kept under observation for about a month and were dewormed with broad spectrum anthelmintic.

Clinical procedures

Calves were examined once daily throughout the period of experimental study and their feed intake, rumen motility and general clinical examination were recorded.

Sampling procedures

To establish base values, rumen liquor was collected on three occasions from each animal. Subsequently six calves were put on exclusive feeding of paddy straw for 92 days and rest of two calves were kept as healthy control. The samples of rumen liquor were collected periodically throughout the period of experimental study.

Analysis procedures

The physical characteristics, pH, qualitative microbial activity tests viz. sedimentation activity test (SAT), glucose fermentation test (GFT), cellulose digestion test (CDT), methylene blue reduction test (MBRT), total bacterial and protozoal count of rumen liquor were determined. The ruminal ammonia nitrogen (NH_3-N), total volatile fatty acid concentration and individual proportion of volatile fatty acids were analysed as described elsewhere (10).

RESULTS

The change of feed from green fodder to paddy straw resulted in abrupt decrease in feed intake which might be as a consequence of abrupt change in diet. Subsequent improvement in feed intake for short duration could be ascribed to adaptation of the animal and ruminal microbiota to the new diet. Paddy straw is resistant to mechanical and microbial comminution in the rumen, whereby its passage is delayed through reticulo-omasal orifice. The rate of feed intake is regulated by rate of passage of undigested residue (1) and also lower nitrogen intake decreases voluntary intake (3). Therefore, inappetance could be due to its low nitrogen content and resistance to comminution.

Table 1. Effect of paddy straw induced alkaline indigestion on rumen liquor biochemicals in buffalo calves (Mean ± SE)

Parameter	Sampling time (days)										
	0	8	16	27	37	50	64	71	78	85	92
pH	7.05 ±0.06	7.79* ±0.15	7.46* ±0.01	7.40* ±0.12	7.44* ±0.04	7.35* ±0.05	7.72* ±0.03	-	7.32* ±0.08	7.32* ±0.06	7.32* ±0.03
TVFA (mEq/L)	74.41 ±3.14	41.98* ±1.56	52.10* ±2.72	51.28* ±3.49	48.91* ±3.01	48.50* ±1.38	49.20* ±2.85	51.50* ±4.18	52.10* ±2.10	56.40* ±5.00	57.50* ±1.76
Acetic acid (Molar %)	68.97 ±1.00	67.12 ±1.10	62.48* ±1.36	66.12 ±0.77	66.37 ±0.87	68.63 ±1.48	68.41 ±1.09	67.14 ±1.58	67.32 ±1.39	67.38 ±0.90	-
Propionic acid (molar %)	20.99 ±0.61	23.91* ±0.80	27.21* ±0.83	24.11* ±0.57	22.76 ±0.64	22.87 ±0.99	23.70 ±0.84	22.48 ±0.46	22.97 ±0.62	22.74 ±0.32	-
Butyric acid (molar %)	10.02 ±0.44	8.94 ±0.51	10.33 ±0.63	9.74 ±0.55	10.84 ±0.52	8.48 ±1.17	7.87 ±0.53	10.37 ±1.18	8.92 ±0.99	10.02 ±0.87	-
NH_3-N (mg %)	15.224 ±1.290	4.748* ±1.001	4.000* ±0.918	3.908* ±0.111	3.873* ±0.334	1.802* ±0.252	1.254 ±0.415	1.802 ±0.193	2.345* ±0.778	2.062* ±0.602	2.012* ±0.492
Urea-N (mg %)	1.653 ±0.284	0.784* ±0.177	1.843 ±1.145	0.820 ±0.142	0.654* ±0.153	0.856 ±0.267	0.647* ±0.112	0.493* ±0.103	0.687* ±0.100	0.730* ±0.109	0.786* ±0.123

*Significant at $P < 0.05$; (-) Not analysed.

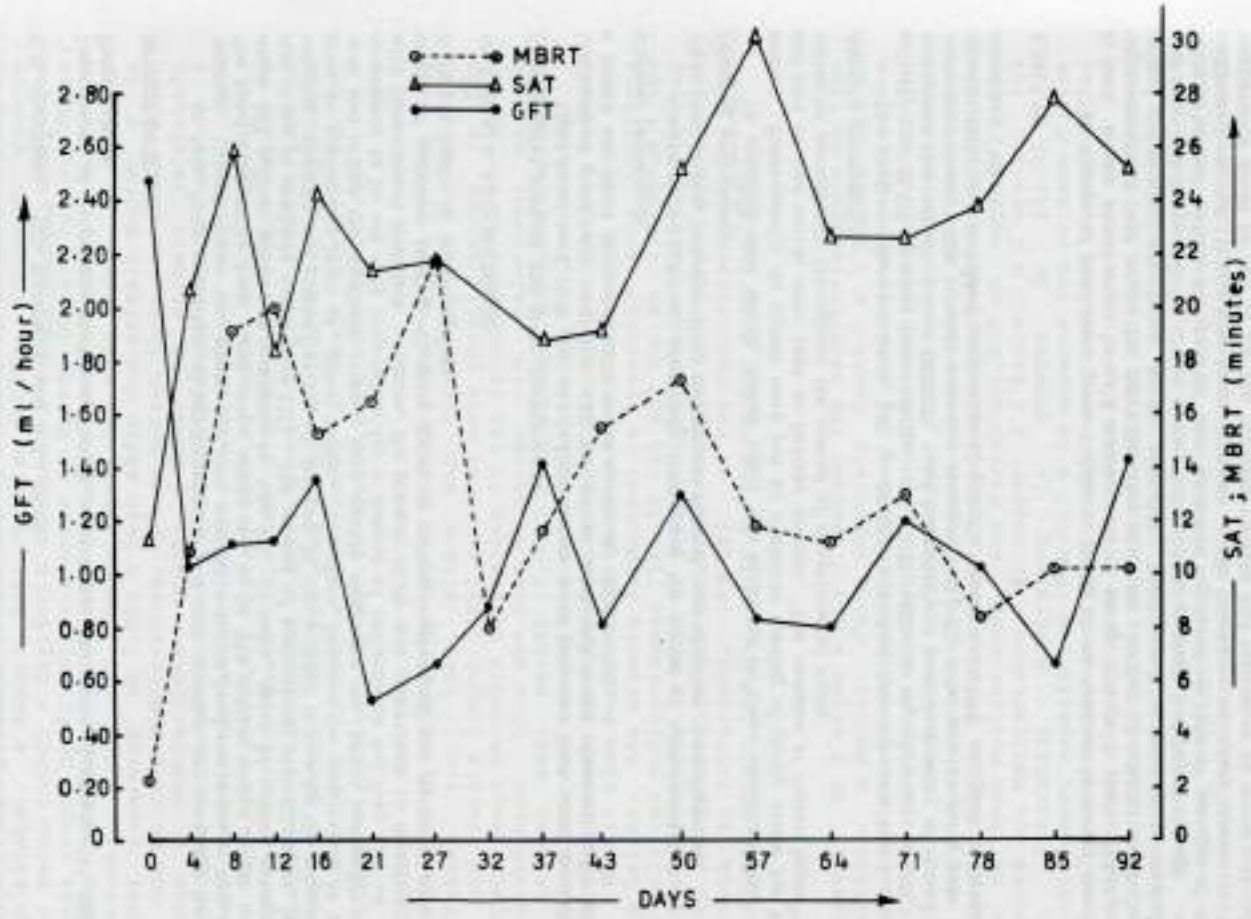

FIG. 1. EFFECT OF PADDY STRAW INDUCED ALKALINE INDIGESTION ON QUALITATIVE MICROBIAL ACTIVITY IN RUMEN LIQUOR OF BUFFALO CALVES

Table 2. Effect of paddy straw induced alkaline indigestion on microbial status in rumen liquor of buffalo calves (Mean±SE)

Sampling time (days)	Bacterial count (10^9)	Protozoal count (10^5)	Differential protozoal count (%)					
			Isotricha	Dasytricha	Entodinium	Diplodinium	Epidinium	Unidentified
0	7.03±0.22	3.54±0.24	25.69±1.97	1.00±0.24	54.30±1.79	13.48±1.53	1.46±0.60	4.33±0.57
8	6.38±0.51	2.91±0.23*	-	-	76.75±1.70*	14.75±1.49	-	8.25±1.31
16	7.19±0.51	2.44±0.32*	2.50±0.64*	-	90.75±1.97*	1.75±0.61*	-	4.75±1.31
27	6.82±0.32	2.07±0.26*	1.16±0.30*	-	81.66±2.64*	10.83±1.13	1.16±0.47	5.00±0.85
37	7.07±0.34	1.81±0.15*	1.66±0.49*	-	83.66±2.34*	9.00±2.30	-	4.66±0.66
50	6.74±0.70	1.12±0.10*	1.00±0.81*	-	72.66±4.65*	23.00±4.63	0.16±0.16	2.83±0.54
64	6.44±1.13	0.60±0.12*	4.00±1.41*	-	49.60±11.42	42.60±12.03*	3.40±1.02	2.00±0.85
71	6.76±0.61	0.95±0.07*	5.26±2.61*	-	39.60±9.26	33.20±9.36	1.20±0.43	2.00±0.57
78	7.51±0.60	1.02±0.10*	1.80±0.48*	-	60.80±6.73	34.20±6.76*	0.50±0.25	2.80±1.06
85	6.00±0.86	0.50±0.10*	2.20±0.86*	-	60.80±7.06	35.80±8.08	0.80±0.37	3.00±1.77
92	6.85±0.59	0.68±0.07*	3.20±0.73*	-	53.20±5.86	40.60±6.67*	0.40±0.24	2.80±1.35

*Significant at P ⩽ 0.05 (-) Not detected

A partial rumen stasis observed could be owing to alkaline pH and overstretching of rumen due to slow passage of digesta. Simultaneous development of mild to moderate impaction might have been due to rumen stasis. Progressive emaciation and unthrifty appearance of calves was ascribed to the effect of ongoing malnutrition because of low digestibility and impaired absorption because of villous atrophy of intestine as observed histopathologically. A well marked weakness of hind quarters could be due to atrophy and hyalinisation of skeletal muscles of thigh region.

The pH of rumen liquor increased, the colour changed to brownish yellow and ruminal contents turned putrid and watery alongwith decline in both concentration and protozoal motility which persisted throughout the period of investigation. The mean SAT, MBRT and GFT values at the start of the experiment were 11.15 ± 0.53 minutes, 2.20 ± 0.07 minutes and 2.47 ± 0.26 ml/hour respectively. However, SAT and MBRT attained a peak value of 27.80 ± 1.77 min and 20.00 ± 1.71 minutes respectively. The GFT values were lower throughout the period of experiment, the lowest being 0.66 ± 0.11 ml/hour. The cellulose digestion test was negative throughout the period of experiment (Fig. 1).

The declining NH_3-N concentration was probably due to lower nitrogen content of diet and enhanced absorption of NH_3 because of a rise in ruminal pH. The decline observed in total volatile fatty acid concentration in rumen liquor of calves thriving on paddy straw might be due to decreased fermentative activity due to alkaline ruminal pH; low digestibility of paddy straw and lower NH_3-N level (4). The molar proportion of acetic and butyric acid did not show any significant change whereas proportion of propionic acid was higher. The observed increase in percentage of propionic acid could be attributed to low crude protein content of diet (2).

The mean total protozoal count declined significantly from the base value of $3.54 \pm 0.24 \times 10^5$/ml to as low as $0.60 \pm 0.12 \times 10^5$/ml of strained rumen liquor (SRL). The decline could be ascribed to high pH of rumen (8) and decreased availability of substrates for protozoal growth and multiplication. The proportion of Entodinia species increased initially which was followed by Diplodinia species. However, holotrichs showed a significant decline. The ecological niche of holotrichs is soluble sugars (7). Therefore the decline observed in proportion of Isotricha and Dasytricha was probably due to absence of soluable sugars in the diet. The initial increase in proportion of Entodinia sp. was probably at the expense of decline in holotrich population. The total bacterial count did not show any significant change because bacterial population is more stable under unfavourable physico-chemical and nutritional environment of rumen (6, 9).

The results suggested development of alkaline indigestion with marked deterioration in rumen microbial fermentation.

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SUMMARY

An experimental study on alkaline indigestion, induced by exclusive feeding of paddy straw in six local bred one to two year old male buffalo calves, was undertaken to study its effect on physical, microbial and biochemical changes in rumen liquor. Physical characteristics, qualitative microbial activity tests revealed marked deterioration in protozoal activity and microbial fermentation. Total protozoal count decreased significantly with a decline in proportion of holotrichs and increase in Entodinia which was followed by Diplodinia whereas total bacterial count did not alter significantly. Significant biochemical alterations in rumen liquor were an increase in pH, decline in ammonia-nitrogen, total volatile fatty acid (TVFA) concentration with an increase in molar proportion of propionic acid.

CUTANEOUS DERMATOPHILOSIS IN CROSSBRED CATTLE

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INTRODUCTION

Dermatophilosis (Cutaneous Streptothricosis) is a pustular, exudative dermatitis of animals and rarely man. The disease was first described by Van Saceghem in 1915(7) as a skin disease in cattle in Belgian Congo. Subsequently, it has been reported from various countries including India and is widely prevalent in cattle in Africa as a serious economic hazard (3, 6, 8). In the present investigation, 2 outbreaks of cutaneous dermatophilosis affecting large population of crossbred cattle at organised dairy farms, involving 110 and 10 animals in first and second outbreak respectively, were rcorded. The present paper describes the characterstic clinical findings, diagnosis, results of therapeutic evaluation and control measures initiated in natural outbreak of dermatophilosis in crossbred cattle in India.

MATERIALS AND METHODS

Clinical procedures

Two outbreaks with skin lesions involving 120 crossbred cattle aged 4-7 years (Red Dane x Sahiwal cross and Holstein Fresian x Sahiwal cross) were observed in the month of May, 1989 at two organised dairy farms in Punjab, India. At one farm (Punjab Agricultural University Dairy Farm) 110 crossbred cattle were found to be affected out of about 1200 animals of all age groups (cattle and Buffalo) while in an other private dairy farm 10 of 10 cattle were affected with skin lesions of the disease. History revealed appearance of cutaneous lesions on coronary region of hind limbs which subsequently involved the upper area of limbs extending to thigh region associated with tail, teats and udder involvement in some of the severely affected cattle. Rectal temperatures, pulse rates, respiration rates and rumen motility of representative animals selected randomly were recorded. The effect of the disease on feed intake and milk yield was also observed.

Sampling procedure

Biopsies from effected skin from 5 cows (two with acute lesions and 3 with chronic lesions of the disease) were collected and preserved in 10 percent formal saline solution for histopathological studies. A part of the biopsy sample was also collected from active lesions aseptically in sterilized test tubes. To demonstrate the organism in active lesions, impression smears were prepared, fixed in methanol and stained by Giemsa and Grams stains.

Microbial procedures

The scrappings from skin lesions of the affected animals were first treated with antibiotic and then inoculated on the slants of the blood agar and serum broth and incubated at 37 C. Identification of the organisms was based on the cultural and microscopic characters as described by Rippon (7).

Pathological procedures

Tissue pieces collected from lesions were examined for histopathological studies as per standard procedures and stained for demonstration of organisms in histopathological sections.

Therapeutic procedures

All the affected animals were treated with parenteral administeration (intramuscular) of long acting tetracycline, oxytetracyline hydrochloride ("Oxyvet-LA" Sarabhai Chemical, baroda, India) @ 20 mg/kg body weight which was repeated at 3 days interval on 2-3 occasions. The afected animals were given a foot bath in 2 percent soluion of copper sulphate. Local application of povidone iodine USP 5% solution containing 0.5% available iodine ("Betadine" Veterinary Wockhardt Private Limited, Bombay, India) in severely affected animals was also carried ot.

RESULTS

Clinical observations

Typical cutaneous lesion's were observed mainly on the hind limbs in all the affected animals. However, the severity of the lesions was more intense in region below the hock joints. The involvement of the forellmbs upto knee joints with appearance of mild form of cutaneous lesions was also recorded in about 50 percent of the animals. Some of the animals also showed lesions on tail, teats and udder region. However, appearance of lesions on facial region, neck and abdominal area as described else where (1,3,8) was not observed in the present investigations. It was observe that limbs of these animals remained soiled with dung and urine which might have precipitated the establishment of the organisms and flare up of the disease in the present study. Initially, the lesions appeared at the coronary region of hind limbs and subsequently spread to involve the upper part of limbs also setting in an acute inflammatory reaction characterised by the appearance of greasy exudate (Fig.1) This was followed by appearance of thick horny encrustations (Fig.2) usually creamish to brownish in coloration with in a period of 5-7 days which led to metting of the hairs in tufts. Removal of the horny encrustations induced a painful response. The involved skin became hard and started peeling off in patches leaving raw erosive surfaces on the skin from which exudate was observed to be oozing out. The thick horny encrustated skin of hind limbs and tail developed alopecia In later stages (Fig.3). Generally, the condition was afebrile but some of the animals revealed mild degree of pyrexia with increased respiration rate probably related to environmental stress (hot climate). Affected animals also showed symptoms of acute lameness with reluctance for movement. The affected cattle revealed partial to complete anorexia of varying duration, moderate decrease in milk yield (40 percent) and delayed onset of oestrus. This is

Figure 1: Acute form of bovine dermatophilosis with swelling and greasy exudation of hind limbs.

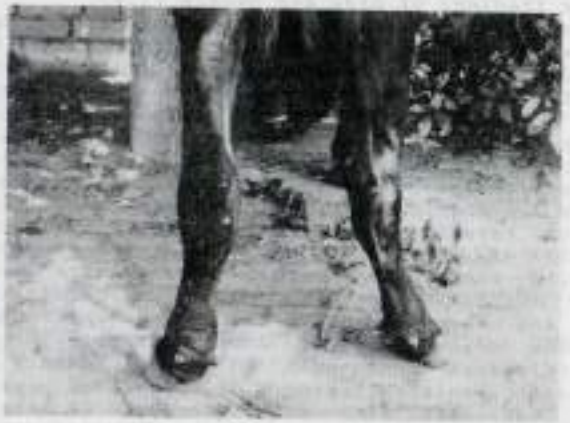

Figure 2: Chronic form of bovine dermatophilosis with horny encrustations particularly involving coronary region of hind limbs.

Figure 3: Chronic form of bovine dermatophilosis extensively involving tail and hind limbs with alopecia and horny encrustations.

similar to the findings of Bida and Dennis (1,4).

Direct microscopic examination of the impression smears and sections prepared from active lesions and stained with Giemsa and Gram's method revealed coccoid organisms in chains which were morphologically indistiguinshable from Dermatophilus congolensis. On cultural examination white round colonies with depressed periphery were observed. The organisms were gram positive and appeared as coccoid filaments or chains of cocci. The organisms were catalase positive and urease positive. These observations are similar to the findings reported earlier (4,7).

Skin sections revealed hyperkeratinization with marked degree of hyperplasia of stratified squamous epithelium. This was associated with congestion, dermal oedema with neutrophilic infilteration through epidermis. In the epidermis there was degeneration and necrosis of cells in the upper portion of stratum spinosum. However, in only 2 biopsy section, Dermatophilus congolensis filaments were seen in the epidermis and hair follicle sheath. Isolated presence of coagulated exudate with degenerated leukocytes were also obsereved in the corneum lucidum. There were focal areas of lymphocytic and neutrophilic infilteration in between the retipegs or even dermal layers. This is similer to the observations recorded earlier (2,5).

Though no mortality was observed in the present investigation but the disease resulted in marked economic losses due to significant decline in milk yield, delayed onset of oestrus associated with prolonged convalesent period. The therapeutic trial comprising of parentral administeration of long acting

tetracyline, oxyvet-LA along with dipping of limbs in 2% copper sulphate solution prepared in a dip tank resulted in marked improvement in condition of the animals with uncomplicated healing of the lesions. This was similar to findings of Blood et al.(3). Local application of povidone iodine solution over the exudative lesions in severe cases also resulted in rapid recovery of the lesions in 7-11 days.

An interesting finding was that in first outbreak involving 110 crossbred cattle, strict quarentine along with therapeutic means prevented the spread of disease from affected herd to the adjacent herds of cattle and buffaloes of all age groups even though, house fly and ticks which are reported to be mechanical carriers of the organisms (3) were prevalent in the farm surroundings during that period of the year. The animal attendants and Veterinarians handling the effected animals also dipped their hands in copper sulphate solution which probably prevented the occurance of the disease in them.

The results of te clinical investigation reveals that Dermatophilus congolensis can lead to severe form of cutaneous affection which can be diagnosed on the basis of impression smears prepared from exudative lesions, tissue sections and growth of the organisms in serum broth and blood agar. The disease responds favourably to parenteral administeration of long acting tetracyclines with local application of copper sulphate and/or povidone iodine sol-ution. The adoption of quarentine measures along with maintenance of hygenic standards in dairy sheds can prevent or minilise the spread of the disease.

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SUMMARY

Two outbreaks of cutaneous dermatophilosis were studied in 2 dairy herds involving 120 crossbred cattle. Typical lesions in severe form were observed maini on the hind limbs opto hock joint. Some of the animals also showed lesions on tail,teats and udder region. Initially the lesions appeared as acute inflammatory reaction leading to swelling of the limbs ollowed by appearance of thick horny encrustations usually creamish to brown in coloratio. The exudative crusts, impression smears and some of the biopsy section revealed the presence of typical

coccoid organisms in chains in Giemsa stain on direct microscopic examination. The Dermatophilus congolensis organisms were cultured from these lesions in serum broth and blood agar. Parenteral treatment with long acting tetracyclines and local application of 2 percent solution of copper sulphate used as a foot bath resulted in complete recovery of the affected animals.

PROTEINOGRAMA DO SORO DE BEZERROS BUBALINOS (Bubalus bubalis L.) DA RAÇA MURRAH NO MOMENTO DO NASCIMENTO

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INTRODUÇÃO

Os ruminantes por possuírem placenta do tipo epitélio-corial,múltipla ou cotiledonária (1) torna os recém-nascidos inteiramente dependentes dos anticorpos recebidos através do colostro (2) o que ocorre também com os bubalinos que possuem o mesmo tipo de placenta (3). O conhecimento do estado imune dos bezerros bubalinos no momento do nascimento, permite diagnosticar os estados de imunodeficiência naqueles animais que tiveram acesso ao colostro e não obtiveram concentrações adequadas de anticorpos circulantes, predispondo-os à infecções como a enterite, debilidade, pneumonia e verminoses (4,5,6,7,3,8,9).

O objetivo do presente estudo foi de verificar as concentrações de proteínas séricas nos bezerros bubalinos no momento do nascimento com especial atenção a fração gamaglobulina que por sua importância protetora está diretamente relacionada aos processos de defesa durante as primeiras semanas de vida.

MATERIAIS E MÉTODOS

Os soros sanguíneos de 17 bezerros bubalinos da raça Murrah, sendo 7 machos e 11 fêmeas, pertencentes ao Centro de Pesquisa Agropecuária do Trópico Úmido (CPATU) da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), nascidos no período de janeiro à abril de 1988, na fazenda Experimental "Dr. Felizberto Camargo" do CPATU, foram estudados visando o conhecimento do estado imune no momento do nascimento.

Colheita das amostras e obtenção do soro

Imediatamente após o nascimento, antes da ingestão do colostro, foram colhidas amostras através de punção da veia jugular o volume de 5 mL de sangue, utilizando-se agulhas hipodérmicas de coleta múltipla (10) e colocados em tubos de centrifugação para obtenção do soro, que após a retração do coágulo foram centrifugados e posteriormente estocados em "freezer" em tubos de teflon arrolhados, à -18 graus Célsius de temperatura.

Determinação da proteína total

Após o descongelamento das amostras à temperatura de 25-27 graus Célsius, determinou-se a concentração da proteína total no soro através de refratometria (11).

Determinação eletroforética das frações protéicas no soro

As frações eletroforéticas foram obtidas em suportes de acetato de celulose gelatinizados em solução tampão de dietilbarbiturato de sódio (veronal) à 8,4% e pH 8,6. As corridas foram realizadas em sistema de semimicroeletroforese com duração de 30 minutos, 230 volts e volume de 1 microlitro. A coloração empregada foi o negro de amido e transparentização recomendada por MOURA (12). Os resultados foram obtidos em percentuais através da leitura em densitômetro integrador automático de eletroforese e à seguir calculados os valores em gramas por cento (g%) a partir da concentração da proteína total, constituindo assim a

proporção de cada fração proteíca no soro.

Análise estatística

As análises estatísticas incluíram a determinação dos valores médios, desvio padrão, coeficiente de variação, intervalo de confiança e amplitude de variação (16) das variáveis proteína total, albumina, alfaglobulina, betaglobulina e gamaglobulina presentes no soro sanguíneo dos bezerros.

RESULTADOS

Na tabela 1 são apresentados os valores médios, desvio padrão, coeficiente de variação, intervalo de confiança e amplitude de variação da proteína total, albumina, alfaglobulina, betaglobulina e gamaglobulina obtidos por refratometria e eletroforese respectivamente. Pode-se observar que os bezerros bubalinos podem nascer hipogamaglobulinêmicos ou agamaglobulinêmicos, já que no presente estudo foi constatado que dos 17 animais acompanhados cerca de 30% nasceram destituídos de anticorpos e os restantes 70% nasceram com concentrações reduzidas de anticorpos quando comparados com a concentração de animais adultos, o que está de acordo com os trabalhos realizados por TENNANT; BUSH; LOGAN (13,14,15) em animais da espécie bovina que possuem o mesmo tipo de placenta dos bubalinos, o que torna ambas espécies inteiramente dependentes do colostro para sobreviverem durante as fases iniciais da vida.

TABELA 1. Proteinograma eletroforético do soro de bezerros bubalinos no momento do nascimento (g/100 mL).

	X	DP	CV	IC		AV
				LI	LS	
Proteína total[1]	6,20	0,29	4,83	6,04	6,35	5,80 - 6,80
Albumina[2]	3,15	0,39	12,51	2,95	3,36	2,20 - 3,80
Alfaglobulina[2]	1,53	0,30	19,63	1,36	1,69	1,00 - 2,00
Betaglobulina[2]	1,04	0,24	23,42	0,91	1,18	0,80 - 1,60
Gamaglobulina[2]	0,71	0,77	10,81	0,31	1,11	0,00 - 3,10

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

Were studied the blood serum of 17 water buffalo calves of the Murrah breed belonging to CPATU/EMBRAPA and born at the period of Jannuary to April of 1988. The separation of the 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 8,6 with run of 30 minutes at 230 volts and a volume of 1 microlitre according to MOURA (1987). To the total protein estimation was used the refractometry method acorrding to JAIN (1986). The results were im g/100 mL: total protein 6,20 ± 0,29 , albumin 3,15 ± 0,39 , alphaglobulin 1,53 ± 0,30 , betaglobulin 3,15 ± 0,39 , gammalgobulin 0,71 ± 0,77. At the birth moment, gammaglobulin concentration in the buffalo calves were very low, what made them a complete dependents of the colostrum to survive.

RÉSUMÉ

Les auteurs ont étudié le sérum sanguin de 17 veaux bubalins de race Murrah, appartenant au CPATU/EMBRAPA et nés entre janvier et avril 1988. La separation des protéines sériques a été réalisée en système de électrophorese, utilisant des bands d'acétate de cellulose gélatinisée en solution tampon de veronal à 8,4% et pH 8,6 en séquences de 30 minutes à 230 volts et volume de 1 microlitre, selon ce que recommande MOURA (1987). Pour la détermination de la concentration de protéine totale, on a utilisé la métode de réfractométrie, selon JAIN (1986). Les résultats obtenus, em g/100 mL, ont été les suivants: protéine totale 6,20 ± 0,29 , albumine 3,15 ± 0,39 , alphaglobuline 1,53 ± 0,30 , betaglobuline 1,04 ± 0,24 , gammaglobuline 0,71 ± 0,77. Au moment de la naissance, les veaux bubalins présentaient des concentrations très

basses de gammaglobuline, ce qui les rendait entièrement dépendants du colostrum pour leur survie.

RESUMEN

Fueron estudiados los sueros sanguineos de 17 terneros bubalinos de la raza Murrah pertenicientes a el CPATU/EMBRAPA nacidos en el período de enero a abril de 1988. La separación de las proteínas séricas fué hecha en sistema de semimicroeletroforese utilizandose cintas gelatinizadas de acetato de celulose en solución buffer de veronal a 8,4% y pH 8,6 con intervalo de tiempo de 30 minutos a 230 volts y volumen de 1 microlitro conforme recomienda MOURA (1987). Para avaliación de la proteína total fué utilizado el método de refratometria segun JAIN(1986). Los resultados fueron los seguientes en g/100 mL: proteína total 6,20 ± 0,29 , albumina 3,15 ± 0,39 , alfaglobulina 1,53 ± 0,30 , betaglobulina 1,04 ± 0,24 , gamaglobulina 0,71 ± 0,77. En el momiento de el nacimiento los terneros bubalinos presentaron concentraciones muy bajas de gamaglobulina lo que los torna enteramente dependientes del calostro para sobrevivir.

RESUMO

Foram estudados os soros sanguíneos de 17 bezerros bubalinos da raça Murrah pertencentes ao CPATU/EMBRAPA nascidos no período de janeiro à abril de 1988. A separação das proteínas séricas 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 com corridas de 30 minutos a 230 volts e volume de 1 microlitro conforme recomenda MOURA (1987). Para avaliação da proteína total foi utilizado o método de refratometria segundo JAIN (1986). Os resultados foram os seguintes em g/100 mL: proteína total 6,20 ± 0,29 , albumina 3,15 ± 0,39 , alfaglobulina 1,53 ± 0,30 , betaglobulina 1,04 ± 0,29 , gamaglobulina 0,71 ± 0,77. No momento do nascimento os bezerros bubalinos apresentaram concentrações muito baixas de gamaglobulina o que os torna inteiramente dependentes do colostro para sobreviverem.

ACTIVIDAD REPRODUCTIVA Y PRODUCCION LACTEA EN GANADO BOVINO: RELACION CON LA CONCENTRACION SERICA DE INSULINA.

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INTRODUCCION

La intensidad productiva a que se ve sometido el ganado vacuno de aptitud láctea determina que, con frecuencia, aparezcan multitud de problemas en la esfera reproductiva que, a la larga, se traducen en pérdidas económicas para el ganadero. La situación de la hembra durante el periodo del postparto inmediato y las características de manejo , tienen una influencia decisiva sobre su capacidad reproductiva posterior (1,2). Ello ha llevado a muchos investigadores a comprobar cuál es el estado endocrinológico de estos animales durante este periodo, y a comprobar si existe alguna relación entre éste, el nivel productivo y la ulterior eficiencia reproductiva.

MATERIAL Y METODOS

El trabajo se llevó a cabo sobre un total de 149 animales pertenecientes a cuatro granjas incluidas en la Cooperativa de Agricultores del Concejo de Gijón. Los animales estudiados eran de raza Frisona, sus edades oscilaron entre los 3 y los 12 años, y los niveles productivos eran MEDIO-ALTOS. Fueron desechadas las novillas con el fin de que el estudio resultara más homogeneo. Para el estudio de la actividad reproductiva de los animales, se calcularon: INTERVALO PARTO-CONCEPCION (IPC): días transcurridos entre la fecha del parto y la de la Inseminación Fecundante; INDICE DE FECUNDIDAD (IF): número de Inseminaciones Artificiales necesarias para que se instaure una gestación; INTERVALO INTERPARTOS (IIP): días transcurridos entre dos partos consecutivos; NIVEL PRODUCTIVO (NP): Kg de leche/lactación.

La recogida de muestras se realizó según la siguiente pauta: TOMA I: 7-8 semanas anteparto; fase de gestación tardía; TOMA II: 0-6 días postparto, coincidente con el momento del parto, o lo más próximo posible a él; TOMA III: 7-23 días postparto, TOMA IV: 24-38 días postparto y TOMA V: 39-53 días postparto. Las extracciones de sangre se realizaron en los vasos coxígeos. Tras la recogida, las muestras

fueron centrifugadas durante 15' a 3000 rpm y el suero recogido fue conservado hasta el momento de su análisis a -20ºC.

La determinación de las concentraciones de Insulina se llevó a cabo mediante el empleo de técnicas de Radioinmunoanálisis,con un contador GAMMACHEM 9612.

Para el estudio estadístico se empleó el test de la t de Student. También se comprobó el grado de correlación existente entre la Insulina, el nivel productivo y la eficiencia reproductiva.

RESULTADOS REPRODUCTIVOS

Cuando consideramos las diferencias que se establecen entre las cuatro explotaciones (Tabla nº 1) podemos comprobar cómo es la Explotación A la que mejores resultados reproductivos presenta, manifestando la producción láctea más baja. Su IF es significativamente inferior ($p< 0,01$) que el de las Explotaciones B y D.

TABLA Nº1- COMPARACION DE LOS RESULTADOS REPRODUCTIVOS DE LAS DISTINTAS EXPLOTACIONES.

	I F	I P C	I I P	N P
	X ± SD	X ± SD	X ± SD	X ± SD
A	1,10 ± 0,30 ab	77,40 ± 16,5 cd	358,90 ± 16,4 fg	4776,89±1454 ijk
B	1,48 ± 0,71 a	81,92 ± 25,8 e	364,26 ± 26,0 h	5944,90±1271 il
C	1,48 ± 0,93	105,51 ± 42,7 ce	386,74 ± 44,4 fh	6815,82±2120 j
D	1,52 ± 0,82 b	97,18 ± 44,5 d	377,78 ± 43,4 g	6797,97±1294 kl

(*) Cifras con índices iguales presentan diferencias significativas
a,b,c,f,i,l:p< 0,01;d,e,g,h:p< 0,05;j,k:p<0,001

El IPC más prolongado correspondió a la Explotación C, y fue significativamente superior a los correspondientes a las explotaciones A ($p< 0,01$) y B ($p< 0,05$).

La Explotación C presenta asimismo el IIP más largo, significativamente mayor que el de la Explotación A ($p< 0,01$) y que el de la Explotación B ($p< 0,05$).

También la duración del IIP en la explotación D fue significativamente superior que la de la explotación A ($p< 0,05$).

Hay que citar también que las explotaciones C y D, que peores resultados reproductivos presentaron, son precisamente las de mayor nivel productivo.

RESULTADOS HORMONALES

TABLA Nº 2.-CONCENTRACION SERICA DE INSULINA (µU/ml) EN LA EXPLOTACIONES ESTUDIADAS.

	EXPLOT. A		EXPLOT. B		EXPLOT. C		EXPLOT. D	
	X	SD	X	SD	X	SD	X	SD
T I	14,48 abc	7,67	10,22 ad	7,13	11,74 ac	5,97	6,14 af	5,22
T II	5,84 a	7,56	5,48 ac	6,02	5,21 af	4,58	6,17 c	5,96
T III	4,25 bd	3,66	5,46 de	4,68	6,40 c	8,83	4,24 bd	4,09
T IV	7,56 c	9,52	8,05	7,96	7,27	7,99	11,00 ad	10,03
T V	13,08 d	11,36	11,99 dc	8,42	10,57 f	5,27	10,50 bcf	5,39
N	24		47		31		47	

Letras iguales en cada columna presentan diferencias significativas
a, c, e : p< 0,05; b: p< 0,001; d, f : p< 0,01

EXPLOTACION A: La máxima concentración de Insulina aparece en la Toma I y es significativamente más alta que la de las Tomas II ($p< 0,05$), III ($p< 0,001$) y IV ($p< 0,05$). Además, la 3ª extracción, momento en que la insulinemia es mínima, también presenta valores significativamente inferiores a los de la Toma V ($p< 0,01$).

EXPLOTACION B: En este caso, la máxima concentración de la hormona se observa en la Toma V, significativamente superior que las de las extracciones 2ª y 3ª ($p< 0,05$ y $p< 0,01$, respectivamente). No obstante dicha concentración es muy similar a la que se encontró en la Toma I que también es significativamente mayor que la de las Tomas II ($p< 0,05$) y III ($p< 0,01$).

EXPLOTACION C: La concentración máxima de Insulina observada en la Toma I es significativamente más alta que la correspondiente a las Tomas II y III ($p< 0,05$). El mínimo tiene lugar en la 2ª extracción, y posteriormente, a medida que avanza el puerperio, la concentración de la hormona se va recuperando para alcanzar en la Toma V valores muy similares a los de la 1ª extracción y significativamente superiores a los de la mínima concentración ($p< 0,05$).

EXPLOTACION D: Esta explotación es la única que no se adapta al patrón seguido por el resto. Los máximos niveles de la hormona se observaron en la 4ª y 5ª Tomas y fueron significativamente más elevados que los de las Tomas I y III (en el caso de la 4ª extracción) y que la I, II y III (en el caso de la 5ª Toma).

La concentración mínima de la hormona, fue observada en la 3ª extracción.

La Insulina no mostró correlaciones significativas con relación a la eficiencia reproductiva de los animales muestreados.

DISCUSION DE LOS RESULTADOS

El comportamiento de esta hormona, en las distintas granjas, sólo difiere en el caso de la Explotación D, que presenta Rangos enormemente amplios y consecuentemente desviaciones altas.

En los tres primeros rebaños, nuestros resultados coinciden con los de BLUM **et al.** (4) que observan niveles máximos de la hormona en los meses anteriores al parto con relación a las concentraciones del puerperio inmediato,y no establecen diferencias significativas entre su concentración , en ambas fases, en animales que manifiestan un puerperio normal. Para SMITH **et al.** (6), las diferencias que aparecen entre la concentración de la hormona antes y después del parto, no son significativas, siendo achacadas a cambios en las dietas. Sin embargo si que observan el mismo hecho que se produce en nuestro estudio, es decir, el incremento progresivo de la insulinemia a medida que avanza la lactación.

El balance energético negativo en que se encuentra la hembra durante el postparto, explicaría la movilización de las reservas para hacer frente al desgaste de la lactación. Además, hay que comentar que los valores que hemos encontrado durante el puerperio son muy similares a los aportados por GIESECKE **et al.** (5) en ganado vacuno de raza Holstein, mientras que en hembras Jersey, las concentraciones son mucho más elevadas.

El caso de la Explotación D, puede ser probablemente debido a una causa alimenticia, lo que concordaría con las tesis de SMITH **et al.** (6) que admiten variaciones en los niveles de Insulina en función de que el animal reciba, en la época previa al parto, mayor o menor cantidad de paja seca.

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RESUMEN

Se estudia el comportamiento reproductivo de 149 vacas, pertenecientes a cuatro explotaciones de producción lechera, su nivel productivo y las concentraciones séricas de Insulina en cinco momentos del ciclo productivo de la hembra. Con excepción de uno de los rebaños, el comportamiento de la hormona presenta un patrón de comportamiento concreto, con descensos en sus niveles tras el momento del parto y una recuperación durante el puerperio. La concentración de Insulina no muestra correlación aparente con el nivel productivo.

RESUME

L'on a étudié les caracteristiques reproductives de 149 vaches qui appartenaient à 4 troupeaux de

production laitière, leurs productions, et les niveaux sériques de l'Insuline pendant 5 moments de son cycle productif. Sauf un troupeau, le reste a eu des niveaux de l'hormone qui s'abaissaient après le vêlage, et qui augmentaient pendant le puerperium. La concentration d'Insuline n'a pas eu de correlation avec la production laitière.

SUMMARY

Caracteristhiques reproductives of 149 cows appartening to 4 herds of milk production, the productive performance and Insuline concentrations over 5 moments of their productive cycle has been studied. Except in one herd, the Insuline concentration decreases at calving and after, it increaes during the puerperium. The hormone concentration does not show a correlation with the productive performance.

ESTUDO SOBRE A AUSÊNCIA DOS DENTES INCISIVOS EM BOVINOS LEITEIROS

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INTRODUÇÃO

A indicação de prótese dentária tem sido de tempos para cá feita com maior frequência dentro da Veterinária, quase sempre na tentativa de melhorar o rendimento dos animais particularmente àqueles de produção, já que nesses animais, a frequência que notamos na ausência de dentes, mostra genericamente estreita correlação com baixos índices de performance.

Entretanto apesar desta afirmação ser de uso comum e aceito pela comunidade científica notamos que a literatura é escassa, relativamente a esse tema particular.

Os métodos de apreender, seccionar e levar os elementos até a boca variam nas diferentes espécies de animais. Os bovinos utilizam principalmente a língua como órgão de apreensão de alimentos e para colocá-los dentro da boca onde são fragmentados pela pressão dos dentes incisivos contra a gengiva do maxilar, segundo DUKES & SWENSON (1).

GETTY (2) informa-nos que a gengiva dos dentes incisivos retrai-se gradativamente com o avançar da idade, expondo, parcialmente a raiz dentária. A frequente ação mecânica não somente retrai a gengiva, mas também os alvéolos se tornam mais raros permitindo aos dentes maior mobilidade e consequentemente favorecendo sua perda. Ainda, o arco dentário perde sua curvatura e se torna mais retilíneo com os dentes não mais se tocando.

PERTIERRA & TUNÕ (4) afirmam que à medida que avança a idade do animal, o espaço entre os dentes, oriundos do desgaste, das suas bordas, torna-se mais notório chegando a perda completa dos dentes. Nesta fase de sua vida, torna-se difícil a obtenção do seu sustento natural e como consequência começa a diminuição da produção. A existência de espaço interdentário também pode permitir que os alimentos provoquem lesões, mais facilmente na gengiva mandibular, devido a deficiência na sua fragmentação. Estas lesões podem ser tão impuras que determinam a perda dos dentes.

Assim, visando contribuir com dados que possibilitem melhor conhecimento sobre as condições da saúde bucal, mais particularmente sobre o índice de mortalidade dentária de nossos rebanhos, especialmente aqueles de animais com característica de produção leiteira, procuramos estudar a incidência da ausência de dentes incisivos em bovinos leiteiros.

MATERIAL E MÉTODO

Para este trabalho, examinamos 342 bovinos, sem raça definida, adul-

tos, fêmeas, em lactação, da região do Triângulo Mineiro - MG, Brasil.

Após adequada contensão dos animais, procedemos ao exame da cavidade bucal, de cada um deles, com auxílio de espelho de BARACH, com a intenção de verificar a ausência de dentes na arcada.

Os animais estudados foram divididos em 3 grupos de acordo com as suas idades. Assim, o grupo A foi constituído de 152 animais com idade compreendida entre 5 e 7 anos; o grupo B com 142 espécimes com idades variando de 8 a 10 anos e o grupo C com 48 bovinos com 11 ou mais anos.

Para a coleta e tabulação dos dados utilizamos a representação dos dentes incisivos pela letra I, seguido da sua ordem, e da letra "D" quando estivesse em correspondência à hemiarcada direita,e da letra "E" quando pertencesse à hemiarcada esquerda, conforme recomendação da Nomina Anatômica Veterinária, já, para a análise estatística valemo-nos do teste x^2 com α = 5%.

RESULTADOS

Dos 342 bovinos examinados, 21 (6,14%) deles mostraram ausência de dente incisivo, sendo que destes, 5 (1,46%) animais pertenciam ao grupo A; 9(2,63%) eram integrantes do grupo B e 7 (2,05%) compunham o grupo C.

De sorte que, foram assinaladas ao todo 53 ausências dentais, distribuídas em: 21 nos animais do grupo B, 19 nos do grupo C e 13 nos do grupo A. A hemiarcada direita apresentou 26 ausências enquanto que à esquerda foi anotada ausência por 27 vezes.

Nos 152 animais do grupo A, a ausência de dentes foi notada 13 vezes nos dentes: I_1D; I_2D; I_1E; I_2E; I_3E; I_4E; sendo que mais frequentemente os dentes I_1D - 5 vezes; I_2D; I_2E; I_3E; I_4E - 1 vez cada, foram anotados como ausentes, enquanto que, em nosso material, os dentes I_3D e I_4D sempre estiveram presentes.

Nos 142 bovinos que pertenciam ao grupo B, a ausência de dentes incisivos foi assinalada 21 vezes dos dentes I_1D; I_2D; I_1E; I_4E. Assim independentemente do animal, os dentes que mais comumente foram marcados como ausentes foram: I_1D - 9 vezes; I_1E - 7 vezes; I_2D - 2 vezes; I_2E - 2 vezes e I_4D - 1 vez. Já, os dentes I_3D; I_4D; I_4E, jamais deixaram de estar presentes nas arcadas examinadas neste grupo de animais.

Nas 48 espécimes enquadradas no grupo C a ausência de dentes foi observada 19 vezes, em todos os dentes incisivos, sendo que independentemente do animal os dentes que foram indicados como ausentes, foram mais frequentemente: I_1D - 4 vezes; I_1E - 3 vezes; I_4E - 3 vezes; I_2D, I_3D, I_2E, I_3E - 2 vezes cada; I_4D - 1 vez.

Independentemente do animal e do grupo etário a que pertencia, os dentes que foram assinalados como ausentes com maior frequência foram: I_1D- 18 vezes; I_1E - 14 vezes; I_2D; I_2E; I_4E - 5 vezes cada; I_3E - 3 vezes; I_3D - 2 vezes; I_4D - 1 vez.

A análise estatística não mostra diferença estatisticamente significante quando confrontamos o mínimo de ausências dentais com o grupo etário dos animais, entretanto indica diferenças significantes quando comparamos o índice de ausência dos primeiros incisivos, tanto à direita como à esquerda, com os demais dentes.

TABELA 1 - Número de ausências de dentes incisivos, segundo o grupo etário, em bovinos leiteiros. MG - 1989.

GRUPO \ DENTE	I_1D	I_2D	I_3D	I_4D	I_1E	I_2E	I_3E	I_4E
A	5	1	-	-	4	1	1	1
B	9	2	-	-	7	2	-	1
C	4	2	2	1	3	2	2	3

DISCUSSÃO

A escassez de dados à disposição na literatura, e a maneira incisiva e frequente com que determinadas afirmações são feitas, nos levaram a crer que as doenças que afetam os órgãos dentais constituiam elemento principal pela diminuição da produção leiteira dos planteis. Entretanto, uma das afecções que comumente é responsabilizada por esta diminuição e performace, a ausência dentária, mostra-se com baixo índice de frequência em nosso material (6,14%) fazendo-nos repensar sobre a importância dada a este fato em particular. Isto não quer dizer que a ausência dentária não deva ser também responsabilizada, mas cremos que sua importância se prende a comparação do animal no padrão individual e não a nível de plantel, como sugere a maioria dos autores. Estas afirmações,na maioria das vezes genéricas, são feitas ao nosso ver, sem fundamento de dados epidemiológicos, ou seja, quase sempre embasados na observação da diminuição de produção, do ponto de vista individual, extrapolando-se estes resultados individuais para todos os rebanhos.

Assim, a indicação de prótese, mostra-se ao nosso ver, acertada para a correção ou atenuação do problema à nível individual, devendo-se à nível de rebanho confrontar-se custos totais de tais condições com benefícios do aumento da produção, já que a ausência dos incisivos é relativamente baixa.

Entretanto, cabe lembrar que talvez se computarmos todas as doenças dos órgãos dentais, e não sòmente ausência, talvez tenhamos índices de comprometimento que sejam significativos, à nível de plantel. Assim,trabalhos que assinalem um perfil epidemiológico da saúde bucal dos bovinos devam ser incrementados e incentivados, na busca constante de mais conhecimentos que permitam afirmações melhor embasadas e fundamentadas.

CONCLUSÕES

Os resultados permite-nos concluir que:

1- Dos 342 animais examinados, 21 deles apresentavam 1 ou mais dentes ausentes.
2- O mínimo de dentes ausentes (53), apresentam distribuição equivalente nos grupos etários por nós compostos (A,B,C).
3- Os dentes que apresentarem-se ausentes com maior frequência e com diferenças significantes entre os demais foram os primeiros incisivos à direita e à esquerda, respectivamente I_1D - 18 vezes e I_1E - 14 vezes. Já, aqueles que apresentaram-se com menor frequência foram I_4D- 1 vez, I_3D - 2 vezes e I_3E - 3 vezes.

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RESUMO

Estudou-se a ausência de dentes incisivos em 342 bovinos, fêmeas em lactação, sem raça definida e com idades variadas e superiores a 5 anos.

O exame da cavidade bucal permite concluir que:

- a ausência de dentes incisivos ocorrem uma ou mais vezes em 6,14% dos bovinos leiteiros;
- o número de dentes ausentes (53), apresenta distribuição equivalente nos grupos etários por nós compostos;
- os dentes mais frequentemente assinalados como ausentes foram os primeiros incisivos, tanto à direita como à esquerda.

SUMMARY

It was studied the incisor teeth lost in 342 crossbred couis over 5 years old in lactation.

The examination of the cavity at the mouth allowed the following conclusions:

- in daily cows the missing of the incisor teeth occurs one or more times in 6.14%;
- the number of absent teeth (53) has a equivalent distribution among the different groups related to the age;
- the preponderant absence was observed in the first incisor, both at right and at left.

ZUSAMMENPASSUNG

Die Untersuchung der Mund der 342 Kühe, die ohne bestimmte Rasse und mehr als 5 Jahre alt sind, hat beweister:

- Die Abwesenheit von einen order mehr Incisivi Zanne kommt zum 6,14% der Laktationsrinder Vorschein;
- Die Verteilung der Nummer der abwesenden Zahne (53) ist gleich wertig in der verschiedende Gruppe;
- Die häufigen abwesenden Zahne waren die erste Incisive sowohl rechts als links.

RESUMEN

Se estudio la ausencia de diente incisivos en bovinos, hembras en lactación, sin raza definida y con varias edades superiores a 5 años.

El examen de la cavidade bucal permite concluiyir:

- La ausencia de dientes incisivos ocurre una o mas veces en el 6,14% de los bovinos lecheros;
- El número de dientes ausentes (53) presenta distribuición igual en las distintas edades por nosotros estudiadas;
- Los dientes mas ausentes fueran los primeiros incisivos, tanto a la derecha como a la isquierda.

REDUCTION DU NIVEAU D'ENGRAISSEMENT DES CARCASSES PAR UNE MEILLEURE GESTION DE LA CROISSANCE CHEZ LE TAURILLON

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INTRODUCTION

Un niveau d'engraissement excessif est le reproche fréquemment adressé par les abatteurs aux producteurs de taurillons en élevage intensif et de fait, une étude réalisée en Région Bretagne en 1988 montre que 20 p.cent des carcasses de jeunes bovins sont considérées comme trop grasses (3-5).

Afin d'éviter le caractère subjectif de l'appréciation visuelle de l'importance des dépots adipeux, compliqué en France par l'habitude d'un dégraissage partiel (1-2), avant pesée et classement des carcasses, les auteurs ont utilisé comme moyen d'appréciation du niveau d'engraissement la pesée du gras éliminé en différents points de la chaîne d'abattage. La nature des opérations de dégraissage réalisées au niveau de 3 postes différents peut se définir comme suit :

Poste 1 : Dégraissage extérieur ou émoussage proprement dit réalisé au niveau de la hanche, du rein, du dos et du pourtour de la queue,

Poste 2 : Dégraissage au niveau du gros bout de poitrine, de la gouttière jugulaire, le long de la paroi costale et au niveau du coeur,

Poste 3 : Dégraissage au niveau des reins, du bassin, le long de la paroi costale et abdominale externe, et au niveau de l'aine.

Le gras récupéré au niveau de chaque poste a été pesé et rapporté au poids de chaque carcasse de façon à déterminer pour chacune d'elles le pourcentage de gras d'abattage (p.cent GA)

$$\text{p.cent GA} = \frac{\text{poids de gras éliminé}}{\text{poids de la carcasse dégraissée + poids de gras éliminé}}$$

L'utilisation d'un critère d'engraissement mesurable et précis a permis d'aborder l'étude de l'importance réelle du phénomène et de son déterminisme (4).

MATERIEL ET METHODE

Choix de l'échantillon

846 carcasses de taurillons appartenant à 41 lots ont été étudiées en tenant compte de la possibilité de contrôler les vitesses de croissance individuelle dans les élevages.

Etude descriptive

La pesée du gras d'abattage a été réalisée telle que décrite ci-dessus, les résultats moyens par carcasse et par portées obtenues sur 846 carcasses ont été étudiés.

Etude analytique

Chaque élevage détenant des animaux destinés à l'un ou l'autre des deux abattoirs a fait l'objet d'une visite avant le départ des lots afin de relever un certain nombre de données regroupées en :

données individuelles : race, résultats des contrôles de croissance, durée d'engraissement,

données collectives : objectif de croissance, apport de céréales, apport protéique, mode de distribution du concentré.

Les données d'ordre alimentaire ont été recueillies par entretiens avec les éleveurs, ils ont pour but de fixer le niveau de complémentation énergétique (sous

forme de céréales) et de complémentation protéique (sous forme de soja ou de concentré protéique titrant en moyenne 35 à 40 p.cent de Matières Protéiques Brutes). L'influence des données individuelles et collectives sur le niveau d'engraissement a été recherchée.

Traitement des données

L'étude des relations entre le pourcentage de gras d'abattage (p.cent GA) et les différentes variables individuelles ou collectives, a été réalisée :

par analyse monofactorielle (Analyse de Variance, Test Chi Carré)

par analyse multifactorielle (analyse factorielle des correspondances)

RESULTATS

Etude descriptive

Niveau moyen d'engraissement (Tableau 1)

TABLEAU 1 : Pourcentage de gras d'abattage et caractéristiques de croissance (846 carcasses)

	Poids de gras (kg)	Poids de carcasses (kg)	Gras d'abattage (p.cent)	GMQ (g)	Durée d'engraissem. (j.)
Moyenne	24,8	351,7	6,6	1101	510
Ecart type	6,9	33,6	1,6	102	-
Mini Maxi	7,3-50,8	258-462	2,0-11,9	773-1378	-

Quantité moyenne de gras éliminé au niveau de chaque poste avant classement

Le poids global moyen de 24,8 kg éliminé par carcasse se décompose en :

Poste 1 : 4,5 kg
2 : 4,8 kg
3 : 16,65 kg

Etude analytique

Etude en fonction du type génétique (Tableau 2)

TABLEAU 2 : Répartition de l'état d'engraissement dans les différents types génétiques (en p.cent de carcasses), effectif 638 carcasses

p.cent gras d'abattage / Type génétique	1,9 - 5,8	5,9 - 7,3	7,4 - 11,9	Effectifs
Croisés Charolais	49,1	33,2	17,7 a	271
Montbéliards	32,5	37,9	29,6 b	169
Normands	18,2	33,3	48,5 c	198

a b c répartitions différentes au seuil de P<0,001

Etude en fonction du poids de carcasse et de la vitesse de croissance pour les 3 principaux types génétiques (Tableau 3)

TABLEAU 3 : Etude du pourcentage de gras d'abattage en fonction du poids de carcasses et du GMQ pour 3 types génétiques (chaque type génétique est divisé en 3 groupes numériquement équivalents)

	Croisés Charolais-Normands			Montbéliards			Normands		
Poids de carcasses (kg)	334,0	365,6	402,4	315,1	345,1	382,3	315,0	344,3	378,0
GMQ (g/j)	1072	1123	1213	995	1107	1193	992	1072	1157
Poids de gras (kg)	20,1	22,6	26,9	21,7	24,6	26,4	23,8	28,2	28,5
Gras d'abattage p.cent)	5,7 a	5,8 a	6,2 a	6,4 a	6,6 a	6,4 a	7 a	7,5 b	7 a
Effectifs	66	67	69	55	57	57	64	68	68

a : différent de b au seuil de P(0,05

Etude en fonction du niveau de GMQ en période de croissance (6-10 mois) et de finition (13-16 mois) (tableau 4)

TABLEAU 4 : Caractéristiques de croissance de 501 taurillons en fonction de leur état d'engraissement

Gras d'abattage (p.cent	Poids de gras (kg)	Poids de carcasse (kg)	GMQ (g/j)			Durée d'engraissement (j)
			global	6-10 mois	13-16 mois	
4,42	16,4 a	352,8 a	1105 a	1278 a	998 a	507
6,47	24,9 b	359,9 b	1125 a	1361 b	968 a	512
8,51	32,7 c	351,4 a	1119 a	1389 b	749 b	506
Test Chi Carré	0,001	0,05		0,001	0,001	

Analyse factorielle multidimensionnelle des paramètres d'engraissement, de croissance et d'alimentation (schéma 1)

INTERPRETATION ET DISCUSSION

Niveau d'engraissement moyen (tableau 1)

Dans l'effectif étudié de 846 carcasses produites dans des conditions homogènes (élevage en claustration sur caillebotis ou pente paillée en raison de 10 sujets par case, alimentation à base d'ensilage de maïs et de concentré (céréales et complément protéique), le poids de gras éliminé par carcasse avant pesée et classement, est en moyenne de 24,8 kg, soit 6,6 p.cent du poids de la carcasse brute (après éviscération).

Ce poids se décompose en 3,5 kg pour la poste 1
en 4,8 kg " 2
en 16,5 kg " 3.

Etude en fonction du type génétique (tableau 2)

La répartition des différents types génétiques par niveau d'engraissement montre une différence significative à P 0,001 : les croisés Charolais fournissent préférentiellement des carcasses de faible niveau d'engraissement tandis qu'à l'opposé, les Normands ont une tendance marquée à l'engraissement : le type génétique Montbéliard se situant en position intermédiaire.

Ces différences sont vraisemblablement dues au niveau de précocité des types génétiques étudiés : en atteignant plus tôt l'optimum de leur développement osseux et musculaire, les Normands ont tendance à se "couvrir" plus précocement (6-7).

Etude en fonction du poids de carcasse et de la vitesse de croissance (tableau 3)

Il est couramment admis que plus les carcasses sont lourdes plus elles sont grasses : de ce fait, l'obtention d'une carcasse peu couverte est souvent considérée comme incompatible avec une vitesse de croissance élevée.

Les résultats présentés dans le tableau 4 montrent que si le poids de gras éliminé augmente effectivement avec le poids de carcasse, **le pourcentage de gras, seul véritable critère de l'état d'engraissement intrinsèque de la carcasse, est totalement inchangé, même pour des niveaux de GMQ moyens de l'ordre de 1200 g/j.**

Etude en fonction du niveau de GMQ en période de croissance 6-10 mois et de finition 13-16 mois (tableau 4)

Si le niveau moyen de croissance ne semble pas influencer le pourcentage de gras d'abattage, il en va tout différemment du profil de croissance.

L'analyse des résultats du tableau 6 montre que pour des poids de carcasses et pour des GMQ moyens identiques, les sujets maigres se caractérisent par des profils de croissance relativement plats (croissance "6-10 mois" modeste, croissance "finition" soutenue) ou contraire, les sujets les plus gras se caractérisent par des profils "en pic" (croissance très élevée entre 6-10 mois et effondrement marqué en finition. Là encore, l'analyse par type génétique montre que cette tendance est encore plus accusée pour les races Montbéliarde et Normande.

Analyse factorielle multidimensionnelle des paramètres d'engraissement, de croissance et d'alimentation (schéma 1)

Dans le système d'axes utilisés (axes 1 et 2), la classification automatique définit 2 groupes de carcasses :

- **Niveau d'engraissement faible ou moyen GRA1 et GRA2** auquel sont associées les caractéristiques suivantes :
  - . CRS 1 et 2 croissance faible ou modérée entre 6 et 10 mois
  - . FIN 2 et 3 croissance moyenne ou forte en finition
  - . CEF 1 apport en céréales faible en finition
  - . PRO 2 apport protéique élevé.

Ces caractéristiques sont associées préférentiellement aux types génétiques "croisés Charolais" et dans une moindre mesure au type Montbéliard qui confirme sa position intermédiaire entre "croisés Charolais" et Normands.

- **Niveau d'engraissement élevé GRA 3** associé aux caractéristiques inverses
  - . CRS 3 croissance élevée entre 6 et 10 mois
  - . FIN 1 croissance faible en finition
  - . CEF 2 apport de céréales important en finition
  - . PRO 1 niveau protéique relativement faible.

On retrouve dans ces résultats la confirmation du rôle négatif joué par le désir de profiter au maximum du potentiel de croissance du jeune sujet : chez les races précoces cet objectif pénalise la croissance ultérieure et favorise le dépôt adipeux sans autre bénéfice qu'un alourdissement apparent de l'animal, constitué par des graisses éliminées en partie avant la pesée commerciale.

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RESUME

Les contrôles réalisés sur 846 carcasses de taurillons montre qu'en moyenne 25 kg de gras, soit 6,6 p.cent du poids de la carcasse, sont éliminés avant le classement commercial d'après la grille communautaire "EUROP".

Ce critère d'appréciation utilisé pour l'analyse multifactorielle du phénomène "engraissement" révèle qu'à côté des causes génétiques et nutritionnelles, la recherche d'un rythme de croissance très rapide entre 6 et 10 mois exerce une influence très marquée sur l'augmentation du niveau d'engraissement chez les races précoces laitières ou mixtes. En outre, un objectif de croissance trop élevé en jeune âge participe fortement à la réduction des performances en période de finition.

SUMMARY

The fatness of 846 bull carcasses measured by weighing the fat eliminated before "EUROP" classification shows that an average of 25 kg i.e. 6,6 p.cent of the crude carcass weight is eliminated for each animal.

The multifactorial approach of the problem gives evidence that beside genetic and nutritional causes, the search of a very high growth rate in the early age of 6-10 months has a strong influence on the increase of the fat level in precocious dairy or mixed type animals and on the reduction of their daily gain during the three last months of their fattening period.

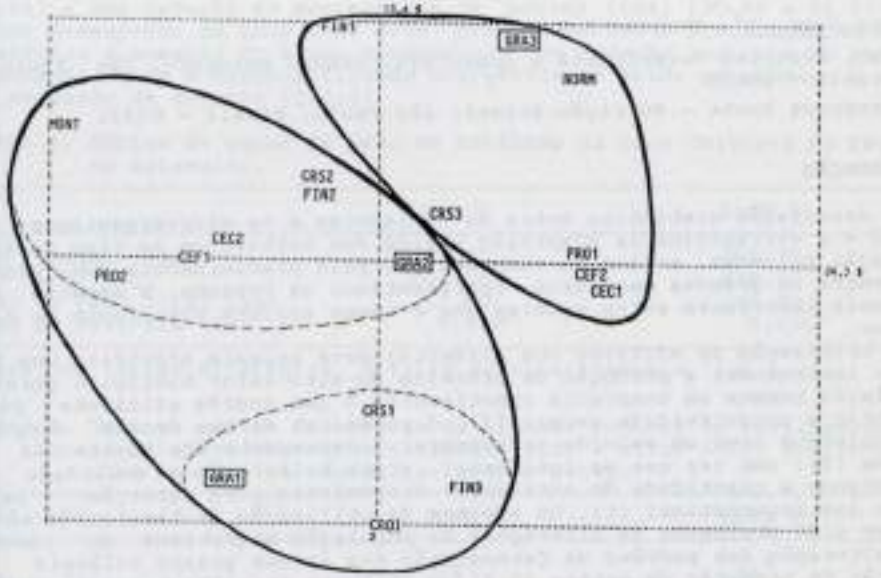
RESUMEN

Se pesaron un promedio de 25 kg de grasa de 846 carcasas de novillos, que representan un 6,6 % de peso de la carcasa - muestras tomadas antes de la clasifcation comercial en grilla como "EUROP".

Este criterio de appreciacion utilizado en el analisis multifactorial del fenomeno "Engrase" revela que, ademas de factores geneticos y nutricionales, la busqueda de un rapido ritmo de crecimiento entre los 6-10 meses ejerce una marcada influencia sobre el aumento de nivel de engrase en las razas lecheras y mixtas.

El objetivo de un muy rapido crecimiento A la edad precos, influye notablemente a la reduccion de resultados en el periodo de terminacion.

SCHEMA 1 : Analyse factorielle multidimensionnelle des paramètres d'engraissement, de croissance et d'alimentation



Code	Signification	Limites de Classes 1	2	3	Effectifs 1	2	3
GRA	Gras d'abattage (p.cent)	1,96 3,20	3,21 7,62	7,63 10,67	133	264	134
CBOI MONT NORM	Croisé Charolais Montbéliards Normands					120 165 133	
CRS	GMQ 6-10 mois (g)	660 1265	1265 1420	1420 1830	168	171	169
FIN	GMQ 13-16 mois (g)	270 800	800 1040	1040 1890	173	172	172
PRO	Apport protéique (kg/j)	0,8 1,0	1,1 1,2		299	233	
CEC	Apport céréales 6-10 mois (Kg/j)	1,2	1,2-2		210	322	
CEF	Apport céréales 13-16 mois (Kg/j)	1-2	2,2-3		274	258	

EFEITO DA SUPLEMENTAÇÃO DA LASALOCIDA SÓDICA NO DESEMPENHO DE BOVINOS EM CRIAÇÃO EXTENSIVA

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INTRODUÇÃO

A associação simbiótica entre os ruminantes e os microorganismos do rumen e a extraordinária adaptação básica dos herbívoros ao tipo de alimentação volumosa, seriam os responsáveis pela predominância dos ruminantes entre os grandes mamíferos, representando os bovinos, a espécie animal mais importante entre aqueles que o homem explora como fonte de alimentos.

A utilização de aditivos nos alimentos para animais significa uma forma de incrementar a produção de proteína de alto valor biológico para uma população humana em constante crescimento. É uma medida eficiente para aumentar a produtividade animal (2). O potencial de uso desses compostos químicos como um meio de se aumentar o desempenho dos ruminantes é enorme (2), uma vez que os ionóforos, atuam melhorando a qualidade ou aumentando a quantidade de nutrientes disponíveis para absorção pelo trato gastrointestinal (6). Os efeitos da utilização da Lasalocida sódica tem sido atribuído às alterações da população microbiana do rumen, com alteração dos padrões de fermentação dos ácidos graxos voláteis e redução da produção de metano (3,4). O presente trabalho tem como objetivo avaliar o efeito do ionóforo, Lasalocida sódia, no desempenho de bovinos da raça Chianina em regime de criação extensiva.

MATERIAL E MÉTODOS

Para avaliar o efeito da Lasalocida sódia como promotor de crescimento, foram utilizados 60 novilhos da raça Chianina, com idade média de 2,5 anos e peso médio de 367 kg. Foram separados ao acaso em 2 lotes de 30 animais, que permaneceram durante 112 dias (setembro a dezembro/1989), em piquetes de Brachiaria decumbens, sob regime de rodízio de pastagem de 7 dias. O lote A (tratado) teve à sua disposição sal mineral misturado com Lasalocida sódica na dosagem de 1,8 kg para cada 50 kg de sal mineral, para um consumo médio de 40 g de sal mineral e 200 mg de Lasalocida sódica. O lote B teve à disposição sal mineral. Os animais foram pesados no início e a cada 28 dias e nos últimos 3 dias do experimento foi coletado líquido do rúmen de 10 animais de cada lote, com auxílio de uma sonda esofágica, para avaliação bioquímica do líquido ruminal (pH, AGV, CH4) (2).

Os dados foram analisados pelo análise de variância e as diferenças entre as médias foram analisadas pelo teste T a um nível de probabilidade de 5% (7).

RESULTADOS E DISCUSSÃO

Os resultados do experimento estão evidenciados na Tabela 1 que mostra que o lote A apresentou um ganho de peso/cabeça/dia de 0,510 kg superior em 16% em relação ao lote B (0,440 kg). Semelhantes resultados foram obtidos em outros experimentos (5). A Tabela 2 evidencia que o lote

A teve uma redução do ácido acético (62% x 65%) e um aumento do ácido propiônico (26% x 22%), em relação ao lote B. Ainda a Tabela 2 mostra que o lote A apresentou uma diminuição de relação acético:propiônico (2,38 x 2,95) e uma redução da porcentagem de metano (CH4) (30,50 x 51,50). O melhor desempenho do lote A se deve, provavelmente, à diminuição de ácido acético e aumento do ácido propiônico, com redução indireta do metano aumentando assim a disponibilidade energética do ácido propiônico e redução da perda de energia (2,3,4).

TABELA 1. Médias de ganho de peso de novilhos da raça Chianina em regime extensivo.

	LOTE A	LOTE B
Peso Inicial (kg)	365,50	368,70
Peso Final (kg)	423,10	418,50
Ganho de Peso Total (kg)	57,60	49,80
Ganho de Peso/dia (kg)	0,510[a]	0,440[b]

Médias com letras diferentes, diferem estatisticamente ($P < 0,05$)

TABELA 2. Valores médias da análise do líquido ruminal de novilhas da raça Chianina em regime extensiva (pH, % molar ácido acético, ácido propiônico, ácido butírico e metano.

	LOTE A	LOTE B
pH	6,5	6,6
Ácido Acético	62[a]	65[b]
Ácido Propiônico	26	22[b]
Ácido Butírico	12	13
Relação Acético:Propiônico	2,38[a]	2,95[b]
Metano (CH4)	30,50[a]	51,50[b]

Médias com letras diferentes, diferem estatisticamente ($P < 0,05$)

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RESUMO

Efeito da Suplementação da Lasalocida Sódica no Desempenho de Bovinos em Criação Extensiva.

Para avaliar o efeito do ionóforo Lasalocida sódica, como promotor de crescimento foram utilizados 60 novilhos da raça Chianina, com idade média de 2,5 anos e peso médio de 367 kg. Foram separados ao acaso em 2 lotes de 30 animais, os quais permaneceram durante 112 dias (setembro a dezembro/1989) em piquetes de Brachiaria decumbens, sob regime de rodízio de pastagem de 7 dias. O lote A (tratado) teve à sua disposição sal mineral, misturado com 1,8 kg de Lasalocida sódica para 50 kg de sal mineral, para um consumo médio de 40 g de sal mineral e 200 mg do ionóforo por dia e o lote B teve somente sal mineral. Os animais foram pesados a cada 28 dias e nos últimos 3 dias do experimento foi coletado líquido ruminal de 10 animais de cada lote, com auxílio de sonda esofágica, para avaliação bioquímica (pH, AGV e CH4). O lote A apresentou um ganho de peso 16% superior ao do lote B.

SUMMARY

Effect of Lasalocid sodium on the performance of Chianina heifers raised under range condition

Sixty Chianina heifers, averaging 2,5 years and 367 kg were randomly divided into two groups of 30, treatment and control. Animals were kept at a Brachiaria decumbens pasture for 112 days (September to December 1989) and were rotated every 7 days. The treatment group was provided with 1.8 kg of Lasalocid sodim per 50 kg of mineral salt for a feed intake of 40 g of mineral salt and 200 mg of ionophore per day. Body weights were recorded every 28 days and in the last 3 days of the experiment ruminal liquor was collected from 10 animals of each group by means of an oesophageal tube to evaluate the pH, VFA and CH4. The treatment group showed an average daily gain by 16% greater (P .05) than the control one.

RESUMEN

Efecto da Lasolocida en el desarrolo de los bovidos.

P ara evaluer el efecto de Lasalocida sodica, en los bovidos fué utilizado 60 novillos de la raza Chianina, con edad media de 2,5 años y peso medio de 367 kg. Fueron separadas en 2 lotes de 30 animales, los cuales se quedaron 112 dias en rodizio de pastoreo de Brachiaria decumbens, de 7 dias. El lote A (tratado) tuvo a su disposición sal mineral, mesclado con 1,8 kg de Lasalocida sodica para 50 kg de sal mineral y 200 mg de ionóforo/dia y lote B tuvo solamente sal mineral. Los animales fueron pesado a cada 28 dias y en los ultimos 3 dias, fué colectado liquido del rumen de 10 animales de cada lote, por medio de sonda esofagica, para analises biquimica (pH, AGV y CH4). El lote A tuevo una ganancia de peso de 16% a mas do que el lote, B.

DESEMPENHO DE BOVINOS E BUBALINOS ALIMENTADOS EM CONFINAMENTO COM DIFERENTES FONTES DE VOLUMOSOS

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INTRODUÇÃO

A terminação de bovinos em confinamento durante o inverno, é uma das alternativas que vem sendo utilizada pelos produtores brasileiros para reduzir a idade de abate. No nosso meio, no entanto, esta prática de terminar os animais se torna mais viável, economicamente, a medida que a dieta utilizada inclui proporções mais elevadas de volumosos do que aquelas usadas em países que tradicionalmente praticam a terminação em confinamento. Entre os volumosos que podem ser utilizados na alimentação dos animais durante o inverno, se destacam a silagem de milho, pelo seu valor nutritivo, e a cana-de-açúcar, que apesar de ter um valor nutritivo mais baixo, mantém o seu teor de sacarose elevado durante o inverno. Bovinos alimentados com dietas que incluem silagem de milho, tem apresentado melhor desempenho do que aqueles cujas dietas incluem cana-de-açúcar (2) (3).

No Brasil a criação de bubalinos para a produção de carne vem aumentando, mesmo na região sul. Os bubalinos tem mostrado bom desempenho quando mantidos em pastagem (1) ou quando confinados (4). MOLETTA (5) verificou que os bubalinos apresentaram melhor desempenho do que os bovinos, quando terminados em confinamento, com dieta alimentar que incluia elevada proporção de cana-de-açúcar.

O presente trabalho teve como objetivo avaliar o desempenho em confinamento de bovinos e bubalinos, cujas dietas alimentares incluiram silagem de milho ou cana-de-açúcar.

MATERIAL E MÉTODOS

O experimento foi conduzido no Departamento de Zootecnia da Universidade Federal de Santa Maria. Foram comparados dois grupos genéticos, bovinos versus bubalinos, e duas fontes de volumosos, silagem de milho versus cana-de-açúcar. O delineamento experimental foi inteiramente casualizado, em um arranjo fatorial de 2x2.

Foram utilizados 12 bovinos da raça Hereford e 12 bubalinos da raça Mediterrâneo, com idade de 20 e 16 meses e peso médio de 286 e 242 kg , respectivamente. Os animais foram agrupados ao acaso em lotes de 3 indivíduos. Para avaliação do consumo e da conversão alimentar, cada tratamento foi composto por duas repetições (2 lotes, cada um com 3 animais); para a variável ganho de peso, cada animal foi considerado uma repetição. Os animais permaneceram confinados em piquetes com cobertura sobre os cochos.

A alimentação em confinamento teve a duração de 112 dias (subdividido em 4 períodos de 28 dias), precedido de um período de adaptação com duração de 14 dias. A dieta alimentar foi calculada para ser isoprotéica,com 12% de proteína bruta. A proporção volumoso: concentrado foi de 78:22 , 76:24, 61:39 e 56:44 no 1º, 2º, 3º e 4º período, respectivamente.

O concentrado foi constituído de farelo de arroz desengordurado, milho em grão moído, farelo de soja e calcário calcítico. Nos tratamentos em que o volumoso era cana-de-açúcar, foi adicionado uréia para igualar o teor de nitrogênio ao da silagem de milho. A cana-de-açúcar era picada no momento do fornecimento aos animais.

Os alimentos eram fornecidos duas vezes ao dia, nas primeiras horas da manhã e no fim da tarde, sendo o concentrado misturado ao volumoso por ocasião do arraçoamento. Os animais tinham livre acesso a água e a uma mistura mineral, composta de sal comum e farinha de ossos (1:1).

No início do período de adaptação os animais foram everminados e banhados (bovinos contra carrapato e bubalinos contra o piolho).

RESULTADOS E DISCUSSÃO

A interação grupo genético x tipo de volumoso não foi significativa ($P > 0,05$) para as variáveis ganho de peso médio diário (GMD), consumo de matéria seca (CMS), consumo de matéria seca por quilo de peso metabólico (CMSM) e conversão alimentar (CA).

Conforme pode ser observado na Tabela 1, os bovinos apresentaram maior GMD do que os bubalinos, tanto com a dieta que incluiu silagem de milho como com a dieta que incluiu cana-de-açúcar. Apesar de não ter sido detectado ($P > 0,05$), o efeito da interação entre espécie x tipo de volumoso a diferença no GMD a favor dos bovinos foi mais acentuada com o volumoso silagem de milho (0,313 kg), do que com a cana-de-açúcar (0,154 kg). A diferença média no GMD a favor dos bovinos foi de 0,233 kg. Outros autores, no entanto, como LORENZONI et al. (4), obtiveram um ganho de peso superior para bubalinos em relação a bovinos quando alimentados com uma dieta contendo 40:60 e 60:40 de volumoso: concentrado, fornecidos alternadamente a cada 36 dias. MOLETTA (5), também obteve ganho de peso mais elevado para bubalinos do que para bovinos, confinados e alimentados com uma dieta que continha elevada proporção de cana-de-açúcar.

O GMD dos novilhos Hereford (1,236 kg) foi ligeiramente superior ao encontrado por QUADROS & RESTLE (6), para novilhos da mesma raça e que foram terminados em confinamento com uma dieta contendo 42,5% de concentrado e 57,5% de volumoso, constituído de cana-de-açúcar mais silagem de milho. O GMD dos bubalinos, por outro lado, foi similar ao obtido por MOLETTA (5), e ligeiramente inferior ao relatado por LORENZONI et al.(4).

Os bovinos apresentaram maior ($P < 0,01$) CMS e CMSM do que os bubalinos (Tabela 2). O CMS foi 2,05 Kg maior para os bovinos quando a dieta incluiu silagem de milho; já na dieta que incluiu cana-de-açúcar a diferença baixou para 1,7 Kg. O maior consumo de matéria seca por parte dos bovinos também resultou em maior consumo médio diário de proteína bruta (1,108 vs 0,894 Kg; $P < 0,05$). A ingestão média diária de matéria seca expressa como percentagem de peso vivo, foi de 2,6 e 2,4% para bovinos e bubalinos, respectivamente. O CMS e CMSM obtidos no presente trabalho, tanto para bovinos como para bubalinos, foram bem superiores aos relatados por MOLETTA (5).

A conversão alimentar média para bovinos e bubalinos, foi similar ($P > 0,05$), conforme pode ser observado na Tabela 2. LORENZONI et al.(4), também verificou conversão alimentar semelhante entre novilhos búfalos e Holandês. MOLETTA (5), no entanto, verificou que bubalinos foram mais eficientes do que bovinos.

TABELA 1 - Valores médios para Peso Inicial (PI), Peso Final (PF) e Ganho de Peso Médio Diário (GMD)

	PI		PF		GMD	
	Média (Kg)	Desvio Padrão	Média (Kg)	Desvio Padrão	Média (Kg)	Desvio Padrão
ESPÉCIE - VOLUMOSO						
Bovino - S.Milho	287,5	10,2	438,8	13,0	1,345	0,175
Bubalino - S.Milho	240,0	17,8	357,6	17,1	1,032	0,106
Bovino -Cana-de-açúcar	285,8	11,5	412,1	16,9	1,128	0,240
Bubalino- Cana-de-açúcar	245,0	10,9	357,3	19,5	0,974	0,114
ESPÉCIE						
Bovinos	286,6	10,4	425,5	32,7	1,236a	0,232
Bubalinos	242,5	14,3	357,5	17,5	1,003b	0,109
VOLUMOSO						
Silagem de milho	263,7	28,4	398,2	44,52	1,189A	0,212
Cana-de-açúcar	265,4	23,38	384,7	33,52	1,051B	0,196

Médias, na mesma coluna, para o mesmo efeito, seguidas de letras diferentes, diferem estatisticamente pelo teste F (a,b; $P < 0,01$) (A,B; $P < 0,05$).

TABELA 2 - Valores médios para Consumo de Matéria Seca (CMS), Consumo de Matéria Seca por Quilo de Peso Metabólico (CMSM) e Conversão Alimentar (CA)

	CMS		CMSM		CA	
	Média (Kg)	Desvio Padrão	Média (g)	Desvio Padrão	Média	Desvio Padrão
ESPÉCIE - VOLUMOSO						
Bovino - S.Milho	10,36	0,367	124	5,0	7,70	0,007
Bubalino - S.Milho	8,31	0,162	116	3,5	8,05	0,466
Bovino- Cana-de-açúcar	8,06	0,205	103	0,7	7,14	1,244
Bubalino- Cana-de-açúcar	6,36	0,466	88	1,5	6,53	0,466
ESPÉCIE						
Bovinos	9,21a	0,733	114a	12,4	7,45	0,733
Bubalinos	7,33b	1,004	102b	16,6	7,30	1,004
VOLUMOSO						
Silagem de Milho	9,33a	0,376	120a	5,8	7,85	0,376
Cana-de-açúcar	7,21b	0,929	95b	8,9	6,86	0,929

Médias, na mesma coluna, para o mesmo efeito, seguidas de letras diferentes, diferem estatisticamente pelo teste F ($P<0,01$).

A conversão alimentar apresentada pelos bubalinos que receberam cana-de-açúcar (6,53) foi similar a obtida por MOLETTA (5) (6,33) que também utilizou cana-de-açúcar como volumoso, porém em maior proporção.

Analisando os volumosos (Tabela 1), se observa que a dieta que incluiu a silagem de milho proporcionou um melhor ganho de peso médio diário aos animais do que aquela que incluiu cana-de-açúcar (1,189 vs 1,051 Kg; $P<0,05$). Diferenças maiores a favor da silagem de milho, no entanto, foram relatadas por BRONDANI (2)e FERREIRA et al.(3), que trabalharam com proporções baixas (44%) e altas (80%) de volumosos, respectivamente.

Os novilhos alimentados com silagem de milho apresentaram maior ($P<0,01$) CMS e CMSM (Tabela 2), e maior consumo de proteína bruta por dia (1,070 vs 0,932 Kg; $P<0,05$) do que aqueles alimentados com cana-de-açúcar.

A conversão alimentar (Tabela 2) apresentou um valor ligeiramente superior nos animais alimentados com silagem de milho mas sem diferença significativa ($P>0,05$). BRONDANI (2) e FERREIRA et al. (3), no entanto, verificaram melhor eficiência alimentar nos tratamentos que incluíam silagem de milho.

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RESUMO

Foi avaliado o desempenho de 12 bovinos Hereford (H) e 12 bubalinos Mediterrâneo (B), confinados durante 112 dias (subdividido em quatro períodos de 28 dias), precedido de uma adaptação com duração de 14 dias. A dieta alimentar continha 12% de proteína bruta. O volumoso foi silagem de milho ou cana-de-açúcar, e representou 78, 76, 61 e 56% do consumo da matéria seca, no 1º, 2º, 3º e 4º período, respectivamente. O concentrado foi constituído de farelo de arroz desengordurado, milho em grão moído, farelo de soja e calcário calcítico. Na dieta com cana-de-açucar foi adicionada uréia. Os animais tinham livre acesso a mistura de cloreto de sódio mais farinha de ossos. Os animais alimentados com silagem de milho apresentaram maior ganho médio diário de peso (GMD) (1,19 vs 1,05 Kg; $P<0,05$), maior consumo médio diário de matéria seca (CMS) (9,3 vs 7,2kg; $P<0,01$) e maior CMS por peso corporal metabólico (CMSM) (120 vs 95 g; $P<0,01$), do que aqueles alimentados com o volumoso cana-de-açucar. Os novilhos H apresentaram maior GMD(1,23 vs 1,00 kg; $P<0,01$), maior CMS (9,2 vs 7.3 kg; $P<0,01$) e maior CMSM (114 vs 102 g; $P<0,01$), do que os

novilhos B. A conversão alimentar não foi afetada significativamente pelo grupo genético dos novilhos, nem pelo volumoso da dieta.

RESUMEN

Se evaluó el desempeño de 12 bovinos Hereford (H) y 12 Búfalos (B), confinados durante 112 dias (quatro períodos de 28 dias) precedido de un período de adaptación de 14 dias. La dieta contenia 12% proteína bruta. El voluminoso fué ensilado de maiz o caña-de-azúcar y representó 78, 76, 61 y 56% del consumo de matéria seca en el 1º, 2º, 3º y 4º período, respectivamente. El concentrado estaba formado por afrecho de arroz magro, maíz molido, torta de soja y calcáreo. A la dieta con caña-de-azúcar fué adicionada urea. Los animales tenian libre acceso a una mezcla de sal y harina de hueso. Los animales alimentados con ensilado de maíz presentaran mayor ganancia diária de peso (GMD) (1,19 vs 1,05 kg; P<0,05), mayor consumo de materia seca por dia (CMS) (9,3 vs 7,2 kg; P<0,01 y mayor CMS por unidad de tamaño metabólico (CMSM) (120 vs 95 g; P<0,01), que aquellos alimentados con dieta conteniendo caña-de-azúcar. Los novillos H presentaran mayor GMD (1,23 vs 1,00 kg; P<0,01), mayor CMS (9,2 vs 7,3 kg; P<0,01) y mayor CMSM (114 vs 102 g; P< ,01) que los novillos B. La conversión alimentaria no fué afectada significativamente por el grupo genético de los novillos ni por el tipo de voluminoso incluido en la dieta.

SUMMARY

The experiment evaluated the feedlot performance of 12 Hereford (H) and 12 Mediterranean buffalo (B) steers, during a period of 112 days (subdivided in 4 periods of 28 days), preceded by an adaptation period of 14 days. The animals were fed with a 12% crude protein diet. The roughage was fresh chopped sugar cane or corn silage, and represented 78, 76; 61 and 56% of the dry matter consumption for period 1, 2, e and 4, respectively. The concentrate included defaded rice bran, ground corn, soybean meal and ground limestone. Urea was added to the diet that included sugar cane. The animals had free access to a salt plus bone meal mixture. Steers fed with corn silage had higher average daily gain (ADG) (1.19 vs 1.05 Kg; P< .05), dry matter consumption (DMC) (9.3 vs 7.2 kg; P< .01) and DMC per Kg of metabolic weight (DMCM (120 vs 95 g; P< .01), than steers fed with sugar cane. The H steers had higher ADG (1.23 vs 1.0 Kg; P<.01), DMC (9.2 vs 7.3 kg; P<.01) and DMCM (114 vs 102 g; P<.01), than the B steers. Feed conversion was not affected (P>.05) by the genetic group or type of roughage included in the diet. The variables studied were not affected (P>.05) by the genetic group x roughage type interaction.

PRODUCTIVE PERFORMANCES OF CALVES BREEDS FED ON DIFFERENT DIETARY ENERGY LEVELS.

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INTRODUCTION

In Italy beef meat is produced by rearing both specialized breeds, mainly of imports, and common ones. Among the latter, dairy and dual-purpose (meat and draught) breeds are usually utilized. In any case, feeding plans, allowing the best productive performances, should be worked out. Poor fodder availability and forage increasing costs justify the utilization of great amounts of concentrate feeds and cereals in calves finishing. For this reason it is of importance to find out the right concentrate feeds/forage ratio in the ration so as to determine the best economical-physio-productive responses in the animals.

All these problems were dealt with by Italian researchers whose studies concerned the choice either of the most appropriate genetic types (1,2,6,8) or the feeding plans (3,4,5,9).

MATERIALS AND METHODS

The trial was carried out on 10 Simmental, 10 Italian Friesian and 10 Apulian calves at 11 months of age approx. and an average live weight of 240 to 275 Kg. All the calves were fed alfalfa hay ad libitum. Half the animals were given 1.4 Kg concentrate feed/100 Kg live weight (high energetic level of the diet), the other half 1.0 Kg/100 Kg live weight (middle energetic level of the diet). During the trial, which lasted 7 months monthly live weight and daily dry matter intake were individually checked. At the end of the trial the animals were slaughtered and subjected to the following measurements: carcass weight, incidence of outer body parts (skin, head, tail, limbs), digestive organs (stomachs and guts) and the other inner organs (lungs, trachea, heart, liver, spleen). 24 hours after slaughtering and chilling at 4°C, the carcasses were divided into choice joints to asess the incidence of first, second and third--class ones. Moreover, from the right side, it was taken a sample cut at the 11th and 12th dorsal ribs which, after testing the caudal area of Longissimus dorsi muscle, was dissected to evaluate the incidence of single tissues (total lean, Longissimus Dorsi, fat, bone).

RESULTS

Simmental calves showed an average daily gain of 1,38 Kg; Italian Friesian ones of 1.01 Kg and Apulian ones of 0.91 Kg. Consequently, at the end of the trial Simmental calves reached a clearly and significantly higher live weight (P< 0.01). Simmental calves also showed the most valid conversion index. In fact, in order to reach a 1 Kg. gain the

animals ingested 7.1 Kg dry matter vs 8.4 and 9.3 Kg of the other two breeds respectively. Such differences proved highly significant ($P<0.01$) when processed by means of the variance analysis.

Dressing percentage did not differ significantly in the three considered breeds, whereas the incidence of the body parts proved different: in Simmental calves the outer parts (skin, head, tail, and limbs), the digestive organs (empty gastro-intestinal tract) were greater, whereas the incidence of the inner parts (lungs, trachea, heart, liver and spleen) did not differ. The carcass composition did not change for the three breeds, whereas the sample cut was more valid for Italian Friesian and Apulian calves presenting a larger amount of muscle than of bone and fat.

The great use of concentrate feeds in the diet sensibly improved ($P<0.01$) the animal weight gain and the conversion index of dry matter but it did not influence dressing percentage, body composition and carcass quality. It also sensibly increased ($P<0.01$) the adipogenetic character in the animal and, in Simmental and Apulian calves, sensibly reduced ($P<0.01$) meat producing capacity. In fact the dissected sample cut evidenced higher amounts of fat and bone in the animals given 1.4 Kg concentrate feeds/100 Kg live weight. The incidence of bone is reduced, thus confirming the carcass refinement due to the high presence of concentrate feeds in the diet as already shown by Montemurro et al. (7).

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SUMMARY

The trial was conducted on 10 Simmental, 10 Italian Friesian and 10 Apulian calves of 11 months of age and an average initial live weight of 240 to 275 Kg. All the animals were fed alfalfa hay ad libitum. Half of them were given 1.4 Kg concentrate feeds/100 Kg live weight, the other half 1.0 Kg. The trial lasted 7 months. Monthly live weight, daily intake of dry matter, dressing percentage, carcass quality and the composition of a sample cut at 11th and 12th dorsal ribs were assessed. The results showed the best weight gain and the most valid conversion index in Simmental calves. The other two breeds presented, on the contrary, a better composition of sample cut due to a large amount of meat. The great utilization of concentrate feeds improved weight gain and conversion index of feeds, but also determined a higher amount of fat in the sample cut.

RESUME

Pour l'epreuve on a utilise 10 veux de race Simmental, 10 de race Frisonne italienne et 10 de race des Pouilles de 11 mois d'âge et du poids de 240 à 275 Kg. Tous les animaux ont recu foin de luzerne ad libitum. A une moitie on a administre 1.4 Kg de concentre/100 Kg de poids vif; à l'autre moitie 1 Kg. L'epreuve a duré 7 mois. On a contrôle les poids vifs mensuels, la consommation quotidienne de substance seche, le rendement à l'abattage, la qualite de la carcasse et la composition d'un morceau echantillon comprendant 11eme et 12eme vertebres dorsales. Les resultats on mis en evidence la meilleure capacite d'accroissement et le plus favorable index de conversion de substance seche des sujets Simmental. Les autrex deux races ont presente, au contraire, une milleure composition du morceau echantillon grâce à une plus elevee incidence de la fraction muscolaire. La large utilisation de concentres a ameliore la capacite d'accroissement des animaux, mais a aussi determine une plus grande accumulation de graisse dans le morceau echantillon.

RESUMEN

Para la prueba hemos utilizados 10 becerros de raya Simmental, 10 de raya Frisona Italiana y 10 de raya Pugliese, de la edad de 11 meses y de el peso vivo medio inicial comprendido entre 240 y 275 Kg. Todos los animales habían recibido beno de mielga ad libitum; a la mitad de ellos vinieron, además, suministradas 1.4 Kg de cebo concentrado por 100 Kg de peso vivo, mientras a la otra mitad 1.0 Kg. La prueba ha durado 11 meses. Hemos pesado los animales cada mes, los consumos cotidianos de sustancia seca, la rendición a la matanza, la calidad de la armazón y la composición de una "biftec selecta" que incluye la XI y la XII vèrtebra dorsal. Los resultados habían evidenciado por los becerros Simmental la mejor capacidad de incremento de peso y de transformación de la sustancia seca. Los otros becerros habián presentado una mejor composición de la "biftec selecta" por una más elevada presencia de la fracción muscolar. La elevada utilizacion de cebo concentrado ha meyorado la capacidad de incremento de los animales y la capacidad de transformación de los alimentos, pero la "biftec selecta" ha resultada mas grasa.

TABLE 1. Daily gain, food conversion indexes, dressing percentage, carcass quality and variance analysis significance.

BREEDS	SIMMENTAL			ITALIAN FRIESIAN			ARYLIAN			VARIANCE ANALYSIS F Significance (4)		
DIFFERN ENERGY LEVEL	H	M	L	H	M	L	H	M	L	Breed	Diet	Breed x Diet
Live weight (kg)												
Final	566.9	558.0	562.4	471.6	446.4	466.0	442.4	431.7	437.0	**	**	**
Daily gain	1.40	1.36	1.38	1.08	0.99	1.01	0.95	0.88	0.91	**	**	**
Dry matter intake												
Daily/head	9.1	10.3	9.7	7.8	9.2	8.5	7.7	9.2	8.5	**	**	**
Daily/head/Kg live weight	6.5	7.6	7.1	7.2	9.8	8.4	8.1	10.4	9.3	**	**	**
Carcass Traits (% live weight)												
Carcass	56.5	56.5	56.5	55.9	54.1	54.8	55.0	55.2	55.1	**		
Body external parts (1)	17.8	18.8	18.3	12.6	12.6	12.6	16.3	16.0	16.1			
Digestive organs (2)	5.1	5.3	5.2	4.5	4.7	4.6	4.3	4.3	4.3	*		
Other organs (3)	3.1	7.8	5.0	3.4	3.5	3.4	3.7	3.6	3.7			
1st quality cuts	23.2	23.6	23.4	21.8	21.8	21.8	22.6	22.8	22.7			
2nd - 3rd quality cuts	33.3	32.9	33.1	33.7	32.3	33.0	32.4	32.4	32.4			
Sample cut composition												
Mean weight (% Total Value)	53.5	54.5	54.0	62.1	59.6	60.8	61.2	63.3	62.2	**		
L. Dorsi " "	37.1	37.0	37.0	40.2	41.0	40.6	40.9	40.4	40.6	*		
Fat " "	23.3	22.8	23.1	19.0.	18.2	18.6	19.4	15.7	17.6	**		
Bone " "	23.2	22.7	22.9	18.9	22.2	20.6	19.4	21.0	20.2	*	*	**
L.D. area " "	44.6	40.9	42.7	38.5	41.9	40.1	40.4	40.8	40.6	*	*	

(1) Skin, head, tail, limbs.
(2) Empty stomachs and guts.
(3) Lungs, trachea, heart, liver, spleen.
(4) ** - P≤0.01; * - P≤0.05.

H - High dietary energy level - 1,4 Kg concentrate feed/100 Kg L.W.
M - Medium " " - 1,2 " " " " " " "
L - Low " " - 1,0 Kg " " " " " " "

DEVELOPMENT AND EMPLOYMENT OF ECOLOGICALLY PURE PREPARATIONS FOR TREATMENT AND PROPHYLAXIS OF GASTROINTESTINAL CALF DISEASES

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INTRODUCTION

Gastrointestinal diseases of newborn calves (at the early postnatal period) accompanied by diarrhea syndrome are widespread due to intensification of animal husbandry. Depending on farm conditions diarrhea syndrome is observed in 35-100% newborn calves, the loss being 12-15% out of the number of sick animals. As a rule, diarrhea causative agents are considered to be enteropathogenic E.coli, Salmonella, Campylobacteria, rota- and corona viruses (4-8). However, there recently appeared reports on etiological importance of proteа, staphylococci, klebsiella, cytrobacteria, pseudomonads (2,3).

Employment of specific (vaccine and hyperimmune sera) antibacterial and chemotherapeutic agents to control diarrhea do not provide the necessary effect. Besides, these agents (particularly antibacterial) exert a negative effect on the animal body and their presence in the products of animal origin is harmful for human beings. Thus, the development and employment of preparations providing sufficiently high effect and being ecologically harmless has become very actual in the treatment and prophylaxis of diarrhea syndrome in young animals.

MATERIALS AND METHODS

Experimental animals

Experiments on studying prophylactic and curative action of new preparations in diarrhea were carried out on 1-10 day old calves.

Preparations

Developed agents for normalization of intestine bacteriobiocenosis-lactobacterin and bacteriophages for supposed to be pathogenic microbes were used.

Lactobacterin is a prep ration produced by lyophilization of 2 lactobacilli strains being re resentatives of normal intestine flora and exerting a marked antagonistic effect on supposed to be pathogenic microbes.

Lactobacterin has been used since the first day of life, 1 dose (10 billion microbial cells) 3 times a day, 3 days running.

Employment of preparations only containing lactobacilli is not effective when dyspeptic syndrome develops in calves; therefore, bacteriophages for isolated feces culture of sick calves have been developed and produced in a sufficient number.

Considering polyetiology of the disease associated polyvalent bacteriophage (polyphage) containing coli, proteа, pyocyanic, staphylococcic and klebciellosis phages with lytic activity not less than 10^{-5} were employed. Polyphage, dose 250-400 ml per one calf 2 times a day, was used prior to clinical recovery in order to treat diarrhea.

Clinical procedures

All of the observed calves were tested for daily control of physiological features of the clinical status applying general and special methods.

Microbiological procedures

Feeces from normal and affected with diarrhea calves as well as in pharmacodynamics by lactobacterin and polyphage effect were collected for bacteriological examinations. Studies and identification of isolated microorganism cultures were carried out by accelerated methods of enterobacteria differentiation and their quantitative analysis in 1 gr of the material (1).

RESULTS

As a result of bacteriological examinations of the material from normal and sick calves 200 strains belonging to 10 general of microorganisms were isolated (Table I).

TABLE I. Microflora of calf intestine

Feeces	Examined samples	Total number of isolated cultures	Percentage of isolated cultures									
			Proteus	Klebsiella	Citrobacter	Intero-coccus	Entero-bacter	Staphylo-coccus	Pseudomonas	Morganella	E.coli	EPEC
Normal	25	73	24,6	13,7	15,1	1,4	6,8	4,1	-	1,4	32,8	-
Sick	175	498	21,1	8,8	4,4	6,4	2,2	21,1	1,6	1,8	12,2	20,3

Data analysis of bacteriological examinations shows that both from normal and affected with diarrhea calves almost similar suggested to be pathogenic microorganisms are isolated except staphylococcus, enteropathogenic E.Coli and Pseudomonas which were isolated only from sick animals. More reliable results were obtained when compared concentration of suggested to be pathogenic microorganisms in the intestinal content both of normal and sick calves. Thus, P.vulgaris and mirabilis K.pneumoniae, C.freundi, Morganella morganii in sick calves were isolated in essentially significant quantity ($1,4 \cdot 10^7 - 5,5 \cdot 10^8$-$10^{10}$ microbial cells in 1 gram of feeces).

During observation of the clinical course of the disease there was established a more severe form in calves from which various associations of the abovementioned groups of supposed to be pathogenic microorganisms were isolated.

In order to select new agents for prophylaxis and treatment of calf diarrhea sensitivity of the isolated cultures to 12 antibiotics, developed bacteriophages and probiotic lactobacterin were studied (Table 2).

Thus, multiple resistance of most bacterial cultures was revealed to a wide set of antibiotics used on farm, which makes it necessary to limit them and replace by more efficient, harmless and ecologically pure preparations - lactobacterin and bacteriophages.

Bacteriophages were used in the form of bi- or polyphage. The polyphage was prepared either directly before administration by mixing monophages in equal correlation or at factory of biological preparations. Results of lactobacterin and polyphage administration are given in Table 3. The data obtained testify efficacy of lactobacterin and polyphage employment in case of prophylaxis (efficacy 87,2 and 69,4%) and

TABLE 2. Phago- and antibiotic sensitivity, antagonistic activity of lactobacterin to supposed to be pathogenic microorganisms

Denomination of cultures	Number of strains	Phagosensitivity, %	Number of antibioticosensitive strains	Antagonistic activity of lactobacterin, %
E.coli (EPEC)	50	76	10	80
P.aerogiosse	8	87,5	5	75
K.pneumoniae	40	100	22	100
Pr.vulgaris	50	66	8	68
St.aureus	50	100	0	86

TABLE 3. Curative and prophylactic efficacy of lactobacterin and polyphage in diarrhea of calves

Way of prophylaxis	Group	N	Fell ill		Average duration of disease	Died		Average weight gain	Protective effect %	Therapeutic effect %
			Number of anim.	%		Number of anim.	%			
Lactobacterin	test	1720	220	12.8	1.7	12	0.7	680	87.2	99.3
	control	1250	1003	80.2	4.8	52	4.1	410	19.8	95.9
Polyphage	test	1042	319	30.6	3.6	25	2.4	570	69.4	97.6
	control	458	371	81	5.0	29	6.3	414	19	93.7

treatment (99,3 and 97,6%) of neonatal calf diarrhea, respectively.

Bacteriological examination of the material from calves receiving lactobacterin and polyphage revealed significantly low importance of quantity of bacteria genus Proteus, Klebsiella, Escherichia isolated from 1 gr of intestinal content, compared to the control animals. It is also established that antagonistic activity of lactobacterin with reference to suggested to be pathogenic microorganisms increases and vice versa lytic activity of bacteriophages decreases on farms where the preparation were used for a long time.

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SUMMARY

Etiopathogenetic importance of suggested to be pathogenic microorganisms (SPM) in diarrhea of calves has been established. Cultures isolated from faeces of sick animals had multiple antibiotic resistance, which allowed to develop ecologically pure and efficient preparations. The new agents - lactobacterin and polyphage - possessed high antagonistic and lytic activity in relation to SPM and were sufficiently effective in prophylaxis and treatment of calf diarrhea diseases. Safety increased and growth rate energy of calves improved when lactobacterin and polyphages were administered.

RESUME

Le role etiopathogenetique des microorganismes pathologenique eventuels (MPE) est etabli a la diarrhee des voeus. Des cultures degagees des feces de voeux attents ont manifeste nombre de resistance antybiotique ce qui a servi de base pour elabores des preparations ecologiquement pures et efficaces. Ces nouvelles preparations - lactobacterine et polyphague - possedent l'action antagoniste et lytique envers les MPE et ils etaient asses efficaces pour la prophylaxie et le traitement des voeux atteints de diarrhee. En employant les sus - dites preparations on a augmente non seulement l'integrite des voeux mais accessi les indices d'energie de leur croissance.

ZUSAMMENFASSUNG

Es wurde athiopathogenetische Rolle der relativ pathogenen Mikroorganismen bei Kalberdiarrhoe festgestellt. Die aus dem Kot der Kranken Tiere ausgeschiedenen Kulturen zeigten bei Antibiotikaanwendung mehrzehlige Wiederstandsfahigkeit, was zur Folge hatte, einige neue Praparate zu entwickeln, die den okologischen Normen mehr entsprechen und auchrtiel effektives wirkten. Die neuen Praparate sind Laktobakterin und Polyfag. Sie besitzen eine hohere antagonistische und zerstorende Wirkung auf die Mikroorganismen. Sie wirken auch prophylaktisch und heilend bei Kalberdiarrnoe. Dank der Anwendung von Laktobakterien und Polyfagen erhoht sich die Lebensfahigkeit der Tiere und verbessernsich die Energiekennzoffer des Kalberwachstums.

PROBIOTIC IN CALVES, A DOUBLE BLIND CLINICAL TRIAL IN VETERINARY PRACTICE IN DENMARK

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INTRODUCTION

For many years antibiotic treatment has been used to regulate the intestinal flora in calves. The antibiotics have either been given via milk-replacements or via concentrates. The theoretical foundation of the effect of antibiotic treatment is described several places in the literature (11), but the effect is very dependent on conditions of production, management, feed quality and environment. In connection with this, the possible negative effects of antibiotic treatment have to be considered such as: Rest-concentrations, resistance, post-antibiotic diarrhoea and toxic side effects, if any. Especially the consumers have become aware of these conditions.

As an alternative to antibiotics and chemotherapeutics with growth-promoting effects new products mainly containing selected bacteria strains from the natural intestinal flora have been developed and marketed extensively during recent years. These products are called biological growth-promoters or probiotics (4, 10).

THE NORMAL INTESTINAL FLORA

The gastrointestinal tract of the new-born calf is sterile without content of bacteria. The first days post partum is consequently of great importance for the establishment of the intestinal flora. Within the first week after birth colonization of each individual section of the intestines takes place each section being dominated by certain bacteria strains. 400-500 different strains can be found in the normal intestinal flora, and Smith (8) has tried to quantify the bacteria in the individual intestinal sections.

The gastrointestinal tract is an open ecosystem where one can distinguish between two different types of micro-organisms: 1. Apathogenic, permanently residing organisms that have developed a symbiotic relation to the host animal, and 2. Potential pathogenic organisms that quickly will be led through the intestinal tract by the peristaltic movements if they do not succeed in colonizing and adhering to the intestinal mucosa. The normal intestinal flora functions as a protective barrier against pathogens by blocking the adhesion of the pathogens to the intestinal mucosa. The colonization of the mucosa acts as a barrier,too. It is believed that this colonization has a stimulating effect on the local immune system by secretion of IgA to the mucosa (3).

The ecosystem of the gastrointestinal tract remains in balance as long as feed and environment are constant (5). A number of environmental changes may cause stress in the calves. These environmental changes can be change of feed, infections and overcrowding of the stables. Also the increased excretion of corticosteroides from the suprarenal glands affects the immune system resulting in changes of the composition of the intestinal flora.

THE PROBIOTIC CONCEPT

A bacteria strain or micro-organism has to meet certain requirements in order to be used as a probiotic. These requirements can be formulated shortly and are based on the present knowledge of adhesion, colonization and function of lactic acid bacteria in the intestinal tract. Together these requirements constitutes the probiotic concept, (2,4, 10).

These requirements explain why not all lactic acid producing bacteria strains are suitable as probiotics for calves.

Jonsson and Olsson (6) inoculated calves with a blend of lacto bacilli isolated from rumen and intestinal epithelium. By prophylactic treatment no significant differences in growth and diarrhoea frequency were seen even though the diarrhoea frequency in the trial was very high.

Compared to this Schumm et. al (9) demonstrated a good effect of adding Streptococcus faecium Cernelle 68 (SF68) to raw milk replacements in four problem herds.

OWN INVESTIGATIONS

This Danish clinical trial is to be seen in the light of the results of the above-mentioned investigations. I found it necessary to test probiotic treatment of Danish calves under Danish production conditions. The objective is to investigate the effect of probiotic treatment of new-born calves measured by:

Average daily gain (in gramme)
Incident of diarrhoea (IR, 0-30 days of age)
Excretion of pathogens (qualitatively)

MATERIALS AND METHODS

5 SDM dairy herds with separate section for calves, good management and without diagnosed infection with E. coli (ETC-K99) and Salmonella. All the calves were in single pens with drinking bowls mounted on the front of the pen. Collective hayrack for 2-4 calves. It has been aimed that management, feed hygiene and environment of the herds were of the same standard approximately in order to estimate subjectively.

Streptococcus faecium Cernelle 68 (SF68) in the form of CALVET (product formulation by Lundbeck Pharma A/S, Denmark) was chosen as probiotic. According to Lowenstein et al. (7) this bacteria in vitro meets the requirements stated in the probiotic concept.

The trial was conducted as double blind, and the trial group were given 5 gramme SF68 (350 million bacteria per gramme) and the control group 5 gramme placebo (the same lactose, that forms the carrier in SF68) from the very first raw milk allocation to 9th day after birth, (4, 10).

Randomization was established by the first calf in each herd being placed in either trial or control group by lot. After this the next calf was placed in the "opposite" group and the subsequent calves were placed according to this principle immediately after calving.

Faeces were taken from calves with diarrhoea prior to treatment with electrolytes and/or antibiotics. At the same time faeces were taken from a "corresponding" calf of same age from the opposite group. The faeces were tested for content of E. coli, Salmonella, Rota virus and Corona virus.

Both groups were fed with the same amount of milk feed and calf-mix, and hay were given according to appetite.

All the calves were weighed at birth, before administration of colostrum, and at 30 days of age at the same time e.g. before morning feeding.

The following notes were taken successively during the trial:

1. Diarrhoea: Faeces test, temperature, course and duration.
2. Treatments: Electrolyte, antibiotic preparation and dose.
3. Other diseases.
4. Number of deaths.

Statistical evaluation:

Diarrhoea: x^2-test (Yates), Mantel-Haenzel method.
Daily gain: Students T-Test.

RESULTS

Occurrence of heifer and bull calves in the trial. The dispersion spread is approximately alike with the exception of herd no. 5 in which the number of heifer and bull calves respectively are very different. Furthermore it should be mentioned that the trial period was characterized by a majority of born bull calves (43% heifer calves and 57% bull calves).

Milk feed administered in the individual herds:

Herd no. 1, 4 and 5:	Full/raw milk from day 0 to about day 60.
Herd no. 2 and 3:	Full/raw milk from day 0 to day 9 and subsequently milk-replacement based on whey containing Virginiamycin (80 ppm.).

Incidents of diarrhoea in the trial is shown in Table 1.

Table 1. Diarrhoea outbreaks in the period 0-30 days after birth.

Herd no.	Trial group treated by by SF68	I.R. in the risk period 0-30 days of age	Control group	I.R. in the risk period 0-30 days of age	x^2-Yates
1	1	0.20	2	0.40	1.9
2	2	0.25	4	0.50	2.4
3	1	0.07	7	0.53	7.4 a)
4	1	0.12	4	0.50	4.6 b)
5	2	0.20	4	0.40	2.2

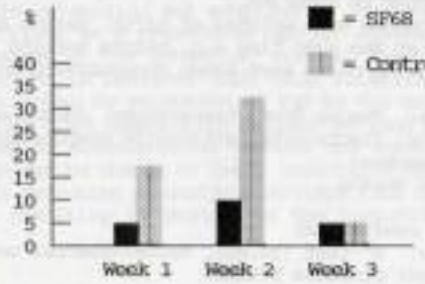
I.R.: Incident level in the risk period 0-30 days of age.
a): $P < 0.01$
b): $P < 0.05$
x^2-Yates: Owing to the size of the herds the stated x^2 values are Yates-corrected.

In order to compare all 5 herds and eliminate differences in these herds, a statistic statement has been made according to the Mantel-Haenzel method.

By this a Odds Ratio is found = 0.21 and $x^2 = 10.38$ ($P < 0.01$).

This shows that the total effect of SF68 treatment in all 5 herds is statistically significant (and that the risk of getting diarrhoea is about 5 times smaller in the trial group than in the control group).

Figure 1. Diarrhoea outbreaks according to age



If the incidents of diarrhoea is grouped according to age, see Figure 1, it will appear that the frequency of diarrhoea in both groups is highest from 7-14 days after birth. However, the level in the group treated with SF68 is much lower.

The qualitative examination of pathogens in faeces did not show any difference between the two groups. An unspecific coli flora was found in all the herds. Herd no. 3 and 4 had an outbreak of rota virus excreting virus in trial as well as in control group.

If the statement of incidents of diarrhoea and time are divided according to excretion of pathogens, the results will be as shown in Figure 2 and Figure 3.

Figure 2. Incidents of diarrhoea in herd no. 1, 2, and 5. + Rota virus

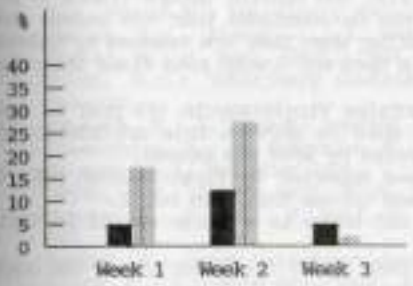
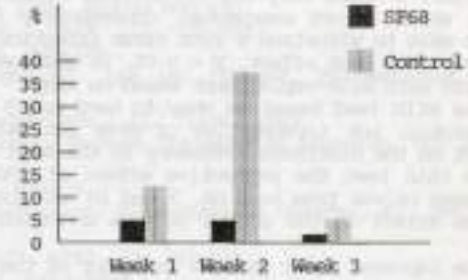


Figure 3. Incidents of diarrhoea in herd no. 3 and 4. + Rota virus.



As regards an evaluation of the daily gain in trial and control groups the results of the trial is stated in Table 2.

Table 2. Gain measured in grams per day from 0-30 days of age expressed by the average daily gain calculated for both groups.

Herd no.	Trial group treated by by SF68	Control group	Difference SF68 - Control	SF68 Daily gain in percentage	T
1	686	580	106	18%	0.53
2	630	609	21	3%	0.50
3	619	603	16	3%	0.12
4	579	525	54	10%	1.44
5	673	640	33	5%	0.86

This way an average gain in the trial group is calculated to 7.8% varying from 3-18%. The difference between trial and control group is statistically tested by a Students T-test. These T-values and corresponding P-values show that the difference in gain was not statistically significant in any of the herds. The feed consumption has not been registered.

DISCUSSION

It is remarkable that the control groups in the 5 herds selected by good management and without specific diarrhoea problems show a relative high average diarrhoea frequency of 47% (44-50%) whereas the trial groups show an average diarrhoea frequency of 17% (8-25%). No deaths have occurred in the trial period.

This test of SF68 demonstrates that it has a reliable effect on the frequency of diarrhoea outbreaks in the trial group. The decrease in diarrhoea outbreaks is bigger than stated by Schumm et. al. (9). It appears from my test that excellent effect has been observed in herd no. 3 and 4 where the calves are "stressed" by the rota virus infection. However, the most remarkable effect has been observed in herd no. 3 that is "stressed" by virus as well as milk replacement based on whey. This observation agrees with the fact that other things being equal the effect of probiotics is best when the micro flora of the tract is out of balance. However, this test shows also effect on the frequency of diarrhoea outbreaks in herds without high disease frequency and without change of feed, compare herds no. 1 and 5.

It appears from Figure 2 that herds no. 1, 2 and 3 show a parallel course as regards the spread of diarrhoea outbreaks in the first 3 weeks after birth whereas the calves treated by SF68 shows a far lower diarrhoea frequency. No difference has been observed between herds no. 1 and 5 using raw milk in the entire suckling period and herd no. 2 being fed with milk replacement based on whey after 9 days.

Figure 3 shows very big differences between trial and control groups (Table 1: $P < 0.05$) which is not unexpected. Consequently it can be concluded that the calves seem to be able to withstand a rota virus infection better when they are treated by Calvet-(SF68). The best effect, $P < 0.01$, is observed in herd no. 3 with rota virus infection and fed with milk-replacement based on whey.

The milk feed based on whey in herd no. 3 contains Virginiamycin (80 ppm) as feed antibiotic, but irrespective of SF68 not being able to survive this antibiotic (?) effect on the diarrhoea frequency in the herd treated by SF68 was proved.

In this test the protective effect of SF68 was superior to Virginiamycin both in stressed calves from herd no. 3 and in not-stressed calves from herd no. 2.

The effect on the growth is more uncertain, and there is a large spread in daily gain.

The improved daily gain is in favour of the Calvet (SF68) treatment in all the herds (Table 2) and can compete with other investigations (2, 4, 9).

SUMMARY

An exposition of the probiotic concept and some possible efficiency mechanisms of probiotics have been obtained. Design and course of a test of Streptococcus faecium Cernelle 68 (SF68) contained in the product **Calvet** (product formulation by Lundbeck Pharma A/S, Denmark) have been discussed. An average diarrhoea frequency in the trial groups of 17% (8-25%) and an average diarrhoea frequency in the control groups of 47% (44-50%) have been demonstrated. The best effect has been observed in the herds having an imbalanced intestinal flora - in this case owing to rota virus infection and milk replacement based on whey.

The effect on the daily growth is not as distinct. However, all the herds show the best growth in the trial groups, 7.8% (3-18%).

It can be concluded that additional investigation of intestinal ecology, quantitative bacteriology and further tests of probiotics are necessary.

ZUSAMMENFASSUNG

Eine Durchnahme des Probiotikum Konzepts und einiger wahrscheinlichen Auswirkungsmechanismen des Probiotikums is vorgenommen. Design und Verlauf einer Überprüfung der Streptococcus faecium Cernelle 68 (SF68) von Präparat **Calvet** (Produktformulierung Lundbeck Pharma A/S, Danemark) sind erhellt worden. Dadurch ist eine durchschnittliche Diarrhöefrequenz in der Versuchsgruppe in Höhe von 17% (8-25%) und in der Kontrollgruppe 47% (44-50%) festgestellt worden. Die beste Wirkung des Probiotikums ist in den Beständen mit unausgeglichen Darmflora konstatiert worden - in diesem Fall wegen Rotavirusinfektion und Vollmilchersatz basiert auf Molke.

Die Wirkung auf den täglichen Zuwachs ist weniger prononciert. Der gröste Zuwachs ist aber in allen Beständen in den Versuchsgruppen festgestellt worden, 7,8% (3-18%).

Daraus is abzuleiten, dass sowohl weitere Forschung innerhalb des Bereiches von Darmökologie und mengenmässiger Bakteriologie sowie mehrere Prüfungen notwendig sind.

RESUMEN

Se ha realizado un examen del concepto probiótico y de algunos mecanismos de efecto probables de los probióticos. Se ha dado cuenta del diseño y curso de una prueba de Streptococcus faecium Cernelle 68 (SF68) del preparado **Calvet** (fórmula de producto Lundbeck Pharma A/S, Dinamarca). A esta relación se ha constatado una frecuencia promedia de diarrea en el grupo de prueba del 17% (8-25%) y en el grupo de control del 47% (44-50%). El efecto mayor se nota en el ganado con la flora intestinal en desequilibrio, por infección de rotavirus y sucedáneo de leche entera basado en suero.

Es menos pronunciado el efecto sobre el crecimiento diario, sin embargo todo el ganado con mayor crecimiento forma parte de los grupos de prueba, o sea el 7,8% (3-18%).

Debemos concluir que hace falta realizar investigaciones adicionales sobre la ecología intestinal, la bacteriología cuantitativa y muchas pruebas de probióticos.

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ETUDE DE LA FORCE DU DIAPHRAGME ET DE SES IMPLICATIONS DANS LE SYNDROME DE DETRESSE RESPIRATOIRE AIGUE CHEZ LES BOVINS

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INTRODUCTION

Les jeunes bovins souffrent fréquemment de maladies respiratoires. Une certaine proportion d'entre elles dégénèrent en syndrome de détresse respiratoire aiguë dont l'issue est souvent fatale, ce qui occasionne une majoration importante des coûts d'exploitation. Il est donc primordial d'essayer d'isoler le ou les facteurs responsables de ce syndrome afin d'en maîtriser plus efficacement la prévention et/ou le traitement. Dans la plupart des cas, l'hypoventilation alvéolaire est attribuée à une maladie pulmonaire ou à un dysfonctionnement des mécanismes de contrôle de la ventilation. La question de savoir si un processus de fatigue des muscles respiratoires peut également être incriminé dans ce phénomène mérite d'être posée. En effet, au cours d'une maladie pulmonaire, la pompe motrice respiratoire doit soutenir un travail exacerbé pendant une période prolongée et sans possibilité de repos. La fatigue éventuelle qui en découlerait pourrait être le chaînon physiopathologique qui distingue une pathologie respiratoire qui évolue favorablement d'une autre qui dégénère en syndrome de détresse respiratoire aiguë. Gustin et al.(6) ont démontré récemment qu'au cours de la respiration dans les conditions de repos, les veaux de race Blanc-Bleu-Belge (BBB) génèrent une pression transpulmonaire plus importantes que les veaux Frisons (F) afin de compenser une résistance pulmonaire totale et une fréquence respiratoire plus élevées. Par conséquent, on s'attend à ce que, parallèlement à cet effort respiratoire plus important, les veaux BBB développent progressivement des muscles respiratoires plus forts que les veaux F. Cependant, au cours d'une épreuve d'hypoxie aigue pratiquée en laboratoire, il a été démontré que les veaux BBB ne sont pas capables de soutenir une hyperventilation compensatrice aussi longtemps que les veaux F (12). De plus, sur un plan clinique, il est bien connu que les maladies respiratoires dégénèrent plus fréquemment et surtout plus rapidement en syndrome de détresse respiratoire aigue chez les BBB par rapport aux F, ce qui augmente significativement les coûts dûs aux maladies respiratoires chez les premiers (4). Ces arguments contradictoires justifient l'intérêt que nous portons à l'investigation comparée de la fonction musculaire respiratoire dans les races BBB et F. La force globale des muscles respiratoires est reflétée par la pression maximum que l'individu peut générer au cours d'un effort inspiratoire maximum développé contre une occlusion des voies aériennes. En pratique, la force maximun des muscles respiratoires est estimée en mesurant les pressions buccale (Pb), oesophagienne (Pes) ou transdiaphragmatique (Pdi) au cours d'un effort inspiratoire maximun. Si ce genre de manoeuvre est facile à obtenir de la part d'un sujet coopératif, ce n'est plus le cas lorsqu'il s'agit d'animaux. Le but de la présente étude était (1) de tester la validité d'un subterfuge destiné à obtenir un effort inspiratoire maximal chez le veau sain et non tranquillisé et (2) de corréler les valeurs des pressions maximum obtenues avec l'âge et la race des animaux.

MATERIEL ET METHODES

Animaux

Vingt-huit veaux F (n=14) et BBB (n=14), âgés de 35 à 134 jours et pesant entre 55 et 125 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 maladie respiratoire. Les animaux avaient été habitués aux conditions et équipements du laboratoire et avaient subi une période de jeûne de 12 heures avant l'expérimentation. Le périmètre thoracique était mesuré immédiatement en arrière des membres antérieurs. Un masque étanche était adapté à la tête de l'animal. Son étanchéité avait été testée jusque 120 cmH2O.

Mesure des paramètres

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 (6, 10) pour estimer de manière fiable et reproductible la pression pleurale chez les bovins F et BBB respectivement. Un second cathéter également muni d'un ballonnet à son extrémité distale était introduit par la deuxième narine et avancé jusque dans le rumen afin de mesurer la pression en arrière du diaphragme (Pru). Les cathéters oesophagien et ruménal étaient connectés, via un tube en "Y", d'une part à un transducteur de pression (Bentley, Trantec M800) et, d'autre part à l'une et l'autre des deux entrées d'un transducteur différentiel (Compliance test Mark III, Gould) respectivement. Pes et Pru étaient obtenues grâce aux deux premiers transducteurs tandis que le troisième mesurait la différence entre Pru et Pes, soit Pdi. Un trou pratiqué dans le masque près des narines permettait, via une unité cathéter-transducteur supplémentaire (Statham PI23D, Gould), la mesure de la pression dans le masque (Pb) A l'exception de Pdi, toutes les pressions étaient mesurées par rapport à la pression athmosphérique. Les quatre unités cathéter-transducteur étaient calibrées mécaniquement au moyen d'une colonne d'eau. Leur linéarité et leur compatibilité de phase avaient été vérifiées entre -100 et +100 cmH2O et jusque 7 Hz respectivement. Tous les signaux étaient enregistrés simultanément sur un polygraphe électrostatique à 12 canaux (Gould ES-1000).

Manoeuvres inspiratoires maximales

Le masque des animaux était bouché hermétiquement lorsqu'ils se trouvaient à la capacité résiduelle fonctionnelle (CRF) qui était repérée grâce au plateau de fin d'expiration sur le tracé de Pes. Les animaux tentaient d'inspirer vigoureusement contre l'occlusion, laquelle était maintenue jusqu'à ce que la contention ne soit plus possible (±70 sec). La force globale des muscles respiratoires était estimée par la valeur minimum de Pb et de Pes enregistrée pendant l'occlusion (Pbmin et Pesmin). La performance diaphragmatique était évaluée simultanément par la valeur maximum de Pdi (Pdimax). La manoeuvre était répétée cinq fois à un intervalle de 8-10 minutes.

Analyse des résultats

Pour chaque manoeuvre, les 3 valeurs extrêmes de Pb, Pes et Pdi ont été moyennées pour donner Pbmin, Pesmin et Pdimax. La reproductibilité des résultats a été testée par une analyse de variance à 2 critères de classificatiuon sur 2 groupes de 5 mesures successives effectuées à 1 jour d'intervalle chez 7 animaux. Les pressions maximales enregistrées chez les 28 animaux ont été analysées par un modèle linéaire fixe hiérarchisé : race, individu (race), poids (≤70, ≤90, ≤110 et <110kg) et âge (1-45, 46-60, 61-85 et 86-134 jours).

RESULTATS

Aucune différence significative n'a pu être mise en évidence entre les mesures des pressions maximales obtenues au cours des occlusions successives ou pratiquées à 1 jour d'intervalle (P≤0.05). D'autre part, le modèle statistique utilisé rend compte de 84.4, 84.1 et 87.8 % de la variation de Pbmin, Pesmin et Pdimax respectivement. A l'exception de la race et de l'âge pour Pbmin et de la race pour Pesmin, tous les effets inclus dans le modèle étaient significatifs. Les moyennes des moindres carrés des effets race et âge sont présentées dans le Tableau 1.

DISCUSSION

Considérations techniques

Comme pour n'importe quel muscle squelettique, les performances mécaniques des muscles respiratoires peuvent être décrites en termes de force et d'endurance. La force des muscles respiratoires des veaux étudiés dans cette étude a été estimée par Pbmin, Pesmin et Pdimax. Avant de discuter les résultats proprements dits, il faut considérer le problème de l'obtention d'un effort maximum chez un sujet non coopératif. Dans cette étude, le problème de la "motivation" du sujet a été contourné en maintenant l'occlusion de sorte que l'animal soit forcé de générer un effort inspiratoire quasi maximum. Si les pressions maximum mesurées au cours d'une telle manoeuvre donnent en elle-mêmes peu de certitudes quant à leur valeur pour estimer la force maximum réelle des muscles respiratoires, le fait qu'elles ne différaient significativement ni d'une occlusion à l'autre, ni d'un jour à l'autre indique que la procédure expérimentale choisie fournissait une information reproductible. Néanmoins, malgré cette excellente reproductibilité, une erreur sur la force maximum pourrait avoir été faite du fait de la nature de la manoeuvre. En effet, pendant les occlusions, tout apport en O2 ou excrétion de CO2 était supprimé, ce qui a immanquablement dû résulter en une diminution de l'apport en O2 et une rétention de CO2 au niveau des muscles eux-mêmes. Donc, les performances mécaniques réelles des muscles respiratoires sont probablement sous-estimées. Cependant, aucune différence significative (t de student, P<0.05) n'a pu être mise en évidence en ce qui concerne les pressions partielles en O2 et CO2 d'échantillons de sang artériel prélevés en fin d'occlusion chez plusieurs sujets F ou BBB d'âges différents. De plus, il a été démontré que les deux races ne diffèrent pas en ce qui concerne l'évolution de leurs gaz artériels pendant une épreuve d'hypoxie aiguë expérimentale (1). Par conséquent, les comparaisons entre races et animaux de différents âges gardent toute leur légitimité. La force globale des muscles respiratoires varie de 41 à 82 et de 57 à 98 cmH2O pour Pbmin et Pesmin respectivement et de 61 à 102 cm H2O pour Pdimax. Ces résultats sont comparables aux performances rapportées pour l'homme adulte (2,5,9).

Effet de l'âge

Les moyennes des moindres carrés de Pbmin, Pesmin et Pdimax (Tableau 1) indiquent que la force des muscles respiratoires augmente progressivement de la première (1-45 jours) à la troisième classe d'âge (61-85 jours). Ce résultat peut être mis en parallèle avec d'autres études qui ont démontré le handicap mécanique du diaphragme du nouveau-né par rapport aux performances de celui d'animaux plus âgés (11). Donc, il apparait qu'entre la naissance et l'âge de 3 mois, le développement des propriétés contractiles des muscles respiratoires dans l'espèce bovine est considérable. Dans le cas du diaphragme, l'augmentation de la force contractile disponible compense donc beaucoup plus que pour simplement contrecarrer le désavantage mécanique progressif lié à un rayon de courbure de plus en plus grand au fur et à mesure de la croissance.

Effet de la race

Les valeurs de Pdimax étaient significativement moindres chez les animaux BBB alors que celles de Pbmin et Pesmin étaient comparables. Donc, contrairement à l'hypothèse de départ, cette étude montre que les muscles respiratoires des BBB ne sont pas plus forts que ceux des F. On peut conclure qu'il n'y a pas d'adaptation fonctionnelle des muscles respiratoires des BBB à un travail respiratoire exacerbé. De plus, la force du diaphragme des BBB est significativement moindre que celle des F. A ce stade, aucune explication fondée ne peut être fournie à ce sujet. Néanmoins, la sélection génétique sévère des gènes de l'hypertrophie musculaire dans la race BBB a provoqué des altérations importantes de la distribution des fibres musculaires (3,7,8) et ce phénomène pourrait avoir modifié les caractéristiques histologiques, biochimiques et donc fonctionnelles du diaphragme des BBB. Ce handicap fonctionnel du muscle inspiratoire principal pourrait expliquer, en partie, le manque d'endurance respiratoire des bovins BBB lors d'une épreuve d'hypoxie aiguë (12).

L'ensemble de nos observations suggère que les performances mécaniques des muscles respiratoires des bovins pourraient devenir critiques au cours des maladies respiratoires chez les animaux jeunes et plus particulièrement ceux de race BBB. Ces résultats suggèrent qu'un processus de fatigue des muscles respiratoires pourrait être incriminé dans la pathogénie du syndrome de détresse respiratoire aiguë chez les bovins. Si des études ultérieures devaient confirmer cette hypothèse, et vu l'importance économique des pathologies respiratoires en spéculation bovine, il faudra probablement incorporer les caractéristiques fonctionnelles des fibres musculaires parmi les critères de sélection des animaux hyperviandeux.

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Tableau 1 : *Moyennes des moindres carrés données par le modèle pour la race et l'âge. Les variables dépendantes sont : la pression buccale minimum, la pression oesophagienne minimum et la pression transdiaphragmatique maximum (Pbmin, Pesmin et Pdimax respectivement).*

		Pb min (cm H2O)	Pes min (cm H2O)	Pdi max (cm H2O)
Race	BBB	-62.9	-73.1	79.2
	F	-62.6	-75.6	87.3
Age	1-45 jours	-49.9	-64.4	63.9
	46-60 jours	-56.6	-71.1	78.8
	61-85 jours	-73	-79.6	94.5
	86-134 jours	-71.4	-82.2	95.8

RESUME

Vingt-huit veaux de race Frisonne (F) et Blanc-Bleu-Belge (BBB), âgés de 35 à 134 jours ont été étudiés. Ils ont été soumis à une occlusion des voies respiratoires réalisée au niveau de la capacité résiduelle fonctionnelle. Des inspirations forcées ont alors été développées par les animaux. Les pressions buccale, oesophagienne et transdiaphragmatique maximum enregistrées sont proposées comme index de la force globale des muscles inspiratoires et du diaphragme respectivement. Malgré la limitation de la méthode, qui est le développement progressif d'une hypoxie et d'une hypercapnie artérielles pendant l'occlusion, des résultats reproductibles ont été obtenus. La force des muscles inspiratoires était significativement augmentée chez les animaux plus âgés par rapport aux plus jeunes, tandis que la force du diaphragme était significativement moindre dans la race BBB par rapport à la race F. Ces résultats suggèrent qu'un handicap musculaire respiratoire pourrait expliquer, en partie, la plus grande sensibilité de la race BBB au syndrôme de détresse respiratoire aiguë.

SUMMARY

Twenty-eight calves of the Friesian (F) and Belgian White and Blue (BWB) breeds, 35 to 134 days of age, were investigated. They were subjected to sustained airway occlusions performed at FRC. The animals vigorously attempted to suck in air against their closed tightly adjusted face mask. The maximal airway opening, esophageal and transdiaphragmatic pressure swings (Paomin, Pesmin and Pdimax respectively) were taken as index off inspiratory muscle strength. Despite obvious limitation of the method, such as the development of a progressive hypoxia and hypercapnia during the occlusions, reproducible values were obtained for Paomin, Pesmin and Pdimax. Inspiratory muscle force increased with age whereas diaphragmatic strength, as reflected in Pdimax, was higher in F than in BBB calves. These results could be related to the greater sensitivity of the BBB calves to ARDS.

ZUSAMMENFASSUNG

Achtundzwanzig 35- bis 134tägige Kälber friesischer Herkunft (F) sowie der weiß-blauen belgischen Art (BBB) wurden untersucht. Bei den Tieren wurde während der Funktionelresidualkapazität ein Atemwegverschluß durchgeführt. Infolgedessen entwickelten die Kälber forcierte Atmung. Die hierbei aufgezeichneten Maximalwerte des Oesophagus- und. Durchzwerchfell-Druckes werden als der Pauschalkraftindex der Atmungsmuskeln und. des Zwerchfells vorschlagen.
Trotz der Beschränkungen dieser Technik wie z. B. der Entwicklung einer fortschreitenden Hypoxia während des Verschlußes wurden wiederholbare Ergebnisse erzielt. Die entwickelte Kraft der Einatmungsmuskeln war bedeutend größer bei den älteren Tieren als bei den jüngeren, während die Kraft des Zwerchfells in der weiß-blauen belgischen Art deutlich kleiner war als bei den Kälbern friesischer Herkunft. Diese Ergebnisse lassen vermuten, daß ein Handikap der Atmungsmuskeln die größere Anfälligkeit der weiß-blauen belgischen Art für den akuten Atemnotsyndrom teilweise erklären könnte.

RECHERCHE D'UN SYNDROME DE COAGULATION INTRAVASCULAIRE DISSEMINEE (CIVD) AU COURS D'UNE PASTEURELLOSE RESPIRATOIRE EXPERIMENTALE A PASTEURELLA HAEMOLYTICA A1 (PHA1) CHEZ LE VEAU.

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INTRODUCTION

Dans le complexe "Broncho-pneumonies Infectieuses Enzootiques des Jeunes Bovins"(BPIE), le rôle de Pasteurella haemolytica A1 (PHA1) est essentiel (4). Cette bactérie dispose de deux facteurs de pathogénicité majeurs : l'endotoxine et la cytotoxine. L'endotoxine lipo-polysaccharidique libérée après mort et lyse de PHA1 interviendrait selon différentes modalités mais surtout en activant les macrophages alvéolaires qui par production de leucotriènes et de platelet activating factor recrutent les polynucléaires neutrophiles. La leucotoxine des PHA1 phagocytée par les polynucléaires neutrophiles ainsi massivement recrutés entraîne la destruction de ces derniers avec libération exponentielle dans le parenchyme pulmonaire non seulement de radicaux libres oxygénés mais également de tout un arsenal enzymatique à l'origine d'une violente réaction inflammatoire.

Les lésions histopathologiques de la pasteurellose respiratoire bovine à PHA1 comportent régulièrement (2) : - des dépots de fibrine dans les alvéoles, les vaisseaux lymphatiques et les espaces interlobulaires infiltrés de macrophages et de polynucléaires neutrophiles,

- des microthromboses capillaires formées d'agrégats cellulaires (macrophages, polynucléaires neutrophiles, plaquettes) et de fibrine à l'origine de troubles ischémiques expliquant en partie les complications de nécrose.

Cet ensemble lésionnel suggère un phénomène de coagulation intravasculaire disséminée (CIVD), processus lié à l'activation in vivo des plaquettes et des facteurs de la coagulation avec formation de thrombine et dépots de fibrine dans les capillaires, artérioles et veinules, en particulier dans certains organes privilégiés comme par exemple les poumons et les reins. Pour ces raisons et, disposant d'un modèle expérimental de pasteurellose respiratoire à PHA1 chez le veau, la recherche d'une CIVD à l'aide de méthodes biologiques a été mise en oeuvre avec l'objectif de mieux cerner la physiopathologie des BPIE, voire de justifier de nouveaux moyens thérapeutiques.

MATERIEL ET METHODES (5)

- **Animaux** : 10 veaux de race française frisonne, âgés de 2-3 semaines, d'un poids moyen de 50 kg, nourris avec un aliment d'allaitement distribué au seau.
- **Schéma expérimental** (Tabl.1)
- **Inoculations** : les inoculum ont été, pour la voie nasale, des cultures agitées de 2h titrant entre 2,3 x 10^7 et 1,8 x 10^8 UFC/ml, pour la voie intratrachéale entre 6,5 x 10^8 et 3,5 x 10^9 UFC/ml. L'inoculation intra-nasale (J0, J1, J2) étant effectuée à 11h, l'inoculation intratrachéale (J0,J1) à 14 h.
- **Examens cliniques standardisés** : il permet sur la base de signes respiratoires et non respiratoires d'obtenir une note moyenne générale de maladie (NMG).
- **Examens hématologiques** :
  - numération des globules rouges et des globules blancs : compteur hématologique semi-automatique SYSMEX modèle CC180.
  - formule leucocytaire
  - numération des plaquettes (Unopettes Becton Dickinson)
- **Explorations de l'hémostase** :
  - recherche de complexes solubles dans le plasma par agglutination d'hématies sensibilisées par des monomères de fibrine issus d'une fibrinogénolyse (F.S. Test, Diagnostica Stago, 92600 Asnières sur Seine),

  - recherche des produits de dégradation du fibrinogène/fibrine dans le sérum par agglutination de particules de latex sensibilisées à l'aide d'anticorps spécifiques des PDF tardifs X,Y,D et E et du fibrinogène humains (SPLI-PREST, Diagnostica Stago, 92600 Asnières sur Seine),
  - recherche de D-dimère de fibrine issu d'une fibrinolyse par agglutination de particules de latex sensibilisées à l'aide d'un anticorps monoclonal (D-Di Test, Diagnostica Stago, 92600 Asnières sur Seine),
  - dosage du facteur V par adjonction au plasma testé d'un plasma contenant tous les facteurs de la voie exogène sauf la proaccélérine.
- **Examen biochimique** : dosage de fibrinogène plasmatique par la réaction du biuret après coagulation par la thrombine (Fibrinogène, Biotrol 75140 Paris)
- **Examens bactériologiques** : recherche et identification de PHA1 dans les poumons des animaux morts ou sacrifiés.
- **Méthodes statistiques** : Microsoft Excel et Stat Works Tmv1 : 0.

RESULTATS

- **Cliniques, nécropsiques et microbiologiques**
  - Tous les animaux ont présenté des troubles respiratoires dès la première inoculation intratrachéale avec élévation de la température rectale, polypnée, abattement et anorexie. Les notes moyennes générales se situant aux alentours de 2 et au-delà.
  - Un des veaux devant être sacrifié à J4 est mort à J2 ; parmi les 6 autres un est mort à J4, deux autres à J6.
  - Tous les animaux morts ou sacrifiés étaient porteurs de lésions de bronchopneumonie fibrineuse et nécrotique à des degrés et à des stades divers d'évolution. A partir des lésions pulmonaires PHA1 a été régulièrement isolée à des concentrations supérieures à 10^6 UFC/g.
- **Hématologiques** : Les tableaux 2 et 3 rassemblent les résultats des comptages leucocytaires et plaquettaires.
- **Hémostase** : la recherche des PDF et du D-dimère a toujours été négative. Les tableaux 4 et 5 rassemblent les résultats de la recherche des complexes solubles et des dosages du facteur V.
- **Biochimiques** : (Tabl.6)

DISCUSSION

Le phénomène de CIVD résulte de la libération dans la circulation d'un activateur de la coagulation qui entraîne la formation de thrombine. Celle-ci attaque le fibrinogène circulant pour le transformer en dérivés solubles intermédiaires entre le fibrinogène et la fibrine (monomères de fibrine) pour former des complexes solubles. Ces polymères solubles se transforment en réseaux fibrineux dans la micro-circulation. Il en résulte une coagulopathie de consommation avec baisse des facteurs plasmatiques (facteurs V, fibrinogène)et des plaquettes, ainsi qu'une fibrinolyse secondaire due à l'activation du plasminogène au niveau des dépots fibrineux qui peuvent aussi être lysés. L'action protéolytique progressive de la plasmine sur le fibrinogène ou la fibrine aboutit à la formation de produits précoces de haut poids moléculaire X et Y et tardifs de plus bas poids moléculaire D et E. Ces produits de dégradation du fibrinogène ou de la fibrine (PDF) peuvent former des complexes solubles par association avec les monomères de fibrine. A noter que les complexes solubles sont encore formés par l'association monomère de fibrine- fibrinogène.

Le diagnostic biologique de CIVD est actuellement bien codifié (7). Il est basé sur les critères suivants : mise en évidence de complexes solubles et de PDF, diminution du nombre de plaquettes et du fibrinogène, diminution de l'activité de facteurs consommables (facteur V)(1).

Dans nos observations de pasteurellose respiratoire expérimentales à PHA1, la formation de complexes solubles apparaît nettement (Tabl. 4), par contre celle de PDF n'a pas été révélée, comme d'ailleurs celle de D-dimère de fibrine. Une première explication de ces anomalies peut résulter de la non spécificité des réactifs utilisés : les immunoglobulines de lapin qui sensibilisent les particules de latex ne reconnaissant que les PDF issus du fibrinogène humain (un commencement de preuves en est fourni par l'absence de réponse au SPLI PREST du plasma de bovins). De la même façon l'anticorps monoclonal de souris anti-D-dimère humain qui sensibilise les particules de latex dans le D-Di Test n'a probablement aucune affinité structurale avec les D-dimère de fibrine bovin. Quoiqu'il en soit, l'absence de PDF ne permet

en aucun cas d'exclure le diagnostic de CIVD surtout si le taux de plasminogène est bas. Par contre la présence de monomères de fibrine dans un plasma prouve spécifiquement qu'il y a eu coagulation intravasculaire disséminée(9).
L'absence de variation du taux plaquettaire et l'augmentation du taux de fibrinogène sont les conséquences du processus inflammatoire attesté par la leucocytose (3). Dans des conditions étiologiques différentes ces deux paramètres permettent de préciser le caractère compensé de la CIVD si les résultats sont sensiblement normaux et décompensé dans l'éventualité inverse.
Dans la pasteurellose respiratoire à PHA1 la CIVD pourrait être l'aboutissement de la somme des processus suivants :
- activation des cellules endothéliales par l'interleukine 1 et le facteur de nécrose des tumeurs produits par les monocytes et les macrophages sous l'effet de l'endotoxine avec apparition d'une hypercoagulabilité locale par déséquilibre entre les substances procoagulantes et anticoagulantes (6).
- altérations de l'endothélium vasculaire par l'endotoxine supprimant ainsi sa thrombo-résistance et le rendant thrombogène par adhésion et agrégation plaquettaire à sa surface (6).
- établissement d'un état de choc entraîné ou provoqué par l'endotoxine générateur d'anoxie et d'acidose induisant une hypercoagulabilité, une altération de la paroi vasculaire avec sécrétion de thromboplastine tissulaire (7).
L'identification par des tests biologiques d'une CIVD dans la pasteurellose respiratoire à PHA1 des bovins suggère l'intérêt clinique de l'héparinothérapie (20 à 40 UI/kg, 3 à 4 fois par jour, par voie intraveineuse) dans la phase aiguë des BPIE des jeunes bovins. Il n'est pas sûr toutefois que les résultats obtenus soient meilleurs que ceux fournis par les antiinflammatoires non stéroïdiens, flumixine méglumine en particulier(8).

REMERCIEMENTS
Marie France PEREZ pour la réalisation des examens hématologiques, biochimiques et d'hémostase.

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RESUME
Dans un modèle expérimental de pasteurellose respiratoire à PHA1 interessant 10 veaux de race française frisonne âgés de 2 à 3 semaines, les paramètres ou éléments suivants ont été recherchés ou mesurés avant et pendant l'évolution de la maladie : présence de complexes solubles, présence de produits de dégradation du fibrinogène et/ou de la fibrine, de D dimère de fibrine, nombre de thrombocytes, taux de deux facteurs plasmatiques consommables (fibrinogène et facteur V). L'intense réaction inflammatoire qui accompagne l'inoculation itérative de PHA1 par voie intranasale et intratrachéale n'a probablement pas permis de caractériser la thrombopénie et l'hyperfibrinogénémie propres aux CIVD. Malgré l'absence de produits de dégradation du fibrinogène et/ou de la fibrine et d'une diminution de l'activité du facteur V, la mise en évidence de complexes solubles dans le sang circulant suffit pour identifier une CIVD.

SUMMARY
In an experimental model of PHA1 respiratory pasteurellosis in 10 French Friesan calves between 2 and 3 weeks of age the following parameters or features were checked or measured before and during the progress of the disease : presence of soluble complexes, presence of fibrinogen degradation products and/or fibrin, of the D-isomer of fibrin, the number of thrombocytes, the levels of consumable plasma factors (fibrinogen, factor V). The severe inflammatory response which accompanies repeated inoculation with PHA1 by intranasal and intratracheal route probably made it impossible to detect the thrombocytopenia and hyperfibrinogenemia characteristic of CIVD. Despite the absence of fibrinogen and/or fibrin and the reduced activity of factor V, the detection of soluble complexes in the bloodstream is sufficient to identify CIVD.

RESUMEN
Se investigaron or midieron en un modelo experimental de pasteurelosis respiratoria a base de PHA1 que incluian 10 terneros de raza francesa frisona de 2 a 3 semenas de vida, los parametros o elementos siguientes antes y durante la evolucion de la enfermedad : presencia de complejos solubles, presencia de productos de degradacion del fibrinogeno y/o de la fibrina, de D-dimero de fibrina, numero de trombocitos, indice de dos factores plasmaticos consumibles (fibrinogeno y factor V). La intensa reaccion inflamatoria que accompana la inoculacion iterativa de PHA1 por via intranasal e intratraqueal probablemente no permitio caracterizar la trombopenia y la hiperfibrinogenemia propias a las CIVD. A pesar de la ausencia de productos de degradacion del fibrinogeno y/o de la fibrina y de una disminucion de la actividad del factor V, la evidenciacion de complejos solubles en la sangre circulante es suficiente para identifiar una CIVD.

Interventions	J0 avant inoculation	J0 après inoculation	J1	J2	J4	J7	J10
examen clinique standardisé	+	+	+	+	+	+	+
Hématologie	+	+	+	+	+	+	+
Hémostase	+	+ *	+	+	+	+	+
Biochimie	+	+	+	+	+	+	+
Sacrifice Autopsie					+ n=4		+ n=6
Bactériologie							

* sauf facteur V

Tableau 1 : Chronologie des interventions.

N° Veau	J0 avant inoculation	J0 après inoculation	J1	J2	J4	J7	J10
1	13,00	31,6	8,8	-			
2	7,5	12,9	17,3	11,2			
3	4,5	26,8	20,3	17,4			
4	9,7	19,6	9,3	7,8			
5	10,2	3,3	14,3	9,5	5,3	7,9	6,9
6	8,9	26,9	16,2	16,5	15,2	14,8	14
7	11,4	15,9	12,2	7,6	6,5	9,7	11
8	8,7	38,6	16,4	20,1	8,9	-	-
9	10,9	17,4	15,6	2,9	-	-	-
10	8,5	13,8	7,4	8,4	8,6	-	-
moyenne	9,4	23,5	13,7	11,26	5,3	10,8	10,63
+ ou - SD	2,5	9,03	4,15	5,58	3,6	3,57	3,56

Tableau 2 : Evolution du nombre des leucocytes (x 10^9/l) (différence significative à 0,001 à J0 entre les deux mesures et à 0,05 à J1).

N° Veau	J0 avant inoculation	J0 après inoculation	J1	J2	J4	J7	J10
1	720	760	600	-			
2	960	920	760	810			
3	720	760	820	920			
4	778	880	790	810			
5	460	520	580	620	580	910	700
6	660	600	580	730	540	720	680
7	460	500	600	660	560	700	640
8	600	640	640	620	680	-	
9	680	680	680	740	-	-	
10	560	620	600	600	620	-	-
moyenne	663,8	688	675	741,11	598	746,66	673,33
+ ou - SD	145,21	141,17	123,22	107,4	554,96	84,29	305,5

Tableau 3 : Evolution du nombre des plaquettes (x 10^9/l) (absence de différence significative).

N° Veau	J0 avant inoculation	J0 après inoculation	J1	J2	J4	J7	J10
1	2	2	3	3	3	3	3
2	2	1	1	3	3	3	3
3	1	2	2	3	3	3	3
4	2	1	2	2	2	-	-
5	0	1	0	2	-	-	-
6	1	1	2	3	3	-	-
7	2	2	2	-	-	-	-
8	1	1	2	2	-	-	-
9	0	2	3	3	-	-	-
10	0	2	2	2	-	-	-
moyenne	1,1	1,5	1,8	2,3	2,8	3	3
+ ou - SD	0,87	0,52	0,78	0,5		0	0

Tableau 4 : Résultat de la recherche des complexes solubles (différence significative avec J0 avant inoculation et J1 à 0,05 puis à J2 à 0,001 et J4 à 0,05).

N° Veau	J0 avant inoculation	J1	J2	J4	J7	J10
1	13,7	13,6	-			
2	13,5	13	13,8			
3	13,5	13,2	13,9			
4	14,2	13,7	13,3			
5	12	12	12	10	11,4	9,5
6	13,1	14,5	14,8	12,5	12	13,2
7	12,4	13,5	14,2	12,4	12,3	14
8	12,9	13,6	12,7	9,2	-	-
9	11,5	14,5	15,5	-	-	-
10	13,4	14,9	15,5	10,5	-	-
moyenne	13	13,5	13,9	10,9	11,9	12,2
+ ou - SD	0,61	1,08	1,21	1,47	0,45	2,40

Tableau 5 : Résultats des dosages du facteur V (*) (absence de différence significative).

N° Veau	J0 avant inoculation	J0 après inoculation	J1	J2	J4	J7	J10
1	4,2	5,9	9,2	-			
2	3,4	4	10,8	10			
3	3,6	4,7	9,2	13			
4	4	5,2	11	9,4			
5	4,1	5,2	7,5	8,3	10,2	12,6	13,6
6	6,1	6,3	9	8,7	10	13	13
7	4,5	5,5	10,4	11,4	11	10,9	11,4
8	5,6	7,1	13	13,1	10,8	-	-
9	3,7	3,3	10	9,1	-	-	-
10	4,1	4,1	9,2	10	10	-	-
moyenne	4,33	5,19	9,91	10	10,4	12,13	12,67
+ ou - SD	0,84	1,14	1,52	1,47	0,48	1,17	1,15

Tableau 6 : Résultat des dosages de fibrinogène (g/l)(différence significative permanente avec J0 avant inoculation à 0,001).

COMPARISON OF TWO METHODS OF ADMINISTRATION OF LIVE IBR VACCINES

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INTRODUCTION

Live vaccines against Infectious Bovine Rhinotracheitis (IBR) are generally administered either intramuscularly or intranasally. Under most field conditions, intranasal vaccination will be acceptable to the operator. However, in certain situations, e.g. when the cattle are not accustomed to being handled or when the facilities for restraint of the animals are poor, intramuscular vaccination will be more practical.

In two experiments the efficacy of two commercial vaccines administered by intramuscular and intranasal route were compared.

MATERIALS AND METHODS

Experiment 1

Animals

15 Hereford-Friesian cross male calves, 16 weeks old and free of antibodies against IBR-virus. The calves were divided into 3 groups of 5 calves and each group was housed in separate pens within the same building.

Vaccination

Vaccine A[1]), a modified live IBR vaccine designed for intranasal administration.

Vaccine B[2]), a live IBR vaccine for intranasal use, containing a temperature sensitive virus strain.

The groups were vaccinated as follows :

Group 1 : Vaccine A intramuscularly
Group 2 : Vaccine B intramuscularly
Group 3 : Unvaccinated controls

Each dose contained 10^6 $TCID_{50}$ vaccine virus.

[1]) Nobi-Bovac IBR (® Intervet International)
[2]) Tracherine (® Norden laboratories)

Challenge

Four weeks following vaccination all calves were challenged intranasally with 7,0 log $TCID_{50}$ of a virulent IBR virus (field isolate 532).

Experiment 2

Animals

12 Hereford-Friesian cross male calves of 14 weeks old and free of antibodies against IBR virus. The calves were divided into 3 groups of 4 and housed as in exp. 1.

Vaccination

The same vaccines were used as in the first experiment but with a different virus dosage.
Group 1 : Vaccine A, intranasally ; 5,0 $TCID_{50}$/dose
Group 2 : Vaccine B, intranasally ; 5,8 $TCID_{50}$/dose
Group 3 : Unvaccinated controls

Challenge

Four weeks after vaccination intranasal challenge with 7,6 log 10 $TCID_{50}$ of a virulent IBR virus (field isolate 532).

Experiment 1 and 2

Clinical procedures

For 3 days before to 10 days after vaccination and challenge all animals were examined. At each examination a quantitative score was given for the following potential signs of IBR : pyrexia, depression, salivation, ocular discharge, nasal discharge and cough to calculate the daily clinical score for each calf.

Serological response

At weekly intervals IBR virus neutralising antibody titres were determined for each calf, using a microtitration assay in bovine embryo lung cells (BEL). Antibody titres are expressed as the reciprocal of the final dilution neutralising 200 - 300 $TCID_{50}$ of IBR virus after overnight incubation.

Challenge virus reisolation

Nasal swabs from each calf were taken daily for 10 days and titrated in BEL 26 cell monolayers in 96 well microtitration plates. Titres are expressed as log10 $TCID_{50}$ per ml.

RESULTS

Clinical reaction to vaccination

No adverse clinical reactions attributable to vaccination were observed in any group in both experiments.

Clinical reaction to challenge

After challenge the average number of "days-ill", i.e. days with a clinical score of 10 or more, was significantly reduced in all vaccinated animals (Table 1).

Table 1 : Mean number of days-ill during the 10 days of challenge in vaccinated and unvaccinated calves

I.M. vaccination	No. of days-ill	I.N. vaccination	No. of days-ill
Vaccine A	0,6[a]	Vaccine A	0,75[a]
Vaccine B	4,0[b]	Vaccine B	2,25[a]
Controls	6,6[c]	Controls	8,5[b]

a,b,c, : means with different superscripts in the same column are different $p < 0,05$, multiple t-test

The mean clinical scores per group of calves are shown in Figure 1 and Figure 2.

Serological response

The serological response to vaccination and challenge is summarized in Table 2 and Table 3.

Table 2 : Antibody titers to IBR in mean 2log VN units in intramuscularly vaccinated calves and unvaccinated control animals

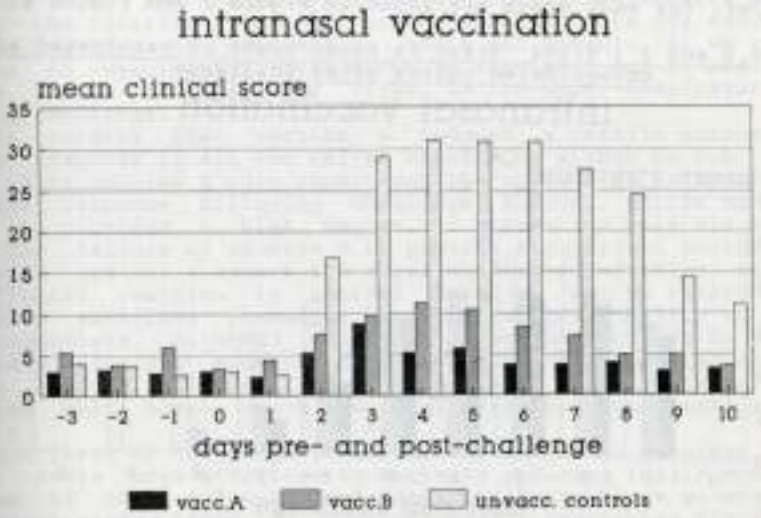
	Weeks pre- and post-vaccination						
	-1	0 (vaccination)	1	2	4 (challenge)	5	5,4
Vaccine A i.m.	<2	<2	<2,6	8[a]	4[a]	8,5	>9,3
Vaccine B i.m.	<2	<2	<2	<2[b]	<2[b]	7,3	>9,2
Unvaccinated controls	<2	<2	<2	<2[b]	<2[b]	<3	<3

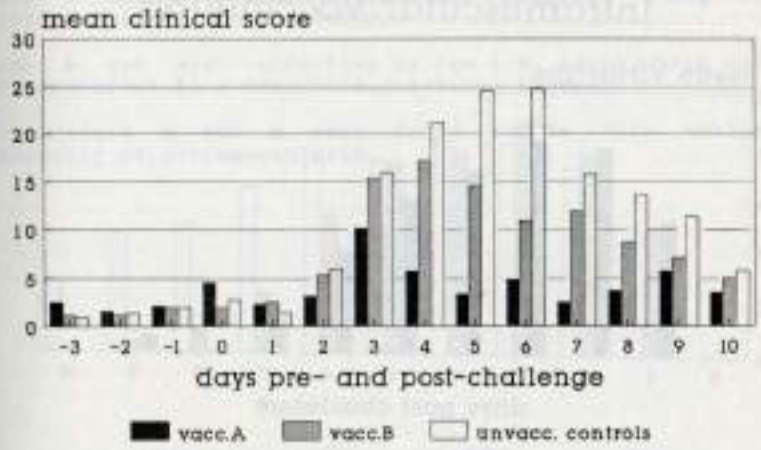
a, b : Means with different superscripts in the same column differ significantly $p < 0,05$, sign-test

Table 3 : Antibody titers in mean 2log VN units to IBR in intranasally vaccinated calves and unvaccinated control animals

	Weeks pre- and post-vaccination						
	-1	0 (vaccination)	1	2	3	4 (challenge)	6
Vaccine A intranasally	<2	<2	<2	2,25	3,3	3,5	7,25
Vaccine B intranasally	<2	<2	<2	<2	<2,6	<2,1	7,25
Unvaccinated controls	<2	<2	<2	<2	<2	<2,1	5,6

Figures 1 and 2 : Mean clinical scores from 3 days before to 10 days after challenge in vaccinated andunvaccinated calves

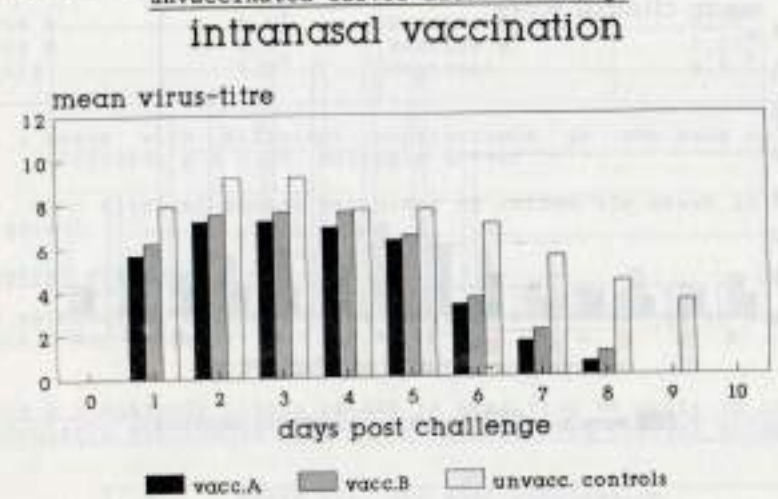




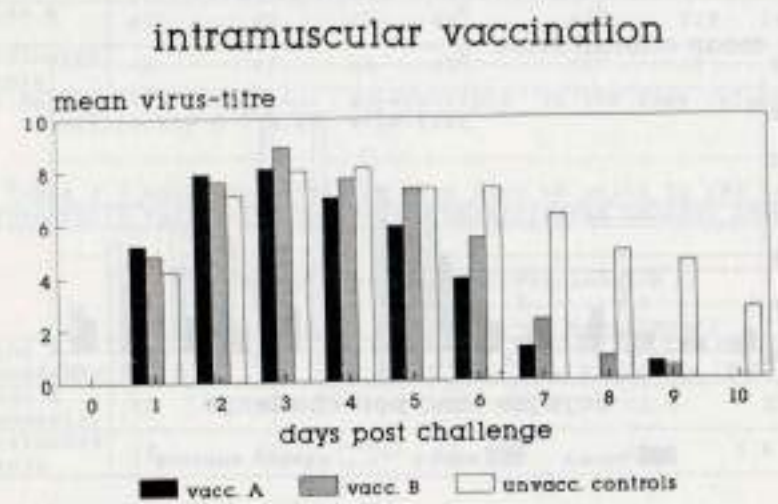
Challenge virus reisolation

The mean titers of challenge virus in nasal swabs, expressed in log 10 $TCID_{50}$/ml, for each group are shown in Figure 3 and Figure 4.

Figures 3 and 4 : Virus titer in nasal swabs of vaccinated and unvaccinated calves after challenge



no virus (0.1 log10 TCID50/ml)
detectable 3 hours after challenge



no virus((0.1 log10 TCID50/ml)
detectable 3 hours after challenge

DISCUSSION

The efficacy of two commercial vaccines (A and B) was assessed by intranasal (i.n.) and intramuscular (i.m.) routes. Parameters measured were (1) the relative growth of vaccine viruses (data not shown), (2) clinical reaction to vaccination (data not shown), (3) VN antibody responses to vaccination (data given in Figures 1 and 2) and (v) the relative growth of challenge virus in the upper respiratory tract following challenge.

It is evident that vaccine A induced a readily measurable VN antibody response in all the calves vaccinated either by i.n. or i.m. routes, while vaccine B only sensitised the animals as is indicated by anamnestic response following challenge. However, unlike vaccine A, vaccine B provided a high degree of protection only via the i.n. route. The failure of vaccine B to provide significant protection by i.m. route against a severe IBR virus challenge infection, as judged from clinical reaction in control animals, may be related to its temperature sensitive phenotype namely to its inability to grow at body temperature. Although vaccine A given intramuscularly afforded significant clinical protection against IBR challenge, vaccine virus was not readily isolated from blood or nasal swabs (data not shown) indicating that there was a low level systemic virus replication at some site.

At the level of challenge virus replication, both vaccines A and B by i.n. route were effective in markedly reducing (daily mean titre reduction of 10 to a few hundred fold) challenge virus growth in the upper respiratory tract and in significantly shortening the duration of challenge virus replication (Figure 3). Similarly vaccine A given by i.m. route significantly reduced the extent and the duration of challenge virus growth in nasal mucosa but vaccine B was not as effective, thus correlating with the clinical findings.

CONCLUSION

1. Vaccine A and B given intranasally provided a high degree of protection against IBR challenge.

2. Vaccine A was also effective by the i.m. route which under some field conditions is a desirable practical benefit.

3. Both vaccines A and B were found to be safe whether given intranasally or intramuscularly.

IMMUNOMODULATORS IN CALF RESPIRATORY DISEASES

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INTRODUCTION

One of the most important factors promoting appearance of calf respiratory diseases is the immunologic status of the animal body. At present it is a matter of common knowledge that calf bronchopneumonia can be caused by 10-15 various viral and bacterial agents while such as adenoviruses, respiratory syncytial, diarrhea viruses bring about secondary immunodeficiency which is able to result in severe pathologies in animals (fig.1,2,4). Polyetiology of calf respiratory diseases makes difficulties for the development and use of vaccine. The world mono-, bi-, polyvaccines and other preparations don't yield desirable results. Improvement of immune status of animals appears to be one of the trends to overcome these infections. Immunomodulators of biological origin should attract great attention in this aspect.

MATERIALS AND METHODS

Preparations

T-aktivin, a preparation obtained by the extraction of bovine thymus is a mixture of polypeptides with molecular mass from 1500 to 6000 dalton. B-aktivin is a preparation from lowmolecular peptide group with molecular mass 1000-3000 dalton isolated from cell culture of porcine bone marrow. Both preparations tested under laboratory and field conditions were innocuous and neither exerted any effect on the idices of the normal immune system of animals nor had any side effect.

Animals

Fifteen - ninety day old calves were used in the experiments on prophylaxis and treatment of respiratory diseases.

Dosage

For prophylaxis T-aktivin was given in the dose of 2-4 mcg/kg (0.02--0.04 ml/kg) subcutaneously once a day for 3 days running, for treatment 4-5 mcg/kg (0.04-0.05 ml/kg) dose was administered once a day for 5-7 days running. B-aktivin in the dose of 0.3-0.5 mg/kg was given both with curative and prophylactic purpose.

Virological research

Indication of viruses was done with the help of immunofluorescent method and examination of sera, taken in 2 week interval for the presence of antibodies to parainfluenza-3, adeno- and infectious rhynotracheitis viruses was carried out by neutrelization and indirect hemagglutination tests (5). Isolation of viruses by 1-2 passages was done in MDBK cell culture.

Immunologic research

Lysozyme dynamics, blood serum bactericidal activity, M and G immunoglobulins, phagocytosis of blood neutrophiles as well as total population of T- and B-lymphocytes in rosette tests were investigated (3,6)

Immunomodulators in experimental calf rhynotracheitis

Experiments on virus infection were carried out on 12 normal, 2-3 month old calves. Infectious rhynotracheitis virus with titre 10^7 50 ml was used for infection. Virus was injected by intranasal and intratracheal ways: 1.5 ml and 5 ml respectively. Group I of 4 calves received B-aktivin once a day 3 days running, 3 mg subcutaneously.

Four animals from group II were injected 100 mcg of T-aktivin subcutaneously once a day 3 days running, group III was given simultaneously T-aktivin - 100 mcg and B-aktivin - 3 mg once a day 3 days running. The controls were injected normal saline solution. For laboratory tests blood was taken prior to infection and on the 3d, 7th and 18th day after infection.

RESULTS

In experimental infectious rhynotracheitis the control calves fell ill on the 2nd-3rd day and the disease lasted for 10 days. The calves receiving B-aktivin fell ill on the 3rd-4th day and were ill for 4-6 days. Those receiving T-aktivin fell ill on the 2nd-3rd day and the disease lasted for 10 days. Those calves given T- and B-aktivin fell ill on the 2nd-3rd day and were ill for 4-6 days. The virus was isolated in all animals throughout the experiment while isolation of the virus was stopped by the 5th day in the group of calves receiving T- and B-aktivin. In the controls the disease was accompanied by decrease of T-lymphocytes (fig.1), significant relaxation of digestion and intensification of absorbing activity of blood neutrophiles.

B-aktivin exerted a positive effect on both humoral and cell factors of the body of calves infected with infectious rhynotracheitis virus. Administration of B-aktivin prevented the decrease of the percent content of mature T-lymphocytes (fig.1), increased immunoglobulin concentration as well as absorbability of blood neutrophiles and prevented decrease of their digestion activity. Administration of T-aktivin brought about increase of immunoglobulin concentration of both classes, and that of lysozyme, prevented decrease of T-lymphocyte population and promoted the greatest activity of the phagocytic process throughout all stages. Complex administration of T- and B-aktivins cut short the terms of diseases and virus discharge from the body. At early stages of the desease a marked increase of phagocyte digestion activity occurs. Percentage content of T-cells (fig.1), immunoglobulin, lysozyme and virusneutralizing cells increased in recovered animals. The data obtained formed grounds for testing prophylactic and therapeutic action of immunomodulators on farms.

Virological research has shown that various combinations of PI-3, infectious rhynotracheitis, adeno-, respiratory syncytial and diarrhea viruses were isolated in 97% of sick calves. Pasteurella, Kleibsiella, protozoa isolated from the rhinopharynx in amount of 10^6 microbial cells per 1 ml played the secondary part in the etiology of respiratory diseases.

Immunologic investigations of 70 animals have revealed immunodeficiency in more than 80% calves. Thus, relative and absolute number of T-lymphocytes was 11.0±1.3% - 15.8±8.2,3% and 0.6±0.1 10^9/l - 0.9±0.1-/l respectively, number of B-lymphocytes fluctuated in the limits of 8-13%. bactericidal and lysozymal activity was at a low level as well (3%±5% and 28±9 mg/ml respectively).

Data on prophylaxis and therapy of calf respiratory diseases with the help of immunomodulators are given in tables 1, 2.

Therefore, immunomodulators demonstrate a high prophylactic effect in calf respiratory diseases. In the USSR immunomodulators are produced

TABLE I. Results of immunomodulators employment for prophylaxis of calf bronchopneumonia

Groups	N	Fell ill		Lost		Average day weight gain, gram	Efficiency, %
		number of animals	%	number of animals	%		
Test T-aktivin	2700	196	7,3	38	1,4	730	92,7
Controls (antibiotics)	1610	629	39,1	169	10,4	480	60,9
Test B-aktivin	110	14	12,7	4	3,6	675	87,3
Controls (antibiotics)	110	49	44,5	9	8,2	550	55,5
Test T+B-aktivin	1170	60	5,1	10	1,1	870	94,9
Controls (antibiotics)	1210	510	42	60	5,0	490	58,0

TABLE 2. Curative efficiency of immunomodulators in calf respiratory diseases

Groups	N	Recovered				Recurrence		Lost		Efficiency, %
		on the 6-7th day		on the 10-14th day		number of anim.	%	number of anim.	%	
		number of anim.	%	number of anim.	%					
Test T-aktivin	280	250	89,3	18	6,4	27	9,5	12	4,3	95,7
Test B-aktivin	270	183	67,7	72	26,6	32	11,8	15	5,7	94,3
Controls (antibiotics)	509	101	19,8	365	71,7	253	49,7	43	8,4	91,6

on industrial scale, 4 mln. Calves have been treated with them. Advantage of T- and B-aktivin compared to the other preparations is as follows: production of preparations is based on meat industry wastes; dependence on administration of a number of antivirus vaccines drops; there is no need to use antibiotics and other medicamental agents; immunomodulators are innocuous and ecologically pure.

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SUMMARY

Widespreed deficiency is proved among 15-25 day old calves resulting in respiratory diseases. Etiological agents are perainfluenza-3, adeno-, infectious rhynotracheitis, diarrhea viruses. It is established that prophylactic efficiency of T-aktivin in calf bronchopneumonia was 92.7% (60.9% in the controls), that of B-aktivin was 87.3% (55.5% in the controls). Prophylactic efficiency was 94.9% in the test group and 58% in the control when T-aktivin and B-aktivin were given simultaneously. Therapeutic effect in calf respiratory diseases was 95.7% while it was 91.6% in the control group where antibiotics were used.

RESUME

On a etabli une large extension de l'immunodeficience chez les voeux a l'age de 15 a 25 jours ce qui provoque l'evolution des maladies respiratoires. Les agents etiologiques sont des virus de paragrippe-3, adeno-, de rinotracheite infectieuse, de diarrhee. On a etabli que l'efficacite prophylactique de T-activine a la bronchopneumonie de voeux etait 92,7% (groupe de controle - 60,9%), l'efficacite prophylactique de B-activine etait 87,3% (groupe de controle - 55,5%). A l'injection combinee aux voeux de T-activine et de B-activine leur efficacite prophylactique dans le groupe d'experience etait 94,9%, dans le groupe de controle - 58%. L'effet therapeutique aux maladies respiratoires chez les voeux etait 95,7%, dans le groupe de controle ou on employait des antibiotiques etait 91,6%.

ZUSAMMENFASSUNG

Es wurde eine Verbreitung von Immunodefiziten unter den Kalbern im Alter von 15-25 Tagen festgestellt, was zur Entwicklung von Respirationskrankheiten fuhrte. Als ethiologische Agens treten Paragrippe-3 Viren, sowie Adeno-, infektiose, Rinothracheitis- und - Dyarrhveviren. Die vorbengende effektive Wirkung des T-Aktivins bei Kalberbronchopneumonie betrug 92,7% (bei Kontrolltieren 60,9%) Die Wirkung von B-Aktivin zeigte 87,3% (bei Kontrolltieren 55,5%) Gleichzeitige Anwendung beider Praparate zum prophylaktischen Zweck zeigte folgende Ziffer: 94,9% in der Versuchsgruppe und 58% in der Kontrollgruppe. Das therapeutische Resultat bei den Kalberrespirationskrankheiten betrug 95,7%, wahrend des der Kontrolltiere mit Antibiotikn Anwendung 91,6% zeigte.

CHANGES IN SERUM INSULIN CONCENTRATIONS DURING THE FIRST 6 MONTHS OF LIFE: A PROSPECTIVE STUDY IN ITALIAN FRIESIAN CALVES(*).

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INTRODUCTION

Somatic growth and development of animals are related with thyroid hormones, pituitary growth hormone, and insulin secretion. Insulin is present in the circulation at all ages and is directly involved in the regulation of metabolism by coordinating the storage and metabolization of carbohydrates, amino acids and fats (1, 5). This hormone increases the movement of glucose into many peripheral tissues (3, 4, 17) including adipose tissue (11) and muscle (10): protein synthesis in skeletal muscle is reduced in absence of insulin (12). Numerous studies report that the serum insulin concentration is to be related to glucose availability (2, 8, 16) and is related to the diet (15).

The purpose of this paper is to describe the changes in serum insulin concentrations in Italian Friesian calves during the first six months of life.

MATERIALS AND METHODS

Animals. Forty five Italian Friesian calves (19 males and 26 females) were included in this study. The animals were born at the end of normal pregnancies (272±3 days).

Table 1 - Chemical composition of the feed used during the trial (a.f.b.)

	commercial milk replacer	weaning mixture	concentrated food
Moisture.....%	4.5	12.0	13.0
Crude protein...."	23.5	16.1	15.7
Ether extract...."	13.0	3.0	5.7
Crude fiber....."	0.5	14.5	9.4
Ash....."	7.0	7.5	9.6

The animals had no clinical problems, were housed in individual boxes until 30th-40th day of life and then reared in single stalls, yoke tying. The calves were fed maternal colostrum twice a day for the first 4 days: on

the 5th day, when the average animal weight was about 36 Kg, they received 8 l of a commercial milk replacer (4 l in the morning and 4 l in the afternoon). At the 30th day (average live weight 70 Kg) the milk replacer was substituted by 400-500 g of a weaning mixture (the animals can eat the weaning mixture till from the second week). After 2-3 months the weaning mixture was gradually substituted by 1-1.5 Kg of concentrated food and 1-2 Kg of lucerne hay (alfalfa). These quantities were gradually increased up to the 6th months (average live weight 170 Kg).(See table 1 for the chemical composition of the employed feed).

Blood samples were collected from all calves by puncture of the right jugular vein in the following way: the first and the second sample were collected at 24 and 48 h after the birth, respectively; the following 10 samples at weekly intervals and the last 4 samples every month up to 6th month. Blood was drawn in the morning (from 8:00 to 9:00 h) with the exception of the first 2 samples which were obviously related to the delivery occurrence. In fact, if the calving took place during the day, the blood sample was collected 2-3 h after delivery and before sucking the maternal colostrum; if the calf birth took place at night, the blood sample was collected 12-14 h after delivery.

The births were distributed over a period of 6 months (January-June 1985) as follows: January (3 males and 4 females); February (3 males and 5 females); March (5 males and 3 females); April (2 males and 7 females); May (3 males and 3 females); June (3 males and 4 females).

Assays. Sera were frozen until analyzed. The assay was carried out in duplicate. All samples belonging to the same animal were run in the same assay to avoid interassay variations. The interassay coefficient of variations was < 5%. Serum insulin concentrations were measured by specific RIA (Insulin Teat IV732, Cambridge Medical Technology, innoVet Division, MA - USA).

The data were analyzed by the following statistical model:

$$INS_{i,j,k,l} = \mu + sex_i + month_j + day_k + (subj_{l,i,j} * day_k)_{i,j,k,l}$$

where: INS = insulin blood level;
sex = sex of calf;
month = month of birth;
day = age of the calf in days: the variable "day" was considered as a continuous ($b*day_k$) and a categorical variable;
subj = subject I.D.

The interaction between subject and day was considered as the error term.

RESULTS AND DISCUSSION

The estimated serum insulin levels are shown in Table 2 and the average observed values are shown in Figure 1. Sex-related differences were observed, females showing higher concentrations than male. No significative difference was observed in relationship to the month of the birth.

Serum insulin concentrations were higher on the first day than on second day (24.02±10.93 µU/ml and 18.18±7.87 µU/ml, respectively; $p<0.01$).

Table 2 - LS-means stimated at 1 day old

Factor		LS-mean	Std.Err.
Male		19.9[a]	0.49
Female		21.4[b]	0.40
Month	January	18.6	0.77
	February	20.3	0.73
	March	20.0	0.73
	April	23.2	0.69
	May	22.4	0.82
	June	20.2	0.78
day	"b"	-0.022	0.077

Serum insulin concentrations differed between the first and the second period of life (2nd-70th vs. 70th-168th). Basal serum insulin values of 15 to 50 µU/ml have been reported in adult cattle (7, 9, 13, 14, 18). A limited number of reports are available on serum insulin concentrations in the early postnatal life, although Erb et al. also found that serum insulin concentrations declined from the first to second day after birth (6). It must be noted that the values we found were higher than those reported by Erb et al. (6): these differences may likely be due to the different breeds considered in these reports. The values we found at 6th months of life were in agreement with those observed by Irvin & Trenkle (9).

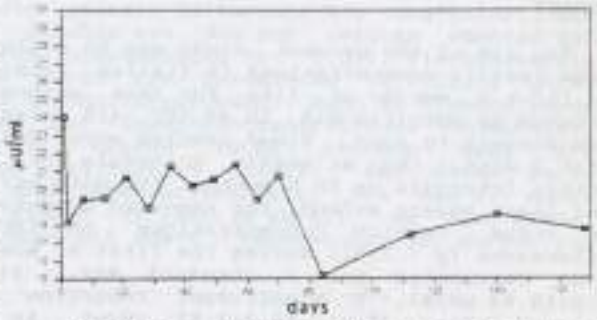

Figure 1 - Serum insulin concentrations

Furthermore, it is interesting to note the fall in serum insulin level at 84 days. At this age in fact the calves change their feed, the weaning mixture being replaced by hay (alfalfa) and meal. The effect of hay on the absorption and consequently on plasma kinetics of nutrients may explain the phenomena observed on insulin serum levels. Later the insulin concentrations increases again and this may be due to the maturation of the digestive trait.

To our knowledge, no longitudinal studies had been so far carried out concerning the changes in serum insulin levels throughout the first 6 months of life in Italian Friesian calves. Our data clearly show that, after the initial decrease occurring during the first two days of life, the serum insulin concentrations remain fairly constant thereafter. These results suggest a complete maturation of islet cell function in the early postnatal life.

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SUMMARY

The aim of the present study was to evaluate the variations of serum insulin concentrations in Italian Frisian calves (IFC) during the first 6 months of life. For this purpose insulin levels were measured by specific RIA in 45 IFC (19 males and 26 females) born from January to June. Blood samples were drawn once a day for the first 2 days, then at weekly intervals for 10 weeks, and then at monthly intervals up to 6 months. The data were analyzed by a split-plot least square method for repeated hormonal measures over time. The serum insulin concentrations in IFC showed significant differences ($p < 0.01$) during the first 6 months of life. The serum value (estimated mean ± standard dev.) 24 h after birth was 24.0±10.92 µU/ml. A significant reduction of insulin level was observed after 48 h: 18.2±7.87 µU/ml. An increase of insulin concentration was observed at first week (19.5±11.18 µU/ml), and the values remained practically unchanged up to 10 weeks. The insulin levels decreased up to 3 months (15.2±5.36 µU/ml), then they increased again remaining around 17-18 µU/ml up to 6 months of life.

SUMÁRIO

O objectivo do presente estudo foi avaliar as variações de concentrações de insulina no soro nos Vitelos Italianos da Frisia (VIF) durante os primeiros 6 meses de vida. Para este fim, os níveis de insulina foram medidos por Radioimunoanálise específicos em 45 VIF (19 machos e 26 fêmeas) nascidos desde janeiro até junho. Foram obtidas amostrar de sangue uma vez por dia durante os 2 primeiros dias, depois com intervalos de uma semana durante 10 semanas e depois com intervalos de um mês até aos 6 meses. Os dados foram analisados por um diagrama duplo com o método dos mínimos quadrantes por medidas hormonais repetidas naquele período. A concentração de insulina no soro nos VIF mostraram diferenças significativas ($p < 0.01$) durante os primeiros 6 meses de vida. O valor de soro (valor ódio estimado ± variante do padrão) 24 h depois do nascimiento era 24.0±10.92 µU/ml. Uma redução significativa do nível foi observado dipois de 48 h: 18.2±7.87 µU/ml. Um aumento de concentração de

insulina foi observado na primeira semana (19.5±11.18 µU/ml) e os valores ficaram praticamente inalteráveis até 10 semanas. Os níveis de insulina diminuiram até aos 3 meses (15.2±5.36 µU/ml), depois aumentaram outra vez ficando cerca 17-18 µU/ml até 6 meses de vida.

ZUSAMMENFASSUNG

Das Ziel dieser Untersuchung war es, die Schwankungen der Serumkonzentrationen von Insulin bei schwarz gefleckten Kälber (SGK) während der ersten 6 Lebensmonate auszuwerten. Zu diesem Zweck wurden mit einem speziellen Radioimmunotest die Insulinkonzentrationen von 45 SGK (19 männliche und 26 weibliche Tiere) untersucht, die in der Zeit von Januar bis Juni geboren waren. In den ersten beiden Tagen wurden täglich eine Blutprobe entnommen, danach 10 Wochen lang einmal wöchentlich und im folgenden bis zum 6. Monat einmal pro Monat. Die Daten wurden mit der Doppelkurvenmethode der kleinsten Quadrate für wiederholte Messungen des Hormonspiegels über die gesamte Zeitspanne hin analysiert. Die Serumkonzentrationen von Insulin bei den SGK zeigten während der ersten 6 Lebensmonate große Schwankungen ($p< 0.01$). Vierundzwanzig Stunden nach der Geburt waren die Serumwerte 24.0±10.92 µU/ml (geschätzter Mittelwert ± Standartabweichung). Nach 48 Stunden wurde ein bedeutendes Absinken der Insulinkonzentrationen beobachtet: 18.2±7.87 µU/ml. In der ersten Woche stiegen dann die Insulinkonzentrationen an (19.5±11.18 µU/ml), und diese Werte blieben 10 Wochen lang praktisch unverändert. Bis zum 3. Monat sanken die Insulinwerte wieder (15.2±5.36 µU/ml), um dann wieder anzusteigen, und blieben dann bis zum 6. Lebensmonat bei Werten von etwa 17-18 µU/ml.

SERUM CORTISOL LEVELS IN ITALIAN FRIESIAN CALVES DURING THE FIRST 6 MONTHS OF LIFE(*).

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(*) Work supported by a Grant M.P.I. (60% and 40%)

INTRODUCTION

Cortisol, with corticosterone, is the main corticosteroid formed in the adrenal (3, 5) and found in jugular (12, 20, 23) venous plasma of dairy cattle. Furthermore, Balfour (3) reported that only cortisol can be detected in calves at birth.

The changes in the plasma concentration of cortisol have been studied in neonate animals of different species. In calf the serum cortisol levels increase during the last days of pregnancy (9, 13), probably in relation to an increased glucocorticoid secretion by the fetal adrenal cortex. The cortisol concentrations progressively decline from birth to 11, 12 days or 20 days of postnatal life (11, 15, 19).

Our study was carried out to investigate the changes in serum cortisol concentrations in Italian Friesian calves from birth to 6 months of life to ascertain when cortisol secretion becomes relatively constant and comparable to that of adult cattle.

MATERIALS AND METHODS

Animals. Porty five Italian Friesian calves (19 males and 26 females) were included in this study. The animals were born at the end of normal pregnancies (272±3 days). The animals had no clinical problems, were housed in individual boxes until 30th-40th day of life and then reared in single stalls,yoke tying. The calves were fed maternal colostrum twice a day for the first 4 days;on the 5th day,when the average animal weight was about 36 Kg, they received 8l of a commercial milk replacer (4 l in the morning and 4 l in the afternoon). At the 30th day (average live weight 70 Kg) the milk replacer was substituted by 400-500 g of a weaning

Table 1 - Chemical composition of the feed used during the trial (a.f.b.)

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Crude protein...."	23.5	16.3	15.7
Ether extract...."	13.0	3.0	5.7
Crude fiber....."	0.5	14.5	9.4
Ash....."	7.0	7.5	9.6

mixture (the animals could eat the weaning mixture till from the second week). After 2-3 months the weaning mixture was gradually substituted by 1-1.5 Kg of concentrated food and 1-2 Kg of lucerne hay (alfalfa). These quantities were gradually increased up to the 6th months (average live weight 170 Kg).(See table 1 for the chemical composition of the employed feed).

Blood samples were collected from all calves by puncture of the right jugular vein in the following way: the first and the second sample were collected at 24 and 48 h after the birth, respectively; the following 10 samples at weekly intervals and the last 4 samples every month up to 6th month. Blood was drawn in the morning (from 8:00 to 9:00 h) with the exception of the first 2 samples which were obviously related to the delivery time. In fact, if the calf birth took place during the day, the blood sample was collected 2-3 h after delivery and before sucking the maternal colostrum; if the calf birth took place at night, the blood sample was collected 12-14 h after delivery.

The births were distributed over a period of 6 months (January - June 1985) as follows: January (3 males and 4 females); February (3 males and 5 females); March (5 males and 3 females); April (2 males and 7 females); May (3 males and 3 females); June (3 males and 4 females).

Assays. Sera were frozen until analyzed. The assay was carried out in duplicate. All samples belonging to the same animal were run in the same assay to avoid interassay variations. The interassay coefficient of variations was less than 5%. Serum cortisol concentrations were measured by specific RIA (Cortisol Test IV807, Cambridge Medical Tecnology, innoVet Division, MA - USA).

The data were analyzed by the following statistical model:

$$CBL_{ijkl} = \mu + sex_i + month_j + b_1 * (1/day)_k + (subj_{ijl} * day_k)_{ijkl}$$

where: CBL = cortisol blood level;
sex = sex of calf;
month = month of birth;
day = age of the calf in days;
subj = subject I.D.

The interaction between subject and day was considered as the error term.

RESULTS AND DISCUSSION

This study represents the first report on the variations of serum cortisol concentrations in Italian Friesian calves in the postnatal life. Serum cortisol values were high on the first day (5.5 ± 1.6 µg/dl,mean ± SD) and sharply declined thereafter (mean value at 48 h: 2.6±0.9 µg/dl, $p < 0.01$). A further decrease occurred at the end of the fourth week (0.8±0.03 µg/dl, $p < 0.01$ vs. the 2nd day value) while the mean serum cortisol levels remained fairly stable thereafter (Figure 1).

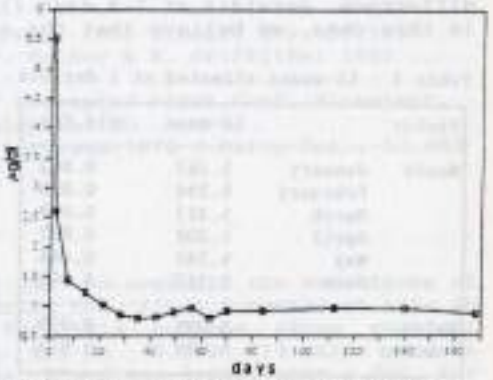

Figure 1 - Serum cortisol levels.

These results are in good agreement with those reported by other authors, who in other bovine breeds also found the highest serum cortisol concentrations shortly after birth (2, 4, 6, 7, 10, 11, 14, 15, 16, 18). This hypercortisolemia can be explained by cortisol hypersecretion which occurring in the fetus, probably to induce parturition (9) and to favour the adaptation to the extrauterine life (17). Alternatively, an accelerated catabolism, rather than a diminished synthesis, may intervene (11). Finally, it cannot be excluded that other factors, such as the hemodilution occurring in the neonatal period, may contribute to these changes (16). The permanence of high serum hormone levels during the first week of life might be explained by the stress due to the absence of the mother and by the replacement of colostrum with a commercial milk replacer; in addition the differences between intrauterine and extrauterine environment should not be forgotten.

It is interesting to note that the mean cortisol values at birth reported in different series were quite scattered, ranging from 5 to 12 µg/dl (2, 4, 6, 7, 10, 11, 14, 15). These differences may be related, on one hand, to the different animal species evaluated, and on the other hand, to differences in the timing of blood sampling. For example, our mean values were similar than those reported by Cabello (6, 7), Goncharova (14) and slightly lower than those reported by Agarwal et al. (2), Bosc & Fèvre (4), Dvorak (10), Eberhart et al. (11) and Hudson et al. (15). The reason of these differences may be attributed, as suggest by Cabello (6) and confirmed by the trend of our estimated means (Table 2), to the fact that serum cortisol concentration quite substantially decreases within the first 24 h after birth.

Table 2 - Regression of cortisol blood level by 1/age. The coefficients b_a and b_s are different ($p < .05$).

	parameter	est.mean	std.err.
all animals	constant	0.798	0.028
	b	4.506	0.099
males	constant	0.672	0.036
	b_m	4.800	0.123
females	constant	0.887	0.040
	b_f	4.279	0.145

Our cortisol data on first day (5.5 µg/dl) are much lower than those reported in male calves by Lopez & Phillips (18) (33.16 µg/dl); this difference persists at 7-8 days (1.4 µg/dl compared to 10.75 µg/dl). In this case, we believe that the explanation is in the different method of analysis used. In fact, for the measurement of serum cortisol concentrations we used a specific radioimmunoassay while Lopez & Phillips (18) employed a competitive protein-binding technique assayed after initial purification by descending paper chromatography.

Table 3 - LS-means stimated at 1 day old

Factor		LS-mean	Std.Err.
Month	January	5.367	0.062
	February	5.294	0.059
	March	5.313	0.059
	April	5.208	0.057
	May	5.247	0.066
	June	5.317	0.064
Males		5.208	0.039
Females		5.374	0.033

Estimated cortisol values in the animals born from January to March were significantly higher than those in the calves born from April to June (5.33 µg/dl vs. 5.26 µg/dl, $p < 0.05$) (see table 3 for the estimated values

of each month). It is conceivable that the different temperature be responsible for these differences, since heat is a factor known to depress cortisol secretion (1, 7, 22).

Finally, the estimated values were slightly lower in male than in female calves (5.21 µg/dl vs. 5.37 µg/dl in the first day after birth). This fact may be due to the effect of gonadal hormones (21).

In conclusion, these results demonstrate that also in Italian Friesian calves the highest serum cortisol levels are found immediately after birth and sharply decline thereafter. The period of birth (winter vs. spring) and the sex of calves in the first day may affect the mean serum cortisol concentrations and must be taken into account when establishing the "normal" values.

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SUMMARY

The aim of the present study was to evaluate the variations of serum cortisol concentrations during the first 6 months of life in Italian Friesian calves (IFC). For this purpose serum cortisol levels were measured by specific RIA in 45 IFC (19 males and 26 females) born from January to June. Blood was drawn, once a day, for the first 2 days, then at weekly intervals for 10 weeks, and then at

monthly intervals up to 6 months. The data were analyzed by a split-plot least square method for repeated hormonal measures over time. Serum cortisol concentration (mean ± standard dev.) 24 h after birth was 5.5 ± 1.6 µg/dl; a significant reduction of cortisol level was observed just after 48 h (2.6 ± 0.9 µg/dl), and continued up to the 4th week of life (0.8 ± 0.03 µg/dl). After this age the cortisol concentration remained unchanged. These data confirmed in IFC a progressively decrease of serum cortisol concentration from the highest values in the perinatal period to the normal adult values after the first month of life. The neonatal hypercortisolemia may be due to the hypersecretion of cortisol by fetal adrenals which precedes and probably induces parturition. The high serum hormone levels in the first week of life may be related to the stress due to the absence of the mother and to the replacement of coloatrum with a commercial milk replacer; in addition the differences between intrauterine and extrauterine environment should not be forgotten.

SUMÁRIO

O objectivo do presente estudo foi avaliar as variações das concentrações de hidrocortisona no soro durante os primeiros 6 meses de vida nos Vitelos Italianos da Frísia (VIF). Para este fim, os níveis de hidrocortisona no soro foram medidos por Radioimuneanálise específicos em 45 VIF (19 machos e 26 fêmeas) nascidos desde janeiro até junho. O sangue foi obtido, uma vez por dia, durante os 2 primeiros diss, depois com intervalos de uma semana durante 10 semanas e depois com intervalos de um mês até aos 6 meses. Os dados foram analisados por um diagrama duplo com o método dos mínimos quadrantes por medidas hormonais naquele período. A concentração de hidrocortisona no soro (valor médio ± variante do padrão) 24 h depois do nascimiento era 5.5 ± 1.6 µg/dl; uma redução significativa do nível de hidrocortisona foi observada logo a seguir às 48 h (2.6 ± 0.9 µg/dl) e continuou até 4ª semana de vida (0.8 ± 0.03 µg/dl). Depois desta idade a concentração de hidrocortisona ficou inalterável. Estes dados confirmaram nos VIF uma diminução progressiva da concentração de hidrocortisona no soro desde os valores mais altos no período pós-natal até aos valores adultos normais depois do primeiro mês de vida. O hiper valor de hidrocortisona neo-natal foi interpretado com a hipersecreção de hidrocortisona pelas glândulas suprarenais fetais que precede e provavelmente motiva o parto. Os altos níveis de hormona no soro na primeira semana de vida foram relacionados com o stress devido à ausência da mãe e com a substituição de colostro por laite comercial substituinte, além disto as diferenças entre ambiente intrauterino e extrauterino não devem ser esquecidas.

ZUSAMMNEFASSUNG

Diese Untersuchung wurde durchgeführt, um die Schwankungen der Serumkonzentrationen von Hydrocortison bei schwarz gefleckten Kälbern (SGK) während der ersten 6 Lebensmonate auszuwerten. Zu diesem Zweck wurden mit einem speziellen Radioimmunotest die Serumkonzentrationen von Hydrocortison bei 45 SGK (19 männliche und 26 weibliche Tiere) untersucht, die in der Zeit von Januar bis Juni

geboren waren. In den ersten beiden Tagen wurden täglich eine Blutprobe entnommen, danach 10 Wochen lang einmal wöchentlich und im folgenden bis zum 6. Monat einmal pro Monat. Die Daten wurden mit der Doppelkurvenmethode der kleinsten Quadrate für wiederholte Messung des Hormonspiegels über die gesamte Zeitspanne hin analysiert. Vierundzwanzig Stunden nach der Geburt waren die Serumkonzentrationen von Hydrocortison 5.5 ± 1.6 µg/dl (Mittelwert ± Standartabweichung); nach 48 Stunden konnte ein starkes Absinken der Hydrocortisonkonzentration beobachtet werden (2.6 ± 0.9 µg/dl), das bis zur 4. Lebenswoche anhielt (0.8 ± 0.03 µg/dl). Danach blieben die Hydrcortisonkonzentrationen unverändert. Diese Ergebnisse bestätigen, daß die Serumkonzentrationen von Hydrocortison bei SGK schrittweise absinken, von den Höchstwerten sofort nach der Geburt bis zu Normalwerten für ausgewachsene Tiere, die nach dem ersten Lebensmonat erreicht werden. Der übermäßige Hydrocortisongehalt bei den Neugeborenen wurde als Hydrocortisonhypersekretion der Nebennieren des Fötus interpretiert, die der Geburt vorausgeht und sie wahrscheinlich einleitet. Der hohe Hormonspiegel im Serum während der ersten Lebenswochen ist auf den Streß zurückzuführen, der durch die Abwesenheit der Mutter und durch die Substitution des Kolostrums mit handelsüblicher Ersatzmilch hervorgerufen wird; außerdem sollte nicht der Unterschied zwischen intrauterinem un extrauterinem Milieu vergessen werden.

ALGUNS PARÂMETROS HEMATOLÓGICOS DE BOVINOS NA REPRODUÇÃO EXPERIMENTAL DO HIPERPARATIREOIDISMO SECUNDÁRIO NUTRICIONAL.

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INTRODUÇÃO

Os parâmetros hematológicos dos animais domésticos são de fundamental importância para o clínico veterinário pois fornecem uma base de comparação com valores obtidos de animais enfermos, proporcionando assim, auxílio para o diagnóstico preciso e tratamento adequado.

Muito embora a literatura mundial inerente ao assunto para a espécie bovina seja bastante ampla, poucas informações se tem sobre estes valores no rebanho mestiço leiteiro brasileiro. Considerando que os valores hematológicos sofrem influência de vários fatores, sobretudo o ambiental e que a maioria das referências descrevem observações de animais criados em condições diversas das nossas, sob clima temperado, o presente trabalho descreve o quadro hematológico de 15 novilhos mestiços leiteiros, clinicamente sadios, durante o período em que foram submetidos experimentalmente ao hiperparatireoidismo secundário nutricional.

Jardin et al.(1) analisaram 162 amostras sanguíneas de bovinos mestiços zebú, procedentes do Estado de Goiás e encontraram os seguintes valores médios e desvios padrões para novilhos: Hemácias (milhões/mm^3) - 7,2 ± 1,37; Hemoglobina (g/dl) - 9,90 ± 1,64; Volume globular (%)- 39,35 ± 7,05; Leucócitos (mm^3) - 15.900 ± 6,04; Mielócitos (%) - 0; Metamielócitos (%) - 0; Bastonetes (%) - 3,33 ± 3,5; Segmentados (%) - 21,55 ± 7,89; Linfócitos (%) 66,61 ± 10,63; Eosinófilos (%) 6,94 ± 3,21; Monócitos (%) 4,59 ± 3,02; Basófilos (%) - 5,00 ± 5,65.

Para Ferreira Neto et al.(2) os parâmetros hematológicos normais em bovinos são: Hemácias(milhões/mm^3)- 5,0 a 10,0; Hemoglobina (g/dl)- 8,0 a 14,0; Leucócitos (mm^3) - 8.000 a 12.000; Bastonetes (%) - 0 a 2,0; Segmentados (%) - 15 a 45; Linfócitos (%) - 45 a 75; Monócitos (%) - 2,0 a 7,0; Eosinófilos (%) - 2 a 20; Basófilos (%) - 0 a 2.

Segundo Duncan(3) os valores hematológicos normais para bovinos são: Hemácias (milhões/mm^3) - 5,0 a 10,0; Volume globular (%) - 24 a 46; Hemoglobina (g/dl) - 8 a 15; Leucócitos (mm^3) - 4.000 a 12.000; Bastonetes (%) - 0 a 2; Segmentados (%) - 15 a 45; Linfócitos (%) - 45 a 75; Monócitos (%) - 2 a 7; Eosinófilos (%) - 2 a 20; Basófilos (%) - 0 a 2.

Rosemberger(4) considera os seguintes valores médios para bovinos adultos e clinicamente sadios: Hemácias (milhões/mm^3) - 5,0 a 8,0; Hematócrito (%) - 30 a 40; Hemoglobina (g/dl) - 8,0 a 12; Leucócitos (mm^3) - 5,0 a 10,0; Eosinófilos (%) - 1 a 10; Basófilos (%) - 0 a 2; Não segmentados (%) - 0 a 3; Segmentados (%) - 25 a 45; Linfócitos (%) - 45 a 65; Monócitos (%) - 2 a 8.

Jain(5) apresenta como valores normais do hemograma de bovinos: Hemácias (milhões/mm^3) - 5,0 a 10,0; Hemoglobina (g/dl) - 8,0 a 15,0; Microhematócrito (%) - 30 a 36; Leucócitos (mm^3) - 4.000 a 12.000; Bastonetes (%) - 0,0 a 2,0; Segmentados (%) - 15,0 a 45,0; Linfócitos (%) - 45 a 75; Monócitos (%) - 2,0 a 7,0; Eosinófilos (%) - 0,0 a 20.

MATERIAL E MÉTODOS

Animais e instalações

Utilizou-se 15 novilhos mestiços leiteiros, não castrados e clinicamente sadios, entre 8 a 12 meses de idade, procedentes da cidade de Nova Serrana, Estado de Minas Gerais.

Durante o período de março à setembro de 1988 os animais permaneceram confinados em baias individuais, pertencentes a Escola de Veterinária da UFMG para a indução experimental do hiperparatireoidismo 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 6),exceto quanto ao cálcio e fósforo, que sofreram modificações e 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 hematológico

Quinzenalmente, colhia-se 5,0 ml de sangue de cada animal por venopunção jugular, os quais foram armazenados em frascos individuais contendo EDTA a 10% e imediatamente encaminhados ao laboratório para exames.

As determinações eritrocitárias e leucocitárias efetuaram-se na câmara de Neubauer, utilizando-se para diluição solução de 0,85% de NaCl e 4% de ácido acético, respectivamente. O volume globular determinou-se pelo método do microhematócrito e o teor de hemoglobina por fotocolorimetria. A contagem diferencial leucocitária realizou-se em esfregaços corados pelo método de May Grünwald-Giemsa.

Análise estatística

Todos os resultados obtidos submeteram-se a análise de variância correspondente a um delinamento experimental de parcelas subdivididas, testando os três grupos (tratamentos). Para comparação de médias utilizou-se o teste de Tukey ($p < 0,05$).

RESULTADOS

Os valores médios hematológicos obtidos nos três grupos não diferiram significativamente entre si a nível de 0,05. O resultado da análise estatística final do quadro hemático estão relacionados na Tab.1.

Foi possível observar que todos os valores encontrados se enquadraram dentro dos parâmetros estabelecidos pelos autores consultados. Conclui-se portanto, que a indução do hiperparatireoidismo secundário nutricional não alterou o quadro hematológico dos bovinos no presente estudo.

TABELA 1. Média e Desvio Padrão dos Valores Hematológicos dos Bovinos Estudados.

Variável	$\bar{X}$	s
Hemácias(milhões/mm^3)	9,63	1,36
Hemoglobina(g/dl)	11,72	1,24
Hematócrito (%)	33,98	2,93
Leucócitos(mil/mm^3)	12,61	2,06
Neutrófilos bastonetes (%)	1,37	1,52
Neutrófilos segmentados (%)	23,44	6,71
Linfócitos (%)	71,22	6,57
Monócitos (%)	2,01	2,03
Eosinófilos (%)	1,42	1,40
Basófilos (%)	0,27	0,55

N = Número de observações = 180

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RESUMO

O presente trabalho teve por objetivo a determinação do quadro hemático de bovinos clinicamente sadios durante o período experimental do hiperparatireoidismo secundário nutricional (HSN). Entre o período de março à setembro de 1988, foram mantidos confinados 15 novilhos mestiços leiteiros, entre 8 a 12 meses 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, colheu-se 5 ml de sangue de cada animal por venopunção jugular, nos quais foram armazenados em frascos contendo EDTA a 10%. As determinações eritrocitárias e leucocitárias foram efetuadas em câmaras de Neubauer, usando-se para diluição solução de 0,85% de NaCl e 4% de ácido acético, respectivamente. O volume globular foi determinado por microhematócrito e o teor de hemoglobina por fotocolorimetria. A contagem diferencial leucocitária foi realizada em esfregaços corados pelo método May Grünwald-Giemsa. Não houve diferença significativa entre os resultados obtidos nos tres tratamentos e os valores médios finais observados foram: Eritrócitos ($x10^6/mm3$)- 9,63 ± 1,36; Hemoglobina - (g/dl)- 11,72 ± 1,24; Hematócrito (%) - 33,98 ± 2,93; Leucócitos - ($x10^3/mm^3$)- 12,61 ± 2,06; Neutrófilos bastonetes (%)- 1,37± 1,52; Neutrófilos segmentados (%)- 23,44 ± 6,71; Eosinófilos(%)- 1,42± 1,40; Basófilos(%)- 0,27 ± 0,55; Monócitos(%)- 2,01 ± 2,03; Linfócitos(%) - 71,22± 6,57. Todos os valores obtidos encontravam-se dentro dos parâmetros estabelecidos pelos autores consultados.

SUMMARY

The objective of this work was to determine the hematological aspects of bovine, clinically healthy during a experimental period of nutritional secundary hiperparathyroidism. From march to september, 1988, 15 yong crossbred 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 minerals were modified to form the groups 1, 2 and 3. In each fifteen days, 5 ml of blood sample were collected from each animal were maintained in small containers with EDTA 10%. The values for erythrocites and leucocites were in Neubauer camera using NaCl 0,85% solution and acetic acid 4% solution, respectively. Globular volume was analised by microhematocrit; Hemoglobin value by photocolorimetry. The differencial leucocite values was made in smear using May Grünwald-Giemsa coloration. The was no major difference between

the values in the three groups and the results found were: Erythrocites (10^6/mm^3)- 9,63± 1,36; Hemoglobin(mg/dl) - 11,72 ± 1,24; Globular volume(%) - 33,98 ± 2,93; Leucocites(10^3/mm^3) - 12,01 ± 0,59; Neutrophils Bands(%) - 1,37 ± 1,52; Neutrophils Segmented(%) - 23,44 ± 6,71; Eosinophils(%) - 1,42 ± 1,40; Gasophils(%) - 0,27 ± 0,55; Monocytes(%) - 2,01 ± 2,03; Lymphocytes(%) - 71,22 ± 6,57. All the results were found to be according to physiological values established by the consulted autors.

RÉSUMÉ

L'objet du présent traveil a été déterminer le cadre henatique de bovins, cliniquemênte sains, durant la pêriode experimentale de l'hyperparatyroidisne nutritionnel secondaire. Durant la période allant de mars â septembre 1988, quinze bouvillons mêtis laitiers agés de 8 a 12 mois ont été maintenus confines dans l'Ecole Vétérinaire de la UFMG. Les animaux ont été divisés en trois groupes afin de recevoir une diête isoprotéique e isocalorique, avec la même composition minérale (selon NRC, 1976), exceptê en ce qui concerne le calcium et lo phosphore qui ont subi des modifications correspondant aux traitements 1, 2 et 3. A chaque quinzaine, on a prêleve par ponction dans la veine jugulaire 5 ml de sang de chaque animal, conservé en flacons contenant de l'EDTA a 10%. Les déterminations éritrocitaires et leucocitaires ont êtê effetuées en chambre de Neubauer, utilisant pur la dilution une solution de 0,85% de NaCl et 4% d'acide acétique respectivement. Le volume globulaire a été déterminé par microhématocrite et la teneur en hêmoglobine par photocolorimêtrie. Le contage différentiel leucocitaire a été realisé par frottis colorés selon la métode May Grünwald-Giemsa. Les résultats ont êtê soumis a l'analyse de variance correspondant a la délinéation des parcelles subdivisées, testant les trois traitements. On a utilisé le teste de Tukey ($P < 0,05$) pour la comparaison des valeurs moyennes. 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: Eritrocites ($x10^6$/mm^3) - 9,63 ± 1,36; Hemoglobine (g/dl) - 11,72 ± 1,24; Hematocrito (%) - 33,98 ± 2,93; Leucocites ($x10^3$/mm^3) - 12,61 ± 2,06; Neutrophiles batons (%) - 1,37 ± 1,52; Neutrophiles segmentes(%) - 23,44 ± 6,71; Eosinophiles (%) - 1,42 ± 1,40; Basophiles (%) - 0,27 ± 0,55; Monocites (%) - 2,01 ± 2,03; Limphocites (%) - 71,22 ± 6,57. Toutes les valeurs déterminees se sont trouvées dans la zone de paramêtres hématologiques établis par les auteurs consultés.

RESUMEN

El presente trabajo tuvo por objetivo la determinación del cuadro hemático de bovinos clinicamente sanos, durante el periodo experimental del hiperparatireoidismo secundario nutricional(HSN). Entre el periodo de marzo à setiembre de 1988, fueron mantenidos confinados 15 novilhos mestizos lecheros, entre 8 à 12 meses de edade 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 al fosforo, que sufriran modificaciones lo que corresponde a lus tratamientos 1, 2 y 3. Quincenalmente cojecse 5 ml de sangre de cada animal por veno punción jugular, los cuales fueron almacenadus en frascos conteniendo EDTA a 10%. Las determinaciones eritrocitarias y leucocitarias fueron efectuadas en cámaras de Neubauer, usandose para diluición solución de 985% de NaCl y 4% de ácido acético, respectivamente. El volumen globular fue determinado por microhematocrito, e el tenor de hemoglobina por fotocolorimetria la contage diferencial leucocitaria fue realizada en esfregaço coloreados

por el método May Grünwall-Giemsa. No Tuvo diferencia significativa entre los resultados obtenidos en los tres tratamientos y los valores medios finales observados, fueron: Eritrócito ($x10^6$/mm^3) - 9,63± 1,36; Hemoglobina - (g/dl) - 11,72 ± 1,24; Hematócrito (%) - 3,98 ± 2,93; Leucócitos - ($x10^3$/mm^3)- 12,61 ± 2,06; Neutrófilos bastonetes (%)- 1,37 ± 1,52; Neutrófilos segmentados (%) - 23,44 ± 6,71; Eosinófilos (%)-1,42 ± 1,40; Basófilos (%) - 0,27 ± 0,55; Monócitos (%)- 2,01 ± 2,03; Linfócitos (%) - 71,22 ± 6,57. Todos los valores obtenidos se encontraban entre los parâmetros fisiológicos establecidos por los autores consultados.

TREATMENT OF EXPERIMENTALLY INDUCED HYPOCHLOREMIC METABOLIC ALKALOSIS IN SHEEP USING HYPERTONIC SALINE

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INTRODUCTION

Metabolic alkalosis can be generated by net gain of base or through net loss of HCl with an accompanying rise in plasma HCO_3^- (5,7). In ruminants, proximal gastrointestinal obstruction produces hypochloremic metabolic alkalosis accompanied by hypokalemia and contraction of plasma volume, due to anorexia and sequestration of gastrointestinal contents proximal to the obstruction (3,8,10). One of us (DFS) has recently described an experimental model in sheep in which diversion (loss) of abomasal contents was used to produce hypochloremic, hypokalemic metabolic alkalosis (9). Diversion of gastric outflow was achieved through a cannula placed in the cranial part of the duodenum. This model eliminated the effects of gastric distention on vascular and respiratory function and the potential for absorption of water and electrolytes from the sequestered fluid (9).

The objective of this study was to determine whether replacement of extracellular fluid (ECF) Cl^- deficit or volume expansion without Cl^- is critical for the correction of experimentally induced metabolic alkalosis in sheep.

MATERIALS AND METHODS

Five adult, female, mixed breed sheep, weighing between 40 and 50 kg were used in the study. Each sheep was fitted with an Ivan and Johnston re-entrant cannula (4,9) placed in the cranial part of the duodenum. Each experimental trial consisted of a 48-hr prediversion period, followed by a diversion period in which gastric contents were diverted to the exterior until the plasma Cl^- concentration reached 60 ± 2 mEq/l. This was followed by a treatment period in which one of three treatments was administered. Treatment I consisted of 6 liters of isotonic Na D-gluconate and was designed to replace volume deficit without replenishing Cl^- deficit and without affecting acid-base balance (6). Treatment II was designed to replace ECF Cl^- deficit and consisted of 2 liters of hypertonic (1.8%) NaCl containing approximately 600 mEq/l each of Cl^- and Na^+. Treatment III was control (no treatment). Assignments of treatment were random and there was a minimum of 3 wk between trials. Treatments were administered over a 12-hr period at a uniform rate.

Comparisons between measured variables were done during the prediversion period, at the end of the diversion period and at the end of the treatment period.

RESULTS

During the prediversion period, sheep demonstrated anorexia, decreased urine and fecal output, tachycardia, and progressive dehydration, depression and weakness (9). Abomasal outflow was greatest during the first 24 hr, then decreased toward the end of the diversion period. Effluent pH ranged 3-5 initially, then increased to a maximum of 8-9 during the diversion period.

The PCV and TP increased during diversion while the plasma Cl^- and Na^+ concentrations decreased in a linear manner. Plasma K^+ decreased early but increased at the end of the diversion period. A severe metabolic alkalosis developed; however, toward the end of the diversion period, sheep developed a superimposed metabolic acidosis with increasing base excess and anion gap. Thirty to 100 hours (mean 60.2 hours) of diversion were required for plasma Cl^-concentrations to decrease to 60 ± 2 mEq/l.

While receiving treatment I (Na gluconate), sheep remained depressed, weak and anorectic. There was a decrease in PCV and TP and a resumption of urination; however, severe alkalosis persisted, and the degree of hypochloremia, hyponatremia and hypokalemia increased. Treatment II (hypertonic saline) produced excellent response. Sheep were more alert, stronger and had lower heart rates than during the diversion period. There was a marked decrease in PCV and TP values. The plasma Cl^-, Na^+ and base excess were also greatly improved. There was improvement in the acid-base status, though the sheep remained moderately alkalotic. Treatment III (control) produced little appreciable change in the attitude and laboratory profile of the sheep. They remained depressed, anorexic and dehydrated.

DISCUSSION

We concluded that our data support the hypothesis that Cl^- is essential for the correction of chloride depletion alkalosis (2). Sheep treated with 1.8% NaCl had return of metabolic parameters to normal or were much improved. While high volume Na gluconate resulted in improved hydration and presumably renal perfusion as evidenced by a resumption of urination and a decrease in PCV and TP, there was no improvement in electrolyte concentrations or acid-base balance.

Relatively small volumes of very hypertonic (7.5%) NaCl have been shown to reverse the pathophysiologic sequelae of severe hemorrhagic shock in several species under experimental conditions, as well as human beings within clinical settings (1). It may be possible that a very low volume of hypertonic saline could be used to correct metabolic alkalosis in cattle. This could greatly facilitate and potentially expand the use and effectiveness of IV fluid therapy, especially under field conditions.

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SUMMARY

During separate trials, 5 sheep with experimentally induced hypochloremic metabolic alkalosis were submitted to 3 treatments to differentiate the role of volume and Cl^- replacement in the treatment of chloride depletion alkalosis. Treatment with 6 liters of isotonic Na D-gluconate improved hydration, but plasma Cl^- decreased further and the sheep became increasingly weak and depressed. Treatment with 2 liters of 1.8% NaCl resulted in return of plasma Na^+ and Cl^- to normal. Metabolic parameters changed little after no treatment (control). We concluded that hypertonic NaCl was effective in the treatment of experimentally induced hypochloremic metabolic alkalosis in sheep.

RESUMO

Durante testes distintos, 5 ovinos com alcalose metabólica induzida experimentalmente, foram submetidos a 3 tratamentos para diferenciar o efeito de reposição de volume e Cl- no tratamento de alcalose por deficiência de cloreto. Tratamento com 6 litros de solução isotônica de D-gluconato de sodio melhorou hidratação, porém Cl^- plasmático diminuiu progressivamente, e os ovinos se apresentaram progressivamente fracos e deprimidos. Tratamento com 2 litros de NaCl 1.8% resultou em retorno da concentração plasmática de Na^+ e Cl^- ao normal. Parâmetros metabólicos mudaram pouco durante testes controles (tratamento nenhum). Nós concluimos que NaCl hipertônico é eficiente no tratamento de alcalose metabólica hipoclorêmica induzida experimentalmente em ovinos.

RÉSUMÉ

Durant differentes expériences, 5 moutons ôu l'on avait produis une alkalose métabolique avec hypochlorurémie, ont recus trois traitements visant a différentier l'importance du remplacement de volume versus le chlorure dans le traitement de l'alkalosis hypochlorurémique. Le traitement avec 6 litres de Na D-gluconate améliorait l'hydration de l'animal, mais le niveau de hypochlorurémie devenait plus sévère et les moutons devenaient progressivement plus faibles et déprinées. Le traitement avec 2 litres de 1.8% NaCl par contre, rendait normal le niveau de chlorure et du sodium. Les paramètres métaboliques ne changeaient guère durant la période de control (pas de traitement). On a donc conclus que la saline hypertonique est efficace dans le traitement de l'alkalose métabolique avec hypochlorurémie, provoquée experimentallement.

EFFETS DU TRANSPORT SUR LES RESERVES IONIQUES EN SODIUM ET POTASSIUM CHEZ LES TAURILLONS A L'ENGRAIS

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INTRODUCTION

Diverses études ont été réalisées aux Etats-Unis (4, 10, 11, 3) en vue de connaître les effets du transport et des stress y associés sur les performances des animaux. Selon Hutcheson et Cole (11), la durée des transports et les distances parcourues sont autant de stress susceptibles de provoquer une hyperactivité du cortex adrénalien et, corolairement, une production accrue d'aldostérone. Une excrétion potassique excessive au niveau urinaire (11) pourrait en résulter et créer un déficit des réserves corporelles. Sachant qu'une teneur élevée en potassium, au niveau cellulaire, est importante pour la synthèse protéique et pour l'activité de plusieurs enzymes (22), la reconstitution de ces réserves durant la période d'adaptation a été envisagée comme une nécessité par certains auteurs. Parmi ceux-ci, Hutcheson et al (10) ont montré que l'addition de potassium permet, durant les premières semaines d'engraissement, d'accroître, de façon notoire, les performances zootechniques ultérieures de taureaux à l'engrais.

En Belgique, la plupart des engraisseurs s'approvisionnent en jeunes bovins nés et élevés hors de l'exploitation. Cette méthode semble la plus rationnelle pour constituer des lots homogènes. Par ailleurs, le sodium est souvent apporté en quantité insuffisante dans les rations pour bovins. Il nous semble donc important de savoir si le transport a un effet réel sur les réserves corporelles en cet ion. En effet, selon Lasmer (13), la carence en sodium peut induire une importante réduction de la quantité d'aliments ingérés et, dès lors, être à l'origine de performances zootechniques suboptimales. Dans cette étude, notre but est donc de mettre en évidence les éventuelles modifications des réserves corporelles, en fonction de transports de courte durée tels qu'on les rencontre dans les conditions de la Belgique, ainsi que la variabilité inter-individuelle des teneurs intra-érythrocytaires en potassium dans la race Blanc Bleu Belge.

MATERIEL ET METHODE

Deux lots, respectivement de 6 et 7 taureaux culards Blanc Bleu Belge (lot I et lot II), d'un poids moyen de 266,8 ± 4,7 kg et 277,6 ± 7,0 kg, ont été utilisés.

Les animaux du premier lot provenaient d'un troupeau allaitant. Jusqu'au moment de leur transport vers l'unité d'engraissement, ils ont été maintenus en prairie et ont reçu environ 2 kg de complément d'élevage par animal et par jour ainsi que du foin et de la paille à volonté. Un complexe minéral apportant 3,4 g de Na par jour fut également distribué.

Les animaux du second lot furent sevrés précocement et maintenus en stabulation libre paillée. Durant leur période d'élevage, leur alimentation consistait en 0,35 kg de pulpes sèches, 0,5 kg de Luzerne déshydratée, 0,5 kg de son, 0,65 kg d'un mélange du commerce dosant 24 % de protéines brutes, 3 kg de céréales et foin à volonté.

La durée du transport de l'exploitation d'élevage à celle d'engraissement fut de 270 et 100 minutes pour les lots I et II respectivement; la distance correspondante étant de 113 et 106 km.

Un examen clinique fut réalisé avant transport et un jour après celui-ci (J 0 et 1 respectivement). Cet examen fut répété après les 8 premiers jours d'adaptation. A ces différentes étapes, les réserves corporelles en potassium ont été estimées sur base de la concentration de cet ion dans les érythrocytes. Celle-ci fut déterminée par la méthode d'Ibsen (12) appliquée par Muylle et al (15, 16, 17). Les prises de sang ont été effectuées sur tubes héparinés et la séparation des phases solide et liquide fut, afin d'éviter toute hémolyse, immédiatement réalisée par centrifugation (1000 g - 20 min.). Le sodium étant un ion extracellulaire, les réserves corporelles en cet élément ont été déterminées dans des prélèvements de salive mixte. La technique de Dobson et al. (5) a été retenue pour ces prélèvements également réalisés aux différentes étapes susmentionnées.

Le test de t de Student fut utilisé pour le traitement des données.

RESULTATS

Les teneurs en potassium intra-érythrocytaire, seules représentatives des réserves corporelles en cet élément, sont déterminées aux jours 0, 1 et 8 et présentées au tableau 1. Dans le temps, ce paramètre physiologique n'a montré aucune évolution significative.

Tableau 1. Teneurs en potassium dans les hématies (mg/l), avant et après transport (moyenne ± erreur standard).

	Jour 0 (Avant transport)	Jour 1 (Après transport)	Jour 8
Lot I	867,0 ± 95,3	872,2 ± 84,8	852,7 ± 88,1
Lot II	609,0 ± 61,8	673,9 ± 72,0	644,2 ± 47,7

Au sein des lots, et pour chaque étape de l'expérimentation, le degré de variation inter-individuelle de la concentration en potassium dans les érythrocytes est exprimé par les coefficients de variation présentés au tableau 2.

Tableau 2. Coefficients de variation inter-individuelle (%) du Potassium dans les hématies.

	Jour 0 (Avant transport)	Jour 1 (Après transport)	Jour 8
Lot I	26,9	23,8	25,3
Lot II	26,9	28,3	19,6

Aux différentes étapes de l'essai, les teneurs en Sodium ont été déterminées dans la salive et sont reprises au tableau 3. Le transport et les manipulations y afférentes n'affectent pas ce paramètre physiologique.

Tableau 3. Teneurs en Sodium dans la salive (g/l), avant et après transport (moyenne ± erreur standard).

	Jour 0 (Avant transport)	Jour 1 (Après transport)	Jour 8
Lot I	2,7 ± 0,1	2,8 ± 0,04	2,7 ± 0,1
Lot II	2,4 ± 0,1	2,2 ± 0,2	2,2 ± 0,1

DISCUSSION

Sachant que la kaliémie ne permet pas de juger des réserves corporelles globales en cet élément (14, 9, 2, 8), que le potassium est un ion intracellulaire et qu'il existe une étroite corrélation entre la concentration en potassium dans les hématies et dans les biopsies musculaires (12), les réserves corporelles en cet ion, aux jours 0, 1 et 8, ont été déterminées sur base de la concentration de celui-ci dans les hématies.

Des prélèvements de salive ont permis de quantifier les réserves corporelles en sodium.

Si le stress de transport affecte l'état clinique des animaux à l'engrais (25, 24), cette étude montre que les effets nociceptifs de celui-ci ne modifient pas significativement les réserves ioniques étudiées. L'hypothèse (11) selon laquelle le stress pourrait induire une sécrétion accrue d'aldostérone et corollairement une déplétion des réserves corporelles en potassium, justifiant l'adjonction de cet élément dans les rations de début d'engraissement, ne peut donc être accréditée.

L'analyse des résultats et des coefficients de variation en particulier permet de constater une très grande variabilité inter-individuelle de la teneur en potassium dans les hématies. Pondérés par leur variation propre dans le temps (Jours 0, 1 et 8 de l'expérimentation), ces coefficients de variation inter-individuelle sont 24,9 ± 2,7 % et 25,4 ± 0,9 % respectivement pour les lots 1 et 2. L'origine génétique et les effets du caractère polymorphique de la teneur en potassium dans les érythrocytes ont été étudiés dans l'espèce ovine (23, 6, 7, 1) et dans l'espèce bovine (7, 18, 20, 21). Jusqu'à présent, la variabilité inter-individuelle de ce paramètre physiologique n'avait pas été constaté dans la race Blanc Bleu Belge.

Plusieurs critères hématologiques (21) ont montré que les animaux dont la concentration en potassium dans les globules rouges est faible semblent capables d'une meilleure adaptation au stress thermique modéré. Partant d'une population (n=62) de taureaux Blanc Bleu Belge cliniquement sains (données non publiées), il nous a cependant été impossible d'établir une corrélation entre la concentration en potassium dans les hématies d'une part et les performances zootechniques des taureaux d'autre part. Néanmoins, la variabilité inter-individuelle des teneurs en potassium dans les hématies de cette population est évidente (coefficient de variation inter-individuelle = 18,9 ± 1,9 %).

En conclusion, le transport des taurillons, en Belgique, n'affecte pas leurs réserves corporelles globales en sodium et potassium. Mesure préconisée aux Etats-Unis, l'adjonction de ces ions à la ration des premières semaines d'engraissement ne semble donc pas se justifier en Belgique. Enfin, la variabilité inter-individuelle de la teneur en potassium dans les érythrocytes des taureaux de race Blanc Bleu Belge tend à confirmer les résultats d'investigations réalisées dans d'autres races bovines. Des études plus approfondies de ce paramètre physiologique pourraient permettre, dans la race Blanc Bleu Belge, la mise en évidence d'un éventuel déterminisme génétique pour ce paramètre.

REMERCIEMENTS

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RESUME

Afin de mettre en évidence l'éventuel effet du transport sur les réserves ioniques de taureaux maigres, cette étude compare les teneurs en sodium salivaire et potassium intra-érythrocytaire avant le transport (J 0), après le transport (J 1) et en début d'engraissement (J 8) pour 13 taureaux culards de 6 à 7 mois répartis en deux groupes (lot I : 266,8 ± 4,7 kg, n = 6; lot II : 277,6 ± 7,0 kg, n = 7). Ces deux groupes d'animaux sont issus de deux exploitations différentes.

Dans les hématies, les teneurs en potassium, déterminées aux jours 0 (lot I : 867,0 ± 95,3 mg/l; lot II : 609,0 ± 61,8 mg/l), 1 (lot I : 872,2 ± 84,8 mg/l; lot II : 673,9 ± 72,0 mg/l) et 8 (lot I : 852,7 ± 88,1 mg/l; lot II : 644,2 ± 47,7 mg/l), n'évoluent pas dans le temps. Pondérés par leur variation propre dans le temps, les coefficients de variation inter-individuelle de la concentration intra-érythrocytaire en potassium sont de 24,9 ± 2,7 % et 25,4 ± 0,9 % pour les groupes I et II. Jusqu'à présent, cette variation inter-individuelle de la concentration intra-érythrocytaire en potassium n'avait pas été constatée dans la race Blanc Bleu Belge.

Dans la salive, les teneurs en sodium ne montrent pas d'évolution dans le temps et sont les suivantes :

lot I : jour 0 : 2,7 ± 0,1 g/l; jour 1 : 2,8 ± 0,04 g/l; jour 8 : 2,7 ± 0,1 g/l;
lot II : jour 0 : 2,4 ± 0,1 g/l; jour 1 : 2,2 ± 0,2 g/l; jour 8 : 2,2 ± 0,1 g/l.

En Belgique, le transport et les manipulations y afférentes n'affectent pas ces paramètres physiologiques. Dès lors, l'adjonction de ces ions, telle que préconisée aux Etats Unis, dans la ration des premières semaines d'engraissement, ne semble pas justifiée dans les cheptels d'engraissement belges.

ABSTRACT

In order to assess the potential consequences of the transport on the body ions, we compared the salivary sodium and red blood cell potassium contents before moving (d 0), after moving (d 1) and at the start of the fattening (d 8) for 13 double-muscled fattening bulls aged 6 to 7 months and distributed in two groups (group I : 266.8 ± 4.7 kg, n = 6; group II : 277.6 ± 7.0 kg, n = 7). These two groups were issued from two differents farms.

The red blood cell potassium contents, determined on day 0 (group I : 867.0 ± 95.3 mg/l; group II : 609.0 ± 61.8 mg/l), 1 (group I : 872.2 ± 84.8 mg/l; group II : 673.9 ± 72.0 mg/l) and 8 (group I : 852.7 ± 88.1 mg/l; group II : 644.2 ± 47.7 mg/l) did not change during the time. The coefficients of variation between bulls for the potassium red blood cell content were 24.9 ± 2.7 % and 25.4 ± 0.9 % in groups I and II respectively. Up to now, this variation between bulls for potassium red blood cell contents was not evident in Belgian White Blue breed.

In the salivary, the sodium contents did not change during this trial :

group I : day 0 : 2.7 ± 0.1 g/l; day 1 : 2.8 ± 0.04 g/l; day 8 : 2.7 ± 0.1 g/l;
group II : day 0 : 2.4 ± 0.1 g/l; day 1 : 2.2 ± 0.2 g/l; day 8 : 2.2 ± 0.1 g/l.

In Belgium, the movings of the Belgian White Blue double-muscled cattle do not modify these physiological parameters. During the first weeks of the fattening, the addition of these ions to the feeding such as recommended in U.S.A. is consequently useless in Belgian fattening units.

ZUSAMMENFASSUNG

Um den eventuellen Einfluß des Transports von jungen ungemästeten Stieren auf ihre Ionengehalte anschaulich zu machen, wurden im Rahmen dieses Versuchs bei 13 6-bis 7 monatigen Mastkälbern, die in zwei Gruppen eingeteilt waren (Gruppe 1 : 266,7 ± 4,7 Kg, n = 6; Gruppe 2 : 277,6 ± 7,0 Kg, n = 7), die Speichelnatrium- und intraerythrozytären Kaliumgehalte vor dem Abtransport (T 0), nach dem Transport (T 1) und am Anfang der Mast (T 8) verglichen. Beide Tiergruppen stammten aus zwei unterschiedlichen Betrieben.

Die intraerythrozytären Kaliumgehalte, die am Tag 0 (Gruppe 1 : 867,0 ± 95,3 mg / L; Gruppe 2 : 609 ± 61,8 mg / L), am Tag 1 (Gruppe 1 : 872,2 ± 84,8 mg / L; Gruppe 2 : 673,9 ± 72,0 mg / L) und am Tag 8 (Gruppe 1 : 852,7 ± 88,1 mg / L; Gruppe 2 : 644,2 ± 47,7 mg / L) ermittelt wurden, schwanken nicht mit der Zeit. Die interindividuellen Schwankungskoeffizienten des intraerythrozytären Kaliumgehalts, gewichtet durch ihre eigene Veränderung im Laufe der Zeit betragen jeweils 24,9 ± 2,7 % bzw. 25,4 ± 0,9 % für die Gruppe I bzw. II. Bis jetzt war diese interindividuelle Schwankung im intraerythrozytären Kaliumgehalte bei der weiß-blauen belgischen Art nie festgestellt worden.

Die Speichelnatriumgehalte ändern sich nicht mit der Zeit und lauten wie folgt :

Gruppe 1 : am Tage 0 : 2,7 ± 0,1 g / L, am Tage 1 : 2,8 ± 0,04 g / L, am Tage 8 : 2,7 ± 0,1 g / L;
Gruppe 2 : am Tage 0 : 2,4 ± 0,1 g / L, am Tage 1 : 2,2 ± 0,2 g / L, am Tage 8 : 2,2 ± 0,1 g / L.

Folglich üben der Transport und die hiermit zusammenhängenden Behandlungen keinen Einfluß auf die physiologischen Parameter aus. Infolgedessen scheint uns also keinen Grund zu geben, in den ersten Wochen der Mast dem Futter der Stiere jene Ionen beizugeben, wie in den Vereinigten Staten angeraten.

THERAPEUTIC EFFECT OF ISOPROTHIOLANE ON BOVINE FAT NECROSIS IN THE JAPANESE BLACK COWS

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Bovine fat necrosis is a disease frequently observed in the Japanese black cows, a typical Japanese native breeds which produce high ranked admirable beef with beautiful marbling. These cows at 4 to 9 years old are highly susceptible to fat necrosis which is character-ized by large necrotic masses in the abdominal fat tissues. These masses compress surrounding gastrointestinal tract, urogenital tract and gonads and then secondary impairment appears in these organs. When so diagnosed, the state is almost terminal. Therefore, economic loss has been enormous in Japanese farmers.

Triglycelide crystaling, deposit of Ca-salt of fatty acid, lack or excess of certain nutrient factors, genetical disorder, escape of lipase from pancreas and liver malfunction have been suspected to be responsible for the disease. But, the exact etiology has not been identified yet.

Isoprothiolane (diisopropyl 1,3-dithiolan-2-ylidenemalonate) has been effectively used as a therapeutic drug to improve fatty liver in dairy cow in Japan. It has been known to have a stimulatory action on protein synthesis in the liver to improve fat metabolism. It is also known to have an interfering effect on fibroblast proliferation seen in the liver after chronic carbon tetrachloride treatment. The present study describes the effectiveness of isoprothiolane to improve fat necrosis in the Japanese black cows.

MATERIALS AND METHODS

Fat necrosis was detected in 97 breeding Japanese black cows by rectal palpation. Isoprothiolane was orally given (mixed with diet or by gavage) to the cows at 50 mg/kg body weight per day for 8 weeks. The cows were kept under normal dietary condition and free from other therapeutic drugs else isoprothiolane.

During pretreatment and treatment periods, the appearances, appetite, abdominal pain and defecation were observed in the cow once every 2 weeks. Size, location and hardness of the fat necrosis were monitored by the rectal palpation on the same schedule. If the situation allowed, the bloods were collected and serum levels of total cholesterol, high density lipoprotein (HDL) - cholesterol complex, neutral lipids, phospholipids (PL), non-esterified fatty acids (NEFA), transaminase activity and blood urea nitrogen (BUN) were measured. Red and white blood cells were also counted.

RESULTS

Administrations were terminated in 2 cows at 4 weeks. Other 2 cows were lost because of selling or wastage. In total, 93 cows were received 8 week administration of isoprothiolane.

Size reductions or softenings of the masses of fat necrosis were detected in 62% of these cows by 4 week after the treatment started. At 8 weeks, in addition to the changes stated above, separations or partial disappearances of the necrotic mass were also detected in 85% of the cows. Successive monitorings for the necrotic fat masses done in 46 cows after sessation of isoprothiolane treatment revealed that the masses disappeared in 19 cows (41%).

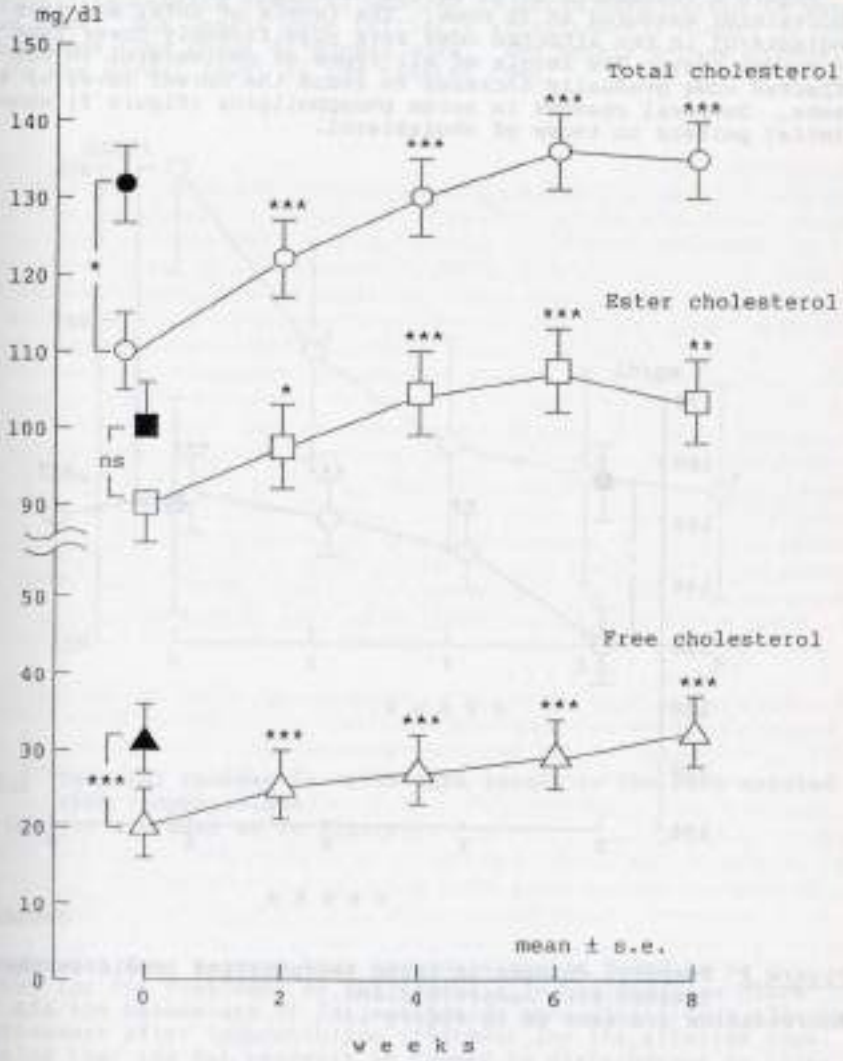

Figure 1 Temporal changes in serum cholesterol levels in the cows treated with isoprohiolane.
Black circle, rectangle and triangle represent normal value.
ns: not significant
*, **, ***: Significantly different at $p<0.05$, $p<0.01$ and $p<0.001$, respectively (data after 2 weeks were compared with each initial value)

Figure 1 represents serum levels of total, ester and free cholesterol measured in 28 cows. The levels of total and free cholesterol in the affected cows were significantly lower than those in normal ones. The levels of all types of cholesterol in the affected cows gradually increase to reach the normal level by 4 weeks. Temporal changes in serum phospholipids (Figure 2) showed the similar pattern to those of cholesterol.

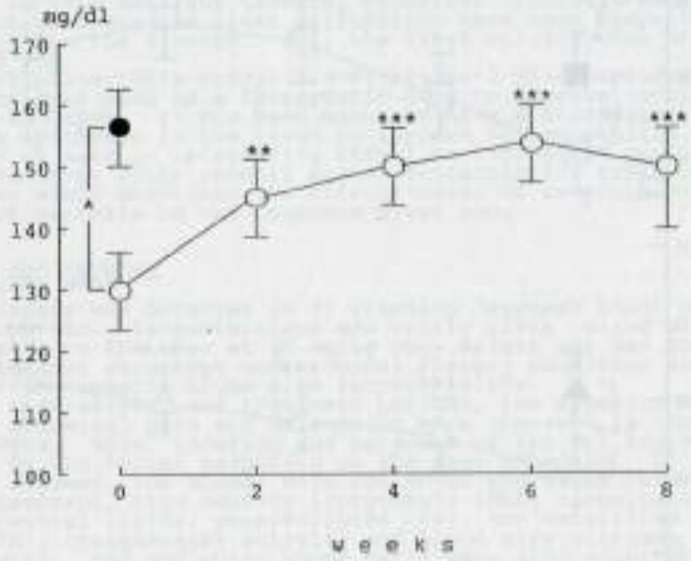

Figure 2 Temporal changes in serum phospholipid levels in the cows treated with isoprothiolane.
Abbreviatins are same as in Figure 1.

Figure 3 represents temporal changes in serum NEFA levels in the treated cows. The levels were initially significantly higher in the affected cows than those of normal ones. The levels became gradually lower to the normal level in the treated cows.

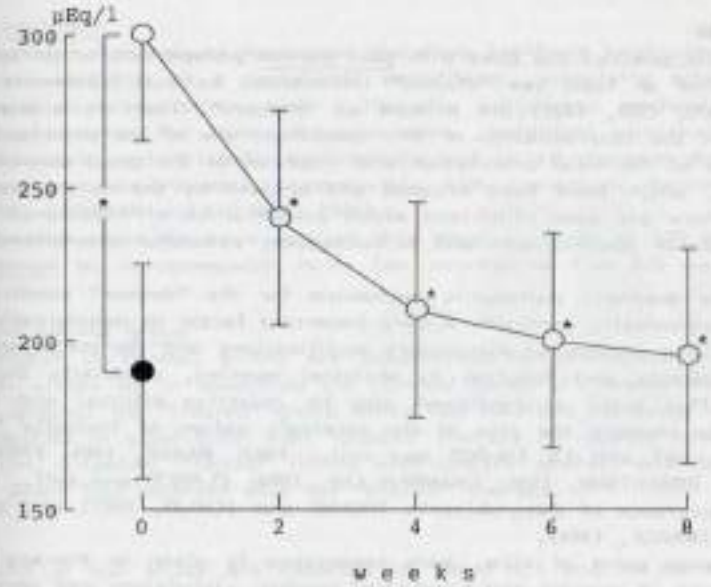

Figure 3 Temporal changes in serum NEFA levels in the cows treated with isoprotiolane.
Abbreviations are same as in Figure 1.

CONCLUSIONS

The present data suggest that isoprotiolane is found to be effective for the treatment on fat necrosis in the Japanese black cows. All the Parameters of fat metabolism show significant effects of improvement after isoprohiolane treatment for the affected cows, suggesting that the fat necrosis is caused by disturbances in liver function.

EFFICACY OF XANTHINOL NICOTINATE IN DOWNER COW SYNDROME

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INTRODUCTION

In Buiatric practice the cows with post partum paraparesis or paraplegia not recovered after at least two "classic" intravenous calcium treatments (GIBBONS and coll.,1970; COX, 1982) are defined as "downers". There is widespread opinion that in the determination of this condition, one of the principal role are modifications of the local circulation and trophism of the great muscular masses in hindlegs, which have been crushed and bruised by the weight itself of the cows, but there are some clinicians which attach a lot of importance to central nervous vascular modifications and to conseguent vasomotor alterations(REYLASZ, 1985).

Whatever the dominant pathogenic mechanism for the "downer" condition is, it is already universally accepted. A very important factor in determination of this syndrome is the process of circulatory modifications and the consequent loss of anatomic integrity and function in skeletral muscles, especially those of the hindlimbs. This event is confirmed also by objective clinical and laboratory findings, for example the rise of the serologic values of tipically "muscular" enzymes, as GOT and CK (BLOOD and coll., 1983; WAAGE, 1984; FRERKING and coll., 1984; ROBERTSON, 1986; CHAMBERLAIN, 1986; CLARCK and coll., 1987), the frequent appearance of myoglobinuria (OSAME and ICHIJO, 1987) and the rise of PCV values (WAAGE, 1984).

This pathogenic point of view, more importance is given to therapy to reduce the compression of muscles and consequent trophic, circulatory and nervous modifications, for example rough muscular massages, the lifting of the cow to quadrupedal position with various support gears (FICARELLI and VEZZANI, 1969), the treatment with antinflammatory and trophic drugs (QUINTAVALLA and coll., 1988).

Following this pathogenic hypothesis, we propose the results of a therapeutical approach on some animals affected by "downer cow syndrome", treated with an active peripheric microcirculation drug,xanthinol nicotinate (COMPLAMIN Italchimici).

MATERIALS AND METHODS

Our research has been done on 49 Italian Fresian cows, between 3 and 7 years of age, breeded in farms in the region of Parma.

The condition of "downer" in each of these has been evaluated on the basis of presence of post-partum paresis, which persist after two treatments with intravenous calcium solutions and without signs of traumatic lesions (pelvis, limbs fractures, etc.) or pathologic conditions of septicemia, endogenous or metabolic disorders (ketosis, acute hepatic insufficiency, etc.) which may interfere with the recovery of motor functions, according to the definition of the before cited COX and GIBBONS.

Laboratory tests

The absence of complications or primary etiologic factors in the animals which have been examined in the present research has been confirmed by specific serologic tests, based on the determination of GOT and Gamma-GT, enzymes which are universally considered usefull for the identification of primary or secundary hepatic lesions or functional insufficiency (UBALDI and coll., 1982).

This serum enzymatic control was made just before the treatment with xanthinol isonicotinate and after 24 and 36 hours from the same treatment.

Therapeutic treatment

For the present research we have used the drug, xanthinol nicotinate, already used in human medicine as a peripheral vasodilator, especially active at the level of tissue microcirculation, with a tipically antispastic mechanism on the precapillar sphyncters. For these characteristics , xanthinol nicotinate is used in diabetic or toxic and metabolic polineuritis and in all diseases due to cutaneous or muscular post-traumatic compression, with consequent dystrophia (GUGLIUCCI, 1988; GOODMAN and GILMAN, 1989).

In all treated cases we have applied this drug at doses of 500 mg/100 kgs l.w. una tantum by intramuscular route (an average of five 500 mg vials for cow).

Treatment and control group

In the region of Parma, births are syncronized for production of Parmesan Cheese which allows us to subdivide the clinical cases of "downer cow" in two homogeneous groups: the "treated" group which has received xanthinol nicotinate as above specified in association with "classic" therapy for downer cows (glucose saline solution, vitamins, "forced" lifting with specific gears), and the "untreated" group, which had received only the "classic" therapy.

RESULTS

The results of our study are reported in Table n°1 and in the Figures n° 1 and 2.

DISCUSSION

The majority of the cows in the "untreated" group had to be slaughtered, because no improvements of the paresis state had been observed within 12 days from the start of "downer" condition: only two cows of this group (8.33%) overcame this illness within the fixed time, while all other cows of the same group had been slaughtered by necessity at different times.

In the downers of "treated" group we observed a total recovery of 2 cows (8.00%) within 8 hours after the treatment with xanthinol nicotinate; after 12 hours of the same treatment another 8 animals regained the standing position. In the days following, we observed other recoveries, even if at a lower rate, until the seventh day from the treatment, bringing the total number of recovered cows to 19 cases (76.0%); if we leave out from the total number of recovered cows 4 cases of relapse at different times after treatment, the incidence of recovery in the xanthinol nicotinate-treated cows is equal to 64.0% of the all treated animals.

Other evaluation and discussion arguments of the here referred results arrive from the behaviour of GOT seric values, which shows a clear drop in cows in which a posteriori results have a good prognosis. In cows with no signs of recovery (slaughtered !) within 12 days after the treatment with xanthinol nicotinate the values of the same enzyme remained high. This finding confirms that this

TABLE 1. Recovery time in treated cows.

8 hours	12 hours	24 hours	2 days	3 days	4 days	7 days	Unrecovered
2	8*	3	2**	2	1	1	5

*= 3 cows euthanased
**= 1 cow euthanased

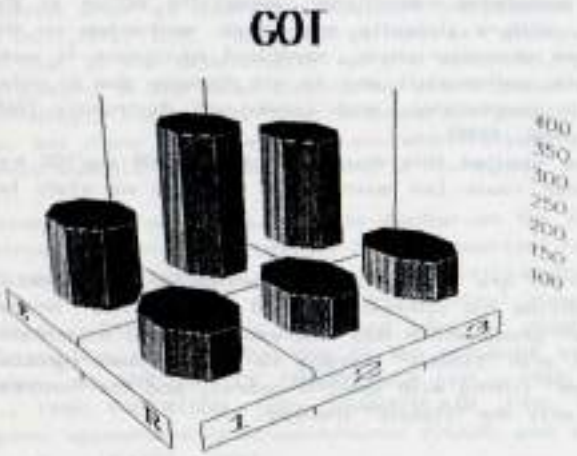

FIGURE 1. Mean values of serum GOT in recovered (R) and euthanased (E) cows at three different times.

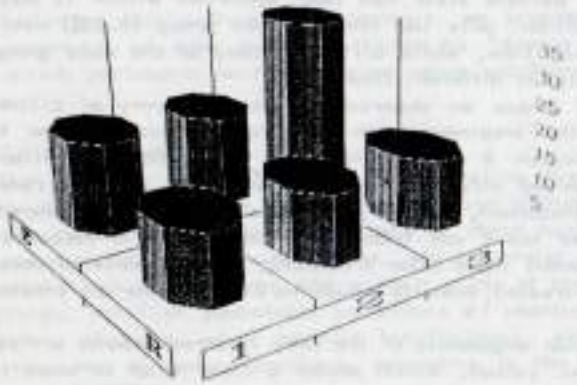

FIGURE 2. Mean values of serum Gamma-GT in recovered (R) and euthanased (E) cows at three different times.

treatment, in the animals with a good prognosis, improve the muscular trophysm, which is reduced by the downer condition itself.

We observed irrelevant modifications in the seric values of Gamma-GT, which may be expected only in the presence of serious hepatic damage, apart from the condition itself of "downer cow".

These results confirm the importance of prolonged decubitus in the etiology of the "downer cow" condition and the effectiveness of xanthinol nicotinate treatment in the therapy of this syndrome.

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SUMMARY

"Downer Cow" Syndrome is a pathological condition whose etiology is not clear. It is characterized by a prolonged recumbency which persists even after two successive therapeutical approaches with calcium. Prolonged recumbency (more than 4-6 hours) can result in ischemic necrosis due to obstruction of the blood supply, especially in a heavy cow. Regarding a research project about "New treatments in Downer Cow Syndrome", 49 Italian Fresian cows have been treated with parenteral injection of xanthinol nicotinate (500 mg/100 kgs b.w.). Xanthinol nicotinate is a vasodilator drugs that has been given in the treatment of peripheral and cerebral vascular disorders. It has also been given in hyperlipidemias. The wanthinol nicotinate therapy gave good clinical response (completed by instrumental verifications as laboratory analysis) in "Downer Cow" Syndrome.

RESUME

Les AA. réfèrent les résultats d'une recherche destinée a vedrifier l'efficacité d'un vasodilatateur périferique, le xanthinol nicotinate, dans la thérapie de la condition pathologique indiquée comme "Downer Cow Syndrome". Ces résultats soulignent la validité de ce médicament dans la thérapie de cette syndrome, où on a determiné une difference très significative entre les animaux ainsi traités (guéris à 64%) et ceux pas traités (pas plus de 1'8%). On a ainsi confirmé l'importance des phénomènes traumatiques musculaires surtout ces da compression et de dégéneration due to alteration de la circulation periphérique dans le déterminisme de cette syndrome.

DISTÚRBIOS CEREBELARES EM CAPRINOS

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INTRODUÇÃO

As patologias do cerebelo já foram fartamente estudadas e caracterizadas (5,7). Todavia, são praticamente inexistentes trabalhos que descrevam patologias cerebelares especificamente em caprinos. Mesmo de forma mais ampla, apenas alguns trabalhos procuram tratar das neuropatias dos pequenos ruminantes. Descreve-se nos ovinos, alguns distúrbios congênitos do cerebelo (3, 6, 9). Cita-se que a betamanosidose e a infecção pelo vírus da "Borderdisease" provocam lesões cerebelares congênitas em caprinos (3). Também descreve-se um caso de hipoplasia cerebelar congênita em caprino (11).

Dentre os processos inflamatórios do cerebelo encontra-se citações a respeito de abcessos encefálicos, listeriose, migração de larvas de parasitas e alguns vírus (1, 3, 5, 6).

Quanto às etiologias que provocam processos degenerativos citam-se a deficiência nutricional de cobre ("swayback") (10) e a intoxicação por algumas plantas, tal como a Solanum fastiagiatum (po. "jurubeba") (8,12).

Este trabalho pretende descrever alguns aspectos sintomatológicos, anátomo-patológicos e etiológicos dos distúrbios cerebelares dos caprinos.

MATERIAL E MÉTODOS

Dentre os caprinos atendidos na Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, foram selecionados aqueles que apresentavam sintomatologia típica de lesão cerebelar. Entre os anos de 1983 e 1986 encontraram-se 4 animais nestas condições. Foram registrados os dados de identificação de cada animal, a saber: raça, sexo e idade.

Além do exame físico geral dos animais, realizou-se o exame clínico específico do sistema nervoso, procurando-se evidenciar os sintomas típicos de lesão cerebelar.

Por punção da cisterna magna no espaço atlanto-occipital colheram-se amostras do líquor. Neste material foi feita a dosagem de proteína, a dosagem de glicose e a contagem de leucócitos e no soro obtido do sangue dos animais fez-se a dosagem de glicose (glicemia), segundo técnicas descritas (2).

De um animal, após o óbito, o encéfalo foi colhido, fixado em formol neutro a 10% e, posteriormente, fragmentos de cerebelo foram processados pela técnica de inclusão em parafina. Cortes de 5 micrômetros foram obtidos, corados pela hematoxilina-eosina e observados em microscopia de luz.

RESULTADOS

A Tab. 1 mostra que os animais não possuiam, em termos de identificação, um perfil comum. Eram de várias raças, de ambos os sexos e com idades variadas.

Tabela 1 - Dados de identificação

Animal	Raça	Sexo	Idade (meses)
1	SRD	fêmea	36
2	Saanen	macho	10
3	Toggenburg	macho	12
4	SRD	macho	3

SRD = sem raça definida

A Tab. 2 mostra que todos animais tinham sintomatologia cerebelar, caracterizada por hiperexcitabilidade (exceto o nº 4), tremor intencional de cabeça, hiperreflexia, perda de equilíbrio (exceto o nº 1) e aumento da reação tônica do pescoço (exceto os nºs 3 e 4).

Tabela 2 - Exame físico

Animal	Excitabilidade	Tremor Intencional	Reflexos	Equilíbrio	Reação Tônica
1	A	P	A	SA	A
2	A	P	A	D	A
3	A	P	A	D	A
4	D	P	A	D	SA

A = aumentado; D = diminuído; P = presente; SA = sem alteração

A tab. 3 mostra os resultados dos exames complementares. A exceção do animal nº 3, todos apresentaram valores proteicos e de células no líquor dentro da faixa normal. Quanto aos valores da glicemia, apenas o animal nº 4 apresentou-se normal. No animal nº 3 encontrou-se um valor percentual da glicose no líquor em relação à glicemia muito baixo.

Tabela 3 - Exames complementares

Animal	Proteína Líquor (mg%)	Células Líquor (/µl)	Glicose Líquor (mg%)	Glicose Sangue (mg%)	Glicose Liq./Sang. (%)
1	15,0	0,6	58,3	N.R.	-
2	32,0	2,4	123,6	136,6	90
3	142,6	12,0	59,6	140,4	42
4	16,6	0,6	39,3	73,2	54

N.R. = não realizada

Quanto a evolução dos casos, apenas o animal nº 4 sobreviveu. O animal nº 1 foi sacrificado após 1 ano tendo se observado uma estabilização do quadro. O animal nº 2 foi sacrificado após 5 meses tendo, também, se observando uma estabilização do quadro. Já o animal nº 3 morreu após 20 dias com o agravamento de seu quadro clínico.

O exame histopatológico do cerebelo do animal nº 2 revelou processos de degeneração e necrose das células de Purkinje. Esses fenômenos foram caracterizados por graus variados de vacuolização citoplasmática e perda das referidas células.

DISCUSSÃO

A sintomatologia encontrada foi semelhante àquela descrita (5) o que permite caracterizar a ocorrência de distúrbios cerebelares em caprinos.

Quanto aos aspectos etiológicos pode-se fazer algumas observações importantes. No que se refere aos problemas congênitos descritos por vários autores (1, 3, 6, 9, 11), apenas o animal nº 4 poderia se enquadrar. A ausência de um processo inflamatório verificada pelos autores normais de proteína e células no líquor indica tratar-se de um quadro degenerativo. A idade do animal (3 meses) reforça, ainda, a possibilidade de um problema congênito. Já o animal nº 3 mostra claramente padecer de um quadro inflamatório. O baixo valor percentual da glicose do líquor em relação à glicemia (42%) sugere a participação de bactérias no processo.

Os animais nº 1 e 2 apresentaram, assim como o nº 4, um quadro degenerativo. Entretanto, em função de sua idade, estes animais estavam acometidos por um processo degenerativo adquirido. O achado histopatológico do animal nº 2 (lesão das células de Purkinje) é muito semelhante àquele descrito (8).

Sabendo-se que a Solanum fastigiatum (pop. "jurubeba"), planta citada por estes autores como causadora de degeneração cerebelar em bovinos, encontra-se com facilidade no Estado de São Paulo, pode-se supor seu envolvimento nestes quadros.

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RESUMO

Em função da escassa literatura a respeito das neuropatias dos caprinos, procurou-se estudar alguns aspectos sintomatológicos, anátomo-patológicos e etiológicos de quatro caprinos portadores de lesão cerebelar. O quadro clínico era caracterizado por hiperexcitabilidade, tremor intencional de cabeça, hiperreflexia, desequilíbrio e aumento da resposta à reação tônica do pescoço. O exame do líquor mostrou 1 caso de encefalite e 3 casos de degeneração não inflamatória. No animal em que foi realizado o exame histopatológico encontrou-se degeneração e necrose das células de Purkinje.

Considerou-se como possibilidades diagnósticas as anomalias congênitas, as infecções bacterianas e as intoxicações por plantas.

SUMMARY

Considering that very little studies on neurological disorders in goats are available, the authors decided to study the symptomatic, pathological and etiologic aspects of cerebellar disorder in four goats. The clinical signs were characterized by hyperexcitability, intentional head tremor, hypereflexy, loss of equilibrium and increase of the neck tonic reaction response.

The cerebrospinal fluid examination showed 1 case of encephalitis and 3 cases of non inflamatory degeneration. In one case the histopathological examination reveled Purkinje cells degeneration and necrosis.

Congenital diseases, bacteria infections and plant poisonig were considered diagnostic possibilities.

RÉSUMÉ

Devant la littérature peu abondante sur les neuropathies des caprins, on a étudié certains aspects symptomatologiques, anatome-pathologiques et étiologiques dans quatre caprins avec lésion du cervelet. La manifestation clinique a été caracterizée pour une intensification de la réflexivité, de la réponse à la réaction tonique du cou et de la excitabilité. On a trouvé, aussi, le déséquilibre et le tremblement intentionnel de la tête. L'examen du liquide céphalo-rachidien a demontré un cas d'encéphalite et trois de dégénération sans inflammation. Dans l'animal où on a réalizé l'examen histopathologique, on a trouvé dégénération et nécrose des cellules de Purkinje. On a considéré comme possibilités diagnostiques les anomalies congénitales, les infections bactériennes et les intoxications pour plantes.

IDENTIFICATION OF MAJOR GENES AFFECTING BIRTH WEIGHT AND DAILY WEIGHT IN CALVES

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INTRODUCTION

Two forms of dwarfism have been reported in domestic animals: proportionate (primordial) and disproportionate (achondroplasia) dwarfism. Disproportionate dwarfism occurs in different types like short-headed (snorier dwarfs), long-headed, compact, compressed or stumpy (Koch et al., 1957). The classic example of disproportionate dwarfism in cattle is the Dexter breed. The most common types of disporportionate dwarfism are characterized by a short, broad and domed head, short legs, deformity of the mandible and soft palate. Hyena disease of cattle is a recently detected type of disproportionate dwarfism (Carrig et al., 1981; Parodi and Espinasse, 1975).
In contrast to disproportionate dwarfism calves showing primordial dwarfism seem to occur only at very low frequencies (Gregory and Spahr, 1979; Koch et al., 1957; Pirchner and Kaiser, 1986). Proportionate dwarfs are miniaturized in body size without any malformations of the skeletal system. The objective of this study is to investigate growth, metabolic parameters and hormones in proportionate cattle dwarfs. The analysis of pedigrees should show if genetic factors contribute to the occurence of proportionate dwarfism.

MATERIALS AND METHODS

During a project, in which disease data were collected by veterinary practitioners in Southern Bavaria, two calves affected by proportionate dwarfism were observed. Thereon farmers were encouraged by an article in a farmer journal to inform our institute on dwarf calves. We could register in totally 37 calves affected by proportionate dwarfism. Also two disproportionate dwarf calves were observed. The diagnosis of proportionate dwarfism is based on following observations: single birth, pregnancy duration in the normal range according to breed, no observable congenital defects of the skeletal system, proportional reduction of body size, however not as a sequel of a clinical disease, birth weight below 20 kg. Pathological examination and radiographs of limbs, head and lumbar vertebrae confirmed the diagnosis proportionate dwarfism, because no malformations like in disproportionate dwarfs could be detected. In table 1 a survey on the recorded primordial dwarfs is given. Proportionate dwarf calves were found in the breeds German Simmental, German Brown Swiss and in crossbreds. In six dwarfs and four control calves blood samples were taken each fortnight starting at an age of two months until an age of 14 months. Bovine growth hormone (bST) and IGF-I plasma levels were determined in a time course over 12 hours each month using RIA. Body weight and body measurements were recorded on a weekly basis.

RESULTS

The distribution of birth weight in dwarfs and normal calves, which are half sibs of dwarfs, is given in table 2. The difference in birth weight is clear cut between dwarf and normal calves. Pedigrees could be traced back to the fourth-sixth generation for the most dwarf calves (Fig. 1). In all pedigrees of dwarf calves common ancestors or ancestors, which are supposed to be carriers of genes for dwarfism, could be found.
The analysis of metabolic parameters and hormones is shown in table 3. Blood parameters like insulin, glucose, bilirubine, urea, GOT, GLDH, AP and minerals were not different between dwarf and normal control calves. Free fatty acids and cholesterol were significantly higher in dwarf calves. Profiles of bST and IGF-I taken in time courses over 12 hours show no clear trend between dwarf and normal control calves (table 4). Bovine growth hormone (bST) plasma levels were not decreased in dwarfs in comparison to control calves. IGF-I levels varied among dwarfs, but showed in most dwarfs similar values as in controls.

In dwarf calves and their mothers chromosomes were studied. No abnormalities in karyotypes of dwarf calves and their mothers could be detected.
The development of body weight and withers height in the first 420 days indicates that control calves show a faster growth than dwarf calves (table 5). The average daily weight gain in dwarfs amounts to 778 g with big individual variations (564-1153 g), whereas in controls the daily weight gain is on average 992 g.

DISCUSSION

The analysis of the pedigrees and the distribution of birth weight in dwarfs and their half sibs indicates that a genetic component causes a major effect on birth weight in dwarfs. Also the postpartal growth seems to be influenced by the same genetic component. However, the defect caused by this genetic component could not detected. The levels of bST and IGF-I are evidently not decreased by dwarfism. The higher levels of free fatty acids and cholesterol in comparison to control calves resemble higher fat metabolism and deposition in dwarfs. The dwarfs showed higher body condition scores (more fat) than controls.

The findings of the pathological and physiological examinations support the conclusion, that hypopituitary dwarfism and isolated growth hormone deficiency are not the reasons for dwarfism in these calves. Suffering from these growth disorders would result in decreased endogenous growth hormone levels. In contrast to our results Pirchner and Kaiser (1986) found proportionate dwarf calves in Austrian Simmentals, which seemed to be caused by an aplasia of the posterior pituitary and a hypoplasia of the diencephalon. Gregory and Spahr (1979) investigated not the reasons for miniature calves. The rare hereditary Syndrome Laron's dwarfism in humans, which is characterized by decreased IGF-I and IGF-II plasma levels and increased growth hormone levels can also excluded as diagnosis for the analysed dwarf calves. An explanation for dwarfism in these calves could be a selective IGF-II defect, because like in human the bovine fetus might constitute an endocrine environment, which is distinct from its adult counterpart. IGF-II seems to be the predominant growth factor in the fetus, whereas bST and IGF-I are responsible for postpartal growth. Growth retardation may also be the result of malfunctioning IGF-receptors. Human patients suffering from this disorder display high levels of human growth hormone and IGF. Further studies are necessary in order to detect the growth factors associated with proportionate dwarfism in cattle.

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Table 1: Frequency of primordial (proportionate) dwarfism

German Brown Swiss:	17 cases out of 34,333 calvings = 1 case per 2020 calvings (0,05%)
German Simmental:	16 cases
Crossbreeds:	German Brown Swiss x Blonde d'Aquitaine 1 case
	German Brown Swiss x German Simmental 3 cases

Figure 1. Pedigrees of calves showing nanosomia primordialis

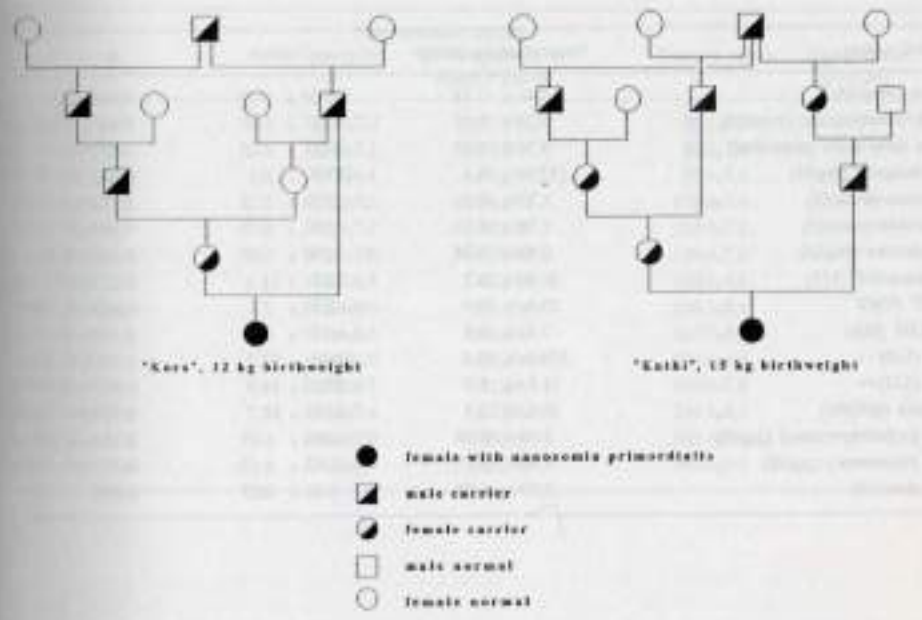

Table 2: **Distribution of birth weight in German Brown Swiss calves with proportionate dwarfism (n=17) and in controls (n=53)**

Birth weight (kg)	Number of calves			
	Proportionate dwarfs		Control	
	male	female	male	female
9 - 11	1*	1*		
12 - 14	2**	2**		
15 - 17	4****	5****		
18 - 20	1*	1*		
36 - 38			2**	3***
39 - 41			4****	4****
42 - 44			4****	5*****
45 - 47			8********	7*******
48 - 50			3***	5*****
51 - 53			3***	2**
54 - 56			2**	
57 - 59			1*	

Table 3: **Blood values in female German Brown Swiss calves with nanosomia primordialis (n=6) and in controls (n=4)**

Blood values	Proportionate dwarfs	Control	P
Urea (mmol/l)	4.46 ± 0.14	4.09 ± 0.37	0.352
ß-Hydroxybutyrate (mmol/l)	0.27 ± 0.02	0.27 ± 0.05	0.985
Free fatty acids (mmol/ml)	0.24 ± 0.02	0.11 ± 0.05	0.031
Cholesterol (mg/dl)	137.9 ± 3.6	117.9 ± 8.5	0.032
Glucose (mmol/l)	5.27 ± 0.12	5.16 ± 0.27	0.711
Bilirubin (µmol/l)	2.76 ± 0.13	2.56 ± 0.35	0.597
Creatinine (mg/dl)	0.99 ± 0.04	0.90 ± 0.09	0.320
Gamma-GT (U/l)	39.8 ± 4.7	54.7 ± 11.1	0.222
GOT (U/l)	29.0 ± 0.9	28.0 ± 2.1	0.667
GLDH (U/l)	7.3 ± 0.8	10.3 ± 2.1	0.167
AP (U/l)	339.9 ± 23.6	246.0 ± 57.7	0.135
CK (U/l)	31.5 ± 5.9	25.0 ± 14.0	0.667
Insulin (µU/ml)	18.6 ± 3.3	21.0 ± 18.7	0.907
T-3 (trijodthyronine) (µg/dl)	2.05 ± 0.09	0.81 ± 1.41	0.384
T-4 (thyronine) (µg/dl)	6.80 ± 0.27	2.42 ± 4.15	0.297
Ca (mmol/l)	2.59 ± 0.03	2.46 ± 0.07	0.090

Table 4: **Bovine growth hormone and IGF-I plasma levels in primordial dwarf and control calves**

		Dwarf calves	Control
Bovine growth hormone (ng/ml)	I	5.4 ± 3.0	
	II	6.8 ± 1.6	
	III	7.1 ± 0.7	4.9 ± 1.2
	IV	8.3 ± 2.7	
IGF-I (ng/ml)	I	442 ± 123	
	II	587 ± 65	
	III	240 ± 22**	451 ± 50
	IV	463 ± 112	

**: p< 0.01

Table 5: **Body weight in female German Brown Swiss dwarf calves (n=6) and in control calves (n=4)**

	Proportionate dwarfs absolute	Proportionate dwarfs in comparison to control calves	Control calves	Significance p<0.001
at birth	13.6±7.0	33.9%	40.1±8.6	***
< 60 days	30.6±7.2	37.0%	82.6±7.8	***
< 90 days	50.6±6.4	50.8%	99.5±7.6	***
<120 days	73.2±7.2	56.3%	130.0±7.8	***
<150 days	92.8±7.2	58.5%	158.5±7.8	***
<180 days	111.2±7.6	59.0%	188.4±7.6	***
<210 days	135.2±6.6	62.5%	216.3±7.6	***
<240 days	151.0±6.6	63.4%	238.3±8.1	***
<270 days	173.4±6.5	64.8%	267.5±8.1	***
<300 days	183.4±6.6	59.2%	309.6±9.7	***
<330 days	209.5±6.7	63.0%	332.8±7.8	***
<360 days	237.8±7.4	67.0%	354.4±8.1	***
<390 days	261.9±7.0	70.8%	369.7±7.6	***
<420 days	274.6±6.7	69.2%	397.0±9.3	***

SUMMARY

In totally 17 German Brown Swiss, 16 German Simmental and 4 crossbreds calves showing proportionate dwarfism were observed in the timeperiod from June 86 to December 89. The frequency of proportionate dwarfism was estimated in German Brown Swiss calves as 0.05%. Birth weight of dwarf calves was below 20 kg, but the pregnancy duration was not shortened. All dwarf calves were single births. The clinical examination gave no evidence for the significant reduced birth weight. The average daily weight gain in the first 420 days amounted in female German Brown Swiss dwarfs to 778g (546g - 1153g) in female and male German Simmental dwarfs to 594g (259g - 858g). The female German Brown Swiss control calves showed an average daily weight gain of 992g.
The analysis of pedigrees indicates that a genetic component is involved in the etiology of proportionate dwarfism. Chromosomal abnormalities in the dwarf calves and their mothers could not detected.
Blood parameters like insulin, glucose, bilirubine, urea, liver enzymes, minerals were not different between calves showing primordial dwarfism and normal control calves. Free fatty acids and cholesterol were significantly higher in calves with primordial dwarfism. Growth hormone levels were not decreased in primordial dwarfs in comparison to the controls. IGF-I levels were similar between dwarf and control calves. The blood analysis and the pathological findings reveal that the dwarf calves show no hypopituitary dwarfism and no isolated deficiency of bST. Possibly a deficiency of IGF-II and/or a defect of IGF-I/II receptors may be the cause for the primordial dwarfism in these calves.

ZUSAMMENFASSUNG

Zwischen Juni 86 und Dezember 89 wurde bei insgesamt 17 Braunviehkälbern, 16 Fleckviehkälbern und 4 Kreuzungskälbern (Fleckvieh x Braunvieh, Braunvieh x Blonde d'Aquitaine) primordialer Zwergwuchs (Geburtsgewicht unter 20 kg) festgestellt. Die Frequenz des primordialen Zwergwuchses wurde beim Deutschen Braunvieh auf 0,05% geschätzt.
Die Trächtigkeitsdauer war bei allen beobachteten Fällen im rassetüblichen Bereich. Alle untersuchten Kälber stammten aus Einlingsträchtigkeiten. Die klinische Untersuchung ergab keine Anhaltspunkte für das stark reduzierte Geburtsgewicht. Die durchschnittlichen täglichen Zunahmen im ersten Lebensjahr lagen bei den weiblichen Braunviehtieren mit primordialem Zwergwuchs bei 778g (546g - 1153g), bei den Fleckviehtieren (männlich und weiblich) mit primordialem Zwergwuchs bei 594g (259g - 858g). Im Vergleich dazu betrugen die durchschnittlichen täglichen Zunahmen bei den weiblichen Kontrolltieren der Rasse Braunvieh 992g.
Signifikant erhöhte Werte konnten bei der Konzentration der Freien Fettsäuren und des Cholesterols für die Tiere mit primordialem Zwergwuchs ermittelt werden, während sich für die Konzentration von Leberenzymen, Insulin, Glukose, Bilirubin, Harnstoff und Schilddrüsenhormonen keine signifikanten Unterschiede zu den Kontrolltieren zeigten. Die Hormonprofile für das bovine Wachstumhormon (bST) und IGF-I bedürfen einer vorsichtigen Analyse. Eine Erniedrigung der Serumhormonspiegel von bST und IGF-I scheint bei den Kälbern mit primordialem Zwergwuchs nicht vorzuliegen. Die Analyse der Pedigrees läßt deutliche Hinweise für eine erbliche Beteiligung erkennen. Anomalien der Chromosomen konnten bei den Zwergkälbern und deren Müttern nicht festgestellt werden.
Als Ursache für den primordialen Zwergwuchs kommen möglicherweise eine Defizienz von IGF-II und/oder ein Defekt von IGF-I/II-Rezeptoren in Betracht.

INFLUENCIA DE FACTORES GENETICOS Y AMBIENTALES EN LAS CARACTERISTICAS SEMINALES DE TOROS DE LAS RAZAS RUBIA GALLEGA, FRISONA Y PARDO ALPINA EN GALICIA (ESPAÑA).

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INTRODUCCION.- Las razas bovinas Rubia Gallega, Frisona, Pardo Alpina y sus cruces constituyen el censo mayoritario de la población vacuna en la región gallega española; y por ello en el C.E.N.S.Y.R.A. de Lugo (Spain) se elabora la mayoría de las dosis seminales que, procedentes de estas tres razas, son utilizadas en forma de semen congelado para atender a las necesidades reproductivas de esta importante región ganadera española (25 % del total). En el presente trabajo se estudian algunos de los factores de variación, genéticos (raza y toro) y ambientales (mes, año, y edad) que pueden incidir, según diferentes autores (GAUTHIER § VARO, 1985; TAYLOR et al., 1985; PARKINSON, 1987), en las características del semen que mayoritariamente se elabora y contrasta en Galicia.

MATERIAL Y METODOS.- Se han utilizado los registros relativos a la producción y calidad de 9.244 eyaculados, procedentes de dos saltos espaciados por 10 minutos en cada sesión de recogida de semen, sobre 23 toros de raza Rubia Gallega (6.636 eyaculados), 15 de Frisona (1.120 eyaculados) y 12 de Pardo Alpina (1.288 eyaculados). El análisis espermático de cada uno de los eyaculados incluyó: volumen, motilidad masal, concentración y número de espermatozoides, realizado según la metodología estandar. Como las dosis seminales se preparan a partir del semen total obtenido en los dos saltos en conjunto, los parámetros analizados en las 4.622 observaciones resultantes fueron: volumen eyaculado medio (VME), concentración media de espermatozoides por eyaculado (CME), número medio de espermatozoides por eyaculado (NMEE), motilidad masal media por eyaculado (MME), motilidad progresiva inmediata en fresco (MPI), a las 4-6 horas de la recogida (MPPH) y despues de congelar y descongelar el semen en pajuelas (MPPCD).

El análisis estadistico se realizó mediante el procedimiento GLM del paquete estadistico SAS, según un modelo matemático en el que se consideraron como efectos fijos: la estación del año (primavera-verano y otoño-invierno), el año (1.974 a 1.984), edad (2 a 9 años), raza (Rubia Gallega, Frisona y Pardo Alpina), toro (1 a 50) y las interacciones entre edad, raza y toro.

RESULTADOS Y DISCUSION.- En la Tabla 1 se resumen los análisis de varianza realizados, comprobándose que todos los factores de variación considerados han influido significativamente en las características seminales y espermáticas investigadas, de conformidad con lo anotado por la mayoría de los investigadores (AMIR et al., 1.982 AL-HAKIM et al., 1.984; SEKONI et al., 1.988) y también por nosotros en un trabajo previo (GUTIERREZ et al., 1.989). Asimismo se observa que los coeficientes de determinación estimados han sido elevados, encontrándose dentro del rango R^2 = 0.3149 a R^2 = 0.4559, al igual que los coeficientes de variación calculados y que oscilaron entre C.V.= 19.624 a C.V.= 50.6503.

Los factores que han permitido explicar los mayores porcentajes de la varianza explicada por el modelo matemático han sido el toro (33.89 % a 45.06 %), el año (33.18 % a 41.17 %) y la interacción edad x toro (14.15 % a 29.23 %), que conjuntamente suponen de un 87.59 % a un 95.97 %. Resulta interesante observar que así como en los parámetros VME, MME, MPI y MPPH el factor de variación que más ha incidido ha sido el toro, en el CME las influencias se equiparan entre los factores toro y año, y en los NMEE y MPPCD es el año el factor que explica el mayor porcentaje de va-

rianza del modelo. La raza y edad explican en conjunto, sólo entre un 2.18 % y 7.87 % de la varianza comentada, mientras que las estaciones del año, al igual que el resto de las interacciones estudiadas han incidido muy poco en el porcentaje de varianza explicada por el modelo matemático utilizado.

En definitiva, se observa que los factores genéticos (raza y toro) y ambientales (mes, año y edad) han explicado porcentajes similares de las varianzas explicadas por el modelo matemático para cada una de las características seminales y espermáticas estudiadas, cuyas medias, y para dichos factores de variación, se resumen en la tabla 2.

De conformidad con TAYLOR et al. (1.982), PARKINSON (1.987) y NAZIR et al. (1.987), los valores de todos los parámetros estudiados han sido significativamente más elevados (P<0.05) en primavera-verano que en otoño-invierno; así, los mínimos invernales observados se corresponden con los fotoperiodos mínimos de otoño-invierno, mientras que las cifras más elevadas de primavera-verano serían paralelas a los períodos de máxima temperatura.

La influencia del año en las características analizadas ha sido significativa, al igual que lo comentan AMIR et al. (1.982) y LANG et al. (1.988), siendo las tendencias observadas acordes con las observaciones de TAYLOR et al. (1.985). Así, debe destacarse la significativa tendencia apreciada en la MPPCD manifestada en la disminución de una unidad entre los años 1.974 y 1.984, situación que debe correlacionarse con las técnicas utilizadas en la congelación y conservación de las pajuelas.

Las tendencias más evidentes observadas en relación con la edad de los toros se han apreciado en las distintas motilidades espermáticas. Mientras que la MME ha evidenciado una tendencia a incrementarse significativamente hasta la edad de 5-6 años, las MPI, MPPH y MPPCD han mostrado una tendencia a la disminución con la edad, observación que se estima muy interesante.

Aunque la raza no ha sido el componente más importante de la varianza explicada por el modelo, su influencia ha sido significativa. Así, se han apreciado diferencias significativas en todos los parámetros estudiados, de conformidad con lo anotado por SCHWAB el al. (1.987) y REKWOT et al. (1.988). La raza autóctona Rubia Gallega ha sido la que ha mostrado un mayor VME si bien, y por el contrario, una calidad espermática inferior ya que para el resto de las características estudiadas, los valores estimados han sido significativamente inferiores (P<0.05) a los calculados para las razas Frisona y Pardo Alpina. El efecto estimado del toro sobre los parámetros estudiados, constata lo observado a este respecto por muchos investigadores.

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RESUMEN.- Se han utilizado 4.622 eyaculados dobles procedentes de 50 toros de las razas Rubia Gallega (23), Frisona (15) y Pardo Alpina (12), y empleados para elaborar dosis seminales congeladas durante los años 1.974 a 1.984 en Lugo (España). Se analiza y comprueba que la estación del año, año de recogida, edad y raza del toro y toro, influyen significativamente sobre las características seminales siguientes: volumen seminal, concentración, número de espermatozoides y motilidad masal por eyaculado, motilidad progresiva inmediata en fresco, a las 4-6 horas de la recogida y después de congelación y descongelación.

SUMMARY.- Data consisted of 9244 eyaculates records for volume, motility, concentration and number of sperms and 4622 total semen production records for immediate progressive motility, 4-6 hours later and after frozing and thwawing have been studied on 23 Rubia Gallega, 15 Holstein Friesian and 12 Brown Swiss bulls from 1974 through 1984. It has been detected a significantly influence of the season, year, age, breed and bull jover all characteristics studied.

RESUME.- Cette étude porte sur les variations saisonnières, de l'année, de l'age, de la race et du taureaux, sur les caracteristiques des 9244 ejaculats et 4622 sperme totaux, des 23 taureaux Rubia Gallega, 15 Holstein et 12 Parde Alpine, pendant les années 1974-1984. Les résultats obtenûs ont mis un evidence des variation significatives du volume, concentration, nombre total de spermatozoides et la motilité massale par ejaculat, et motilité progressive immediate, aprês 4-6 heures et aussi congelation et decongelation en function de tous les facteurs etudiée.

TABLA 1.- Análisis de factores de variación que influyen en características seminales del ganado vacuno (n = 4622 observaciones)

Factores de variación		V M E (ml)		C M E (2)		N M E E (3)	
ORIGEN	G L	R^2 (1)	F	R^2 (1)	F	R^2 (1)	F
Estación	1	1.03	32.49**	0.27	9.22**	0.99	28.77**
Año	10	28.38	89.44**	35.14	118.25**	40.81	119.01**
Edad (E)	7	3.30	14.83**	2.22	10.68**	2.08	8.65**
Raza (R)	2	4.57	11.97**	4.36	73.33**	0.64	9.30**
Toro (T)	49	45.06	28.98**	38.41	26.38**	33.89	20.17**
E x R	14	1.88	4.22**	1.99	4.79**	1.06	2.20**
E x T	107	14.15	4.17**	16.20	5.09**	19.06	5.22**
R x T	10	1.05	3.30**	1.28	4.29**	1.13	3.30**
E x R x T	4	0.58	4.60**	0.13	1.09^{+}	0.24	1.77^{+}
$\mu \pm$ E.T.		5.3516 $\pm$ 0.0225		0.9545 $\pm$ 0.0056		5.4957 $\pm$ 0.0409	
R^2		0.4164		0.4324		0.3976	
C.V.		28.5705		39.8585		50.6503	

V M E = Volumen medio / eyaculado

C M E = Concentración media / eyaculado

N M E E = Núm. medio espermatozoides / eyaculado

G L = Grados de libertad

R^2 = Coeficiente de determinación explicado por el modelo matemático

C.V. = Coeficiente de variación

(1) Valores relativos al % de varianza explicada por el modelo (R^2)

(2) (Espermatozoides / mm^3) x 10^6

(3) Espermatozoides x 10^9

+ $P > 0.05$

** $P < 0.01$

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TABLA 1 (continuación).- Análisis de factores de variación que influyen en características seminales del ganado vacuno (n = 4622 observaciones)

Factores de variación		M M E (2)		M P I (2)		M P P H (2)		M P P C D (2)	
ORIGEN	G L	R^2 (1)	F	R^2 (1)	F	R^2 (1)	F	R^2 (1)	F
Estación	1	0.57	20.94**	0.01	0.19^{+}	0.20	4.31*	0.24	6.15*
Año	10	30.86	114.19**	23.18	47.07**	25.08	53.84**	41.17	103.77**
Edad (E)	7	2.26	11.95**	3.90	11.30**	3.51	10.76**	1.38	4.97**
Raza (R)	2	5.06	93.72**	2.41	24.49**	2.12	22.75**	0.80	10.99**
Toro (T)	49	42.08	31.78**	39.44	16.34**	39.86	17.46**	40.57	20.87**
E x R	14	2.58	6.81**	1.18	1.72^{+}	1.28	1.95^{+}	0.51	0.92^{+}
E x T	107	15.27	5.28**	29.23	5.55**	27.29	5.47**	14.23	3.35**
R x T	10	0.98	3.62**	0.60	1.21^{+}	0.61	1.31^{+}	1.04	2.62**
E x R x T	4	0.34	3.16*	0.05	0.24^{+}	0.05	0.28^{+}	0.06	0.34^{+}
$\mu \pm$ E.T.		2.8269 $\pm$ 0.0145		4.6406 $\pm$ 0.0134		3.6631 $\pm$ 0.0109		1.9730 $\pm$ 0.0075	
R^2		0.4559		0.3149		0.3270		0.3633	
C.V.		34.9226		19.6224		20.2974		25.8715	

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M M E = Motilidad media masal / eyaculado

M P I = Motilidad progresiva en fresco inmediata

M P P H = Motilidad progresiva después 4-6 horas

M P P C D = Motilidad progresiva después de congelación y descongelación

G L = Grados de libertad

R^2 = Coeficiente de determinación explicado por el modelo

C.V. = Coeficiente de variación

(1) Valores relativos al % de varianza explicada por el modelo (R^2)

(2) Escala 1 a 5

+ P 0.05

* P 0.05

** P 0.01

TABLA 2.- Valores medios de parámetros seminales de toros de las razas Rubia Gallega, Frisona y Pardo Alpina, en función de diversos factores de variación (n = 4.622 observaciones)

Factores variación	VME (ml)	CME (1)	NMEE (2)	MME (3)	MPI (3)	MPPH (3)	MPPCD (3)
Prim. + Ver.	5.48 a	0.97 a	5.73 a	2.90 a	4.65 a	3.69 a	1.99 a
Otoñ. + Inv.	5.23 b	0.94 b	5.28 b	2.76 b	4.64 a	3.64 b	1.96 b
Año: 1.974	3.81 e	0.92 cd	5.53 bc	2.37 e	4.94 a	4.00 a	2.80 a
1.975	4.41 d	1.18 a	5.83 b	2.43 de	4.98 a	3.99 a	2.34 b
1.976	4.65 d	0.66 e	3.35 e	2.05 f	4.92 a	3.93 ab	2.20 c
1.977	5.16 c	0.73 e	3.81 e	2.64 d	4.94 a	3.81 bc	2.09 d
1.978	5.24 c	0.85 d	4.40 d	3.17 b	4.37 c	3.33 de	2.06 d
1.979	4.62 d	1.17 a	5.05 c	3.27 b	4.27 c	3.26 e	1.94 e
1.980	5.45 c	0.99 bc	5.30 bc	2.89 c	4.37 c	3.40 de	1.87 ef
1.981	5.98 b	0.85 d	5.87 b	2.54 ce	4.22 c	3.36 de	1.68 g
1.982	6.45 a	1.03 b	7.03 a	3.25 b	4.34 c	3.47 d	1.70 g
1.983	6.15 ab	1.18 a	7.39 a	3.66 a	4.64 b	3.71 c	1.78 fg
1.984	5.41 c	1.14 a	7.35 a	3.17 b	4.80 ab	3.81 bc	1.77 fg
Edad años: ≤2	5.04 c	1.05 a	5.70 b	2.79 bc	4.85 a	3.86 a	2.04 ab
3	5.34 b	0.89 c	5.01 cd	2.66 c	4.70 ab	3.80 a	2.10 a
4	5.43 b	0.82 d	4.76 d	2.71 c	4.68 bc	3.68 b	2.03 b
5	5.38 b	0.94 bc	5.38 bc	3.04 a	4.72 b	3.68 b	2.03 b
6	5.78 a	0.92 bc	5.70 b	2.91 b	4.45 d	3.48 c	1.88 a
7	5.46 b	0.84 c	5.15 c	2.50 d	4.09 e	3.21 c	1.67 e
8	5.31 b	0.96 b	5.40 bc	2.90 b	4.37 d	3.43 c	1.77 d
≥9	5.45 b	1.06 a	6.34 a	3.05 a	4.57 c	3.62 b	1.92 c
Raza:							
Rubia Gallega	5.51 a	0.90 b	5.46 b	2.71 c	4.57 c	3.61 c	1.93 c
Frisona	4.53 c	1.09 a	5.19 b	3.33 a	4.77 b	3.74 b	2.05 b
Pardo Alpina	5.22 b	1.09 a	5.96 a	3.01 b	4.89 a	3.88 a	2.10 a

VME = Volumen medio / eyaculado
CME = Concentración media / eyaculado
NMEE = Núm. medio espermatozoides / eyaculado
MME = Motilidad media masal / eyaculado
MPI = Motilidad prograsiva inmediata en fresco
MPPH = Motilidad progresiva después 4-6 horas
MPPCD = Motilidad progresiva después de congelación y descongelación
(1) (Espermatozoides / mm^3) x 10^6
(2) Espermatozoides eyaculado x 10_9
(3) Escala 1 a 5

- Letras distintas corresponden a grupos distintos (P > 0.05)

CONTROL AND REGISTRATION OF HEREDITARY DISEASES IN DANISH CATTLE

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INTRODUCTION

With the widespread use of artificial insemination (AI) in modern cattle breeding, hereditary diseases have become of greater importance, because individual AI-bulls can sire a large number of calves. This intensive use of bulls can spread a hereditary disease to a large number of calves within a short period of time, if the bull is carrier of such a disease, and as the sons often are used as AI-bulls as well, a large number of animals with clinical disease suddenly can appear.

CURRENT HEREDITARY DISEASES

These problems were already recognized in Danish cattle breeding years ago, as several calves with congenital hereditary paralysis appeared (2,7). During recent years several hereditary diseases have occured. In the Danish Holstein-Friesian Breed the adema disease (A46) (1,3) and bovine syndactylism (9) have been recognized. In the Red Danish Milkbreed the weaver syndrome was diagnosed few years ago, and in 1988 a new hereditary disease, the recumbent calf syndrome (bovine hereditary spinal muscular atrophy) appeared (6,10). In the Danish Jersey Breed recto-vaginal constriction was diagnosed in 1985 (5). The latest hereditary disease recognized in Danish cattle is the 1/29 Robertsonian translocation in the Blonde d'Aquitaine Breed (4,8). Intensive breeding and selection programmes, performed by the national cattle breeding organizations, have effectively combatted these diseases and several of them only appear seldom now.

Common for the hereditary diseases mentioned are, that they have all been imported to Denmark through live animals or semen. Partly due to this, partly due to the increasing demand for certification on hereditary diseases by export, a registration system for hereditary diseases was instituted in January 1989.

THE CONTROL AND REGISTRATION SYSTEM

The basis for the registration is reports from breeders, advisers, inseminators, meat inspectors and veterinary practitioners. These persons contact either the National Committee on Danish Cattle Husbandry (NCDCH) or the National Veterinary Laboratory (NVL). A special card for report on possible hereditary cattle disease has been distributed to advisers, inseminators, meat inspectors and veterinary practitioners. The card (English version) is shown in figure 1.

All reports are passed on from the NCDCH to the NVL for evaluation together with the animals herd book leaf. The results of the evaluation are sent back to the NCDCH for registration.

The reports can be classified in three main categories: 1. Cases of known hereditary diseases 2. Cases with a possible hereditary background and 3. Cases not indicating a hereditary etiology. Examples of the first group are the diseases mentioned previously, the second group

are diseases with symptoms similar to known hereditary diseases, but not yet diagnosed in Denmark, and diseases appearing after certain parent combinations. The third group consists mainly of spontaneous abnormalities. Cases from the first and second group are examined, and a correct diagnosis is ensured, often by autopsy at the NVL. Correct paternity is ensured by blood sampling both calf and parents. When a hereditary disease is diagnosed, steps are taken by the NCDCH to stop the spread of the disease by AI-bulls. In case of e.g. bovine syndactylism or recto-vaginal constriction breeding tests are performed.

Whenever a hereditary disease is diagnosed in AI-bulls, the Danish Veterinary Service is informed and possible semen export from the bull is suspended.

REPORT ON POSSIBLE HEREDITARY CATTLE DISEASE

Reporter's name:_____ **phone number:**_____

Address:_____

Owner's name:_____ **phone number:**_____

Address:_____

Property CCR.-No:_____

Animal CCR.-No:_____

Birth date:____/____**19**_____ **Mother's CCR.-No:**_____

Father:_____ **Herd book No:**_____

Grandfather:_____ **Herd book No:**_____

Symptoms:_____

_____

_____ **The animal is dead/alive**

Owner's veterinarian:_____

Figure 1 Card for reporting possible hereditary cattle disease. (CCR.-No: Central Cattle Registration Number)

A new animal disease act, which came into force December 1989, places the Danish Veterinary Service in a central position in the control and registration of hereditary diseases as well as of infectious diseases, since the act made it possible to legislate on hereditary diseases. Furthermore, the Danish Veterinary Service in collaboration with the NCDCH established a committee in 1988 "The committee concerning control and registration og hereditary cattle diseases", which elaborates direktions for registrations of hereditary diseases and proposes investigations. The committee is composed of representatives of the Danish Veterinary Service, the Danish Veterinary Association, the NCDCH and the AI societies, respectively.

CONCLUSIONS

The instituted Danish control and registration system has already shown its validity. Several bulls have been diagnosed as carriers of the recumbent calf syndrome on basis of progeny autopsy (10) as well as the control of other hereditary diseases has made progress. Furthermore, a so far in Denmark unknown hereditary disease, the syndrome of arthrogryposis and palatoschisis in the Charolais Breed, has been diagnosed.

Based on the Danish experiences with import of bovine genetic material, it is emphasized that future planning of international trade regulations should reflect the importance of requirements concerning hereditary diseases.

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SUMMARY

The occurence of actual hereditary diseases in Danish cattle is viewed. Within the last two decades several new hereditary diseases have been diagnosed. The system used in Denmark to control hereditary diseases in cattle is described. A new Danish animal disease act has made it possible to legislate on hereditary diseases. The importance of international trade requirements concerning hereditary diseases is mentioned.

SOMMAIRE

On retrace l'occurence des maladies héréditaires actuelles du bétail danois. Au cours des deux dernières décades plusieurs nouvelles maladies héréditaires ont été diagnostiquées. On décrit le système utilisé au Danemark dans le contrôle sur les maladies héréditaires. Un nouveau code danois des maladies des animaux a rendu possible de donner des lois sur les maladies héréditaires. On fait mention de l'importance des demandes commerciales internationales relatif aux maladiee héréditaires.

ZUSAMMENFASSUNG

Das Vorkommen aktueller Erbkrankheiten in dänischen Viehbeständen wird geschildert. Innerhalb der letzten Jahrzehnte sind mehrere neue erbliche Krankheiten diagnostiziert geworden. Das in Dänemark angewandte Süstem Erbkrankheiten zu kontrollieren wird beschriben. Ein neues dänisches Gesetz für animalische Krankheiten hat es ermöglicht erbliche Krankheiten zu kontrollieren. Die Wichtigkeit internationaler Handelsforderungen hinsichtlich Erbkrankheiten wird genannt.

ACCOUNTING FOR HETEROGENEOUS VARIANCES IN ESTIMATING THE BREEDING VALUES OF BEEF CATTLE.

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INTRODUCTION

The mixed model methodologies (1) are actually widespreading for estimating the breeding values of many species. Several procedures are available for solving such models, even for very large data sets, but in any case the variances associated to the random effects must be known at least at proportionality (2). In fact, for a general linear mixed model:

$$y = Xb + Zu + e$$

where y is the observation vector, X Z are the known incidence matrices respectively of the fixed (b) and random (u) unknown effects, the solution for $\hat{u}$ (i.e. the breeding values) is found based on the mixed model equations (MME)

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z+kA^{-} \end{bmatrix} \begin{bmatrix} b \\ u \end{bmatrix} \quad \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

The scalar k is the ratio var(e)/var(u) and A^{-} is the inverse of the relationship matrix. An error in the supplied values of the variances can cause a bias of the estimates breeding values and a rank exchange of the parents when the progeny size is limited (3).

Beef cattle is breed in a great variety of environments, with different mating and feeding methods. Therefore it is logical to expect that the environmental variances change even within regions. Moreover the use of A.I. is not so common in many countries and consequently the offspring size is often limited. Furthermore also the genetic variance changes when selection is applied. For these reasons an error in the estimated breeding values of beef cattle is likely to occur if the variances used in the MME are wrong.

An obvious solution would be to partition the Z'Z matrix according to a criterion for which inside the partitioned matrices the variances are supposed to be homogeneous, then to solve the MME with a method that allows the contemporary estimation of both u and k, such as the EM-REML (4). The method is correct, but unfeasible for even medium size data sets.

An other problem encountered in beef cattle is that some registered traits are strongly affected by the dam, such as the weaning weight. In these cases it is advisable to consider also

the dam effect, as happens in the Animal Model (5). But the customary absorbtion-like process of the dam effect cannot be carried out if the variances are unknown. The previous solution can be applied, but the computational work became, of course, heavier.

We present a method, based on the SWEEP algorithm (6), for solving the MME, that is appliable for medium size data sets and allows the contemporary estimation of the breeding values and the k-ratios of the Z'Z matrix partitioned according to the different conditions of breeding and of selection.

METHOD

One of the most common methods for inverting large square matrices is to apply the SWEEP algorithm N-1 times to the "augmented" MME, where N is the rank of the MME. The term "augmented" means that the last row (and column) of the MME is made up by (X|Z)'y and y'y .

The components of the variance are iteratively found by

$$var(e) = y'(y-X\hat{b}-Z\hat{u})/(N-p^*)$$

$$var_2(\hat{u}) = [\hat{u}'\hat{u} + var_1(e)\ trace(C_{22})]/q$$

where: p^* is the rank of X, q is the rank of $[Z'Z+kA^-]$ and C_{22} is the portion of the inverted MME corresponding to Z'Z, var_1 and var_2 are two successive estimate of the variances.

Looking at the preceding formulas it is evident that not all the inverted matrix is needed, but only the diagonal (for computing the trace) and the last row (for computing the quadratics). Therefore a considerable time saving can be achieved if a procedure is applied to find only the needed elements and disregarding the rest of the MME.

Considering the SWEEP algorithm as described by Goodnight (6):

1) let D = a_{kk}, i.e. the diagonal element the SWEEP is based on,
2) divide the row k by D,
3) for every other i_{th} row with i<>k, let B = a_{ik} and subtract B*row_k from row_i,
4) set a_{kk} = 1/D.

The algorithm we found modifies the step 3, considering in a different way the rows preceding from the ones following the sweeping element:

3) for every i_{th} row with i<k sweep only the diagonal element,
3')for every i_{th} row with i>k, let B = a_{ik} and subtract B*row_k from row_i.

In such way there is a substantial time saving, although for arrays saved in triangular mode a temporary vector has to be created for saving the a_{ik} elements for i>k, but only one row at time.

NUMERICAL EXAMPLE AND DISCUSSION

The example is in part taken from Cunningham & Henderson (7). The model is:

$$y = Z_1u_1 + Z_2u_2 + Xb + e$$

The Z_1 Z_2 and X incidence matrices are each made up by 2 levels.

The MME are saved in triangular way and a guess for k (1.0) has already been added to the diagonal; the fixed factor follows the random ones and one level has been zeroed for finding a generalized inverse (8):

```
 5.00
 0.00   9.00
 0.00   0.00   7.00
 0.00   0.00   0.00   5.00
 2.00   3.00   5.00   2.00  12.00
10.00  30.00  21.00  15.00  48.00 500.00
```

After the first iteration round, the augmented MME inverted with the usual SWEEP algorithm (9) are:

```
 0.23
 0.02   0.13
 0.05   0.04   0.23
 0.03   0.02   0.05   0.23
-0.07  -0.06  -0.12  -0.07   0.17
-1.64  -2.59  -1.41  -2.11  -2.23 243.00
```

If they are instead inverted by our algorithm, they result:

```
 0.23
 0.00   0.13
 0.00   0.00   0.23
 0.00   0.00   0.00   0.23
-0.47  -0.33  -0.71  -0.40   0.17
-1.64  -2.59  -1.41  -2.11  -2.23 243.00
```

As it is evident the diagonal and the last row are conserved, while the inner elements are different. Therefore the components of the variance can be computed as well as the genetic values, although the covariances between them cannot be computed, but this is usually not needed. In our examples the convergence is reached after few iterations and the final values are:4.35 and 2.69 for the variance of the random factors and 12.10 for the error term.

As previously mentioned, for the rows preceding the sweeping element the only element needed is the diagonal one, therefore a straightforward practice would be to put the larger block of the MME as first and to drop all its elements but the diagonal ones.

In beef cattle the obvious candidate for that block would be the incidence matrix of the cows. Thus the analysis of maternally influenced traits (such as weaning weight) would be greatly improved at the only expense to add a diagonal vector to the MME. On the other hand the customary absorption process cannot be applied, since the cow variance component is any way to be computed.

Using the proposed strategy, it is then possible to apply the EM-REML procedure to medium size data sets. If the computer runs out of memory, it is still possible to carry out the analysis, since the sweeping is done one row at time and then only one vector needs to be stored in memory.

CONCLUSION

The algorithm presented consents to contemporarily evaluate the variances and the breeding values in shorter times than the actual routines. The matrices can eventually be partitioned to account for etherogeneity of the variances and the evaluation of the breeding values and the ranking of the parents can be improved, while saving substantial computer resources.

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SUMMARY

In estimating the breeding values by mixed model methodologies it is quite customary to assume that the variances of the random components (usually the parent and the error factor) are normally distributed with the same variances across the design. Heterogeneous variances can indeed occur as consequence of selection and/or different conditions of breeding. Ignoring this fact can yield biased estimates mainly in beef cattle, where the progeny size is usually limited and a strong dam effect occurs for some registered trait. A solution can consist in a simultaneous estimation of the variances and the breeding values by EM-REML. An algorithm based on a modified SWEEP routine is presented, which allows to take into account the dam effect and the eventually heterogeneous variances, while not overwhelming the computer resources. The algorithm consists in a partial absorption of the dam effect, which is indeed estimated for computing the variance component.

ZUSAMMENFASSUNG

Bei der Bestimmung der genetischen Werde mit Methodologien, di gemiscte Modelle benutzen, ist es üblich anzunehemen, daß die Varianzen der zufälligen Komponenten (normalerweise die Abstammung und der Fehlerfactor) gewöhnlich mit den selben Varianzen über das Schema verteilt sind. Heterogene Varianzen können wirklich als Folge von Selection und/oder von unterschiedlichen genetischen Bedigungen auftreten. Wird diese Tatsache nicht beachtet, können sich daraus Fehlbestimmungen ergeben; dies vor allem bei Schlactrindern, deren Zahl der Nachkommenschaft normalerweise begrenzt ist, und bei denen für einige registrierte Merkmale ein starker Einfluß mütterlicherseits auftritt. Dieses Problem kann durch eine gleichzeitige EM-REML Bestimmung der Varianzen und der genetischen Werte gelöst werden. Es wird ein Algorithmus vorgestellt, der auf einer modifizierten SWEEP-routine Basierte, die es ermöglicht, den Einfluß mütterlicherseits und eventuelle heterogene Varianzen zu berücksichtigen, ohne die Möglichkeiten des Computers zu überschreiten. Der Algoritmus besteht in einer teilweisen Absorption des Einflußes mütterlicherseits, der dagegen bei der Verarbeitung der Varianzkomponente berücksichtigt wird.

SUMARIO

Avaliando os valores genéticos através de metodologias com modelos mistos é muito usual assumir que as variações dos componentes casuais (habitualmente a ascendência e a factor de erro) sao normalmente distribuidas com as mesmas variações através do esquema. As variações heterogéneas podem de facto ocorrer como consequencia de seleção e/ou diferentes condições genéticas. Ignorando este facto pode levar a avaliações falsas, sobretudo em bovinos para abate, nos quais o numero da progenitura é habitualmente limitado e nos quais um forte efeito materno ocorre para alguns traços registrados. Uma solução pode consistir numa avaliação simultânea das variações e dos valores genéticos por EM-REML. Um algoritmo baseado numa SWEEP routine modificada é apresentado, o qual permite tomar em consideração o efeito materno e as eventuais variações heterogeneas, sem dominar os meios do computador. O algoritmo consiste numa absorção parcial do efeito materno o qual é de facto estimado para avaliar o componente de variação.

ESPONDILITE ANQUILOSANTE EM TOURO SANTA GERTRUDIS COMO CAUSA DE INCAPACIDADE DE SERVIÇO: DESCRIÇÃO DE UM CASO

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INTRODUÇÃO

A espondilite anquilosante é uma artrite da coluna afetando as articulações vertebrais e Sacro ilíacas levando a anquilose e completa rigidez da coluna. Também conhecida espondilose rizomélica, espondilite anciloposítica, artrite reumatóide da coluna, espondilite deformante e doença de Strumpell-Marie.

Os osteofitos vertebrais foram descritos em vários animais (3, 8, 10, 11, 12, 13, 14) e no homem (9). Segundo alguns autores atribuem maior desenvolvimento de osteofitos pela postura ereta (4, 6, 9).

Demonstraram em um trabalho experimental (6) que ratas em postura ereta bípede, desenvolveram mais osteofitos, que ratas quadrúpedes.

O objetivo do presente trabalho é relatar o caso por nós estudado, tendo em vista a carência de informações na literatura nacional sobre o assunto, especialmente no Estado da Bahia. Assim evidenciando, a motivar a descrição destes casos, visando contribuir para um melhor conhecimento dos fenômenos osteopatológicos relacionado com a intensidade da atividade sexual e incapacidade de serviço.

MATERIAL E MÉTODO

Um touro da raça Santa Gertrudis de treze anos de idade, 1.125 kg de peso vivo, sendo utilizado em monta a campo. Este touro empenhou suas atividades sexuais desde os dezoito meses (Figura 1) até o dia da morte. Durante sua vida reprodutiva, segundo um cálculo simples, se estimou-se um mínimo de 1.728 saltos. Os saltos realizados por este touro eram violentos, acompanhados em muitas ocasiões de dois ou três passos com os menbros posteriores.

Depois da necrópsia, os músculos da coluna vertebral foram removidos e as vértebras foram preparadas segundo técnicas convencionais para observar melhor os osteofitos.

Estes foram classificados e registrados segundo classificação proposta por GLOOBE & NATHAN (1973).

RESULTADOS

Os osteofitos encontrados, ao longo da coluna vertebral, eram das quatro categorias classificadas em trabalho anterior por (4, 6). Havia osteofitos com pontas soltas de hiperostases; (primeiro grau); protusões ósseas projetadas horizontalmente ao longo das bordas craneal e caudal das vértebras (segundo grau); havia osteofitos que se curvavam um em direção a outro, em direção do disco intervertebral, de maneira que lembra o bico de papagaio (terceiro grau); apareceram osteofitos entre quais se formaram uma crosta óssea contínua, chamada ponte (quarto grau). A maioria dos osteofitos encontrados pertencian ao terceiro e quarto graus.Não foi encontrado osteofitos nas vértebras cervicais. Na região torácica e lombar entre T_6 e T_{13} e entre L_4 e L_6 prevaleceram os osteofitos ventral do corpo (Figura 2). A nível de T_5 a T_7 a ligação osteofítica foi demais lateralmente (Figura 3). Se tomaram em consideração só osteofitos ventrais do corpo vertebral.

Figura 1 - Touro já mostrando incapacidade de serviço, durante a presença de uma vaca em estro.

Figura 2 - Aspecto ventro-lateral das vértebras lombares L_4 e L_5. Se observa o alto grau de desenvolvimento de osteofitos (quarto grau). Indicado por seta.

Figura 3 - Vértebras torácicas, aspecto direito. Se observa osteofitos laterais de maneira de crista contínua tipo ponte, entre a T_5 e T_6 e entre T_6 e T_7 (indicado por seta).

DISCUSSÃO E CONCLUSÃO

Em pessoas, os osteofitos tem sido encontrados esporadicamente à partir dos vinte anos de idade, em alguns casos depois dos quarenta, em cem por cento das colunas os tem. Com a idade, aumenta o grau de desenvolvimento dos osteofitos.

Entre os trinta e os quarenta anos de idade se encontram osteofitos de terceiro grau e aos oitenta anos em 100% das colunas tem osteofitos de quarto grau (9).

A estrutura histológica dos osteofitos é a de um tecido ósseo mais compacto e mais forte que o resto do corpo vertebral.

Devido a posição vertical, há grande pressão sobre a coluna vertebral e todo o peso do corpo se recai sobre ela e se acumula principalmente sobre as últimas vértebras torácicas e lombares. Essas vértebras responden com a formação de osteofitos. Os osteofitos se desenvolve como mecanismo de proteção e defesa contra as pressões, para fortalecer a coluna vertebral (4).

Os osteofitos tem sido encontrados em cão (3, 10), em gato (10), em cavalo, em urso e gorila (11, 12) respectivamente e em bovino (14) e em ratos (4, 6) em um estudo único em seu gênero, pela primeira vez, foi possível comparar colunas vertebrais de animais da mesma espécie, quadrúpedes com bípedes. Utilizou ratas que foram transformados em bípedes, por meio da amputação dos membros anteriores aos dois dias de nascidos. Para estimular a bipedestação se mantiveram dentro de gaiolas altas e se alimentava na parte superior em uma caixa, que os abrigava a caminhar em posição erecta para obter seu alimento. As ratas foram sacrificadas por etapas sucessivas até a idade de vinte meses. Este experimento demonstrou que os dois grupos de animais tinham distribuição similar de osteofitos, alguns casos com maior grau de desenvolvimento no grupo bípede.

A maior pressão sobre as vértebras nestes bípedes explica claramente o maior desenvolvimento dos osteofitos.

Os osteofitos vertebrais não só, pos si mesmo, uma entidade patológica, a não ser que se desenvolva como um mecanismo de defesa contra a pressão na posição vertical. As vezes se apresentam como resposta a certas situações patológicas como por exemplo a osteoporose, onde a coluna se parece mais fraca e menos capaz de suportar pressão.

Se comprovou, que demais idade, as fêmeas tinham menor grau de formação osteofíticas (9, 10).

Em comparação com os dados em bovinos (14), este touro de monta, apresenta maior grau de desenvolvimento dos osteofitos.

O touro deste estudo, foi sujeito a postura erecta com movimentos bruscos e forçados associados a salto, pelo menos três vezes por semana durante 12 anos. Não havia evidências claras em redução da força do salto durante a ejaculação com a idade. (1, 2) reportaram um comportamento sexual normal em touros de monta de idade avançada.

Estes resultados confirmaram uma vez mais a teoria (7) e de outros investigadores, em que a posição vertical promove a formação de osteofitos; e denota que há uma correlação entre a postura erecta e o grau de desenvolvimento dos osteofitos vertebrais. E se sugere estudar um grupo mais grande de touros e relacionar a intensidade da atividade sexual com a formação de osteofitos.

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RESUMO

Foi descrito um caso de escrescência óssea vertebrais em um touro de monta da raça Santa Gertrudis em atividade sexual. A maioria dos osteofitos eram do quarto grau, segundo a classificação de GLOOBE & NATHAN (1973) devido a união completa entre as vértebras adjacentes. O presente trabalho tem objetivo de contribuir para a patologia dos bovinos ao relatar a ocorrência de um caso de espondilite anquilosante em um touro de monta e sua relação com atividade reprodutiva. Os autores atribuem este severo grau do fenômeno a posição erecta do touro, durante os repetidos saltos, e também devido a avançada idade e possível causa hereditária.

SUMMARY

A case of vertebral osteophytes was described in a bull of higt sesual activity. Most of the osteophytes were of fourth degree GLOOBE & NATHAN (1973) classification, fused osteophytes an the two adjacent vertebral. The objective of this work is report a case of spondilosis ankilosing in a herd bull and relationship with your reproductive performance. The authors attribute the sever development of osteophytes of this case, to the pressure exerted on the spine during the repeated and vigorous mounting, besid the increase of age anel possibility hereditary motive.

RESUMÉN

La descripción de un caso de excrescencia ósea vertebral en un toro de monta de la raza Santa Gertrudis en actividad sexual. La mayoría de los osteófitos eran de quarto grado, según la classificación de GLOOBE y NATHAN (1973) debido a la unión completa entre los vertebras adjacentes. El presente trabajo tiene como objetivo la contribución para la patología de los bovinos al relatar la ocurrencia de este caso de espondilitis anquilosante en um toro de monta e su relación con la actividad reproductiva. Los autores atribuyen este severo grado del fenómeno a la posición erecta del toro, durante los repetidos saltos y también debido a la avejentada edad y posible causa hereditaria.

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ARTROPATIA DEGENERATIVA TARSIANA SECUNDARIA EN BOVINOS PARA CARNE Y CARNE/LECHE : ANATOMIA PATOLOGICA E HISTOPATOLOGIA.

J.L. Queirolo.

Ejercicio liberal. Bvd. Artigas 1941, Montevideo-Uruguay.

INTRODUCCION.

Es conocida la importancia que la correcta funcionalidad articular tiene en la actividad social y sexual del vacuno(4).La importancia del tren posterior del vacuno es destacada en la actividad de monta y en la vida de relación.La patología articular que nos ocupa la hemos observado en vacunos de diversas razas en Brasil,Argentina y Uruguay.Es importante conocer exactamente las características anátomo-patológicas e histopatológicas de las articulaciones problemas a los efectos de:
--utilizar una terminología patológica precisa y definida.
--conocer la futura evolución de la misma,de hecho no posee el mismo pronóstico un proceso inflamatorio que uno degenerativo.
--aplicar medidas preventivo-terapeuticas en los animales afectados.
Observemos la poca uniformidad de criterios entre los distintos autores al referirse a patologías tarsiana en toros.En 1960 Groulade y Sorel estudiaron una patología tarsiana en toros Normando del centro de inseminacion de Laigle en Francia.La consideraron como una osteoartritis,clasificándola como artritis seca, pues en muy pocos casos se encontró aumento de líquido articular.En 1961,Burdenjuk,utilizó el término osteoartritis társica dandole gran importancia a la nutricion en su etiología.Van Pelt, en 1966 realizó estudios referidos a una patología articular que llamó enfermedad articular degenerativa,artritis degenerative,osteoartrosis,osteoartritis,etc..Este trabajo se realizó en razas Holstein,Shorthorn y Aberdeen Angus.En 1970 el mismo autor estudia la articulación del carpo y distales en Shorthorn,Aberdeen Angus,Holstein y Guernesey.En 1977, Weaver estudió las articulaciones femoro-tibio-rotuliana y coxo femoral aplicando el término osteoartritis.Sin embargo al contrario de la patología encontrada por Groulade, aquí había gran ácumulo líquido articular.Resulta evidente de esta reseña la escaza uniformidad de criterios que ha existido en cuanto a englobar la alteración que nos ocupa dentro de un cuadro anátomo-patólogico e histopatológico definido.Lo expresado aquí complementa el estudio realizado por el autor titulado Neopatología minimisante de la producción.

MATERIAL Y METODO.

Se realizan estudios macroscópicos articulares e histopatológicos de 25 muestras de capsulas articulares tarsianas seleccionadas de tarsos patólogicos de una muestra total de 800. Las mismas fueron extraídas con bisturi del fondo de saco articular externo e interno y consistieron en triángulos de 2 cm. de lado.Se recogieron además muestras de líquido articular de tarsos problema,estas se tomaron por punción y

aspiración.Se extrajeron cartílagos articulares con sus respectivos tejidos óseos subcondrales.Las muestras se fijaron en formol(10 %).Los cartilagos y huesos, a los efectos de ser sometidos a estudios histopatológicos fueron descalcificados.Las coloraciones realizadas fueron:
-Hematoxilina-Eosina.
-Orceina
-Sudan III,Hemalumbre de Delafield
-Sudan Black-B

RESULTADOS

Anatomía Patológica: se procedió a estudiar las lesiones visibles de la pieza articular tarsiana.Se encontraron lesiones a nivel de los cartílagos articulares.Algunos presentaban erosion de su superficie mostrando el tejido oseo subcondral epifisario.Otras lesiones más avanzadas en el tiempo poseían pequeñas formaciones de color blanco-amarillento,solidas,fijas,de escasos milímetros de diámetro que emergían del centro del cartílago erosionado(2,3).Se extraen estas pequeñas formaciones, se las descalcifican y colorean con Hematoxilina-Eosina.El estudio histopatológico nos muestra que se trata de tejido de regeneración que comienza a completar la zona erosionada(1,5).En la membrana sinovial de las articulaciones observadas se encuentran un número alto de"villis" los que aparecian sésiles y pedunculados.En mas de una oportunidad se observaron villis del tamaño de una nuez.Casos de hiperplasia e hipertrofia de villis sin evidencias de excesivo ecúmulo líquido articular, tanbién se observó.No todos los animales con hiperplasia vellosa poseian cartilagos articulares lesionados.En cuanto a los liquidos articulares recogidos de las articulaciones problema la mayoría eran de color amarillo pálido,existian flóculos en todas las muestras.

Histopatología: los resultados fueron,
--un 66 % de los cortes mostraban infiltrados a celulas mononucleares.
--del porcentaje anterior un 70 % poseía un grado leve de infiltración celular, un 10 % moderado y un 20 % intenso.
--no se observaron polimorfonucleares.
--proliferación vascular fue observada en 70 % de los cortes.
--infiltrado perivascular se encontró en el 6 % de las muestras.
--ecúmulo importante de tejido adiposo se encontró en la membrana sinovial de todos los cortes.
--la fibrosis local no es constante en todas las muestras.

Tabla 1. Capas celulares de membrana sinovial y grados.

Número de capas celulares de la membrana sinovial.	1 a 2	3 a 4	5	6 a 7	8 o más...
	0	1	2	3	4
	Grados.				

--de acuerdo a lo expresado en la Tabla 1 se clasificaron los cortes _ histopatológicos en:

- 46 % de los cortes presentaban grados 3 y 4.
- 54 % de los cortes presentaban grados 0,1 y 2.

CONCLUSIONES

Basándonos en los resultados expuestos podemos expresar que las cápsulas articulares tarsianas observadas no se apartan en forma importante de lo que es el tejido sinovial normal,teniendo en cuenta solamente el aspecto cualitativo.Sin embargo encontramos cambios importantes cuantitativamente hablando.Esto lo expresamos,pues en los cortes estudiados encontremos:

-aumento en el contenido adiposo subsinovial.
-mayor presencia de tejido de tipo fibroso que lo normal.
-incremento significativo en la cantidad de células sinoviales.No debido al incremento de estratos celulares, ya que hemos visto que se mantiene dentro de los límites normales de 1 a 5, sino por el aumento del número total de células.Esto debido a que el importante acúmulo adiposo local y la fibrosis proyectan los tejidos subsinoviales a la luz articular,a manera de"dedos de guante" lo que aumenta el total de células.Esta modificación cuantitativa es de vital importancia pues determina un incremento en la producción total de ácido hialurónico. Este ácido al ser volcado a la luz articular aumenta la osmolaridad local lo que desencadena un pasaje progresivo de líquidos desde los vasos sanguíneos hacia la luz.Así se dilata la articulación.
-no se encuentran evidencias de compresión vascular que pudiera explicar pasaje de líquidos hacia la luz por éstasis compresivo adiposo o fibroso.
-la presencia predominante de mononucleares a nivel capsular nos indica que se trata de un proceso crónico.
-la destrucción del cartílago articular la explicamos:
  --clínicamente por la amplitud exagerada del ángulo tibio-tarso-metatarsiano que recarga las presiones locales en el cartílago y por la alteración de las características básicas del líquido sinovial que nutre y lubrica el cartílago.
  --histológicamente por el incremento celular sinovial subsinovial lo que lleva a una mayor producción de enzimas cartílago destructivas.

Basándonos en la Anatomía Patológica y en la Histopatología decimos que esta patología es una artropatía degenerativa.Si a esto le agregamos los datos clínicos y estadísticos encontrados por el autor diremos que es una artropatía degenerativa secundaria tarsiana.Al llamarla secundaria nos referimos a factores etiológicos tales como conformación y obesidad.

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RESUMEN

La Anatomía Patológica e Histopatología de esta grave afección que acorta la vida reproductiva de machos Hereford,Aberdeen Angus,Shorthorn,Normando,Charolais,Fleckvieh.etc.es estudiada.Se examinan 25 muestras de capsulas articulares seleccionadas de tarsos patológicos de una muestra total de 800. Las mismas fueron extraídas con bisturí de los fondos de sacos articulares tarsianos.Se extraen muestras de líquidos articulares de los tarsos problema, los cartílagos articulares fueron descalcificados y sometidos a las tinciones correspondientes.Los resultados histopatológicos demuestran el porcentaje de infiltrado celular local así como hiperplasia de villis.Se clasifica la membrana sinovial patológica en distintos grados según la proliferación de las celulas sinoviales de superficie.La importancia de la fibrosis y del tejido adiposo local se discuten.La Anatomía Patológica local revela lesiones cartilaginosas y oseas así como alteraciones de la cápsula articular.Este estudio permite afirmar que se trata de una artropatía degenerativa tarsiana.Esta terminología concreta permite eliminar los términos poco exactos que se utilizan a nivel internacional para definir esta patología.Eliminemos el término sinovitis, pues en estos casos se trata de una patología degenerativa.

SUMMARY

Pathological Anatomy and Hystopathology of this serious pathology which shortness reproductive life in Hereford,Aberdeen Angus,Normando ,Fleckvieh and Charolais is studied. 25 samples of joint capsule are examined.The samples were selected from a lot of 800 pathological hocks.Samples of joint liquid were taken from pathological hocks.Joint cartilages were decalcified and subjected to usual tissue processing. Histopathological results show percentage of local cellular infiltrate and villis hyperplasia.Synovial membrane is classified in several de-

grees according to proliferation of surface synovial cells. Importence of fibrosis and local fatty tissue are discussed. Local pathological anatomy shows cartilage and bone injuries and alteration of the joint capsule. This study allows to afirm that is a degenerative hock arthropathology. The terminology eliminates innacurate words commonly used internationally for defining this problem. "Synovitis" must not be used, as in this cases, there is a degenerative problem.

RÉSUMÉ

Nous avons observé sur des taureaux Hereford, Aberdeen Angus, Shorthorn, Normando, Charolaise et Fleckvieh, une arthropathie du jarret. Nous étudierons successivement: 25 synoviales articulaires du jarret des bovines avec arthropathie, les lésions anatomiques, macroscopiques et microscopiques. À l'examen macroscopique de l'articulation nous avons observé de proliferations osseuses, avec des lésions cartilagineuses. L'éxamen histologique a montré : fibrose dans le tissu conjonctivo-vasculaire sous endothelial. La synovial est scléro-lipomateuse, riche en vaisseaux et avec infiltration lymphocytaires. Les caractères anatomiques, macroscopiques et microscopiques nous autorisent à affirmer qu'il s'agit d'une arthropathie dé enerative du jarret et non pas d'une arthrite.

NEOPATOLOGIA MINIMIZANTE DE LA PRODUCCION: ARTROPATIA DEGENERATIVA TARSIANA SECUNDARIA EN TOROS.

J.L. Queirolo. Bvd. Artigas 1941, Montevideo-Uruguay.

INTRODUCCION

Trabajos científicos realizados indican que 45 % de toros con diversas patologías articulares en el tren posterior poseían valores de capacidad de servicio bajos, de 0-1 y 2. Teniendo en cuenta el número de servicios en 40 minutos en prueba a corral se estima que ese porcentaje de toros con baja capacidad de servicio logran entre 4 % y 40 % de concepción al primer servicio y entre 4 % y 67 % de preñez después de 10 semanas(1), lo que habla por si solo de la importancia de evitar problemas articulares. En ciertas regiones de Uruguay así como en Argentina, Brasil y Paraguay la explotación ganadera es extensiva, esto hace que durante la temporada de servicio el toro camine muchos kilómetros en busca de hembras en celo. Al encontrarlas las monta promedialmente entre 6 a 8 veces(1). Luego de varios minutos de monta y desmonta el toro con problemas articulares resiente sus articulaciones y siente dolor. Es interesante señalar un trabajo realizado en el Uruguay(4) sobre 1621 toros de más de 1 año de edad de los que 24,3 % poseían patologías del tren posterior, siendo el 8,9 % de ellas a nivel articular, fundamentalmente en el tarso. La patologia que nos ocupa es hoy un problema importante de los toros de razas tales como Hereford, Aberdeen Angus, Pleckvieh, Charolais, Normando. La misma se ubica a nivel tarsiano. La consideramos como una alteración, básicamente, evolutivo-ponderal.

MATERIAL Y METODO

Se realizan estudios estadísticos sobre un total de 539 toros Hereford, en sus variedades mocha y astada. Se vinculan así parámetros tales como peso, altura al anca y edad con la presencia o ausencia de la patologia articular en estudio. La edad de los toros varió de los 9 meses a mas de 25 meses de vida. El rango de peso fue hasta los 1100 kilos. La altura llego 1,54 metros.

RESULTADOS

Evolución Zootécnica.

La raza Hereford, en sus variantes mocha y astada son mayoría dentro de las razas para producción cárnica en el Uruguay. Resulta pues simple entender como ciertos problemas infecciosos, no infecciosos y genéticos que afectan a los vacunos, tienen en nuestro país un campo propicio para castigar duramente a la raza. Llama la atención las modificaciones de frecuencia genética tan pronunciada que ha experimentado la raza Hereford, en un lapso breve de 10 años aquí. Estos cambios son prueba veraz de la enorme maleabilidad zootécnica de ella y es el fruto de la selección por parte de los criadores quienes en esfuerzo continuo seleccionaron a favor de ciertas características con material genético de dis-

tintas regiones del mundo.Algunas de estas caracteristicas fueron: ganancia de peso,altura al anca, largo del animal(3).Lamentablemente se dejó de lado consideraciones anatómicas y reproductivas de enorme importancia.El"tipo" de bovino para carne no era ya redituable económicamente por su alta conversión de nutrientes en grasa, grasa que ya no es aceptada por el consumidor quien desea más musculo en su compra.Así surgió el "new type" con modificaciones extremas,aunque no en todos los casos, en órganos de la reproducción y locomoción.Nos interesa fundamentalmente el cambio posicional del coxal, con elevación del tuber ischii con respecto al tuber coxae.Surgen así dos puntos de giro: a nivel sacro-ilíaco y coxo-femoral.Este último origina un ángulo tibio tarso metatarsiano anterior mas abierto, tipificandose a un toro así como"perdo de garrones",postlegged o straight legged.Se recargan entonces las presiones en cabeza de femur y tarso.

Aspectos clínicos.

Las principales caracteristicas clínicas de esta patología son:

-.- a la palpación notamos una deformacion articular fría, de contenido fluctuante.Así al presionar sobre algun fondo de saco articular ,el líquido se proyecta a otros.
-.- no hay calor ni edema.El dolor a la palpación-presión en oportunidades se presenta y en otras no.
-.- la claudicación inicial es leve o nula aunque a medida que la patología se acentúa el trastorno locomotor se hace más evidente,surgiendo una claudicación mixta de tal manera que el animal desplaza el miembro hacia adelante a los efectos de disminuir las presiones
-.- afecta indistintamente tarsos derechos-izquierdos o ambos simultaneamente.
-.- mayor incidencia en machos y animales adultos de razas carniceras.
-.- inicialmente el estado general del animal no se modifica.
-.- en las primeras etapas de desarrollo,esta patología, es de dificil visualización,debiendo entonces recurrirse a la palpación.
-.- hemos observado que la quietud y la estabulación del toro hacen que el problema se agrave.La disminución de peso y el ejercicio diario mejoran el pronóstico.
-.- en etapas finales de esta artropatía el toro afectado se niega a caminar permaneciendo echado gran parte del tiempo.

Las maniobras semiológicas de inspección y palpación pueden ser aplicadas aquí.Incluso la palpación-auscultación la hemos aplicado en mas de una oportunidad, escuchándose el ruido de pasaje líquido de una cavidad a otra a través de un orificio estrecho.Las maniobras citadas ayudan a diferenciar una alteracion inflamatoria de una artrosis y de una hidroartrosis.

Aspectos estadísticos.

De la observación de 900 toros de razas tales como Hereford,Aberdeen Angus,Normando,Pleckvieh y Charolais surgen modificaciones corporales evolutivas semejantes entre sí,Se considera para este estudio

una muestra de 539 toros de raza Hereford.Se procede a agrupar la población animal en 5 grupos etarios indicándose el porcentaje de animales afectados en cada grupo.

Tabla 1.Porcentaje de artropatía en cada grupo etario.

	meses				
	0-13	13-17	17-22	22-25	mas de 25
% de machos con artropatía.	1,9	9,5	6,9	6,9	15,5

Se realiza el ensayo de independencia conocido como "Tabla de contingencia",utilizando un nivel de significación del 5 %. Se busca la dependencia o no entre artropatía tarsiana y el hecho de pertenecer a los grupos etarios establecidos.Se estudian 154 toros en dos grupos : de menos de 13 meses y de más de 13 meses.Un estudio similar al anterior se practica con 528 toros agrupados en 5 categorías de edad,según Tabla 1.Tambien se estudia la dependencia entre artropatía/peso y analogamente entre artropatía/altura al anca.

Tabla 2.Tabla de contingencia.Valores de J y J_c(crítico) para artropatía/grupo etario,artropatía/peso y artropatía/altura al anca.

		J	J_c
artropatía/grupo etario (menos de 13 meses y más de 13 meses).	154 toros	8,19	3,84
artropatía/grupo etario (5 grupos según Tabla 1)	528 toros	15,1	9,49
artropatía/peso	539 toros	13,6	11,1
artropatía/altura anca	426 toros	5,17	9,49

CONCLUSIONES

Los datos de la Tabla 1 nos muestran la tendencia que tiene la artropatía en estudio a presentar mayor incidencia en animales mas viejos. El 9,5 % de enfermos en la categoría de 13 a 17 meses no guarda relación con el resto de los valores porcentuales ascendentes debido al menor número de animales integrantes de este grupo.

De los resultados estadísticos de la Tabla 2 decimos que:

-.-existe dependencia entre la presencia de artropatía tarsiana y el hecho de pertenecer a los grupos etarios establecidos.En términos generales entre artropatía y edad. Al incrementarse la edad la posibilidad de padecer este problema es mayor.

-.-existe dependencia estadística entre artropatía y peso vivo. Clinicamente se hace notorio que los animales de más peso son los mas afectados. Recordemos , de acuerdo a los estudios anátomo e histopatólogicos realizados por el autor, la importancia que el acúmulo adiposo local posee en la génesis de este problema. El sobre peso y el ángulo tibio-tarso-metatarsiano excesivamente abierto pueden o no presentarse simultáneamente en un mismo toro y cualquiera de ellos en forma aislada desencadenar la patología en estudio

-.-no existe dependencia estadística entre artropatía y altura al anca. Es decir que el hecho de ser más o menos alto no influye en la presencia o no de la enfermedad.

Diremos que esta afección que nos ocupa es, desde el punto de vista clínico, una Hidroartrosis tarsiana. Admitamos que como consecuencia de las demandas del mercado internacional de la carne el "tipo" de ganado para carne modificó su fenotipo. Consideramos que han existido criterios no balanceados en la cría de ganado "new type". Estan los productores que piensan que el animal más alto es el mejor, otros que lo es el más largo, otros el más pesado. Todos estos aspectos son importantes, pero el querer destacar uno de ellos, llevará a descuidar otras áreas relevantes tales como fertilidad, crecimiento, masculinidad, carcasa adecuada y belleza productiva. Este último término, basicamente, implica la armonía de partes a los efectos de lograr , sin problemas, el objetivo para lo cual fue creado en el tiempo de vida útil lo más amplio posible. Si un animal va a vivir 5 o 6 años en el rodeo necesariamente debe tener belleza productiva, máxime en sistemas extensivos. La artropatía estudia de separa al reproductor del concepto señalado de belleza.

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RESUMEN

Se destacan los principales aspectos clínicos, estadísticos y zootécnicos de esta grave patología que afecta tarsos de toros, disminuyendo su actividad reproductiva y por ende la producción global del predio. Las afecciones más frecuentes que afectan la actividad del toro en cría extensiva se discuten aquí, resaltando la importancia del tren posterior En manejos extensivos el toro debe de caminar varios kilómetros diarios en busca de hembras en celo, de aqui la importancia de esta alteración. Se discute la evolución zootécnica que ha experimentado el ganado para producción cárnica en el mundo. La misma ha aparejado cambios clínicos tales como el giro del coxal, lo que origina un tren posterior más recto y un ángulo tibio-tarso-metatarsiano más abierto. Los síntomas de la patología en estudio son ausencia de dolor, calor y edema. No hay claudicación inicial, pero sí en etapas posteriores. Lamentablemente estos signos, aparentemente benignos y leves, enmascaran una afección grave. Los daños capsulares y cartilaginosos se inician desde un principio y los mismos difícilmente involucionen. Finalmente el toro cae imposibilitado de caminar. El autor observa 900 toros de razas tales como: Hereford, Aberdeen Angus, Fleckvieh, Normando, Charolais. En todas ellas hay modificaciones evolutivas similares. Se estudia estadísticamente la presencia de artropatía en toros hasta los 30 meses, utilizando el método estadístico conocido como Tabla de contingencia (nivel de significación del 5 %) se encontró dependencia estadística entre la artropatía tarsiana y edad así como con el peso vivo. No se encontró dependencia estadística entre artropatía y altura al anca. La mala conformación del miembro posterior es tambien un factor de riesgo.

SUMMARY

Principal clinical, statistical and zootechnical aspects of this serious pathology affecting bulls' hocks are considered. This pathology decreases bulls reproductive activity and thus ranch overall production. In an extensive production system, the bull must walk several kilometres daily, in reach of females, the importance of this pathology is high. Zootechnical evolution of meat production is discussed. Such evolution has brought several clinical changes as turning of coxal, wich makes a straighter legged bull, and a wider hock angle. The clinical signs of this artropathology are: absence of pain, no initial lameness, but present in later stages, difficult visualization, absence of local heat. This sign although slight, mask a serious pathology. Injury in joint capsule and cartilage starts from the beggining and is unlikely that it goes back. Finally the bull rests with no posibility of walking. The author observes 900 bulls of breeds such as Hereford, Aberdeen Angus, Charolais, Fleckvieh, Normando. In all of them there are similar evolutionary changes. The presence of the artropathology is studied statistically for bulls up to 30 months. At significance level of 5 %, relations between artropathology/living weight and lumbosacral height were studied. The conclusions are that artropathology depends on age and living

weight, and is independent from lumbosacral height. Bad conformation of the hind leg is also a risk factor.

RÉSUMÉ

Nous étudierons successivement les caractères anatomiques, macroscopiques et zootechniques des taureaux avec arthropathie du jarret, âgés de 25 à 30 mois. L'angle tibio-metatarsien s'ouvre jusqu'à devenir rectiligne dans les membres postérieurs. Le taureau effectue difficilement un saut. Cet effort semble être pénible, puisque si l'animal réussit à se lever, cette douleur articulaire crée un reflèxe conditionné et l'animal lors d'ultérieuries saillies, n'ébauche même plus le saut et paraît indifférent. On rencontre toujours très abondent l'engorgement des culs - de sacs synoviaux, sans oedème périarticulaire et sans chaleur. Les possibilités des mouvements articulaires sont limitées. Nous avons observé 900 taureaux Hereford, Normande, Aberdeen Angus, Charolais et Fleckvieh, et nous retrouverons, dans tous eux, les mêmes caractères évolutifs. Nous étudierons, statisquement, les suivantes relations: arthropathie / poids vif (avec dépendance), arthropathie/ âge (avec dépendance), arthropathie/ hauteur (sans dépendance)

MEDULLOBLASTOMA IN CALF. ANATOMO-PATHOLOGICAL, HISTOLOGICAL, IMMUNO-HISTOCHEMICAL AND ELECTRON-MICROSCOPICAL FINDINGS.

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INTRODUCTION

In domestic animals medulloblastomas have been recorded chiefly in calves and puppies (1,2,3,4,5,6,9) as malignant neoplasm probably arisen from undifferenziated cells of the external granular layer (8).

Table 1 is a tabulation of studies on medulloblastomas, all related to very young animals, published since 1960. All cases occurred in the cerebellum with or without local extension to the fourth ventricle, meninges and adjacent brain. No intra and extracranial metastases are described.

Table 1 - Literature review of medulloblastoma in calves.

Authors	Year	Country	n°Cases	Age
McGavin M.D.	1961	Australia	1	2 months
Shaham S. & Coll.	1964	Israel	1	2 "
Fankhauser R. e Coll.	1968	Switzerland	3	4 weeks/6 months
Cowart E.C.	1969	U.S.A.	3	1/6 months
Jolly R.D. & Coll.	1969	U.S.A.	3	6 weeks/4 months
Ciarba A. & Coll.	1981	Costa Rica	1	5 weeks
Fankhauser R. & Coll.	1982	Switzerland	2 (twins)	2 months
Guarda F. & Coll.	1987	Italy	2	2-20 days

This paper records the anatomo-histopathological and electron-microscopical findings of a medulloblastoma in the brain right hemisphere with metastases in the right lung apical lobe. The himmunohistochemical researches carried out to show the specific cellular neoplastic structure are reported.

MATERIALS AND METHODS

Animal history

The tumor was observed in a 2-months old female chianina calf. Progressive ataxia and difficulty to feeding, tendence to move and fall to the right side, contraction of neck was recorded. Consaguinity in the fecondation was pointed out. The worsing of the nervous syndrome led an early slaughtering.

Anatomo-histopathological investigations

The calf was autopsied as soon after slaughtering. Samples from neoplasm in brain and in lungs, from adjacent cerebral and lung tissue were collected in 10% formol saline and processed by standard methods for histopathological studies. The sections were stained with routine methods

and by silver impregnation techniques. Different nervous structures were studied by the following specific stain methods: Gömori, Lynch, Luxol Fast Bleu, Holzer, Del Rio Hortega.

Immuno-histochemical procedures

The 3 microns thick sections were tested with a group of monoclonal and polyclonal antibodies with specific cross-reaction to animal antigens (Dako & Amercham). Two antibody series were tested:

a)- polyclonal anticyto-keratine
- monoclonal antidesmine
- anti-neurofilaments (160-200 Kd)
- anti-glial fibrillar acid protein (GFAP)

b)- anti-non specific enolase(NSE)
- anti-endothelial factor VIII
- anti-neurofilaments 68 Kd
- anti-histiocytic Mac387

The reactions for monoclonal antibodies were carried out by new fuxin stain for APAAP (Alkaline Phosphatase Anti-Alkaline Phosphatase) method; the reactions for polyclonal antibodies were carried out by carbazol for ABC (Avidin Biotin Complex) method.

Electron-Microscopy procedure

Several pieces of tumor were post-fixed in osmium tetroxide for 2 hours, dehydrated in acetone and embedded in epon. In order to get informations about the general structures of the tumor, semi-thin sections were cut and stained with bleu-methylene methods. The ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens 101 electron microscope.

RESULTS

Gross Pathology

A single, large, mandarin-like mass, about 4.5x3x2.5 cm in the cerebral right emisphere was found. The lesions caused distention of the third ventricle with relieved distortion of the brain stem, compression of the mesencephalon and of the cerebellum. A mild internal hydrocephalus was observed.

The external surface was lobulated with numerous nodular protuberances. External and cut surface was grey-pink. Rare hemorrhages were ascertained. The neoplastic mass was soft, more friable than normal brain. Also in the apical lobe of right lung a mass very similar in the gross form, colour and consistence was ascertained. It was about 7x6x3.5 cm and necrosis foci were more severe. A second white, bright, maize-like nodulus was observed in the middle lobe of right lung.

Histopathology

Microscopically the tumor appeared densely cellular and uniform, showing a closely packed cellularity; rare and small foci of necrosis and liquefaction occurred. The tumor cells usually appeared uniformly distributed but in some areas appeared arranged in clumps or drifts. The feature of pseudorosettes has been rarely observed.

The tumor cell usually had a rounded form with a piriform nucleus and a scant amount of clear cytoplasm. The cell sometimes showed an elongated feature with a carrot-shaped nucleus, placed within a rim of eosinophilic cytoplasm. The cell showed a high mitotic rate, with atypic polynuclear forms. Chromatine appeared mainly in discrete clumps. Piknosis and carry

orrhexis were common and widely distributed.

In neoplastic tissue a scant stroma and a small vascular network with design of lobular-like areas were ascertained.

The metastatic lung neoplastic cells showed a histologic appearance which closely looked like the brain mass. There was a greater proportion of elongated cells and more evidence of mitotic activity. The peripheric fibroblastic hyperplasia appeared more active.

Gömori and Lynch stain methods have revealed thin reticular network. Luxol Fast Bleu, Holzer and Del Rio Hortega stain methods have respectively ascertained the absence of myelin, astrocytes, microglia and oligodendroglia both in tumoral mass and in metastatic noduli.

Immuno-histochemistry

The first series of antibody reactions ascertained:
- clear presence of neurofilaments in the neoplastic cells of brain and lung masses;
- positivity for the vimentine in stromal tissue of brain tumor and metastatic noduli;
- high positivity for cytokeratin only in bronchiolar cells incarcerated in the neoplastic tissue of metastatic noduli;

The second series of antibody reactions ascertained:
- positivity for the vascular and histiocytic antigens, but non in neoplastic evolution;
- negativity for neurofilaments 68Kd and for neuroendocrin components.

Electron-microscopy

The main tumor cells were round, polygonal or elongated. The nuclei were usually highly lobulated and showed one or often two large nucleoli. Cells appeared in circle, forming a pseudo-rosetta, whose centre showed many cytoplasmic processes in cross sections. The perinuclear cytoplasm was usually a small rim containing only a few organelles.Microtubules were often found; the rough endoplasmic reticulum was highly developed and formed dilatate cisternae. The mitochondria were small and few. Fat droplets were sometimes found in circumscribed cytoplasmatic areas of a few cells.

A few very dark cells were observed. The nucleus showed a highly condensed finely granulated chromatin. The cytoplasm was only a small rim around the nucleus and contained numerous swollen mitochondria. These dark cells were more often seen within lung metastases.

The intracellular spaces of the tumor were considerably wider and often filled by a great amount of fibrils.

DISCUSSION

The age incidence, the localization and gross appearance of tumor mass but above all the histological, immuno-histochemical and electron-microscopical findings of our case were highly suggestive of medulloblastoma.

The precise origin of medulloblastomas is still uncertain but is believed to arise from undifferentiated neuroepithelial cells and particularly from primitive cells of the external granular layer (Rubistein, 1972).

Our immuno-histochemical researches confirm the neuronal characteristic of neoplastic cells and exclude the theories of mesenchimal or glial

histogenesis.

On the basis of electron-microscopical observations the nature of the tumor cells is more clearly showed in the cyto-architecture than in the cyto-morphology. Our investigations confirm a unique cytogenesis of medulloblastoma; the dark cells observed may be regarded as necrobiotic granular cells as is proved by their swollen mitochondria. Our histopathological, immuno-histochemical and electron-microscopical findings compared with those of other similar cerebral tumors (gangliocytomas, arachnoid sarcomas and retinoblastoma) suggest and verify the neuronal origin and the characteristic cellular structure of the observed medulloblastoma.

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SUMMARY

Medulloblastoma is a rare malignant tumor of calves normally localized in the cerebellum; rare metastases are reported. This report describes a medulloblastoma in the brain of a two months old chianina female calf, affected by nervous syndrome since the birth. A rounded, mandarin-like mass, about 4x3x2.5 cm was found in the right brain connected to the third ventricle. Two extracranial metastases were observed at the cranial lobe of the right lung. On histological examination the neoplasm showed a thick cellularity, scant stroma and few vessels. The tumoral cells had elongate or pearlike form with dense, dark, ovoid nucleus and scant cytoplasm. Immuno-histochemical researches by mono and polyclonal antibodies and electron-microscopical observations have contributed to characterize the nature of neoplastic growth in brain as well as in lung.

RESUME'

Le medulloblastome, rare tumeur des jeunes bovins, est localisé dans le cervelet et rarement forme metastases intra ou extracraniales. Notre publication décrit un medulloblastome observé dans un veau femelle chianina de deux mois atteint de symptomatologie nerveuse depuis naissance. Nous avons observé une masse arrondie, de forme et volume tel un mandarine dans l'hémisphère droit de l'éncephale attachée au troisiéme ventricule et presque pareil dans le lobe apical droit du poumon. Les recherches

histologiques montraient une remarquable cellularité, peu de stroma et de vascularisation. Le cellule cencéreuse, souvent rangée en filières, montrait une forme allongée, le noyau ovoidal, et peu de cytoplasme. Les recherches immuno-histochimiques avec les anticorps mono-policlonales et les observations électron-microscopiques ont permis de caracterisé le tumeur cérébral et les métastases pulmonaires.

RESUMEN

El meduloblastoma es un raro tumor maligno de los terneros, suele localizarse en el cerebelo y raramente se ha citato la presencia des metástasis intra o extracraniales. En este trabajo ilustramos un caso de meduloblastome en une ternera de raza chianina de dos meses de edad, que desde el nacimiento pedacia de un sindrome nervioso. En el hemisferio derecho del cerebro y connectado al tercer ventriculo, se notaba una masa redonda grande como una mandarina. Además se comprobó en el lobo apical del pulmón derecho la presencia de dos formaciones tumorales similes a la masa encontrada en el cerebro. El exámen histológico del tumor permitia apreciar una intensa densidad cellular, escaso estroma y pocos vasos. Las células neoplásticas presentaban el cuerpo a veces alargado otras veces piriforme con nucleo ovoideo de color cargado y poco citoplasma. Las probas inmuno-histoquimicas efectuadas empleando anticuerpos mono y policlonales y la observaciones ultra-estructurales han ayudado a caracterizar la naturaleza de la neoplasia primaria y aquellas de la localizaciones pulmonares.

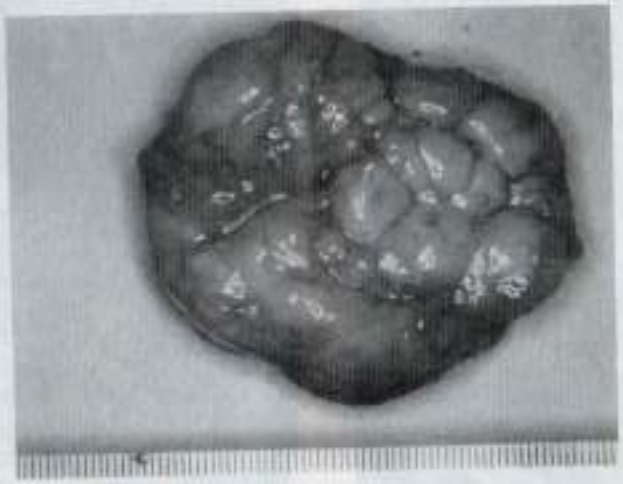

Fig.1- Appearance of the cerebral neoplastic mass.

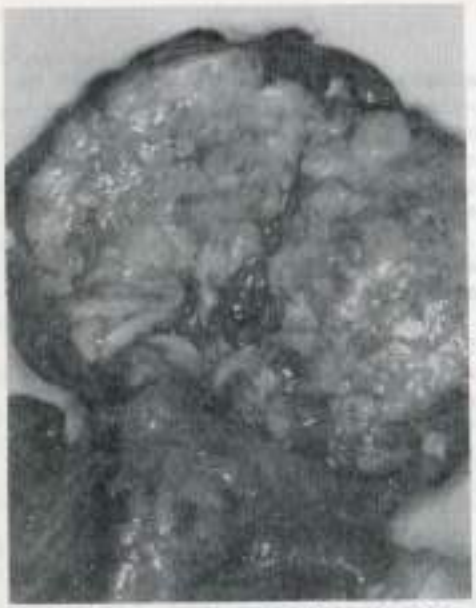

Fig.2- Cut surface of the metastases in the apical lobe of right lung.

Fig.3- H-E stain. Low magnification. Histological appearance "in mosaic" of neoplastic growth.

Fig.4- Carbazole stain.High magnification. Positivity of immunohistochemical ABC method to neural filaments (200 Kd)

Fig.5- Nuclear membrane invaginations, few mitochondria, and some droplets are observed in neoplastic cells.

VERTEBRAL EPIPHYSIOLYSIS IN NEWBORN CALVES

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INTRODUCTION

Dystocia related bone injuries in calves are well known. The injuries can affect various parts of the skeleton e.g. ribs (9), mandible (8), caput femoris (3) and columna (2,4,5,7). Vertebral epiphysiolysis (VE) in newborn calves has been diagnosed in several breeds including both beef and dairy breeds and in different parts of the world. Vertebral epiphysiolysis has previously been diagnosed in Danish Charolais cattle (6). Vertebral epiphysiolysis is most commonly located in the thoracolumbar junction (5,7), but has been found between the 11th thoracic vertebra and the fourth lumbar vertebra (7). A foetal osteoporotic condition in these calves has been proposed (1), but others have not been able to confirm this (2). A connection between traction force applied on the calf and vertebral epiphysiolysis has been proposed (7). The patogenesis could involve a genetic predisposition (5).

MATERIAL AND METHODS

From 65 farms on Zeeland, all stillborn calves and all dying during or shortly after the birth were autopsied. The total number of cows and pregnant heifers in the study was approximately 3400 with a distribution of 44% Danish Holstein-Friesian (DHF), 21% Red Danish Milkbreed (RDM), 22% Danish Jersey breed (DJ) and 13% beef cattle, mainly Hereford and Aberdeen Angus. Calves were sent for autopsy from June 1989 to February 1990. Autopsy was performed with special regard to VE. In all calves the columna was sawn through longitudinally. On calves with VE radiologic examination was made on the 5th right costa and on the columna from T10 to L5. X-ray examination was also made on a control group consisting of stillborn calves with similar autopsy findings except VE and without infections. Samples for histopathology were taken from the distal epiphysis of the 5th right costa and from the corpus of the 13th vertebra. Bone samples were fixed in 10% neutral buffered formalin, decalcified with EDTA, paraffin embedded, sliced and stained with hematoxylin-eosin.

From all calves samples from lung and spleen were taken together with content of the abomasum for bacteriology. Search for bovine virus diarrhoea virus was performed on samples from lung and spleen. Search for antibodies was made on liquid from the thoracic cavity.

RESULTS

A total number of 100 calves were autopsied and 6 cases of VE were detected. The breed distribution of the calves are shown in table 1. Breed, sex, weight and location of the VE are shown in table 2.

	DHF*	RDM**	DJ***	Beef cattle	Total
Number of stillborn calves	53	33	4	10	100
Number of stillborn uniparous calves	40	21	3	8	72
Calves with VE	4	2	0	0	6

Table 1. The distribution of stillborn calves in Danish cattle breeds with special regard to vertebral epiphysiolysis (VE). *: Danish Holstein-Friesian breed, **: Red Danish Milkbreed, ***: Danish Jersey breed

Calf	Breed	Sex	Weight (kg)	VE location
1	RDM**	female	35.6	T13, ant. epiphysis
2	DHF*	male	46.5	T13, post. epiphysis
3	DHF	male	41.3	T13, post. epiphysis
4	DHF	female	39.6	T12, post. epiphysis
5	DHF	female	38.0	T13, post. epiphysis
6	RDM	male	29.4	L2, ant. epiphysis

Table 2. Breed, sex, weight and location of the vertebral epiphysiolysis (VE) in 6 stillborn calves. *: Danish Holstein-Friesian breed, **: Red Danish Milkbreed, ant: anterior, post: posterior.

Almost all calves with VE were born during the summer months: calf 1 and 2 in June, 3 and 4 in July, calf 5 in August. Calf 6 was born in October. Calf 2,3 and 4 were from the same stock - the largest in the study with 340 cows. The calves 1, 5 and 6 were from stocks with 234, 75 and 28 cows, respectively. All calves except no. 6 were born by heifers. Calf 6 was born by a cow after 2nd pregnancy.

Manual traction force was applied in all cases of VE. Mechanical traction force was not used in any case. The heifer, which gave birth to calf 6, had a torsion of the uterus. A fracture of the columna was not suspected in any case neither by the breeder nor by the veterinarian.

The mean body weight (MBW) of the RDM and DHF calves with VE was 32.5 and 41.4 kg, respectively. In this study the MBW of the stillborn uniparous RDM calves was 40.8 kg and for DHF it was 37.8 kg. One of the RDM calves with VE (number 6) was born two weeks before estimated calving date.

Calf 3 and 4 were sired by the same bull. The other four calves were sired by different bulls.

Pathology

The significant major autopsy finding was a vertebral epiphysiolysis. In one case the lesion consisted of both epiphysiolysis and fracture of the corpus vertebra. This calf (case 6) also had a rupture of vena cava caudalis with excessive bleeding to the abdomen. In all cases bleeding was found in the ventral columna musculature of the thoracolumbar region and in the meninges of the spinal cord. Bleeding was never observed in the dorsal columna musculature.

Three calves had a total and three calves had a partial foetal pulmonal atelectasis. Subepicardial and subpleural bleedings were common, together with oedema and stasis in the head, neck and distal parts of the front legs. Histopathology made on the fractured vertebrae revealed acute bleeding with no repair processes.

Radiology

On laterolateral radiographs seperation of the involved epiphysis was observed. Only one of the cases showed combination of epiphysiolysis and fracture of the metaphysis (Salter-Harris type II).

The density of the ribs and compacta at all was lower than in the normal calves, and the compacta was thinner. Furthermore the contour of the compacta was less distinc than in the control calves.

Microbiology

The organs of the 6 calves with VE were either sterile, or an unspecifik coliform flora was found. Infection with bovine virus diarrhoea virus was not detected in any case of VE.

DISCUSSION

The incidence of VE among stillborn calves has not previously been determined. In the present study the incidence among stillborn calves of the large Danish dairy breeds (DHF and RDM) was 7.0%. RDM and DHF individuals are almost similar in size and weight, and the relative number of uniparous stillborn calves in the two breeds was equal in this study. No cases of VE were diagnosed in the Danish Jersey breed, and this breed had a significant lower number of stillborn calves than the other two dairy breeds (table 1). No cases of VE were found among the stillborn calves of the beef cattle breeds. This might be due to the relative small number of calves autopsied. Schuh and Killeen (7) found, that the majority of VE occurred in beef cattle (7). VE has previously been diagnosed in a Danish Charolais calf, which had a recumbent position and died 10 hours after birth (6).

In all cases of VE in this study, an anamnesis of dystocia and manual traction force was given. This is similar to the results found by Schuh and Killeen (7), and it seems that dystocia and traction force play a major role in the patogenesis of VE.

The distribution of VE in this study is similar to previous findings (4,7) with a majority at the thoracolumbar junction.

The radiographic findings might be indicative for an early stage of osteoporosis or osteodystrofia fibrosa. The histopathological studies made on bones from calves with VE and the controlgroup have not yet demonstrated pathological changes. Therefore further studies have to be carried out, before a propose for the pathogenisis is advanced.

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SUMMARY

A study was made on the incidence of vertebral epiphysiolysis (VE) in newborn calves. In a population of approximately 3400 cows consisting of 44% Danish Holstein-Friesians (DHF), 21% Red Danish Milkbreed (RDM), 22% Danish Jersey (DJ) and 13% beefcattle, all stillborn calves and all calves dying during or shortly after the birth were autopsied from June 1989 to February 1990. In DHF and RDM a total number of 6 calves with VE were diagnosed, and this reveals an incidence of 7.0% of all the stillborn uniparous calves. No cases were found in DJ and in beef cattle breeds. X-ray examination was made on bones from calves with VE and from a control group.

SOMMAIRE

On a fait des études sur l'occurence d'épiphysiolysis vertebralis (VE) chez les veaux nouveau-nés. Dans une population de l'ordre de 3400 vaches, comprenant 44% de holsteinois-frison danois (DHF), 21% de la race laitière rouge danoise (RDM), 22% Jersey danois (DJ) et 13% de bétail de boucherie, on a fait l'autopsie de tous les veaux mort-nés et tous les veaux mourants pendant ou peu de temps aprés la naissance de juin 1989 jusqu'à fevrier 1990. Chez les DHF et RDM on a diagnistiqué VE chez 6 veaux, correspondant à une frequence de 7.0% de tous les unipares veaux mort-nés. Chez les DJ et chez les races de boucherie aucun cas n'a été démontré. Radioscopie était effectuée sur les os des veaux avec VE et sur des veaux d'un group témoin.

ZUSAMMENFASSUNG

Eine Untersuchung über dei incidens vertebraler Epiphysiolysis (VE) bei neugeboreren Kälbern wurde durchgeführt. Von Juni 1989 bis Februar 1990 wurde von einer Population von ca. 3400 Kühen, 44% dänisches Holstein-Friesen Vieh (DHF), 21% rotes dänisches Milchvieh (RDM), 22% dänische Jersey (DJ) und 13% Fleischvieh, alle todtgeborenen Kälber und alle Kälber, die während oder kurz nach der Geburt starben, obduziert. Bei dem DHF und RDM wurden insgesamt 6 Fälle von VE diagnostiziert. Diese Anzahl entspricht einer Incidens von 7.0% aller einzelgeborenen Kälber, die todtgeboren wurden. Weder bei den DJ noch bei dem Fleischvieh wurde Fälle von VE registriert. Sowohl von Kälbern mit VE als auch von einer Kontrolgruppe wurden röntgenologische Untersuchungen der Knochen vorgenommen.

BLOOD GAS AND ACID-BASE STATUS OF MECONIUM-STAINED AND UNSTAINED NEWBORN CALVES DELIVERED BY CAESAREAN SECTION

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INTRODUCTION

At birth all fetuses are exposed to hypoxia and hypercapnia, owning to disturbances of the uteroplacental circulation by rupture of the fetal membranes and by the uterine contractions. With the decrease of oxygen supply, a reflectory shifting of the fetal circulation occurs. Blood supply to the vital organs (heart,brain) remains unchanged, while in the non-vital organs (muscles, skin, lungs, gastro-intestinal tract, etc.) vasoconstriction develops. In the non-vital organs anaerobic glycolysis starts with lactic acid accumulation in the blood and primary metabolic acidosis develops. In this stage, heart function is normal, because the oxygen supply still covers the needs of the heart and brain.

Disturbance of fetal oxygen supply, mainly its chronic form, leads in 85 - 95% of the human cases to meconium passage. The amount of meconium is proportional to the severity of hypoxia. First, a small amount of meconium escapes into the amniotic fluid, later meconium lighter in color is discharged from the higher parts of the intestinal tracts and the amniotic fluid becomes thick with meconium (1). The meconium-stained amniotic fluid is one of the classic signs of early intrauterine hypoxia which necessiates immediate initiation of parturition or depending on the fetal acid-base status and heart function, of intensive care when required with immediate surgical intervention (2).

In contrast, Duifhuizen and others (3) reported that the pH, blood gas tension and viability of newborn lambs were identical both in the meconium-stained and in the meconium-unstained lamb. According to another study the percentage of meconium-stained calves was 9.1% and only two of them were acidotic immediately after birth (4).

To obtain more information on the problem in the bovine veterinary practice, we investigated the blood gas and acid-base status of meconium-stained and unstained newborn calves delivered by Caesarean section during the first 60 min of life.

MATERIALS AND METHODS

A total of 91 complicated parturitions of Dutch Friesian and Dutch Red-and-White cows were studied at the Clinic of Veterinary Obstetrics in Utrecht, The Netherlands. Cows with dystocia had been transported to the Clinic directly by the owners or after a veterinary examination had taken place on the farm. Indication for a Caesarean section included was oversize fetus in anterior presentation (n=88), or in posterior presentation (n=3). Caesarean sections were begun and done as described previously (4).

Blood samples were taken from the jugular vein of the newborn calf immediately after birth and were repeated 10 and 60 min after birth. Blood sample processing and the methods used to measure and calculate pH, PCO2, PO2,base excess (BE) and actual bicarbonate (HCO3) have all

been described previously (5).Newborn calves were assigned to one of the following three groups according to their blood pH values at birth: Group 1: blood pH above 7.2 (normal); Group 2: blood pH 7.2 to 7.0 (slight to expressed acidosis); and Group 3: blood pH below 7.0 (severe acidosis).Similar groupings were used earlier (4). The statistical significance of differences between groups was assessed by analysis of variance and by paired-t-test.

RESULTS

The blood gas and the acid-base status of meconium-unstained newborn calves delivered by Caesarean section are given in Table 1. The acid-base parameters of the newborn calves at the different collection time were calculated according to grouping of the calves immediately after birth. The metabolic parameters (BE and HCO3) and PCO2 values of calves immediately post partum also showed a significant difference among the groups. At the same time no appreciable difference was found among the PO2 means of the different groups. Except for the PCO2 means, the significant differences among the groups 10 and 60 min after birth were similar to those observed immediately after birth. During the first ten min after birth a significant rise in PO2 values could be detected. At the same time the methabolic parameters showed a futher decrease in all groups while the PCO2 values were very similar to those measured immediately after birth. Between 10 and 60 min after birth the pH and the metabolic parameters showed a significant rise in all groups. Except for HCO3 in Group 3, these parameters reached or exceeded the initial ones 60 min after birth.

The distribution of the meconium-stained calves among the three groups was as follows: Group 1: n=7, Group 2: n=1, Group 3: n=3 (Figure 1). Three out of 11 meconium-stained calves (No.2,3 and 8) were extracted in posterior presentation. The BE values 60 min after birth reached the initial ones in two calves (No.2 and 4) or exceeded them in another two cases (No.1 and 3). The BE values in 4 out of 7 newborn calves (No.8 to 11) showed a futher decrease 60 min after birth while these values in the remaining calves were similar to those measured 10 min after birth (No.5 to 7).

DISCUSSION

At birth the degree of hypoxia and hypercapnia as well as the resulting respiratory and metabolic acidosis depends not only on the length of the period between complete separation from the dam's circulation and the start of spontaneous respiration, but also on the degree of disturbance of the diaplacental gas exchange during parturition. Except for placental and umbilical cord anomalies, the mode of obstetrical assistance (traction or Caesarean section) has been found to be a great influence on blood gas and acid-base status of newborn calves (5,6).

In our investigation, the respiratory and metabolic parameters of the meconium-unstained newborn calves showed significant differences among the groups immediately after birth. In contrast, no differences in the degree of hypoxia were found among the groups, since newborn calves are probably soon equalized by the the start of spontaneous respiration immediately after birth. Similar data have been reported by other researchers (7). During the first ten min after birth a significant shift of the acid-base balance towards metabolic acidosis

TABLE 1. Blood gases and acid-base balance of meconium-unstained newborn calves (Mean ± SD)

Parameter	Group 1 (pH > 7.2)	Group 2 (pH 7.2-7.0)	Group 3 (pH < 7.0)
	Immediately after birth		
n	44[a]	22[b]	14[c]
pH	7.259±0.032[bc]	7.123±0.058	6.884±0.123
PCO2 (kPa)	7.9 ±0.9[bc]	9.1 ±1.2	11.3 ±2.0
PO2 (kPa)	2.9 ±0.6	3.3 ±1.0	3.2 ±1.3
BE (mM/L)	-1.0 ±2.6[bc]	-7.8 ±3.2	-16.8 ±5.0
HCO3 (mM/L)	25.7 ±2.4[bc]	21.6 ±3.0	15.4 ±3.0
	10 min after birth		
pH	7.216±0.039[bc]	7.113±0.078	6.865±0.145
PCO2 (kPa)	8.5 ±0.8[c]	8.9 ±1.1[c]	10.6 ±1.8
PO2 (kPa)	4.0 ±0.6[c]	4.3 ±0.6[c]	5.1 ±0.7
BE (mM/L)	-2.4 ±3.4[bc]	-7.8 ±4.1	-19.2 ±5.2
HCO3 (mM/L)	24.9 ±2.8[bc]	20.7 ±3.5	13.3 ±2.7
	60 min after birth		
pH	7.268±0.040[bc]	7.188±0.068	6.939±0.136
PCO2 (kPa)	7.7 ±0.8[c]	8.0 ±0.8	8.6 ±0.9
PO2 (kPa)	3.9 ±0.5[c]	4.2 ±0.5[c]	5.1 ±1.0
BE (mM/L)	-0.8 ±3.0[bc]	-5.3 ±4.4	-16.8 ±5.9
HCO3 (mM/L)	25.6 ±3.4[bc]	22.1 ±3.9	13.9 ±3.8

a: bc $P < 0.05$
b: c $P < 0.05$

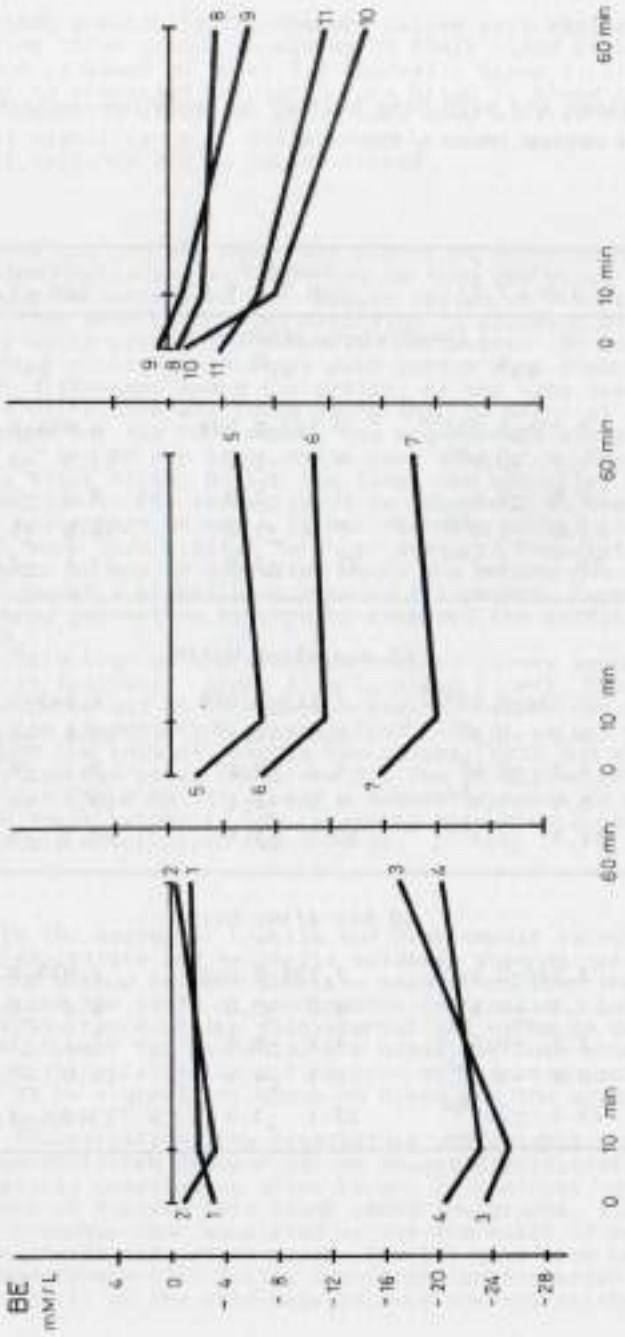

Figure 1 Base excess values of meconium-stained newborn calves / n= 11 /

could be detected by some researchers (8), while we, among others (9,10) observed no significant change. Ten min after birth spontaneous normalization of respiratory and metabolic acidosis takes place (11) but in our study the significant differences among the groups remained unchanged until 60 min after birth.

In contrast, only 36.4% (n=4) of the meconium-stained newborn calves showed the above mentioned acid-base changes during the first 60 min after birth. At the same time the other ones showed a very expressed metabolic change during the first 10 min of life which continued in 4 cases 60 min after birth as well, while in 3 ones the symptoms of a slow compensation of metabolic parameters could be detected. Respiratory values (PCO2 and PO2) changed similarly as in the meconium-unstained newborn calves.

Premature delivery causes a respiratory distress syndrome together with a progressive increase in the combined respiratory and metabolic acidosis within the first hour of life (12). In contrast, except one calf (No.3) the meconium-stained newborn calves showed an expressed metabolic change 10 min after birth and instead of spontaneous normalization of it 60 min after birth a futher decline in 4 cases (No. 8 to 11) could be detected. Delayed compensation of the metabolic acidosis in such cases needs futher investigation.

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SUMMARY

Blood gas and acid-base status of meconium-stained (n=11) and meconium-unstained newborn calves (n=80) delivered by Caesarean section was determined during the first hour of life. The newborn calves were assigned by venous blood pH value at birth to three groups as follows: Group 1: blood pH above 7.2; Group 2: blood pH 7.2 to 7.0; and Group 3: blood pH below 7.0.

During the first 10 min of life a non-significant shift of the acid-base balance toward metabolic acidosis could be detected in the meconium-unstained newborn calves. After 10 min spontaneous normalization of respiratory and metabolic acidosis took place but the significant differences among the groups remained unchanged until 60 min after birth. In contrast, except for 3 newborn calves the meconium-stained calves showed a delayed compensation of the metabolic acidosis (n=3) or a further decline of that (n=4) 60 min after birth.

RÉSUMÉ

On a déterminé la composition des gases sanguines et le status d'acid-basique des veaux, reçu par l'opération césarinne (dividé à deux group, c.-à-d. les individus, pollués /n=11/ et exempt du méconium /n=80/) pendant la 1er heure de la vie. Les veaux nouveau-nés ont été divides dans 3 groups selon les valeur du sang veneuse du temps de la naissance: group 1: pH au-dessus de 7.2; group 2: pH 7.2-7.0 et group 3: pH au-dessous de 7.0.

Pendant les premières 10 minutes après la naissance on a pu déterminé une décalage non significative vers d'une acidose métabolique chez les veaux, exempt de méconium. Après 10 minutes une normalisation spontanée s'est présentée concernant de l'acidose respiratorique et métabolique, mais la difference des groups s'est conservée à 60 min apres la naissance. Au contraire de celui, à l'exception de 3 veaux, les individues, pollués avec de méconium ont montré une compensation plus lente de l'acidose métabolique (n=3) ou même une diminution plus lente (n=4) que 60 minutes après la naissance.

ZUSAMMENFASSUNG

Das Blutgas und der Säure-Basen-Status von durch Meconium verschmutzten neugeborenen (n=11) und unverschmutzten neugeborenen Kälbern (n=80), die mit Kaiserschnitt zur Welt gebracht wurden, wurden in der ersten Lebensstunde bestimmt. Die neugeborenen Kälber wurden nach dem pH-Wert im venösen Blut bei der Geburt in drei Gruppen eingeteilt: Gruppe 1: pH-Wert über 7,2; Gruppe 2: pH-Wert 7,2-7,0; und die Gruppe 3: pH-Wert unter 7,0.

Während der ersten 10 Minuten nach der Geburt konnte eine geringgradige Verschiebung des Säure-Basen-Haushalts gegen die metabolische Azidose festgestellt werden. Die respiratorische und die metabolische Azidose normalisierten sich spontan bei den durch Meconium unverschmutzten Kälbern, aber die beträchtlichen Unterschiede zwischen den Gruppen blieben 60 Minuten nach der Geburt unverändert. Dagegen kompensierten die durch Meconium verschmutzten Kälber mit Ausnahme von 3 neugeborenen Kälbern die metabolische Azidose verzögert (n=3) oder 60 Minuten nach der Geburt nahm deiser Wert weiter ab (n=4).

A STUDY ON FETAL LUNG MATURITY IN DAIRY COWS

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INTRODUCTION

By employing thin-layer chromatography (TLC),Gluck et al. (1967) determined the lecithin / sphingomyelin (L/S) human amniotic fluid. As this L/S is indicative of fetal lung maturity, use of this ratio as a determination method for projecting fetal lung maturity has enjoyed extensive applications.

Further, incidence of respiratory distress syndrome (RDS), indicative as the main cause of fetal mortalities in human, is closely correlated to L/S of amniotic fluid during terminal pregnancy (Gluck et al., 1971).

Previous studies on volume plotted against expansion-contraction pressure by filling the lungs with physiological saline and air, indicate the existence of surfactant in lungs of cats (Neergaard, 1929). Moreover, attempts to measure influences on lung surfactant substances on the stabilization effects of alveoli (Pattle, 1955), and surface tension (Clements, 1957) have been reported. While fetal lung maturity time in rabbits and the relationship between surfactant and glucocorticoids (GC) in sheep fetuses (Liggins and Galeves, 1971) have been studied, no attempts have been made to investigate on fetal lung surfactant in cows.

Our present study determined surfactant,L.S values in amniotic fluid (AF), bronchoalveolar lavage fluid (BALF), lung tissue fluid (LTF) and amniotic cortisol (AFK) level in fetuses of dairy cows. Correlationship of fetal lung maturity, L/S and AFK levels have been clarified, and projection on fetal lung maturity was attempted.

MATERIALS AND METHODS

BALF, LTF and AF in fetuses of dairy cows.

After measuring crown-rump length, body weights of 137 fetuses from 118 Holstein-Friesian pregnant cows sacrificed at the Shibaura abattoir, Tokyo, AF was successfully extracted from 94/118 cows. Physiological saline (10 ml) was introduced into lungs and rinsed thoro ughly after having recorded the lung tissue weight. Saline in the lungs was collected, centrifuged and the supernatant was sampled as the BALF. To lung tissues from 57/118 cases, 5 ml physiological saline was added, homogenized and centrifuged. The supernatant was extracted as the LTF. The extracted AF, BALF and LTF were stored at -20 °C before assay.

Further, AF from 9 other pregnant cows was collected during parturition (secondary amniotic rupture). LTF from 12 cows and procedured similarly to fetal lung tissues described above.

Determination of lecithin (L) and sphingomyelin (S).

After the extraction oh total fat with Bligh-Dyer solution ($CHCl_3$: CH_3OH : H_2O = 1 : 2 : 0.8), AF (63/137 fetuses), BALF (60/137) and LTF (54/137) were dissolved in 100 ul chloroform, and from this solution 50 ul was spotted in a TLC. Using a mobile phase ($CHCl_3$: CH_3OH : CH_3COOH : H_2O = 50 : 25 : 8 : 4), the highly saturated solution was performed with 0.05 % Rodamin B-EtOH solution, and the L and S portions were extracted

(Bligh and Dyer, 1959).

On L-S separation, 0.5 ml of perchloric acid and 0.5 ml HNO_3 (65 %) were heated at 195 °C for 20 min according to Hoeflmayr-Fried method (Fukui and Kujo, 1973) with slight modifications. After cooling to room temperature, 5 ml distilled water and 0.5 ml ammonium molybdate were added to the solution, mixed well and agitated thoroughly with 0.5 ml of a reducing agent (hydroquinone 2g, acidified $NaHSO_4$ 7g, H_2O 100 ml) prior to centrifugation at 3,000 rpm from 10 min. The supernatant was measured with a spectrophotometer (Hitachi 100-10) at 660 nm wavelength.

Determination of amniotic cortisol (AFK) level.

Cortisol levels in the AF (63/137 fetuses) were determined using the procedure described by MAKINO (1973). The antiserum against cortisol-21-hemisuccinate-BSA was purchased from Teikoku Hormone Mfg. Co.(Tokyo,Japan). Cross-reactivity with steroids such as cortisone, aldosterone and corticosterone were 28.1, 2.9 and 2.9 %, respectively. Sensitivity of the assay was 10 pg/tube; intra- and inter-assay coefficients of variations were < 8 % and < 12 %, respectively.

Statistical analysis.

Variations of L/S of LTF, BALF and AF with respect to fetal age (FA) were verified and compared by converting the values the multiple regression equation. Moreover, correlationships on L, S values, L/S of AF, BALF and LTF to fetal weight, crown-rump length, lung tissue weight and AFK were verified.

All determined parameters were subjected to factor analysis (Sarle and Sall, 1982), and their gross correlationships were derived.

RESULTS

1.Changes of various parameters that accompanied fetal development with respect to time in dairy cows:

A standard plot of fetal age (FA) against fetal crown-rump length of cows was studied with track records on 54 Holstein-Friesian dairy cows, and verified with a multiple regression equation. Estimated FA (in days) was derived according to the multiple regression ($y=31.0099+3.72606x+0.01333x^2$), and used as the FA (Table 1-1). FA, estimated fetal age day (FAD), and the other parameters are indicated in Table 1-2 to 1-4.

(i) Relationships between L and S values and L/S of fetal LTF with respect to FA (Table 1-2).

In contrast to S value, L value registered an elevating tendency against FA increase. From FAD 150, the L/S began to increase, registering 2.29 ± 0.68 on FAD 210 - 240 and elevated further to 3.85 ± 1.59 on 240 - 270.

Table 1-1 Changes in fetal crown-rump length, body weight and lung tissue weight against fetal age.

FA	Estimated fetal age(days)	CRL(cm)	Fetal body weight(kg)	Lung tissue weight(g)
90~120	109.6± 4.9	23.9± 1.5	0.45± 0.05	15.6± 8.8
121~150	133.3± 10.1	31.9± 3.5	1.14± 0.28	40.3± 12.3
151~180	163.3± 8.9	41.3± 3.4	1.72± 0.61	130.1± 53.9
181~210	197.4± 8.8	55.9± 3.9	3.08± 1.13	221.6± 54.8
211~240	226.1± 7.2	69.9± 3.9	5.08± 1.62	457.2± 155.9
241~270	253.8± 7.8	87.0± 5.68	7.88± 1.68	733.6± 198.3

Parameters were taken at estimated fetal age (in days) derived from the standard plot of fetal crown-rump length (fetal age (in days) according to the multiple regression equation (see Fig 1).

Table 1-2 Changes in lecithin (L), sphingomyelin (S) values and L/S of LTF against fetal age.

FA	L-value	S-value	L/S
90~120	-	-	-
121~150	8.51± 0.2	0.72± 0.45	8.77±5.47
151~180	8.98± 0.4	0.84± 0.30	1.22±0.49
181~210	8.47± 0.4	1.11± 0.51	1.48±0.52
211~240	8.79± 1.1	1.34± 0.89	2.29±2.20
241~270	8.64± 1.8	1.80± 1.51	3.85±1.59

L, S values are expressed in mean ± standard deviation (M±S.D.) in μg/ml.

(ii) Relationships of L, S value and L/S with FA in BALF (Table 1-3).

In contrast to S value, L value of BALF elevated to a significant value ($p<0.001$) as FA increased. When compared on each month-age, there were significant differences verified on the month-ages, especially after FAD 210 - 240, L values indicated sudden increases. L/S ascended sharply to 1.82 ± 0.77 on FAD 220 - 230 and increased further to 2.99 ± 0.851 on FAD 240 - 270, accordingly.

(iii) Changes of L, S values and L/S in amniotic fluid against amniotic cortisol (AFK) level in bovine fetuses (Table 1-4).

L value of AF registered significant increases against the progress in pregnancy day (PD). However, when L value of secondary amniotic rupture on parturition was compared to that of AF, the former registered a value of 22.81 ± 23.85 mcg/ml, whereas the latter recorded 15.24 ± 5.80 mcg/ml on FAD 240 - 270, respectively. There were no significant differences between the 2 values. In contrast to L, S values indicated no changes during pregnancy and varied at a constant value as pregnancy progressed. Elevated L/S , accompanied with FA changes, were influenced by increasing changes in L values, registering 1.69 ± 0.52 on FAD 210 - 240 and to peak at 3.73 ± 0.44 on parturition.

AFK levels, though indicated slight changes before FAD 220, registered a sharp increase thereafter (Fig. 2).

2. Gross correlationships of various parameters that accompanied fetal development:

Growth correlationships of L, S values, L/S and AFK levels with regards to body weight, crouw-rump length, lung tissue weight, BALF, LTF and AF of fetuses were investigated. Lung weight increased with respect to fetal weight gains, indicating a close correlationship (r=0.946) with the linear regression equation of $y=0.779+0.032x$ AS regards to lung weight increases against fetal weight gain, a secondary regression equation of $y=-15.55+6.21x-4.46x^2$ was procured. L/S of BALF indicated a cross correlationship with crown-rump length (r=0.792), lung tissue weight (r =0.793) and AFK levels (r=0.547).

3. L, S values and L/S of adult bovine lung tissue registered 3.57 ± 1.10 mcg/ml, 0.95 ± 0.77 mcg/ml and 3.94 ± 1.20, respectively. When the L/S on FAD 210 was compared to that of adult bovine lung tissue, the former indicated a significantly low value with no significant difference in L/S registered on FAD 240, and thereafter.

4. Maturity term of lungs in relation to surfactant level:

To clarify the maturity term of fetal lung in dairy cows, changes in L/S and AFK level with respective to pregnancy progress were focused. The AFK-L/S index, with L/S taken as the nominator and AFK level considered as the denominator (AFK / L/S), was expressed for comparison purposes between the L/S and AFK level. This index was when plotted against FA (Fig. 3). The AFK-L/S index elevated gradually from pregnancy day (PD) 150 only to decline suddenly after reaching the peak value on FAD 220 - 230. This index represented the intersection point of plots in L/S increase against AFK level elevation. Further, L/S and AFK levels were expressed as $y=1.6109+0.3589x$ (r=0.755) and $y=0.907-0.1892x$ (r=-0.056) with the linear regression equation ($y=10.2+27.5x$), respectively, indicating a negative correlation before FAD 220 - 230. However, the same parameters indicated a positive correlationship thereafter. This may be closely related to significant changes in lung surfactant biosynthesis at the turning point of FAD 220 - 230 (Fig. 4,5).

Table 1-3 Changes in L, S values, L/S of BLFA against fetal age.

FA	L-value	S-value	L/S
90~120	5.9± 1.2	13.9± 6.82	0.73± 0.43
121~150	11.9± 7.9	16.7± 3.60	0.81± 0.32
151~180	19.9± 4.0	22.7± 5.30	0.89± 0.15
181~210	21.7± 16.1	23.4± 10.60	0.93± 0.89
211~140	43.2± 18.7	24.9± 4.45	1.82± 0.77
241~270	84.0± 22.7	29.5± 3.79	3.39± 0.85

L, S values are expressed in M± S.D. in μg/ml. Refer Table 1-2 for abbreviations

Table 1-4 Changes in L, S values, L/S and cortisol(FK) level of amniotic fluid against fetal age.

FA(months)	L-value	S-value	L/S-ratio	AFK(ng/ml)
90~120	6.38± 3.31	2.61± 1.40	0.12± 0.05	0.77± 0.39
120~150	3.57± 2.99	5.26± 1.87	0.46± 0.49	0.58± 0.37
150~180	2.71± 2.88	6.39± 10.3	0.55± 0.40	0.64± 0.15
180~210	6.48± 2.19	5.38± 1.61	1.24± 0.72	1.24± 0.72
210~140	7.22± 1.62	4.44± 0.86	1.69± 0.52	0.48± 0.39
240~270	15.54± 5.86	5.96± 1.33	2.71± 0.59	2.40± 1.19
Parturition	23.61± 3.88	6.26± 1.01	3.73± 0.44	–

L, S values are expressed in M±S.D. in μg/ml. Refer Table 1-2 for abbreviations. AFK levels were expressed in ng/ml

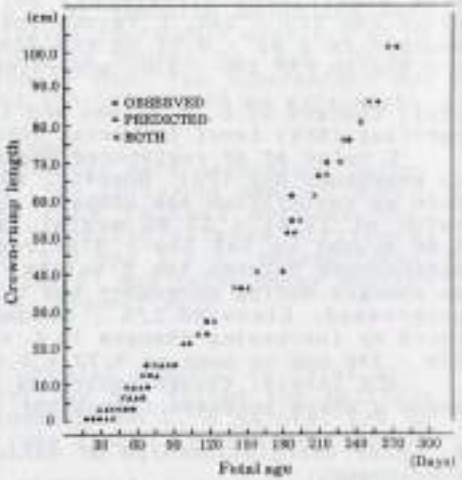

Fig. 1 Standard plot of fetal crown-rump length against track-recorded fetal age (in days), and the relationship between these 2 parameters was expressed as $Y=31.0099+3.7260x-0.0733x^2$. Estimated fetal age was therefore derived from this multiple regression equation.

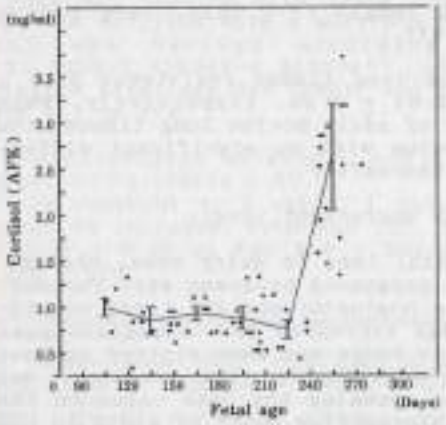

Fig. 2 Changes in AFK (cortisol) against estimated fetal age day (FAD) derived from the regression equation expressed in Fig. 1. After FAD 230, the AFK level increased sharply.

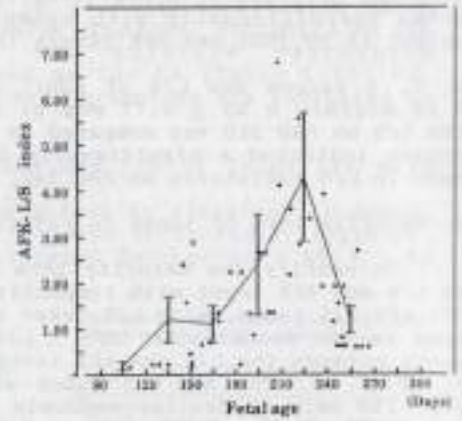

Fig. 3 Changes in the AFK-L/S index (where AFK-L/S index = (L/S) × AFK level) against estimated FAD. The peak value of this index appeared between FAD 220~230, and declined thereafter.

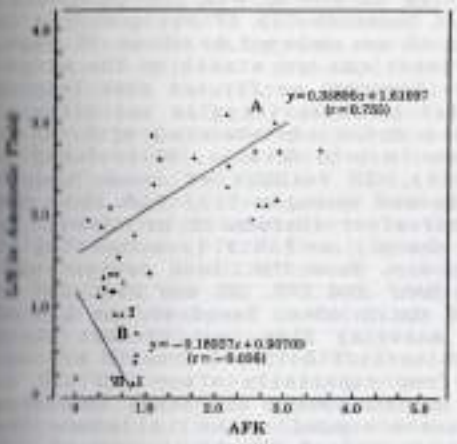

Fig. 4 Changes of L/S in amniotic fluid against amniotic cortisol (AFK) levels before (Plot A: $y=0.35886x+1.61097$; $r=0.755$) and after (Plot B: $y=-0.18927x+0.90709$; $r=-0.096$) the peak on FAD 120 of FK-L/S index (refer to Fig 3).

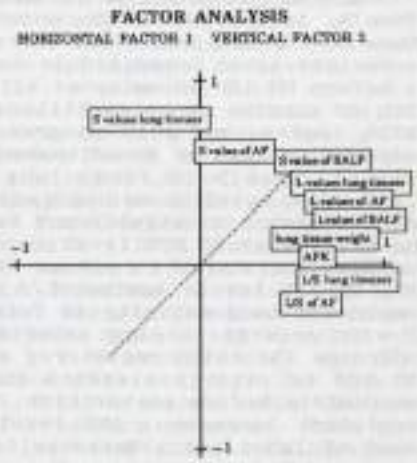

Fig. 5 Factor analysis on all determined parameters was performed to express their gross correlationships. Refer to text for abbreviations.

DISCUSSION

L value and L/S of fetal lung tissue increased from FAD 120 and thereafter when the tissue were still not matured. these value elevated sharply on parturition, especially after FAD 240. There was significant difference for the L/S on FAD 240 but that for lung tissue was not obtained. Based on such findings, lung maturity was thought to occur on FAD 240 and thereafter. However, the S value remained constant throughout the above-mentioned period. As regards to L values of BALF, the increases were about parallel to changes of levels in lung tissue.

Though L and S values of human AF varied at low values till pregnancy week (PW) 32, L value elevated sharply from PW 34, 35 and persisted at high values till PW 42 (Sato,1978).

L values of AF in dairy cows indicated significant increases from FAD 150. However, L values increased sharply from especially FAD 240 to parturition time.

It has been demonstrated that when L/S of fetal AF in human exceeded 2, lung maturity ensued (Gluck et al. 1972). However, L/S that registered a more 0.13 ± 0.55 around FAD 100, increased to 1.69 ± 0.50 on FAD 220 and 2.71 ± 0.59, on FAD 250, respectively. The value elevated further to 3.73 ± 0.44 on parturition in fetuses of dairy cows.

Increases of L value and L/S of AF in dairy cows manifested earlier than those seen in human, and both indicated similar tendency in increases after FAD 240 - 250.

When L value were expressed in a decreasing order, the 3 parameters ranked as follows; LTF > BALF > AF. The index indicated L/S between 2 parameters of LTF, BALF and AF st any one time to express a highly significant correlationship during the progress of pregnancy. Moreover, when expressed in the order of highest to lowest L/S ; LTF (3.85 ± 1.59) > BALF (2.99 ± 0.85) > AF (2.71 ± 0.59) on FAD 240 - 270 accordingly, indicating a rank-order similar to that of L values.

From the above findings, bovine fetal lung at FAD 240 synthesized

about the same surfactant level as adult dairy cows.

Surfactant produced in lungs shifted to the AF via the pulmonary alveoli. As such, when the onset of L/S increases in AF was compared to those of LTF and BALF, the increase in AF was delayed by about 15 days. Surfactant level contained in lungs fetuses can not stabilize the alveoli before PD 120 (Brumley et al, 1967). However, as fetuses administered with GC survive on parturition on PD 118 in sheep (Liggins and Gnieves, 1971), and as maturity progresses on a fetus administered with GC is compared to that of an untreated fetuses in twin fetuses, GC is found to related closely to fetal lung maturity. In fetuses of cows, though plasma AFK level dose not indicate marked changes till FAD 210, the level elevates to significant value thereafter (Harada et al, 1980). In the present study AFK level increased sharply on FAD 210, coinciding to readings registered in plasma FK. Moreover, from the close correlationship of AFK levels against L/S of AF, BALF and LTF, GC was produced to complement lung maturity in fetuses of dairy cows. Based on the L/S of AF with regards to lung maturity and maturity time, our present study indicates the ratio registered a low value at FAD 125, increased arround FAD 150 to clearly elevated sharply from especially after FAD 240 to immediately before parturition. Thus, in cases where AFK level indicates persistent increases, AFK level increases ensued so as to induce the onset of labor pain. Moreover, when the ratio of AFK level against L/S was investigated, the peak appeared around FAD 220. From this, the intersection point of L/S increase and the sharp elevation point of AFK was found to locate around FAD 220. Interestingly, this intersection point also corresponded to the lung maturity point. Moreover, when the L/S before and after FAD 220 were compared, a positive correlationship was obtained. From the above findings, while the site of gaseous exchange is located in the placenta, lung maturity time of fetuses in dairy cows occurs after AD 220 - 230, and is believed to prepare the fetus for respiratory challenge out side the mother's body.

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SUMMARY

The respective relationships on surfactant of amniotic fluid, lecithin/sphingomyelin (L/S) ratio of lung tissue (54 fetuses), bronchoalveolar lavage fluid (60 fetuses), amniotic cortisol (63 cases) of 137 fetuses from 118 dairy cows were studied, and their influences on fetal lung maturity were evaluated.Lecithin and sphingomyelin levels were measured with thin-layer chromatography (TLC) whereas amniotic cortisol was evaluated with a radioimmunoassay method. Fetal lung tissue weight increased proportionally with respect to corresponding body weight gain (62/137 fetuses), and there was correlationship found in the corresponding increases (y=0.0321x+0.7790, r=0.946). L value L/S of lung tissues increased sharply from after the fetal age day 240 until parturition. Positive correlationship between the L/S of bronchoalveolar lavage fluid (60/137 fetuses) and fetal crown-rump length (137/137 cases), lung tissue weight (119/137 cases) and amniotic cortisol (63/137 cases) were r=0.792, r=0.793 and r=0.547, respectively. Moreover, L value and L/S elevated with respect to fetal age increases. Especially after the fetal age day 220-270, the L/S increase was marked, registering a value of 1.82 ± 0.77 and elevated further to 2.99 ± 0.85 around fetal age day 240 - 270. In a decreasing order of values obtained, L/S for the 3 parameters ranked as follows; lung tissue fluid>bronchoalveolar lavage fluid>amniotic fluid, during the course of pregnancy progress. From the above findings, when the point of intersection between amniotic cortisol level and L/S of amniotic fluid was considered as the time of fetal lung maturity, the L/S before fetal age day 220 was taken as positively correlated.

DETECÇÃO DE TOXINAS TERMOESTÁVEIS EM DISTINTAS AMOSTRAS DE MORAXELLA BOVIS.

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INTRODUÇÃO

Ceratoconjuntivite infecciosa bovina (CIB) é uma enfermidade contagiosa, que tem como agente etiológico MORAXELLA BOVIS pertencente a Família Neissereaceae, sendo Gram negativa, oxidase positiva, não fermenta aos carboidratos, não reduz nitrato a nitrito e morfologicamente apresenta-se como diplobacilo ou diplococo, geralmente aos pares (Lennette et al.,1974; Fraser & Gilmour, 1979).

Essa bactéria ainda sintetiza fímbrias, exotoxinas e endotoxinas responsáveis pela adesão e alterações oculares (Araújo, 1986; Franco, 1987). Tem sido detectada a produção de colagenase, desoxirribonuclease e dermonecrotoxina por várias cepas, (Franco, 1987). Esta enfermidade se caracteriza clinicamente, por apresentar uma evolução rápida, observando-se inicialmente lágrima, seguindo-se opacidade, congestão vascular, ruptura da córnea e cegueira uni ou bi-lateral (Blood et al, 1983).

O processo infeccioso envolve seguramente uma série de outros fatores intrínsecos do agente agressor, tais como, síntese de enzimas hidrolíticas e outras toxinas responsáveis por danos celulares locais que comprometem a função ocular. Notadamente o mecanismo deste patógeno não está devidanebte elucidado.

Araújo (1989), através de estudos de virulência de M. bovis, verificou a endotoxidade do lipopolissacarídeo, demonstrando claramente o elevado potencial endotóxico desta estrutura de superfície. Ainda, observou que 57,14% de amostras estudadas, eram ST positivas, achados inéditos, nesta bactéria, sugerindo um novo fator de patogenicidade. O presente trabalho teve por objetivo isolar novas amostras de M.bovis bem como, detectar a presença de toxina termoestável.

MATERIAL E MÉTODOS

Isolamentos de M. bovis diretamente do saco conjuntival de bovinos com infecção e quadro clínico recente de CIB. Utilização de amostras isoladas e mantidas em laboratório caracterizadas bioquimicamente (Fraser & Gilmour, 1979).

A demonstração da toxina termoestável foi de acordo com o teste em camundongos recém-nascidos (Dean et al., 1972).

Avaliou-se a pureza da toxina termoestável nos materiais obtidos pelo método de Kilsten & Fystro (1982).

Inoculando-se intraperitoneal, camundongos adultos (brancos, suíços) avaliando-se o potencial tóxico (Araújo, 1986).

RESULTADOS

De um total de 17 coletas de material do saco conjuntival de bovinos com CIB, 14 isolamentos foram obtidos caracterizando-se bioquimicamente, mantendo-se liofilizadas, essas amostras somando-se mais três padrões que estavam mantidas em laboratório, foram avaliadas quanto a presença de toxina termoestável obtendo-se somente 2 positivas (ST+); em todos os materiais, através do "Limulust Test", verificou-se ausência de endotoxina;em camundongos adultos, através da inoculação de 0,5 ml foi possível observar alteração fisiológica ficando as fezes amolecidas geralmente as 12-16 horas da aplicação. Os materiais acima referidos, são sobrenadantes obtidos pelo método de Dean et al., (1972).

DISCUSSÃO

Araújo (1986) encontrou 57,14% das amostras isoladas de M.bovis apresentavam toxina termoestável, no presente trabalho apenas 2 das 15 amostras foram positivas, indicando um neor percentual ST+. Destaca-se no entanto, que M. bovis, algumas amostras são capazes de produzir uma toxina do tipo ST, sendo estas, detectadas pelo método de DEAN. Pelo que ficou demonstrado, no teste intraperitoneal em camundongos adultos, estas toxinas assemelham-se as produzidas por E. coli e Y. enterocolíticas (Nunes & Nicciardi, 1981).

A capacidade de produção de toxinas ST, somam-se a outras endo e exotoxinas descritas por Pugh et al., (1973); Franco & Gil Turnes (1984) e Araújo (1988).

Os trabalhos de Konpal e Deibel (1975) e Trabulsi (1981) demonstraram a ação da toxina termoestável de Salmonella enteritidis e E. coli com leituras de duas horas e trinta minutos e quatro horas, respectivamente. Segundo Nunes e Nicciardi (1981), o melhor tempo para a leitura da ação de ST de Y. enterocolítica foi após duas horas de inoculação. Para M. bovis, indicam um tempo ótimo de leitura nas 4 horas para o diagnóstico (Tabela 1).

O efeito da ST de enteropatógenos é ativar o Guanilato ciclase aumentando a concentração intracelular de Guanina 3', 5' monofosfato a nível de mucosa, é sugestivo que o ST de M. bovis atue a nível da mucosa da conjuntiva ocular facilitando a ação de outros fatores de patogenicidade.Faz se necessário isolar, concentrar e definir o efeito biológico da ST de M. bovis na córnea bovina buscando-se elucidar a importância deste fator no mecanismo de virulência deste patógeno primário da CIB.

TABELA 1. Teste de toxinas das amostras de M. bovis (Dean)

Amostras	Leitura 2 h	Leitura 4 h
1	0,071	0,081
2	0,061	0,064
3	0,069	0,056
4	0,070	0,082
5	0,065	0,065
6	0,060	0,059
7	0,070	0,058
8	0,063	0,066
9	0,065	0,061
10	0,058	0,059
11	0,061	0,065
12	0,063	0,062
13	0,073	0,055
14	0,071	0,057
15	0,062	0,058
16	0,059	0,068
17	0,064	0,060

Y. enterocolítica em 2:30 hs - 0,088
E. coli em 2:30 hs - 0,120
Controle do meio 0,062

Pela diferença de peso intestino/carcaça realizou-se os cálculos, onde valores superiores a 0,080 desta relação, foi considerado postivo (Koupal & Deibel, 1975).

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RESUMO

Dezessete amostras de Moraxella bovis isoladas de vários surtos de Ceratoconjuntivite infecciosa bovina, foram sumetidas ao teste do camundongo recém-nascido para detectar atividade similar a da toxina ST de E. coli. Duas amostras foram positivas. As toxinas produziram diarréia discreta em camundongos adultos inoculados intraperitonealmente.

SUMMARY

Seventeen Moraxella bovis isolates recovered from several outbreaks of infectious Bovine Keratoconjunctivitis were subjected to the infant mouse test to detect E. coli ST toxin activity. Two isolates were positive. The toxins produced mild diarrheae when by the intraperitoneal route in adult mice.

RESUMEN

Diecisiete cepas de Moraxella bovis aisladas de varios brote de queratoconjuntivitis infecciosa bovina fueran sometidas al test de ratón lactante para detectar actividad similar a la de la toxina ST de E. coli dos cepas fueran positivias. Las toxinas produjeram diarrea discreta en ratchas adultas inoculadas intraperitonealmente.

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ESSAI D'APPRECIATION DE L'EFFICACITE IN VITRO D'UNE ASSOCIATION DE TROIS ANTIBIOTIQUES (MASTIJET FORT ND) PAR L'ETUDE CINETIQUE DE L'ACTIVITE BACTERICIDE

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INTRODUCTION

L'efficacité d'une spécialité destinée à une administration intramammaire doit être démontrée au cours d'essais cliniques (4).

Cependant, le choix des antibiotiques inclus dans la spécialité doit être préablement justifié par la démonstration de leur activité sur la plupart des bactéries pathogènes majeures dans le développement des mammites. Une telle étude est généralement réalisée en mesurant les CMI de chaque antibiotique vis-à-vis de chaque bactérie.

Quand la préparation contient plus d'un antibiotique, l'intérêt de l'association peut être démontré par la mise en évidence d'une synergie entre les antibiotiques ou par l'élargissement du spectre d'activité de la spécialité.

La détermination des interactions entre antibiotiques est généralement établie par la méthode de titrage en échiquier, avec détermination de l'index des fractions de concentrations inhibitrices (Index F.I.C.) (1-3).

Cette technique étant mal adaptée à l'étude des associations comportant plus de deux antibiotiques (2) nous avons donc utilisé une autre méthode d'analyse fondée sur la cinétique de bactéricidie. Nous présentons ici, les premiers résultats obtenus avec cette méthode dans l'évaluation d'une association comportant trois antibiotiques : la néomycine, la bacitracine et la tétracycline, qui sont les principes actifs d'une préparation intra-mammaire commercialisée en France ("Mastijet Fort" ND).

MATERIAL ET METHODE

1. Bacteries

Trois souches bactériennes d'épreuve appartenant aux principales espèces bactériennes responsables des mammites bovines (Streptococcus uberis, Staphylococcus aureus et Escherichia coli) ont été tirées au sort dans un lot de souches isolées à partir de cas cliniques. Ces souches étaient conservées par congélation à -80°C.

2. Antibiotiques

La technique a été réalisée avec les principes actifs suivants : Néomycine (s.f. de sulfate) titrant 680 µg/mg; Bacitracine titrant 75 U/ml; Tétracycline (s.f. de chlorhydrate titrant 983 µg/mg.

3. Détermination des vitesses de bactéricidie

Les CMI de ces antibiotiques vis-à-vis des trois souches bactériennes sont préalablement déterminées par la technique des dilutions sériées en milieu liquide.

L'étude cinétique de bactéricidie est basée sur l'estimation de la croissance bactérienne en fonction du temps.

Les antibiotiques, seuls ou en association par 2 ou par 3, sont placés en contact avec l'inoculum bactérien, en milieu liquide Mueller Hinton. Les tubes contiennent le ou les antibiotiques (aucun antibiotique dans les tubes témoins) en quantités équivalant à des fractions de CMI (½ ou ¼). La concentration bactérienne initiale est de l'ordre de 10^5 UFC/ml. La vitesse de bactéricidie d'un antibiotique est estimée par le comptage bactérien à 1, 3, 6 et 24 heures après le début de l'incubation à 37°C.

Dès la mise en contact du germe de l'antibiotique (T0), puis chacun des temps T1, T3, T6 et T24 h., des échantillons sont prélevés de chaque tube. Des dilutions de raison 10 sont ensuite réalisées dans les cupules d'une plaque de microtitrage. Les différentes dilutions sont ensemencées sur un milieu gélosé (gélose Columbia) à l'aide d'un inoculateur manuel, équipé de 96 aiguilles qui libèrent une quantité de 1 µl de l'échantillon par cupule. Compte tenu de la technique utilisée, nous ne pouvons apprécier une concentration bactérienne inférieure à 10^2 UFC/ml. Pour l'interprétation, nous considérons qu'une activité bactéricide complète est obtenue quand le nombre de bactéries survivantes après 24 heures est inferieure ou égale à 0,01 p. cent de la concentration initiale. De plus, une association est considérée comme synergique quand le pourcentage de survie est inférieur à celui obtenu avec l'antibiotique seul le plus actif (1). Nous considérons cependant cette différence significative seulement su un écart de 2 log. est observé entre les deux valeurs.

RESULTATS

Les CMI de chaque antibiotique vis-à-vis de ces souches pathogènes sont présentées dans le Tableau. Elles sont exprimées en mg/l.

Tableau I :
CMI des trois antibiotiques vis-à-vis des souches étudiées en (mg/l)

Antibiotique	E. coli 1700	Staph. aureus 660	Strept. uberis 1408
Néomycine	16	4	32
Bacitracine	>128	8	1
Tétracycline	4	0,25	0,125

Les cinétiques de bactéricidie, mesurées en présence de chaque antibiotique pris isolément, et de leur association par deux ou par trois, sont représentées dans les figures 1, 2 et 3. Notons que la tétracycline est bactériostatique; néomycine et bacitracine sont bactéricides, mais cet effet n'apparait pas forcément aux concentrations étudiées (cas en particulier de la bacitracine).

Pour Escherichia coli, nous avons testé seulement l'effet de l'association néomycine-tétracycline (figures 1a et 1b), la bacitracine étant inactive aux concentrations usuelles. Aux concentrations étudiées (½ et ¼ de CMI), la néomycine excerce une activité bactéricide précoce, mais transitoire n'empêchant pas la recroissance bactérienne, vers la 6ème heure. L'adjonction de la tétracycline, bien que ralentissant l'activité bactéricide de la néomycine, la prolonge dans le temps en empêchant cette croissance.

L'action des trois antibiotiques sur Staphyloccus aureus (figure 2a, 2b et 2c) est présentée ici à une concentration équivalent à ½ CMI. Comme pour Escherichia coli, la néomycine exerce une activité bactéricide précoce et transitoire, suivie d'une recroissance bactérienne après 6 heures. L'association néomycine-bacitracine permet d'obtenir une bactéricidie précoce (visible à la 3ème heure) et prolongée dans le temps; cet effet n'est pas modifié par l'adjonction de la tétracycline.

Ces antibiotiques sont également testés à ½ CMI sur Streptococcus uberis (figures 3a, 3b, 3c). Notons qu'avec ce germe, la néomycine exerce une activité bactéricide plus progressive, sans recroissance bactérienne tardive comme nous l'avons observé avec les germes précédents. Mais là encore, l'association néomycine-bacitracine permet obtenir un effet bactéricide précoce (visible à la 6ème heure). cet effet n'est pas modifié par l'adjonction de la tétracycline.

DISCUSSION

Malgré les limites techniques de la méthode et le faible nombre de souches étudiées, les résultats obtenus donnent une image représentative de l'action des trois antibiotiques :

- Aux concentrations étudiées (½ ou ¼ de CMI), aucun effet antagoniste n'est observé, même si dans l'association tétracycline-néomycine il semble que l'action bactéricide de la néomycine puisse être retardée par la présence de tétracycline.
- L'effet respectif des antibiotiques varie avec le germe : Pour Streptococcus uberis et Staphylococcus aureus, le couple néomycine-bacitracine, nettement synergique, représente l'association dominante; l'effet bactéricide est obtenue au bout de trois à six heures de contact et la présence de tétracycline n'interfere pas sur cette activité.

Pour Escherichia coli, c'est le couple néomycine-tétracycline qui intervient, avec un effet synergique net permettant aux concentrations étudiées un effet bactéricide dès la 6ème heure. La bacitracine n'est pas active sur ce germe aux concentrations usuelles et nous avons contrôlé que sa présence ne modifie pas l'activité des deux autres antibiotiques.

La cinétique de bactéricidie est largement utilisée en médecine humaine et ses avantages et inconvénients sont bien connus (1-3). Compte tenu de ses performances, elle nous semblait la meilleure approche pour analyser les effets respectifs de chaque antibiotique au sein d'une association multiple comme celle que nous avons étudiée ici. Dans ce sens, les résultats obtenus nous apparaissent encourageants.

CONCLUSION

L'étude cinétique de bactéricidie peut être une méthode de choix pour apprécier l'efficacité in vitro d'une association antibiotique. Appliquée à l'étude de la triple association néomycine-tétracycline-bacitracine (Mastijet Fort ND), elle nous a permis de mettre en évidence deux associations synergiques; néomycine-bacitracine vis-à-vis de Streptococcus uberis et Staphylococcus aureus et néomycine-tétracycline vis-à-vis d'Escherichia coli, et l'absence d'antagonisme en présence du troisième antibiotique. Dans cet exemple, la synergie se traduit en outre par une amélioration de l'activité bactéricide. Au vu de ces résultats, la triple association antibiotique de Mastijet Fort nous parait disposer d'atouts majeurs pour une bonne efficacité clinique.

Des essais cliniques conduits sur le terrain avec cette triple association antibiotique ont permis de vérifier le bien fondé de cette approche pharmacodynamique, notamment vis-à-vis des souches de Staphylococcus aureus.

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RESUME

Les interactions susceptibles de se manifester au sein d'une triple association néomycine, tétracycline et bacitracine ont été recherchées in vitro par étude de la cinétique de bactéricidie. Cette recherche s'inscrit dans le cadre de l'évaluation pharmacodynamique d'une spécialité intramammaire destinée au traitement des mammites bovines cliniques.

Le taux de survie de trois souches de bactéries pathogènes majeures (Streptococcus uberis, Staphylococcus aureus, Escherichia coli) a été mesuré après 1, 3, 6 et 24 heures d'incubation en présence d'1, 2 ou 3 antibiotiques à des concentrations correspondant à la moitié ou au quart de leurs CMI respectives. L'association néomycine-bactracine présente un effet synergique vis-à-vis des souches de Streptococcus uberis et Staphylococcus aureus et l'association néomycine-tétracycline vis-à-vis d'Escherichia coli. Le troisième antibiotique ne perturbe pas ou même renforce l'activité des deux autres.

SUMMARY

The interactions of a triple combination containing neomycin, tetracycline and bacitracin have been studied by determining in vitro kinetics of the bactericidal activity. The study was part of the pharmacodynamic evaluation of an intramammary product for the treatment of bovine mastitis. The survival rate of 3 strains of pathogens (Streptococcus uberis, Staphylococcus aureus and Escherichia coli) was measured after 1, 3, 6 and 24 hours of incubation in the presence of 1, 2 or 3 antibiotics at MIC or lower levels.

The combination neomycin-bacitracin shows strong synergic activity against Streptococcus uberis and Staphylocuccus aureus when compared to each antibiotic alone. The combination neomycin-tetracyclin shows a similar activity againt E. coli. The third antibiotic does not influence the activity of two other ones.

Figure 1 : Escherichia coli 1700

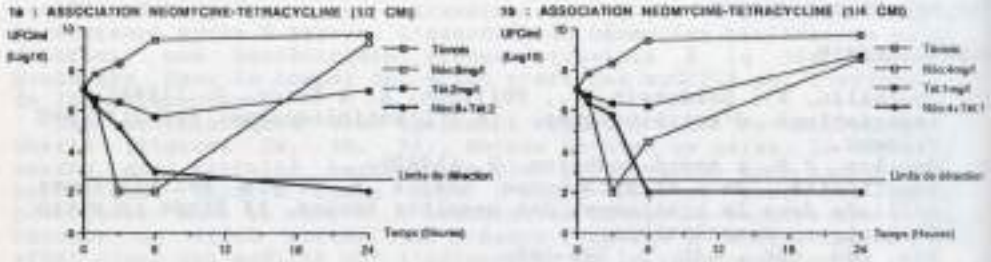


Figure 2 : Staphylococcus aureus 660

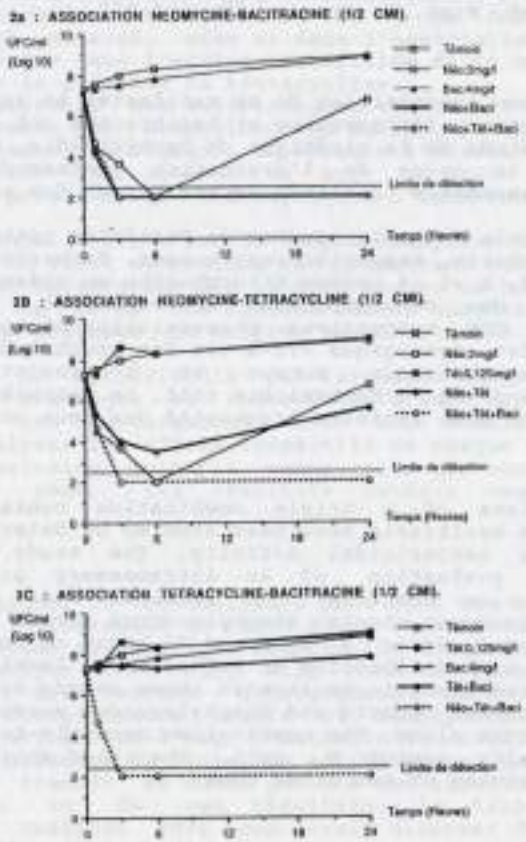
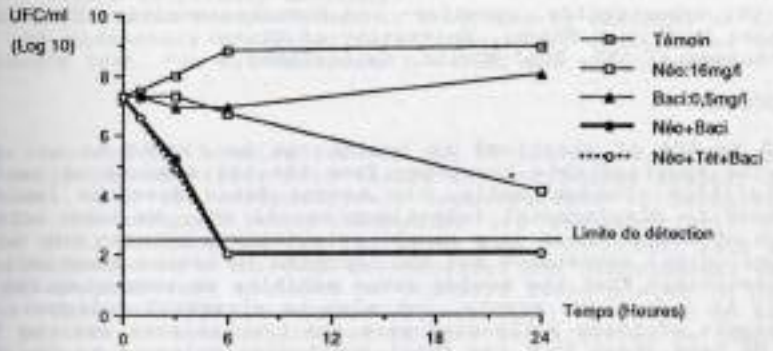
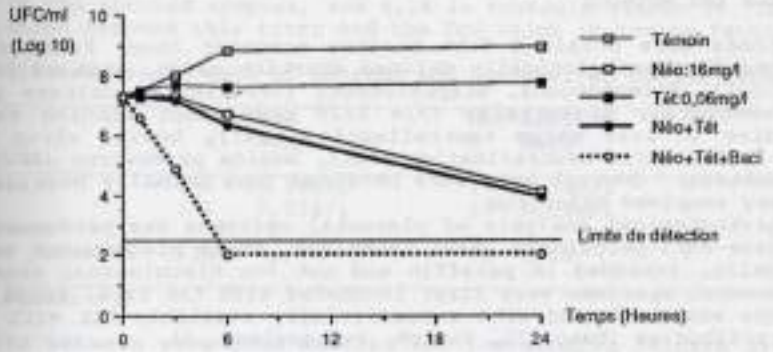


Figure 3 : Streptococcus uberis 1408

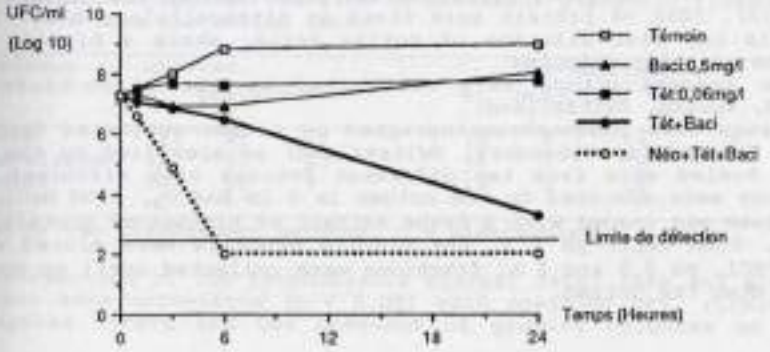
3a : ASSOCIATION NEOMYCINE-BACITRACINE (1/2 CMI)



3b : ASSOCIATION NEOMYCINE-TETRACYCLINE (1/2 CMI).



3c : ASSOCIATION TETRACYCLINE-BACITRACINE (1/2 CMI).



ANTIBODIES TO PLACENTAL EPITOPES IN BOVINE ABORTION

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INTRODUCTION

Only 30 to 50% of abortions in bovine can be traced to an infectious agent. The abortion rate increases from the third month of gestation up to parturition. Concomitantly, the bovine fetus develops immunological competence to diaplacental infectious agents (1). We have adressed the question of wether there is a causal relationship between the development of immunological competence and the increase in bovine abortions. We have shown previously that the bovine fetus exhibits an immunological response not only to infectious agents, but also to placental epitopes. The primary targets of these antibodies were the trophoblasts and the fibrinoid layer. We have shown that the fetal antibodies belonged to the IgG class (2).

MATERIALS AND METHODS

Placentomas were obtained from healthy pregnant cows. Fetal serum was collected from pathologically defined abortion cases, such as infections with E.coli, Streptococci, Staphylococci (verified by culture in selective substrata), Rickettsiae (>/= 1:20 complement binding reaction), Chlamydiae (> 1:20 serum neutralisation test), bovine virus diarrhoe (BVD), (> 1:4 serum neutralisation test), bovine parvovirus (BPV), (>1:27 Immunodottest). Control sera were obtained from normally born calves before they received colostrum.
Immunohistochemical analysis of placental epitopes was performed using a peroxidase-anti-peroxidase (PAP) technique. Whole placentomas were fixed in formalin, inbedded in paraffin and cut for histological examination. The placental specimen were first incubated with the fetal serum. The PAP technique was performed with a commercially available kit with an anti-rabbit antibodies (Dako, IG, Zurich, Switzerland, 2).
A crude extract of placentoma proteins was prepared by ammonium sulfate precipitation. The precipitate was disolved in 50mM Tris/HCl, pH 7.5, 10mM 2-merceptoethanol, 10mM sodium bisulfite, 0.001mM pepstatin, 250mM saccharose (4). Antibody titration was performed by the immuno-dot-technique (3). 50ng of protein were fixed on nitrocellulose BA85. The final titer is the last dilution of bovine serum, where a binding reaction could be visually detected.
Protein concentrations were determined by the micro-biuret method (BioRad, Zurich, Switzerland).
Fetal sera were immunochromatographed on a CNBr-activated Sepharose 4B column (Pharmacia, Dübendorf, Switzerland) as specified by the manufacturer. Pooled sera from ten different fetuses with different abortion aetiology were adsorbed to the column in 0.1M $NaHCO_3$, 0.5M NaCl, pH 8.3. The column was loaded with a crude extract of placentoma proteins in 0.2M $NaHCO_3$, 0.5M NaCl, pH 8.3. The binding proteins were eluted with 0.1M Glycin/HCl, pH 2.5 and 5 ml fractions were collected until no protein was coming down the column.

SDS-polyacrylamide gel electrophoresis was performed as described by LAEMMLI (6). The gel was stained with Coomassie blue.
Westernblotting was performed as described by WALKER (8). Fetal serum was used as the source of primary antibody.
Radial immunodiffusion was performed as described by MACCHINI et al. (7)
Means of the parameters evaluated were compared by analysis of variance and Chi square test. For all analyses, $P < 0.05$ was significant.

RESULTS

Our study involved 127 aborted fetuses and 18 calves, which had not yet received colostrum. The sera were tested for antibodies against BPV (41 seropositive), BVD (38 seropositive), Leptospirosis (0 seropositive), Rickettsiae (26 seropositive), Chlamydiae (26 seropositive). In each serum, the total protein and the IgG value was quantified. Each aborted fetus was subjected to a thorough pathological and histological examination. Organ samples were cultured on standard blood plates in order to screen for other bacteria ("unspecified bacteria"). As shown in table 1, the total protein in sera of aborted fetuses was elevated as compared to sera from non-aborted fetuses. Likewise, the IgG values in sera from aborted fetuses were elevated as compared to sera from non-aborted fetuses (Table 1). The mean titer of bovine fetuses to placental epitopes was 7,16 in the aborted samples, and 6,18 in controls (Table 1). There is no correlation between this titer and the IgG value in bovine fetuses.

Table 1: Differences in sera of aborted and non-aborted bovine fetuses

	aborted		non-aborted		
	n	mean	n	mean	
Total protein	118	45,59g/l	17	39,35g/l	*
IgG	131	0,37g/l	17	0,23g/l	*
Titer against placentoma proteins	114	7,16	16	6,18	*

* = significant

Differences between sera from aborted and non-aborted fetuses with regards to antibody binding capacity were only detectable in immunohistological examinations involving trophoblasts (Table 2).

Table 2: Immunohistochemical reaction of different placental tissues with IgG from the bovine fetus

positive tissue reaction[1]	aborted			non-aborted			
	n	postive	%	n	positive	%	
trophoblasts	127	91	72	18	8	44	*
connective tissue	127	32	25	18	3	16	
blood vessels	127	11	9	18	3	16	

1= as seen by two independent persons, * = significant

A positive reaction of the trophoblasts against fetal sera and elevated serum titers were correlated ($p < 0.05$) with positive BPV, rickettsiae and chlamydiae titers and the presence of growing colonies on blood

plates. No correlation between trophoblast reactivity and positive BVD titers was found (Table 3).
Immunoreactive placentoma proteins were purified about 500 fold in a one step purification on CNBr- activated- Sepharose- 4B. The proteins were separeted using SDS-polyacrylamide gel electrophoresis and visualized by Coomassie blue staining. 8 proteins were identified which migrated at 65, 63, 58, 55, 30, 25, 24 and 23 kD, respectively. These proteins were transferred to nitrocellulose and probed with pooled sera from aborted bovine fetuses. The Westernblots were positive for 65, 63, 58 and 55 kD proteins.

Table 3: Correlation of titers against placentoma epitopes and some infectious agents

	aborted			non-aborted			
	seropositive for	and reaction against placentoma		seropositive for	and reaction against placentoma		
	n	n	%	n	n	%	
TOTAL	128	-	-	18	-	-	
BPV	41	16	39	9	2	22	*
BVD	38	5	13	9	1	11	
Rickettsiae	26	3	12	11	0	0	*
Chlamydiae	26	1	4	11	0	0	*
unspecified bacteria	28	8	30	11	0	0	*

* = significant

DISCUSSION

We have found that the bovine fetus expresses elevated IgG levels not only in response to uterine infections, but also an autoimmune response to trophoblast epitopes (2). This reaction was demonstrated by immunohistochemistry and by immunodot titration. An other important finding of this study is that a significant percentage of abortion cases involving infectious agents like BPV, Rickettsiae, Chlamydiae and "unspecified bacteria" exhibited an autoimmune response (Table 3). Apparently, the infection *per se* rather than the presence of a specific infectious agent was causally related to the immune response, since 4 out of 5 tested infectious agents correlate with fetal antibodies against placental tissue. The specific reaction is directed against the trophoblast. The proteins recognized by the fetal IgG have a molecular weight of 50, 30, 25, 24 and 23 kD. Moreover, the 65, 63, 58 and 55 kD proteins were positive in a westernblot, probed with fetal serum.
Unlike in humans, the placentation in bovines involves a fetomaternal barrier of the type that is impenetrable to antibodies. Thus, the bovine fetus is a good model for the study of immunological factors in the pathogenesis of abortions.
In up to 70% of cases studied, the causative agent of abortion remains uncertain (5). There are two reasons for these findings: First, the abortion occurs several weeks after a presumed fetal infection, and by the time of abortion, the infectious agent is already eliminated. Secondly,

there are a lot of permissive abortion agents and factors. Their presence may be indicative rather than causative for abortion.
We conclude that abortion in bovine may involve a two step mechanism. First, the presence of a permissive infection, secondly this induces the fetal immune system to produce an autoimmune response against trophoblast epitopes. The development of an autoimmune response in the bovine fetus may be related to the fact, that at this time of gestation, the priming of T cells is not yet completed.

ACKNOWLEDGEMENTS

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SUMMARY

IgG isolated from aborted bovine fetuses were found to be directed to trophoblasts. Some infectious agents are likely to play a permissive role in this immune reaction. Main biochemical characteristics of the immunoreactive proteins in the placentoma are shown.

ZUSAMMENFASSUNG

IgG von abortierten, bovinen Feten reagieren mit Trophoblasten. Verschiedene Erreger lösen diese Reaktion aus. Einige biochemische Eigenschaften der durch die IgG erkannten Proteine im Plazentom wurden gezeigt.

RESUME

Les IgG de foetus bovins avortés réagissent avec les trophoblastes. Divers germes provoquent cette réaction. Certaines propriétés biochimiques des proteines ayant été reconnues par les IgG dans le placentome ont été mises en évidence.

OBSERVATIONS ON BOVINE PYELONEPHRITIS.

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INTRODUCTION

Contagious bovine pyelonephritis is a specific infection of the urinary tract of cattle caused by Corynebacterium renale and characterized by chronic purulent inflammation in the bladder, ureters and kidneys (4). Although the disease is widespread it seldom constitutes an important problem in a herd. The present study describes an outbreak of pyelonephritis in a dairy herd and investigates some of the epidemiological and diagnostic aspects of the disease.

MATERIALS AND METHODS

Data are from the first author's routine practice in seven Israeli Holstein herds from September 1986 through April 1988.

Clinical examination and treatment regimen.

A postparturient examination was carried out on all cows 5 to 12 days postpartum when the state of the uterus and the reaction of the urine was evaluated. Cows with either retained placenta for more than 18 hs or primary metritis were treated with 2 gm Chlortetracycline tablets (Chlortetraoblet, Vitamed) introduced into the uterus every other day. Pyelonephritis was diagnosed on the basis of presence of blood and pus in the urine, enlargement of the left kidney, and necropsy in slaughtered cases. Clinical cases were treated with 6 daily injections of 6 m. I.U. of penicillin G intramuscularry. Blood was collected at the end of the clinical examination from either the mammary or the coccygeal vein. Urine samples were taken via a metal catheter. All samples were examined on the day of collection for total and differential wbc, serum creatinine and urea, and urine pH, blood and protein content as described previously (14). Urine was cultured for the presence of bacteria by the method described by Buxton & Fraser (5).

Scope and methods of statistical analysis.

2089 cows were used for the evaluation of the annual prevalence rates of pyelonephritis in the various farms in 1987. 48 cows with 51 cases of pyelonephritis were recorded and 36 out of 48 cows were examined for both postparturient uterine diseases and urine reaction and were used for the evaluation of the associations between pyelonephritis and those traits. A case control method was applied, the pairs matched to farm, parity, and date of calving. Data were analysed by a retrospective study using the Mantel and Haenszel technique (12).

Association of the disease with parity was established by comparing the risk of the disease in one parity group with the risk of all other groups pooled together as used by Erb & Martin (8) and calculated in subcategories of the various months and postparturient uterine diseases according to the Mantel Haenszel method.

32 samples were examined for creatinine and urea concentrations. 23 blood samples were counted for total and differential wbc. 16 urine samples were cultured for the presence of bacteria. 9 urine samples were analysed for haematuria and proteinuria and were used for the determination of urine reaction and Specific Gravity.

152 cows calving from September through December 1986 in farm #3 were used for the evaluation of some epidemiological aspects of the disease.

Urine samples of 38 cows from farm #3 which had calved in December 1986 and were still in the herd in March 1988 were cultured for the presence of C. renale.

Coefficient of linear correlation between creatinine and urea values was computed by a standard "r" test (2).

RESULTS

1. Prevalence rate.

The overall prevalence rate for pyelonephritis in 1987 was 1.6% for 2089 cows in 7 farms. Farms prevalence rates are described in Table 1.

Table 1. Prevalence rate of pyelonephritis (1987).

farm	n cows	% affected
total	2089	1.6
1	347	.6
2	353	.6
3	305	6.2
4	299	.3
5	263	.8
6	278	1.4
7	244	1.6

2. The association with parity.

The overall rates of prevalence of pyelonephritis in 1987 were .3%, 1.8%, 1.3% and 2.7% for 742 first, 572 second, 377 third, and 398 fourth and subsequent calvers respectively. Odds ratio for the various parity groups suffering from pyelonephritis when compared to all other groups pooled together were .1 ($p<.01$), 2.3 ($p<.02$), .8, and 2.2 ($p<.05$) for first, second, third, and fourth or subsequent calvers respectively.

3. The association of pyelonephritis with postparturient urine reaction and state of uterus.

Of 19 cows with postparturient uterine diseases at calving 15.8% had pyelonephritis compared with 62.3% of 53 cows with no such a history. The odds ratio of cows with no postparturient uterine diseases having pyelonephritis was 8.9 compared to those which had those diseases ($p<.01$) in 36 pairs matched to farm, parity, and date of calving and calculated in subgroups of urine reaction. Of 63 cows which had a basic reaction of the urine at calving 52.4% had pyelonephritis compared to 11.1% of 9 cows with aciduria. The odds ratio of cows with basic reaction of the urine having pyelonephritis was 2.3 compared to those with aciduria (NS) in 36 pairs matched to farm, parity, date of calving and calculated in subgroups of postparturient uterine diseases.

4. Recovery rate.

Of 48 cows with pyelonephritis diagnosed in the study 11 (22.9%) died or were culled without treatment. Of 37 treated cows 5 (13.5%) died or were culled. Loss from pyelonephritis was therefore 33.3% of affected animals. 3 of the 32 recovering cows (9.4%) had a relapse (after 299, 313, and 486 days respectively).

5. Prognosis and laboratory tests.

Urinalysis.

All 9 urine samples examined were positive for protein and blood cells (>300 mg/dl and large amount respectively). pH of all urine samples was >8.5. Specific Gravity was in the range of 1.010 - 1.015.

Biochemistry.

The mean value of creatinine for 32 samples examined was 1.93 mg/100 ml ± 1.65. 46.8% of the affected cows had creatinine level of more than 1.5 mg/100 ml. The odds ratio of cows with serum creatinine of more than 1.5 mg/100 ml dying or being culled was 104.0 (p<.01) compared to those with lower concentration. The mean value of serum urea for 31 samples examined was 150.1 mg/100 ml ± 107.6 SD. 48.3% of the affected cows had urea level of more than 100.0 mg/100 ml. The odds ratio of cows with serum urea of more than 100.0 mg/100 ml dying or being culled was 60 (p<.01) compared to those with lower concentration. Serum creatinine and urea concentrations were correlated. (r=.572, p<.0001).

Haematology.

The mean value of total WBC for 23 cows sampled was 8296 Leukocytes/ml ± 3360 SD. The mean percentage of neutrophils of the total WBC was 55.7% ± 16.3 SD. In 58.3% of the affected cows that percentage was more than 50%. No odds ratio dying or being culled was associated with the relative percentage of neutrophils.

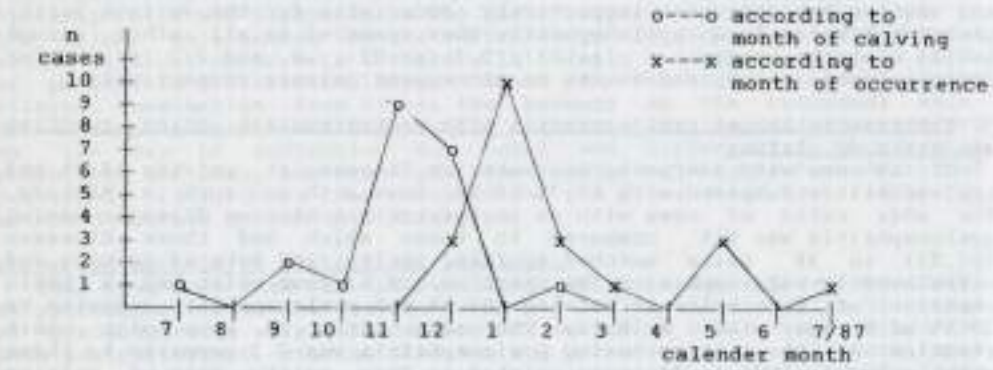
Bacteriology.

16 urine samples were cultured for bacteria. In 12 of the samples (75.0%) C. renale was isolated. E. coli was isolated from the kidney of one slaughtered cow.

6. Pyelonephritis in farm #3.

The distributions of cases of pyelonephritis classified according to month of calving and that of disease occurrence are described in fig 1.

Fig 1. Monthly distribution of cases in farm #3.



The mean interval from calving to the onset of clinical cases was 82.8 days ± 70.3 SD for 19 cows. The cumulative percentages were 31.5%, 47.4%, and 73.6% for intervals of 30, 60 and 90 days respectively.

Corynobacterium renale was isolated from urine samples of two out of thirty eight apparently healthy cows calving in December 1986 and cultured in March 1988 (5.3%). One of those developed a clinical pyelonephritis a month later.

DISCUSSION

It is claimed that, although pyelonephritis is widespread, clinical cases appear sporadically and the disease seldom constitutes an important problem in a herd or an area (4). In a survey of Israeli abattoirs (1) nephritis was diagnosed in 3.3% of 849 cows emergency

slaughtered in 1987. The annual incidence rate of pyelonephritis described presently (.5% to 1.5% in the various farms but farm #3), probably represents the common incidence for similar herds in the country. The increased incidence in farm #3 can be defined therefore as an outbreak and investigated accordingly.

In agreement with previous studies (3,11) few cases were recorded in heifers. 2nd calvers were in the present study in a higher risk for the disease compared to all other parity groups pooled together (O.R.=2.3).

It has been agreed that although the disease could be blood born it is primarily an ascending one under natural conditions. It was suggested (6) that adherence of C. renale to the urinary system is a required virulence factor in the establishment of Corynebacterium infection and that the vulva may be an important portal of entry for the bacteria (10). It was suggested (11) that such an ascending infection is strongly associated with parturition, being the outcome of secondary cystitis following parturition diseases. The data in the present study supply some evidence on the subject. The distribution of cases in farm #3 (fig. 1) fits better the period of calving than that of the disease occurrences. In view of the long and ununiform intervals between calving and the onset of cases a "point epidemic" cannot be ruled out.

Pyelonephritis was in the present study closely associated with the postparturient traits investigated. Cows with no postparturient uterine diseases at calving or with basic reaction of the urine were at risk of having pyelonephritis compared to those with aciduria to those with such diseases at calving (O.R being 8.9 and 2.3 resp.). C. renale is basophilic and its growth in the urine is correlated to the urine reaction (15). The prompt treatment of uterine diseases with tetracycline soon after calving practiced could explain the lower risk of treated animals for the disease. As aciduria is strongly associated with uterine diseases in the postparturient cow (13) the risk associated with the two factors should be calculated independent of one another. Although of the two associations so established only that between pyelonephritis and the absence of postparturient uterine diseases was statistically significant, these relationships strongly imply that the disease is associated with calving.

Infected animals are known to carry C. renale for a long period without evidence of clinical disease (3,15). It is reasonable to assume that the onset of clinical cases or relapses follow some stress such as pregnancy. 73.6% of the cases in the present study appeared within 90 days from calving and before pregnancy. The stress associated with peak production at this stage of the lactation may be responsible for the onset of clinical cases.

The clinical diagnosis of pyelonephritis is relatively simple. Laboratory tests are nevertheless of value in both diagnosis and prognosis. Although the infectious process associated with the disease is expected to cause neutrophilia, this parameter was not constant in the present study and could serve neither for prognosis nor for diagnosis. Both creatinine and urine proved to be effective as a measure for the involvement of the kidneys and of prognosis. Values of creatinine and urea higher than 1.5 mg/100 ml and 100.0 mg/ml respectively were of grave prognosis. The two enzymes were correlated as described previously in other kidney diseases of cattle (7). It was suggested that the levels of those enzymes depend on the degree of urinary tract involvement, high values are associated with kidney damage while lower ones with cystitis (9). It was suggested on the basis of negative serological antibodies that penicillin therapy was less effective when the kidneys were involved (10). In view of these findings, the poor prognosis of cows with either a high serum urea or creatinine concentrations displayed in the present study could be readily understood.

The urine pH of cows infected with pyelonephritis is high due to the ammonia produced from the activity of urease (3). In view of the lower pH values of normal urine in the population under study (13) those higher than 8.5 should be further investigated.

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SUMMARY

Epidemiological, diagnostic and prognostic aspects of 51 cases of bovine pyelonephritis were investigated. The annual prevalence rate for 2089 Israeli Holsteins was 1.6% in 1987. The mean interval from calving to onset of cases was 82.9 days. Multipara were at higher risk to contract the disease. Loss from pyelonephritis was 33.3% of all affected cows, and relapse occurred in 9.4% of apparently recovered animals. Both serum creatinine and urea concentrations were of high prognostic values. Odds ratios being culled for cows with levels of creatinine above 1.5 mg/100 ml and of urea above 100.0 mg/100 ml were 104.0 and 60.0 respectively compared with those with lower values. Haematological results were of no diagnostic value. Odds ratio for cows with no post parturient uterine diseases having pyelonephritis was 8.9 compared with those which had such diseases and were treated at calving with antibiotics. It was concluded that cows were infected at calving, that the infection was ascending, and that the onset of clinical cases were associated with the stress of peak lactation. Biochenical tests are of limited diagnostic, but good prognostic values.

SUMARIO

Se investigaron los aspectos epidemiologicos, diagnostico y pronostico de 51 casos de Pielonefritis Bovina. El nivel de prevalencia anual en 2089 vacas Holstein Israeli fue del 1.6% en 1987. El intervalo medio entre el parto yel comienzode los casos fue de 82.9 dias. El riesgo de contraer la enfermedad fue mayor en multiparas. Les bajas por pielonefritis fueron del 33.3% de todas las vacas afectadas, y una reincidencia del 9.4% en animales aparentemente recuperados. Las concentraciones de creatinina y urea en suero fueron de alto valor

prognistico. Los peligros relativos observados en vaca con niveles de creatinina de alrededor del 1.5 mg/100 ml y urea de alrededor de 100.0 mg/100 ml fueron 104.0 y 60.0 respectivamente comparado con aquellos de valores menores. Los resultados hematologicos no tuvieron valor diagnostico. Los peligros relativos en vacas que no presentaron enfermedades uterinas, post partum, sufriendo de pielonefritis fue de 8.9 comparado con aquellas que si enfermaron y fueron tratadas con antibioticos al parir. La conclusion es que las vacas se contagiaron durante el parto, la infeccion fue en ascenso, y el comienzo de los casos clinicos estuvo asociado con el stress registado en el pico de lactancia. Los tests son de valor diagnostico limitado pero de buen valor prognistico.

ZUSAMMENFASSUNG

Es wurden epydemiologische, diagnostische und prognostische ansichten von 51 fällen von Bovine Pyelonephritis hinterfragt. Die jährliche erkrankungs rate für 2089 Israelische Holsteins war 1.6% im Jahre 1987. Die durchschnitt liche pause zwischen der kalbung bis zum anfang der krankheit war 82.9 tage. Maltipara hatten ein höheres risiko krank zu werden. Der verlusst wegen pyelonephritis war 33.3% von allen erkrankten kühen, und ein rückfall von 9.4% der angeblich gesundgewordenen tieren. Serum creatinin und urea konzentrationen waren von hohen diagnostischen wert. Die relative gefahr für kühe mit creatinin werten von 1.5 mg/100 ml und urea 100.0 mg/100 ml waren 104.0 und 60.0 jeweils, mit kühen mit niederigen werten verglichen. Die hematologischen resultate waren von keinem diagnostischem wert. Die gefahr bei kühen welche keine gebärmutter krankheiten, nach dem kalben zeigten die unter pyelonephritis litten war von 8.9 mit denen verglichen welche nach der kalbung erkrankten und dan kein kalben mit antibiotica behandelt wurden. Daraus wird entnomen dass die kühe sich wärend dem kalben ansteckten die infektion aufwärts eindrang und der anfang der krankheit mit dem stress des höhepunkts der milchproduktion zusammenhängt. Biocemische tests sind von bergenzten diagnostischen wert aber von guten prognostischen wert.

The urine pH of cows infected with pyelonephritis is high due to the ammonia produced from the activity of urease (3). In view of the lower pH values of normal urine in the population under study (13) those higher than 8.5 should be further investigated.

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SUMARIO

Se investigaron los aspectos epidemiologicos, diagnostico y pronostico de 51 casos de Pielonefritis Bovina. El nivel de prevalencia anual en 2089 vacas Holstein Israeli fue del 1.6% en 1987. El intervalo medio entre el parto yel comienzode los casos fue de 82.9 dias. El riesgo de contraer la enfermedad fue mayor en multiparas. Les bajas por pielonefritis fueron del 33.3% de todas las vacas afectadas, y una reincidencia del 9.4% en animales aparentemente recuperados. Las concentraciones de creatinina y urea en suero fueron de alto valor

prognistico. Los peligros relativos observados en vaca con niveles de creatinina de alrededor del 1.5 mg/100 ml y urea de alrededor de 100.0 mg/100 ml fueron 104.0 y 60.0 respectivamente comparado con aquellos de valores menores. Los resultados hematologicos no tuvieron valor diagnostico. Los peligros relativos en vacas que no presentaron enfermedades uterinas, post partum, sufriendo de pielonefritis fue de 8.9 comparado con aquellas que si enfermaron y fueron tratadas con antibioticos al parir. La conclusion es que las vacas se contagiaron durante el parto, la infeccion fue en ascenso, y el comienzo de los casos clinicos estuvo asociado con el stress registado en el pico de lactancia. Los tests son de valor diagnostico limitado pero de buen valor prognistico.

ZUSAMMENFASSUNG

Es wurden epydemiologische, diagnostische und prognostische ansichten von 51 fällen von Bovine Pyelonephritis hinterfragt. Die jährliche erkrankungs rate für 2089 Israeliache Holsteins war 1.6% im Jahre 1987. Die durchschnitt liche pause zwischen der kalbung bis zum anfang der krankheit war 82.9 tage. Maltipara hatten ein höheres risiko krank zu werden. Der verlusst wegen pyelonephritis war 33.3% von allen erkrankten kühen, und ein rücktall von 9.4% der angeblich gesundgewordenen tieren. Serum creatinin und urea konzentrationen waren von hohen diagnostischen wert. Die relative gefahr für kühe mit creatinin werten von 1.5 mg/100 ml und urea 100.0 mg/100 ml waren 104.0 und 60.0 jeweils, mit kühen mit niederigen werten verglichen. Die hematologischen resultate waren von keinem diagnostischem wert. Die gefahr bei kühen welche keine gebärmutter krankheiten, nach dem kalben zeigten die unter pyelonephritis litten war von 8.9 mit denen verglichen welche nach der kalbung erkrankten und dan beim kalben mit antibiotica behandelt wurden. Daraus wird entnomen dass die kühe sich wärend dem kalben ansteckten die infektion aufwärts hineindrang und der anfang der krankheit mit dem stress des höhepunkts der milchproduktion zusammenhängt. Biocemische tests sind von bergenzten diagnostischen wert aber von guten prognostischen wert.

THE ROLE OF PREGNANCY IN THE RETENTION AND DISTRIBUTION OF ^{137}Cs BETWEEN MOTHER AND FETUS IN THE CATTLE

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INTRODUCTION

The study of ^{137}Cs with respect to pregnancy in the cattle and in other animals, of comparative concern too, was hindered by the difficulty of obtaining representative samples of pregnant contaminated subjects and the relative fetus, as resulting from the available bibliography we found; only one article on goats (1).

The occasion of our study, about the contamination of ^{137}Cs in the cattle, the fetus and about the processes that govern the exchange between mother and fetus, occurred after the radioactive fall-out coming from Chernobyl (USSR). On that occasion we had the opportunity of sampling a representative number of pregnant cattle slaughtered 7-8 months after the Chernobyl accident and fed with contaminated fodder.

MATERIALS AND METHODS

Sampling

The study was carried out on 24 cattles of italian Friesian race, pregnant from 2 to 9 months: 14 of them came from their same stock-farm placed in the north Italy (group A), and 10 cattles came from 8 stock-farms placed in other regions of north Italy (group B).

The group A was formed by 4 dairy-cattles and 10 heifers: these animals were born in the same stock-farm and lived in the same ambiental conditions with the same food regine qualitatively different, but in all cases with contaminated fodder.

The cattles of the group B were fed with fodder exposed to the radioactive fall-out too, harvested in the own stock-farms.

Just before the slaughter we withdrew a sample of blood from all the 24 cattles and during the slaughtering, occurred since november 7th to december 5th 1986, about 7 months after the beginning of the fall-out; we sampled a pillar diaphragm, index of the mean level of ^{137}Cs of the whole scheletric musculature (2).

Besides this tissue we sampled myocardium, kidney, liver, placenta, amniotic liquid and 26 fetuses (2 couples of twins), all alive.

We withdrew blood from the fetuses, without the ones in the first stages of growth, either directely from the cardiac cavity or from umbilical cord; then all the fetuses, except the #245 2 months old, were dissected to sample muscles (thigh), myocardium, kidney and liver.

Finally we sampled, in the same stock-farms, specimens of the different kind of fodder utilized to feed the cattles.

Radiometric analysis

This study was carried out radiometrically measuring (3) the activity of the radionuclides of all the biological samples (the fetus #245 was counted by "total body").

To prepare of the samples for the radiometric analysis, we did the normal procedures with relation to the volume to measure, using different counting container of 250 ml and 20 ml inserted in NaI(Tl) wells of appropriate diameters. Part of the samples of weight near 100 g were treated by wet ashing (1). The samples of fodder were counted with a Marinelli geometry by a NaI(Tl) crystal; all the results of the fodder were expressed by Bq/kg of dry weight. The counting time, with maximum values up to 80000 s, was choosen to obtain significant results for the samples with low activity too.

The subsequent analysis of the spectrum γ recordered was done utilizing an appropriate software that take into account the different counting geometry and that utilize a particular approximation of the gaussian function describing the peak.

The internal coherence of the measured activities was controlled by the known ratio between ^{137}Cs and ^{134}Cs,corrected for the radioactive decay. All the measured values were divided by the original weight to utilizing only data in concentration expressed by Bq/kg of fresh weight.

Statistical analysis

To process the data statistically we utilize adimensional indexes, which permit the comparison between dishomogeneus contaminated samples. Pratically, we normalized the values of the various organs of the mother and fetus, dividing them by the values of the muscle of the respective mother.

We divided the animals in statistical groups according to their category (dairy-cattle or heifer) and in dependence to the months of pregnancy (<3 months, 3-6, 6-8, >8).

We omit from the statistical processing the data of the cattle with the lowest contamination (cattle #1 #2), because of the largest measurement error associated.

For the statistical analysis we used a specific software (5) that processes data with the test of analysis of variance and the comparison between means with the "t" Student test and RECWP test.

RESULTS

In order to obtain comparable values of ^{137}Cs contamination we used ratios to normalize the dishomogeneity of the contamination.

About the comparison of the maternal organs we processed statistically the ratio kidney mother/muscle mother (K_m/M_m) and liver mother/ muscle mother (L_m/M_m).

Table n.1 shows the absolute contamination value of ^{137}Cs for the maternal organs and the ratio K_m/M_m and L_m/M_m.

In order to compare the fetal organs we used the ratios between the fetal organ themselves and the ratios between the muscle, the kidney and the liver of the fetus with the muscle of the respective mother (M_f/M_m; K_f/M_m; L_f/M_m). In table n.2 are shown the absolute contamination values of the fetal organs and their ratios with the muscle of their respective mother.

Contamination pattern of the maternal organs

As regards the K_m/M_m ratio, a very significant difference has been evidenced between heifers and cows ($P<0.0001$). Generally a mean larger contamination value appears in the kidney than in the muscle; a mean ratio of 2.40 and 1.26 has been determined for heifers and cows respectively.

Analogously the L_m/M_m ratio is significantly larger ($P<0.0029$) in the heifer than in the cow, with a mean value of 1.16 and 0.74 respectively. The liver tends to retain more than the muscle in heifer, while the opposite acts for cows.

Contamination pattern of the fetal organs

All the values of the fetal organs analysed, with the exception of the heart, have resulted comparable and homogeneous (α=0.05); they virtually have the same value within the fetus.

The ratio of the ^{137}Cs concentration of the fetal heart and of the fetal muscle ranges from 1.27 at the beginning of pregnancy to 0.73 at its terminal period, resulting significant the difference between the first and the last period ($P<0.0038$).

The fetal organ contamination pattern (tab. n. 2) have proved to significantly depend on the pregnancy stage ($P<0.0001$); at the beginning the values are very low and tend to be comparable with the muscle of the mother at the end.

Table n.1 137-Cs activity (Bq/kg) in mother organs

N.	G	C	M	muscle	kidney	liver	Km/Mm	Lm/Mm
245	B	C	2	179.33±1.88	232.18±2.83	128.31±1.09	1.29	0.72
100	B	C	2.5	167.80±1.92	296.91±3.53	163.01±1.26	1.77	0.97
14	A	H	3	51.44±1.40	129.24±2.34	54.06±0.59	2.51	1.05
15	A	H	3	56.54±1.15	132.51±2.82	54.56±0.65	2.34	0.96
6	A	C	4	53.68±1.10	50.99±1.14	36.85±1.13	0.95	0.69
9	A	H	4	58.72±1.27	138.79±2.29	65.74±0.55	2.36	1.12
12	A	H	4	58.54±1.51	148.43±1.65	64.77±1.34	2.54	1.11
124	B	C	4	8.88±0.37	12.00±0.72	5.79±0.34	1.35	0.65
10	A	H	4.5	56.66±2.09	163.03±2.51	69.74±0.61	2.88	1.23
11	A	C	4.5	86.87±1.60	204.01±2.68	132.06±0.95	2.35	1.52
7	A	H	5	59.36±1.16	149.19±2.59	80.22±0.78	2.51	1.35
15	B	C	5	121.06±1.79	170.08±3.69	79.78±0.75	1.40	0.66
49	B	C	5	85.95±1.57	83.49±2.51	58.51±0.65	0.97	0.68
16	A	C	5.5	62.53±1.20	69.79±1.11	37.98±0.84	1.12	0.61
1	B	C	6	3.63±0.46	1.50±0.43	0.32±0.41	0.40	0.09
2	B	C	6	1.09±0.35	2.00±0.33	0.50±0.23	1.83	0.46
4	A	C	6	49.59±1.05	41.41±1.01	22.60±1.09	0.84	0.46
48	B	C	7.5	34.54±1.61	34.84±1.20	17.53±0.90	1.00	0.51
5	A	H	8	49.49±1.05	153.27±2.82	74.07±0.69	3.10	1.50
13	A	H	8	82.01±1.42	163.73±2.17	97.77±1.15	2.00	1.19
129	B	C	8	12.16±0.47	11.83±0.48	6.97±0.41	0.97	0.57
3	A	H	8.5	51.62±1.06	139.73±2.43	72.96±0.74	2.71	1.41
8	A	H	8.5	85.25±1.85	90.92±1.64	58.05±0.63	1.07	0.68
137	B	C	9	10.40±1.11	12.18±0.71	8.28±0.46	1.17	0.80

legenda:
N = number of the animal C = category (C=dairy cattle; H=heifer)
G = group (A or B) M = months of pregnancy

^{137}Cs transfer from mother to fetus

In order to model the transfer of the contamination from mother to fetus in the cattle, the ratios of the fetal organs and maternal muscle have been fitted with a mono-exponential relation, without taking into account the diet or other related effects. The following relation, function of the fetus age (t), holds significantly with A=1 and K=0.231 month^{-1}:

$$F(t)/M(t)_m = A \cdot (1-e^{-K \cdot t}),$$

where F is the mean concentration of the various fetal organs; the kinetic constant, so assessed, turned out to be 0.231 month^{-1}, equivalent with a biological half life of 90 d.

Table n.2 ^{137}Cs activity (Bq/kg) in fetal organs

N.	G	C	M	muscle	kidney	liver	Mf/Mm	Kf/Mm	Lm/Mm
245	B	C	2	30.89±3.17 (*)					
100	B	C	2.5	45.25±3.40	72.03±22.92	70.20±4.04	0.27	0.43	0.42
100b	B	C	2.5	46.87±1.96	19.99±24.83	43.31±8.32	0.28	0.12	0.26
14	A	H	3	28.41±0.85	20.81±1.52	29.33±0.91	0.55	0.40	0.57
15	A	H	3	29.70±0.51	30.17±1.15	29.79±1.36	0.53	0.53	0.53
6	A	C	4	54.84±1.62	70.37±2.27	53.94±0.76	1.02	1.31	1.00
9	A	H	4	---	41.01±1.93	28.10±1.60	-	0.76	0.48
12	A	H	4	30.45±1.07	45.86±1.93	36.41±0.74	0.52	0.78	0.62
124	B	C	4	7.74±0.74	6.50±1.32	9.91±0.25	0.97	0.73	1.12
124b	B	C	4	6.87±0.34	5.77±1.52	9.91±0.25	0.77	0.65	1.12
10	A	H	4.5	41.72±1.71	61.77±1.58	55.98±0.90	0.74	1.09	0.99
11	A	C	4.5	38.56±0.56	64.66±1.84	61.22±1.20	0.44	0.74	0.70
7	A	H	5	27.54±0.56	59.38±1.54	39.24±0.78	0.46	1.00	0.66
15	B	C	5	126.20±1.54	139.25±2.48	115.57±1.93	1.04	1.15	0.95
49	B	C	5	67.65±0.72	76.54±2.03	82.00±0.93	0.79	0.89	0.95
16	A	C	5.5	68.75±0.74	54.34±1.02	64.87±0.91	1.10	0.87	1.04
4	A	C	6	52.22±0.91	47.71±2.12	51.54±0.81	1.05	0.96	1.04
1	B	C	6	2.91±0.21	1.19±1.97	3.82±0.32	0.80	0.33	1.05
2	B	C	6	2.90±0.34	2.18±2.01	2.12±0.24	2.66	2.00	1.94
48	B	C	7.5	111.28±0.80	74.01±1.55	69.42±0.62	3.22	2.14	2.01
5	A	H	8	47.98±0.75	48.16±1.15	42.36±0.58	0.97	0.97	0.86
13	A	H	8	73.32±0.89	60.93±0.91	58.05±0.67	0.89	0.74	0.71
129	B	C	8	16.15±0.37	12.61±0.88	14.78±0.24	1.33	1.04	1.22
3	A	H	8.5	42.00±0.58	38.38±0.75	34.54±0.64	0.81	0.74	0.67
8	A	H	8.5	101.69±0.95	83.36±0.90	84.22±0.73	1.19	0.98	0.99
137	B	C	9	18.98±0.42	10.32±0.96	12.56±0.26	1.83	0.99	1.21

legenda: (*) "total body" counted
N = animal number C = category (C=dairy-cattle; H=heifer)
G = group (A or B) M = months of pregnancy

DISCUSSION AND CONCLUSIONS

From the results presented above, a significant difference of K_m/M_m and L_m/M_m between heifer and cow can be drawn. This difference however could be related to variables not analysed in this paper, as, for instance, the disbomogeneous diet probably occurred between the two categories of the cattles examined.

As regards the ^{137}Cs contamination, although concentration differencies among organs does exist, we didn't prove any consistent

relation with the pregnancy period, so that we can draw the conclusion that pregnancy doesn't affect the ratios between the organs within the mother. The explanation of the ratio greater than unity must be researched into other effects, and we think that diet could be a major one.

On the other hand, ^{137}Cs is homogeneously retained in the fetus in all the organs considered, with the exception of the heart. ^{137}Cs concentration in fetus, compared with natural values, results very low at the beginning of pregnancy, and then it increases so to get similar in the late period. The time dependance agrees with a mono-exponential curve.

In the fetal heart, ^{137}Cs tends to concentrate just from the first stage, probably because of its precocious activity, then it gets back to a lower value than the other organs, restoring also in the fetal system the heart/muscle ratio known for the mother (2,8).

In the light of the data worked out till now, we can conclude that ^{137}Cs moves from mother to fetus with a pattern well described from the equation presented: the placenta-measurements, that we are actually analysing, will help to implement our compartment model in order to get a finer comprehension of the ^{137}Cs transmission in this important biological system, when environmental radio-contamination occurs.

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SUMMARY

The authors have analysed the relationship between ^{137}Cs radioactivity and pregnancy in the cattle with radiometric analysis of muscle, kidney and liver of 24 cows at various stages of pregnancy, feeded with fodder exposed to radioactive fall-out. Also the relative fetal muscle, kidney, liver and myocardium have been analysed. The automatic spectra analysis and the statistical one have given the following results: there is no correlation between ^{137}Cs retention and the pregnancy stage in the cattle sampled; the absolute ^{137}Cs concentration is always larger in the kidney than in the muscle (K_m/M_m=1.7), while it is just minor in minor in the liver (L_m/M_m=0.8). The fetal concentration in muscle, kidney and liver is generally homogeneous, whereas the myocardium tends to retain more ^{137}Cs in the first stages of the pregnancy as against the other tissues. In the last stage of pregnancy it presents a lower concentration. The Authors have examinated the obtained results and suggested a monoexponential equation which gives the value of the ^{137}Cs exchange between mother and fetus in the cattle.

ZUSAMMENPASSUNG

Die Autoren haben das Verhaltnis zwischen ^{137}Cs und der Schwangerschaft untersucht. Sie haben auch radiometrische Analyse des Muskels,der Niere und der Leber von 24 trachtigen Kuhen vorgenommen, die mit radioaktivem Fall-out ausgesetztem Fotter gefuttert worden sind. Der Muskel,die Niere, die Leber und der Herzmuskel der jeweiligen Fetussen sind auch untersucht worden.Die automatische Analyse der Spektra und die statistische Analyse haben hervorgehoben,dass kein Verhaltnis zwischen der ^{137}Cs Retention und der Schwangerschaftsstufe in allen analysierten Kuhen besteht. Die absolute ^{137}Cs Konzentration ist immer hoher in der Niere als in dem Muskel (Km/Mm=1.7), wahrend sie in der Leber etwa niedriger ist (Lm/Mm= 0.8). Die ^{137}Cs Konzentration in dem Muskel,der Niere und der Leber des Fetusses ist normalerweise homogen,wahrend in der ersten Schwangerschaftsstufe der Herzmuskel die Tendenz zur hoheren ^{137}Cs Retention, im Gegensatz zu den anderen Geweben,hat.In der letzten Schwangerschaftsstufe hat der Herzmuskel eine kleinere Konzentration davon. Die Autoren haben die Ergebnisse uberpruft und stellen eine Monoexponentialgleichung, die den Wert des ^{137}Cs Austausches zwischen Kuh und Fetus feststellt.

RESUMEN

Los Autores han estudiado el raporte entre el ^{137}Cs y la prenez en el bovino con analisis radiometricas de el musculo, rinon y higado de 24 bovinas a distintos periodos de prenez alimentados con foraje expuesto a "fall-out" radioactivo y sobre el muscolo,rinon,higado y miocardio de los respectivos fetos. La analisis automatica de los espectros y la analisis estatistica han evidenciado: en las madres no existe una dependencia de la ritencion de ^{137}Cs en los organos examinados con el periodo de prenez y el valor absoluto de ^{137}Cs es siempre major en el rinon respecto al muscolo (Km/Mm=1.7) y ligermente inferior en el higado (Lm/Mm=0.8): en los fetos las concentraciones de ^{137}Cs en el musculo, rinon y higado son generalmente homogeneas, mientras el miocardio parece retener majormente el cesio en los primeros estadios de la prenez, respecto a otros tejidos, mientras en la fase final tiene niveles de contaminacion inferiores. Los autores discuten de los resultados optenidos y proponen una equacion mono-exponencial que exprime el valor de intercambio de ^{137}Cs entre la vaca y el feto.

ETUDE ECHOCARDIOGRAPHIQUE DE LA FONCTION ET DE LA MORPHOLOGIE CARDIAQUE CHEZ LE VEAU HYPERVIANDEUX

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INTRODUCTION

La sélection du gène hyperviandeux a permis d'augmenter de façon importante les profits économiques issus de la production intensive de viande bovine (6). Cependant, les très hauts niveaux de performances zootechniques réalisés par les bovins de conformation hyperviandeuse (ou culards) ne peuvent être atteints qu'au prix d'un métabolisme aérobie parfaitement fonctionnel. Cela implique qu'aucun des maillons de la chaîne de transport de l'oxygène ne joue un rôle limitant dans la réalisation de ce métabolisme aérobie. Or, il a été démontré que dans certaines circonstances, le système cardio-vasculaire, et plus particulièrement la pompe de ce système que constitue le coeur, peut constituer une étape limitante du transport de l'oxygène chez les sujets culards de la race blanc bleu belge (BBB), comme en témoignaient des index cardiaque et d'éjection systolique inférieurs chez ceux-ci en comparaison à des sujets de conformation standard de la race frisonne (F) lors d'un effort musculaire sur tapis roulant (5) ou lors d'une épreuve d'hypoxie aiguë expérimentale (1).

L'échocardiographie constitue un moyen simple, non invasif, répétable et fiable d'investiguer à la fois la morphologie et la fonction cardiaque (4), et ce dans de nombreuses espèces animales (7) dont le Bovin (8).

Dans cette étude, cette technique a dès lors été appliquée dans le but de caractériser et de comparer l'évolution de divers paramètres échocardiographiques chez des veaux de conformation hyperviandeuse et standard au cours de la croissance.

MATERIEL ET METHODE

Animaux

17 veaux de race F (poids 35 à 135 kg ; âge 9 à 138 jours ; 12 mâles et 5 femelles) et 8 veaux culards de race BBB (poids 45 à 144 kg ; âge 15 à 119 jours ; tous mâles), considérés comme sains lors d'un examen clinique et électrocardiographique approfondis, ont été utilisés dans cette étude. Ils ont régulièrement subi un examen échocardiographique au cours de leur croissance. Au total, 53 protocoles ont été récoltés chez les veaux F contre 45 chez les veaux BBB.

Protocole échocardiographique

Les échocardiogrammes étaient enregistrés comme décrit dans une étude antérieure (2). Les paramètres les plus appropriés dans le cadre de cette étude comparative, à savoir ceux qui caractérisent la morphologie et la fonction du ventricule gauche (VG), ont été sélectionnés. En résumé, les images étaient récoltées selon 2 modes échographiques, temps mouvement (TM) et bidimensionnel (2D), au moyen d'un échocardiographe (Sono Layer, modèle SAL 77B, Toshiba, Tokyo, Japan) couplé à une sonde sectorielle d'une puissance d'émission de 5 ou de 3 MHz selon la taille du patient investigué. Les veaux étaient examinés debout, sans tranquilisation ni anesthésie préalable. Dans un premier temps, une vue parasternale droite du VG selon le long axe du coeur (LAx) était recherchée dans le mode TM. Dans cette vue (TMLAx), l'épaisseur du septum interventriculaire et de la paroi libre du ventricule gauche (IVS et LVFW respectivement) ainsi que le diamètre interne du VG (LVID) étaient mesurés en systole et en diastole. Un léger pivotement cranio-caudal

du transducteur permettait ensuite d'obtenir une vue LAx et dans le mode 2D du tractus d'éjection du VG. Dans cette vue (2DLAx) et pendant la fermeture diastolique des valvules aortiques, le diamètre de l'oreillette gauche (LA) était mesuré, ainsi que celui de l'aorte à 3 niveaux : (1) au point d'attachement des valvules aortiques sur la paroi de l'aorte (Ao_{va}), (2) au niveau du diamètre maximal des sinus de Valsalva (Ao sinus) et (3) au niveau du diamètre minimal de l'aorte juste en aval des sinus de Valsalva (Ao_{min}). La mise en place du curseur TM à travers les valvules aortiques permettait alors d'obtenir une vue TMLAx de la racine de l'aorte, et dans cette vue son diamètre (Ao) ainsi que LA étaient mesurés. Une rotation de la sonde à 90 degrés permettait d'obtenir ensuite une vue en mode 2D du VG dans un plan perpendiculaire au long axe du coeur (SAx). Dans cette vue (2DSAx), IVS, LVFW, LVID, la circonférence interne et externe (LVIC et LVEC respectivement) ainsi que la surface interne et externe (LVIS et LVES respectivement) du VG étaient mesurés en systole et en diastole. Le curseur TM était alors dirigé perpendiculairement au diamètre du VG et IVS, LVFW et LVID étaient à nouveau mesurés en systole et en diastole.

Chacun des paramètres était mesuré 5 fois sur 5 cycles cardiaques différents et la valeur moyenne sur ces 5 mesures était retenue.

Calculs

Les paramètres suivants ont été calculés selon les formules décrites antérieurement (2) : Les rapports LA/AO et IVS/LVFW ; le pourcentage d'épaississement du septum interventriculaire (% Δ IVS) et de la paroi libre du VG (% Δ LVFW) ; la fraction de raccourcissement du VG (% Δ LVD) ; la variation systolique de la surface interne du VG (FAC) ; la surface du myocarde en systole et en diastole (LVMSs et LVMSd respectivement) et le pourcentage de variation systolique de la surface du myocarde du VG (% Δ SM).

Le raccourcissement circonférentiel du VG (% Δ circ) était obtenu selon la formule [LVICd - LVICs/ LVICd].100.

Analyse des résultats

Chaque paramètre échocardiographique a été analysé par un modèle linéaire fixe hiérarchisé incluant les effets race, poids, âge et individu (inclus dans la race).

RESULTATS

Le tableau 1 illustre le résultat de la comparaison des différents paramètres échocardiographiques dans les 2 races de veaux étudiées. Les résultats sont donnés sous forme de moyennes des moindres carrés, c'est à dire qu'ils représentent la valeur moyenne du paramètre obtenue dans chaque race après avoir éliminé les effets de l'individu étudié ainsi que de l'âge et du poids des animaux, grâce à l'utilisation d'un modèle linéaire fixe hiérarchisé.

Dans l'ensemble, la plupart des paramètres d'estimation de la morphologie cardiaque mesurés en diastole ne différaient pas entre les 2 races. Par contre, leur comparaison, lorsqu'ils étaient mesurés en systole, faisait apparaître des différences significatives entre les 2 groupes de veaux.

Par exemple, la figure 1 illustre la comparaison des mesures de IVS en systole dans la vue TMLAx dans les 2 races de veaux.

Quant aux paramètres d'estimation de la fonction cardiaque, ils prenaient des valeurs significativement plus élevées chez les veaux de race F que chez les veaux de race BBB (Tableau 1).

Tableau 1. Comparaison de la moyenne des moindres carrés ± erreur standard (X ± ES) de divers paramètres échocardiographiques chez des veaux de race frisonne (F, n = 53) et blanc bleu belge (BBB, n = 45). Voir texte pour légendes.

Paramètres	Mode et vue	X ± ES F	X ± ES BBB	
IVSs (mm)	TMLAs	19.6 ± 0.3	17.7 ± 0.4	**
	2DSAs	18.6 ± 0.2	17.5 ± 0.4	*
	TMSAs	19.1 ± 0.2	17.3 ± 0.4	***
IVSd (mm)	TMLAs	12.0 ± 0.1	12.2 ± 0.2	
	2DSAs	11.8 ± 0.1	12.3 ± 0.2	
	TMSAs	11.7 ± 0.2	11.4 ± 0.2	
LVIDs (mm)	TMLAs	28.3 ± 0.5	28.3 ± 0.7	
	2DSAs	29.4 ± 0.5	32.8 ± 0.8	*
	TMSAs	27.2 ± 0.5	31.6 ± 0.8	***
LVIDd (mm)	TMLAs	49.2 ± 0.5	47.9 ± 0.8	
	2DSAs	46.7 ± 0.5	47.9 ± 0.8	
	TMSAs	48.1 ± 0.5	50.0 ± 0.7	
LVPWs (mm)	TMLAs	20.3 ± 0.3	19.4 ± 0.4	
	2DSAs	18.1 ± 0.3	15.9 ± 0.4	***
	TMSAs	19.8 ± 0.3	17.9 ± 0.5	**
LVPWd (mm)	TMLAs	12.1 ± 0.3	13.8 ± 0.4	**
	2DSAs	11.2 ± 0.2	11.6 ± 0.2	
	TMSAs	11.0 ± 0.3	11.7 ± 0.4	
Ao_{Vg} (mm)	2DLAs	18.9 ± 0.5	18.8 ± 0.6	
Ao_{sinus} (mm)	2DLAs	32.7 ± 0.3	30.1 ± 0.3	***
Ao_{min} (mm)	2DLAs	23.5 ± 0.2	22.1 ± 0.2	***
Ao	TMLAs	31.4 ± 0.3	29.4 ± 0.4	***
LA (mm)	2DLAs	25.4 ± 0.3	27.6 ± 0.3	***
	TMLAs	25.2 ± 0.2	27.2 ± 0.3	***
LVESs (cm^2)	2DSAs	5.8 ± 0.2	7.9 ± 0.4	***
LVISd (cm^2)	2DSAs	17.8 ± 0.5	18.2 ± 0.7	
LVICs (mm)	2DSAs	92.7 ± 1.8	112.4 ± 2.7	***
LVICd (mm)	2DSAs	158.2 ± 2.0	163.2 ± 3.1	
LVESs (cm^2)	2DSAs	35.0 ± 0.6	40.5 ± 0.8	***
LVESd (cm^2)	2DSAs	41.4 ± 0.7	41.0 ± 1.1	
LVECs (mm)	2DSAs	208.9 ± 1.7	218.6 ± 2.6	**
LVECd (mm)	2DSAs	228.7 ± 2.0	230.8 ± 2.9	
LVMS (cm^2)	2DSAs	29.2 ± 0.5	32.6 ± 0.8	
LVMSd (cm^2)	2DSAs	23.5 ± 0.5	22.9 ± 0.7	**
% IVS (%)	TMLAs	62.7 ± 2.4	45.3 ± 3.4	***
	2DSAs	58.1 ± 1.9	43.1 ± 2.9	***
	TMSAs	62.6 ± 1.7	52.0 ± 2.5	**
% LVD (%)	TMLAs	42.0 ± 0.7	41.2 ± 1.1	
	2DSAs	36.6 ± 1.0	31.8 ± 1.5	*
	TMSAs	43.1 ± 0.8	37.1 ± 1.2	***
% LVPW (%)	TMLAs	70.0 ± 2.9	45.9 ± 4.1	***
	2DSAs	62.8 ± 2.8	39.3 ± 4.2	***
	TMSAs	79.7 ± 3.1	55.4 ± 4.6	***
% Δ SM (%)	2DSAs	26.9 ± 2.5	21.3 ± 3.8	
% Δ circ (%)	2DSAs	41.0 ± 1.1	31.7 ± 1.7	***
FAC (%)	2DSAs	67.0 ± 1.3	57.4 ± 1.9	***
IVS/LVPW	TMLAs	1.01 ± 0.02	0.95 ± 0.03	
	2DSAs	1.07 ± 0.01	1.07 ± 0.02	
	TMSAs	1.07 ± 0.02	1.01 ± 0.03	
LA/Ao	2DLAs	0.78 ± 0.01	0.92 ± 0.01	**
	TMLAs	0.81 ± 0.01	0.93 ± 0.01	***

* = valeur significativement différente de la valeur correspondante chez les veaux de race F, modèle linéaire fixe hiérarchisé, $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

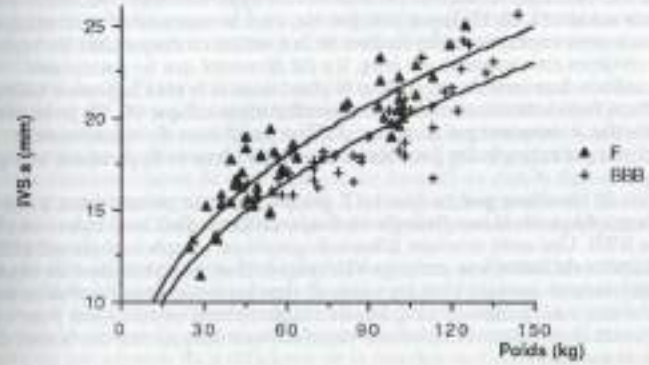

Fig. 1. Evolution de l'épaisseur du septum interventriculaire mesuré par échocardiographie en systole (IVSs) en fonction du poids vif chez des veaux de race F (n = 53) et BBB (n = 45). Voir texte pour légendes.

DISCUSSION

L'analyse des résultats de cette étude permet de constater que la sélection du gène culard dans la race BBB n'a pas été accompagnée d'une hypertrophie et/ou hyperplasie des fibres musculaires cardiaques décelable par échocardiographie. En effet, l'épaisseur des parois du VG, lorsqu'elle était mesurée par ultrasons en diastole et donc pendant la phase de relaxation des fibres musculaires ventriculaires, n'était pas significativement différente dans les 2 groupes de veaux étudiés.

D'autre part, l'hypertrophie musculaire des bovins hyperviandeux n'a pas non plus été accompagnée d'un rétrécissement significatif des cavités ventriculaires décelable à l'échocardiographie. En effet, ni le diamètre, ni la surface, ni la circonférence du VG mesurés par échocardiographie n'étaient significativement plus petits chez les veaux BBB que chez les veaux F.

Ces résultats sont étonnants au vu de ceux obtenus par Ansay & Hanset (3), qui ont démontré que si la sélection du gène culard dans la race BBB était accompagnée d'une augmentation de 20 % du poids de muscle par kilo de poids vif, par contre elle était accompagnée parallèlement d'une réduction de 15 % du poids de coeur relatif. Il est donc surprenant que cette différence pondérale cardiaque ne se soit pas accompagnée chez le culard d'une différence cardiaque morphologique décelable par échocardiographie.

D'un autre côté, la comparaison de tous ces paramètres échographiques d'estimation de la morphologie cardiaque mesurés en systole montraient des différences significatives entre les 2 groupes d'animaux. Ainsi, les veaux F présentaient en systole des parois ventriculaires significativement plus épaisses et des dimensions du VG significativement plus petites que les veaux BBB. L'explication de ce phénomène ne doit pas se trouver dans une différence de morphologie cardiaque entre les 2 races de veaux. En effet, une analyse plus détaillée des résultats permet de constater que chez les veaux F, la plupart des paramètres d'estimation de la fonction cardiaque prenaient des valeurs significativement plus élevées que chez les veaux BBB. Les valeurs d'épaisseur systolique des parois ventriculaires plus élevées chez les veaux F étaient donc probablement le résultat d'un épaississement plus important de ces parois pendant la systole chez les veaux F que chez les

veaux BBB. Inversément, les dimensions systoliques ventriculaires plus larges chez les veaux BBB que chez les veaux F étaient probablement le résultat d'un raccourcissement moindre des fibres myocardiques chez ces premiers. Ces valeurs de fonction cardiaque inférieures chez les bovins hyperviandeux confirment donc les observations faites par d'autres auteurs (1, 5). De façon plus précise, c'est la contractilité myocardique qui semble devoir être mise en cause pour expliquer le rôle limitant de la fonction cardiaque chez les veaux culards de la race BBB dans certaines circonstances. En effet, il a été démontré que les paramètres fonctionnels que nous avons utilisés dans cette étude, et dont le plus connu et le plus largement utilisé est le % Δ LVD constituent des indices fiables d'estimation de la contractilité myocardique (4). Ce point nécessite une investigation plus approfondie, notamment par la mesure d'autres paramètres d'estimation de la contractilité myocardique, comme par exemple des paramètres dérivés de mesures de pressions tels que dP/dt.

La comparaison du diamètre de l'oreillette gauche dans les 2 groupes de veaux permet quant à elle de relever un élément échocardiographique de la morphologie cardiaque différent dans les 2 races : en effet, LA était plus large chez les veaux BBB. Une autre structure échocardiographique morphologique qui diffère entre les 2 types de bovins est le diamètre de l'aorte à sa sortie du VG, celui-ci étant plus petit chez les veaux BBB que chez les veaux F. Ce rétrécissement aortique chez les veaux de type hyperviandeux pourrait se traduire par une augmentation de la résistance à l'éjection du sang lors de chaque systole ventriculaire. Pour vérifier cette hypothèse, il serait nécéssaire de comparer la résistance vasculaire systémique chez des bovins de conformation hyperviandeuse et standard.

En conclusion, cette étude a permis de constater que la sélection du gène culard n'a pas été accompagnée d'une modification significative de l'épaisseur du myocarde et/ou d'un rétrécissement de la cavité du VG décelables par échocardiographie. Par contre, elle a été accompagnée d'une part d'un rétrécissement du diamètre du tractus d'éjection du VG, ce qui pourrait se traduire par une augmentation de la résistance vasculaire systémique et donc de la post-charge du coeur gauche, et d'autre part par une chute de la force de contraction du myocarde, ce qui pourrait en partie expliquer que chez les bovins hyperviandeux, la fonction cardiaque pourrait jouer un rôle limitant dans la chaîne de transport de l'oxygène lors d'une demande métabolique accrue. Il serait opportun de compléter ces informations par une investigation de la fonction cardiaque du bovin hyperviandeux par des techniques invasives.

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RESUME

La fonction cardiaque a été suspectée comme facteur limitant potentiel du métabolisme aérobie chez les bovins hyperviandeux lorsque la demande métabolique, déjà très élevée à son niveau basal pour assurer la réalisation de performances zootechniques de haut niveau, est encore accrue dans certaines circonstances particulières.

Dans cette étude, 29 paramètres échocardiographiques différents, fonctionnels et morphologiques, ont été comparés chez des bovins de conformation hyperviandeuse de race blanc bleu belge (BBB) et des bovins de conformation standard de race frisonne (F) au cours de la croissance. Les dimensions du ventricule gauche (VG) mesurées en diastole ne différaient pas dans les 2 groupes d'animaux. Par contre, l'épaisseur des parois et le diamètre interne du ventricule gauche mesurés en systole étaient respectivement significativement inférieure et supérieur chez les veaux BBB . D'autre part, le diamètre de l'aorte était significativement plus petit chez ces derniers. Enfin, les paramètres fonctionnels avaient des valeurs significativement plus élevées chez les veaux F.

Ces résultats montrent que si l'échographie ne permet pas de mettre en évidence de différence de la morphologie du VG entre les 2 types de bovins au cours de leur croissance, par contre elle permet de constater que d'une part le diamètre de la racine de l'aorte et que d'autre part la contractilité myocardique sont réduits chez les bovins de conformation hyperviandeuse. Cette dernière constatation pourrait en partie expliquer les mécanismes de la déficience de la fonction cardiaque du bovin culard à répondre à une demande métabolique accrue.

ABSTRACT

The cardiac function has been suspected as a potential limiting factor of the aerobic metabolism in double muscled cattle when metabolic request, already very high in basal conditions to steady very high zootechnic performances, is more increased.

In this study 29 functional and morphological echocardiographic parameters were compared in double muscled calves of the belgian white and blue (BWB) breed and in calves with standard conformation of the friesian (F) breed during growth. Left ventricular (LV) dimensions measured during diastole were not different between the 2 groups of animals. On the opposite, systolic LV wall thicknesses and internal diameter were significantly lower and higher respectively in BWB calves. On the other hand, aortic diameter was significantly lower in the latest and functional parameters were significantly higher in F calves.

It was concluded that echocardiography does not allow to display morphological LV differences between the 2 breeds of calves during growth. On the opposite, it allows to etablish that root aortic diameter and myocardic contractility are reduced in double muscled. This last could partly explain the mechanisms of the lower cardiac efficiency of double muscled cattle to respond to an increased metabolic request.

ZUSAMMENFASSUNG

Man vermutete, das kardiovaskuläre System sei ein mögliches Hindernis des aeroben Metabolismus bei den Mastkälbern, wenn die Metabolismusforderung, die für zootechnische Höchstleistungen an ihrem Grundwert schon sehr hoch ist, unter gewissen Umständen noch erhöht ist.

Im Rahmen dieses Versuchs wurden 24 verschiedene echokardiographische Parameter funktioneller und morphologischer Art bei Mastkälbern der weiß-blauen belgischen Art (BBB) und bei normalgebauten Kälbern friesischer Herkunft (F) im Laufe ihres Wachstums verglichen.

Die Messungen der linken Herzkammer, die während der diastolischen Phase gemessen waren, sind gleichbedeutend in den zwei Gruppen der Tiere.

Dagegen war bei den Mastkälbern die Stärke der Herzwände bzw. der innere Durchmesser während der systolischen Phase jeweils bedeutend kleiner bzw. größer. Anderseits war der Durchmesser der Aorta bedeutend kleiner bei den Mastkälbern. Schließlich lagen die Werte der funktionellen Parameter bei den normalgebauten Kälbern deutlich höher.

Aus diesen Ergebnissen können wir schließen, daß die Morphologie der linken Herzkammer beider Rinderarten während des Wachstums vergleichbar zu sein scheint. Dagegen scheinen der Durchmesser der Aorta und die Zusammenziehbarkeit des Myokards bei Mastkälbern niedriger zu sein. Diese letzte Feststellung könnte die Mechanismen der unter gewissen Umständen bei den Mastkälbern auftretenden Herzfunktionsschwäche teilweise erklären.

BOVINE LEUKEMIA VIRUS INFECTION: DEVELOPMENT OF NEW DIAGNOSTIC METHODS BASED ON DNA-RECOMBINANT TECHNOLOGY.

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INTRODUCTION:

Bovine Leukemia Virus,BLV, is a C-type lymphotropic retrovirus which is associated with a disease complex termed enzootic bovine leukosis (8,14,15,16).BLV infection of cattle is worldwide in distribution.In Sweden 25-30% of all dairy herds are housing one or more positive animals.The prevalence of BLV infected individuals is estimated at 5-10% (7).For the diagnosis, serological tests are preferred, since the direct detection of the virus by the conventional techniques (e.g. virus isolation,electron microscopy, competition RIA, antigen-capture ELISA) is rather difficult and ineffective (9,10).The Swedish BLV-eradication programme, starting this year, is based on testing of serum and milk in an indirect ELISA, developed by Nils Juntti and his team at the National Veterinary Institute (7).Additional simple and sensitive methods of direct detection would be of great importance, because the serological methods are of no value for diagnosis in certain cases.For example,young calves from infected cows frequently possess maternal antibodies and are positive in serological tests.Furthermore, adult cattle may carry the provirus without having detectable level of antibodies.In such cases, direct methods of virus detection are needed.Considering this, our approach has been to apply a Polymerase Chain Reaction, PCR, assay for the detection of BLV proviral DNA sequences (11,13).Sets of primers and probes have been selected from the gp51 region of the BLV genome (6,12).Simple and double PCR were run on cultured cells as well as on separated peripheral blood mononuclear cells of cattle.

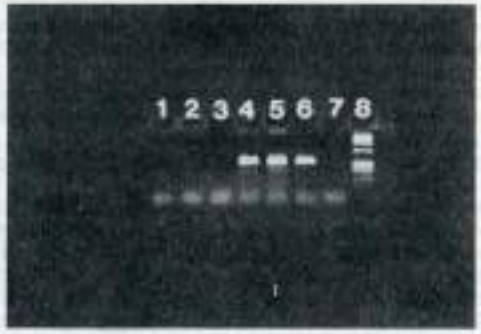

Fig.1.Electrophoresis of the products of the BLV-PCR assay.

Figure 1.Continued: Peripheral blood mononuclear cells of uninfected cattle (1 to 3) and of infected cattle (4,5). Ovine fetal kidney cells infected with BLV (6).Negative control of PCR (7) and size marker (8).

The preliminary results show that PCR is a reliable and sensitive technique for rapid detection of BLV infection.

EXPERIMENTAL DESIGN AND SAMPLING PROCEDURES:

A dairy farm,with tank-milk absorbance value in the BLV-ELISA indicating a low to moderate infection rate in the herd,was selected for the experiment.The farm is well managed, the hygienic standard high, and the general condition of the animals is good.85 animals were sampled initially: 38 lactating cows, five cows in the dry period, 33 heifers and nine young cow-calves.Individual blood-samples were drawn from the jugular vein of all 85 animals, using evacuated blood collection tubes without anticoagulant. Individual milksamples were collected from the 38 lactating cows.All samples were tested in the indirect ELISA used in the eradication programme.Five cows were positive for antibodies to BLV in serum and milk, one heifer and two newborn calves had antibodies to BLV in serum.The newborn calves were the offspring of seropositive dams.
All seropositive animals and their neighbours, with whom they had nose-to-nose contact, were selected for a second sampling;a total of 20 individuals.Serum and heparinized blood was collected.Peripheral blood mononuclear cells were prepared by the Ficoll-Paque separation method (Pharmacia, Uppsala,Sweden).Serum samples were tested in agargel immunodiffusion test, AGID, and indirect ELISA.The lymphocyte-preparations were tested by a double PCR method.The PCR-products were analysed by gel-electrophoresis.

RESULTS AND CONCLUSIONS:

In Table 1 the results from the second sampling are shown. The tests correlate to a high degree. Six of the animals are infected with BLV, whereas the two newborn calves, who have been fed colostrum from their infected mothers are virus free and merely possess maternal antibodies to BLV. The PCR assay proves to be a practical and rapid method for the detection of BLV (1 to 5).

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TABLE 1:

	Number	BLV-antibodies in i-ELISA serum	BLV-ab:s in i-ELISA milk	BLV-ab:s in AGID serum	Virus-detection with PCR
cows	1	-	-	-	-
	2	+	+	+	+
	3	+	N.L.	+	+
	4	-	N.L.	-	-
	5	-	-	-	-
	6	+	+	+	+
	7	-	-	-	-
	8	-	-	-	-
	9	+	+	+	+
	10	-	-	-	-
	11	+	+	+	+
	12	-	N.L.	-	-
heifers	13	+	N.L.	+	+
	14	-	N.L.	-	-
In the	15	-	N.L.	-	-
same pen	16	-	N.L.	-	-
since Oct.	17	-	N.L.	-	-
-89	18[a)]	-	N.L.	-	-
Newborn cow-calves	19[b)]	+	N.L.	+	-
	20[c)]	+	N.L.	+	-

N.L.= Not Lactating

a)daughter of number 9

b)daughter of number 6

c)daughter of number 11

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SUMMARY:
For the diagnosis of BLV infections in a dairy herd serological tests and a direct method of virus detection based on PCR were applied.Antibodies to BLV in serum were detected with an indirect ELISA and AGID-test, and BLV antibodies in milk with the same i-ELISA.For direct detection of virus in peripheral blood mononuclear cells a PCR assay was applied. Six out of 20 blood samples were positive in the PCR.The results obtained with the methods correlated to a high degree. Furthermore seropositive newborn calves who had been fed colostrum from infected mothers were found to be virus free. The PCR is thus a useful and rapid method for the viral detection of BLV.

RESUMEN:
Un ELISA indirecto fué aplicado para la detección de anticuerpos contra leukosis bovina (BLV) en sueros y leche de una hato lechero.La técnica de immunodifusión en agar (AGID) fué aplicada también para la detección de anticuerpos en sueros de esos animales.
Para la detección directa de BLV en celulas perifericas mononucleares se aplicó la técnica de "Polymerase Chain Reaction" (PCR).De 20 muestras de sangre seis fureon positivas en PCR.

Terneros recién nacidos sueropositivos a BLV y alimentádos con colostro de madres infectadas, fueron negatios en PCR. Los resultados indicaron una alta corelación entre las técnicas aplicadas.
La técnica de PCR ha demonstrado ser rápida y eficáz para la detección de BLV.

ZUSAMMENFASSUNG:
Um in einem Milchkuhbestand die Infektion mit dem BLV zu diagnostizieren, wurden serologische Teste als auch das PCR-Verfahren angewandt. In den Serumproben wies man Antikörper gegen das BLV im indirekten ELISA und im AGID-Test nach, und in den Milchproben mit gleichem ELISA. Um das Virus in mononukleären Zellen des peripheren Blutes nachzuweisen, wurde das PCR-Verfahren angewandt. Von 20 Blutproben erweisen sich in der PCR sechs als positiv. Die Ergebnisse der verschiedenen Verfahren simmten weitgehend überein. Neugeborene, seropositive Kälber, an die man das Kolostrum infizierter Mütterkühe verfüttert hatte, erweisen sich als virusfrei. Somit ist die PCR ein brauchbares und schnelles Verfahren, um die Infektion mit dem BLV nachzuweisen.

METABOLIC PROFILES IN CALVES: EFFECT OF MEAL.@

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INTRODUCTION

The knowledge of absorption mechanisms is closely connected with the study of blood constituents which are influenced by many factors: age, productive level, genotipe, feed, enviroment, blood drawing time and error during analysis.

The right meaning of blood drawing must be representative of the subject or the group analysed. In order to define reference values it is important to limit the possible causes of variability so to obtain precise and comparable values that make metabolic profile a very important tool in physiological and nutritional studies (1,2,3). Single refernce values can be worth when blood constituents are constant for different factors in different age, however for blood components which do not show stability it is necessary to give values by season diurnal rhythm, breed, stage of growth, age, reproductive status and, for milking cows (4), stage of lactation (5,6,7,8).

The objective of this experiment is to evaluate the influence of the time of drawing (hour), the absorption kinetics, the variability among days that may be see as the precision resulting from repeated series of analyses.

MATERIALS AND METHODS

Animals. Four Friesian calves 12.th weeks old were employed. They were only fed by a commercial milk replacer * (skinned milk 67%) given in two daily meals (at 8 a.m. and 6 p.m.). Blood samples were collected for 6 days from all calves at 8, 9, 10.30, and 12 a.m. and 2, 4, 6 p.m. by punture in the giugular vein using vacutainer tubes.

Part of the blood sample was used to determinate PCV values (centrifuge 12000 round/min x 10') and haemoglobin (method CMHB and B.B.R. reagent). The remaining blood after centrifugation (centrifuge 3000 round/min x 15') was stored at -20° C until analysis. These serum samples were analysed by centrifugal

* Milk replacer composition: crude fat 18%, crude protein 28%, crude fiber 0.2%, ash 6.5%, N-free extract 47.3, moisture 4.5%

microanalyzer (Multistat III - I.L.) in order to determinate the following constituents:

Blood constituent	Method	Producer
Glucose	Trinder	I.L.
Total protein	Biuret	I.L.
Albumine	Green-bromocresol	R.A.I
Urea	Urease-UV	I.L.
Creatininie	Jaffè	I.L.
Cholesterol	Trinder	I.L.
Triglycerides	GPO-Trinder	R.A.I
NEFA	Trinder	Boehringer.
Calcium	Cresoftaleine	I.L.
In .Phosphorus	Phosphomolibdate	I.L.

I.L. Instrument Laboratory - Paderno Dugnano (Milano); R.A.I. Reagents Applications Inc. - San Diego California (USA); Boehringer -Milano.

The analytic control for quality was made by using liofilized human serum Precinorm U (Boehringer.M.). For the analysis of α-aminic-N was employed the Goodwin (9) method with DNFB after deproteination by percloric acid 1 M.

Assay. For the statistic analysis were employed tests of kurtosis and skewness on the collected values and for glucose and NEFA it was necessary to transform the data to obtain a Gaussian distribution. We used the analysis of variance to evaluate the effect of the sampling hour and the day of drawing upon the blood constituents on repeated measures.

Repeatability of blood constituents at different sampling time, of average values in a same day and correlation coefficients between values obtained after 48 and 120 hours were elaborated using StatView II - Abacus Concepts In.- and Pc Machintosh II. Then we used the Precinorm test in order to check the quality and to satisfy the precision exigency of the executed analysis.The average analitic error was estimated by 40 double determinations. Reproducibility is defined as the degree of closeness of a measure to the real value. In Tab.1 it is shown how much the observed values differ from the real value. This variability source must be added the one coming from the inaccuracy of the method adopted which determines a random error when the analysis is repeated on the same specimens.

Table 1: Reproducibility of some analytical methods employed.

Blood constituent	Error (%)*
Glucose	2,3
Total protein	3,7
Albumine	1,7
Urea	1,6
Creatininie	3,7
Cholesterol	3,0
Triglycerides	7,7
Calcium	2,4
In .Phosphorus	2,9

* = $\sqrt{(\Sigma d^2/2n)}$x100/x, where d= difference between duplicate analysis, n= number of duplicate analyses (40)

The precision is so major as minor is the observed variance and is generally expressed as standard deviation term in any analitic procedure executed. If the reproducibility method is inadequate we can improve the technique or repeat the analysis. About the improvement of the technique we can see that any passage or operation brings its error contribution to the total variation of the analysis result. About the repetition of the analysis this is based on the execution of a series of doubled analysis for at least 30 specimens. The precision of a test is generally indicated as "95% limits" resulting from $\mu \pm 2$ S.

$$s = \sqrt{\Sigma d^2 / N}$$

d = difference between duplications
N = n. total determinations

It is customary to express the analitycal error as percentage of the average value of the analysis results. In this way we obtain the C.V. calculated as (10)

C.V. = 100 x (std err / average) %

the 95% limits became ± 2 C.V.

RESULTS AND DISCUSSION

The results of the analysis are shown in Tab. 2 while in Tab.3 we present the ANOVA results. Only glucose, triglycerides, α-aminic-N and NEFA show significant variations due to the hour of sample after meal. The meaning of the first three can be explained by the relation existing between the absorption kinetic of nutrients and the modifications in hematic levels after meal.
For glucose we found increasing values until 2 hours after meal then a decrease within 6 hours, until the values at fast.This observation is explicable with the quick alimentary passage in abomasus. Triglycerides at the beginning of the meal became lower and reach the highest level 8 hours after meal. The reduction after meal is ascribed to the slow fats passage from abomasus to the intestine. This trend agrees with the one found by Toullec (11)while other Authors report sudden changes within 3 hours from meal. The α-aminic-N serum concentration is caracterized by two maximum peaks, the first one 1 hour after meal with 55 mg/L, the second one 8 hours after the meal (57 mg/L) with a minimum value 2.30 hours after meal.

Table 2: Average values for different sampling time (mean and S.E., n=24).

Blood constituents	0	1	2,5	4	6	8	10
PVC (L/L)	29,4 ±0,3	28,6±0,3	28,3±0,3	28,3±0,3	28,7±0,2	28,5±0,3	27,4±0,3
Haemoglobine (g/L)	9,5±0,1	9,4±0,1	9,3±0,1	9,4±0,1	9,3±0,1	9,4±0,	9,3±0,1
Glucose (mmol/L)	1,73±0,03	2,12±0,03	2,09±0,03	1,92±0,03	1,75±0,03	1,78±0,02	1,76±0,02
Total protein (g/L)	62,9±0,7	62,3±0,9	62,9±0,7	60,6±0,7	61,8±0,7	62,5±0,8	63,3±0,8
Albumine (g/L)	35,1±0,3	35,0±0,4	35,2±0,4	34,4±0,4	34,6±0,4	34,8±0,3	34,9±0,5
Urea (mmol/L)	3,88±0,13	3,95±0,11	4,04±0,13	3,83±0,12	3,72±0,10	3,68±0,1	13,82±0,1
α-aminic N (µg/L)	49,7±0,95	53,9±1,3	44,3±1,3	45,9±1,4	51,3±1,4	54,6±2,0	53,3±1,4
Creatininie (mmol/L)	81±3	84±4	83±4	81±3	81±3	80±3	82±4
Cholesterol (mmol/L)	3,30±0,10	3,31±0,10	3,39±0,13	3,26±0,11	3,27±0,12	3,27±0,11	3,35±0,09
Triglycerides (mmol/L)	0,22±0,01	0,17±0,01	0,12±0,01	0,14±0,01	0,24±0,01	0,27±0,02	0,27±0,01
NEFA (mEq/L)	13,0±0,7	8,5±0,6	6,7±0,6	7,1±0,5	10,1±0,5	11,7±0,6	13,1±0,6
Calcium (mmol/L)	2,38±0,04	2,41±0,04	2,51±0,06	2,42±0,04	2,50±0,05	2,40±0,04	2,49±0,04
InPhosphorus (mmol/L)	2,69±0,05	2,70±0,06	2,68±0,06	2,64±0,05	2,66±0,04	2,69±0,05	2,75±0,05

Table 3: Effect of hour, day and interaction AB (ANOVA).

Blood constituent	Hour(A)	Day(B)	AB
PCV	n.s.	***	n.s.
Haemoglobine	n.s.	***	n.s.
Glucose	***	n.s.	*
Total protein	n.s.	n.s.	n.s.
Albumine	n.s.	***	n.s.
Urea	n.s.	***	n.s.
a-aminic N	***	n.s.	n.s.
Creatininie	n.s.	***	n.s.
Cholesterol	n.s.	***	n.s.
Triglycerides	***	**	n.s.
NEFA	***	***	***
Calcium	n.s.	***	n.s.
In.Phosphorus	n.s.	***	n.s.

* p< 0.05 ** p< 0.01

About NEFA we observe a high early concentration followed by a drop until minimum values in coincidence with the drawing made 2.30 hours after meal. Then the values increase to a new peak with the last specimen. The effect of the day of drawing shows significant variations for all haematochimic parameters (Tab.3) except for glucose, total protein and α-aminic-N (12,13,14,15,16,17,18). These significant results underline that uncontrolled circumstances cause fluctuations in haematic parameters of animal groups when they are housed in the same environment.

Table 4: Repeatability in blood constituents.

Blood constituent	Repeatability
PCV	0.56 **
Haemoglobine	0.65 **
Glucose	0.74 **
Total protein	0.93 **
Albumine	0.50 *
Urea	0.30 *
a-aminic N	0.40 *
Creatininie	0.93 **
Cholesterol	0.76 **
Triglycerides	0.60 **
Calcium	0.80 **
In.Phosphorus.	0.41 *

* p< 0.05 p< 0.01

The values for repeatability of the haematic constituents are reported in Tab.4. The significant values suggest to verify if with standard enviroment or feeding their dosage can still give usefull informations about individual methabolic characteristics.
About α-aminic-N, the influence of distance from meal on repeatability in different hours emerges. This parameter with the drawing at fast is 0.00 but increase until 4 hours from meal (0.25),

then decreases again until 0.00 at 10 hours. The repeatability of a character allows
- to evaluate the degree of genetic determination of the character;
- to know the precision increase we can obtain by using multiple measures. For the computation we can use the following formula (10):

$$r = \frac{Vg + Veg}{Vp}$$

where
Vg = genotypic variance
Veg = enviroment-genotypic variance
Vp = phenotypic variance

CONCLUSIONS

We found that some factors connected to the absorption kinetic and particularly for some metabolites, can cause variability of the haematochimic parameters concentration. In order to inprove the estimation of individual values it should be advisable to make several drawings in a short time. Furthermore, expecially in young calves, the absorption kinetics were found linked to the milk somministration.

SUMMARY

Blood constituents levels were studied in four milk replacer fed male Fresian Calves after 1, 2.5, 4, 6, 8, 10 hours after the morning meal. Six complete series of blood specimens were obtained every second of third day. The following parameters were analyzed: PCV, Hb, glucose, triglycerides, cholesterol, NEFA, Total Protein, albumine, urea, creatinine, α-NH_2, Ca and inorganic P. A significant effect of sampling time was observed only for glucose, a-NH_2, triglycerides and NEFA. Day to day variation affected significantly all the blood constituents except glucose and a-NH_2. Individuality (repeatability) of blood constituents levels changes acccording to the distance from the meal, this effect depending on the particular parameter.

SUMARIO

Os niveis dos constituintes do sangue foram estudados em quatro vitelos frisianos machos alimentados com substituintes do laite, depois de 1, 2.5, 4, 6, 8, 10 horas a seguir à refeiçao da manha. Séis séries completas de amostras de sangue foram obtidas em cada segundo ou terceiro dia. Foram analisados os seguintes parametros: Hematocrito, Hemoglobina, Glucose, Trigliceres, Colesterol, NEFA, Proteinas totais, Albumina, Ureia, Creatinina, α-NH_2, Ca e P inorganico. Um efeito significativo de factor tempo foi observado somente para glucose, α-NH_2, trigliceres e NEFA. A variaçao do dia a dia afectou significativamente todos os constituintes do sangue excepto glucose e α-NH_2. A individualidade (possibilidade de repetiçao) dos niveis dos constituintes do sangue muda segundo a distancia da refeiçao, dependendo este resultado do parametro particular.

ZUSAMMENFASSUNG
Bei vier mit Ersatzmilch gefütterten männlichen Friesland-Kalbern wurde das Blutbild 1, 2.5, 4, 6, 8 und 10 Stunden nach der morgendlichen Fütterung untersucht. Jeden zweiten oder dritten Tag wurden jeweils 6 komplette Blutprobeserien hergestellt. Folgende Mebgroben wurden analysiert: Hamatokritwert, Hamoglobin, Glukose, Triglyceride, Cholesterin, unveresterte Fettsauren, Gesanteiweib, Albumin, Harnstoff, Kreatinin, α-NH2, Ca und anorganisches P. Der Zeitabstand hatte nur auf die Werte von Glukose, α-NH2, triglyceride und unveresterte Fettsauren einen nennbaren Einflub. Alle Blibesteandteile zeigtan von einem Tag zum anderen grobe Schwankungen, mit Ausnahme der Glukose und α-NH2 Werte. Das individuelle Blutbild (Wiederholbarkeit) andert sich je nach dem Zeitabstand von der Fütterung; diese Wirkung ist jedoch für die einzelnen Mebwerte unterschiedlich.

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PARAMETROS HEMATOLOGICOS EN HEMBRAS BOVINAS DE RAZA RUBIA GALLEGA.

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INTRODUCCION

Hemos pretendido "conocer" esta raza bovina Rubia Gallega de producción eminentemente cárnica, bajo la visión de su fisiología, mediante la determinación de su perfil hematológico, intentando reflejar en lo posible las variaciones fisiológicas-reproductivas, que se manifestarán durante el periodo de gestación y puerperio.

MATERIAL Y METODOS

Agrupamos 102 hembras Rubia Gallega, inscritas todas ellas en el Libro Genealógico de la raza lo que nos permitió aunarlas en un standard de conformación y producción. Las hembras eran de diferentes edades y número de partos, clínicamente sanas, explotadas en régimen de semiestabulación a excepción de las dos semanas anteriores y posteriores al parto, ya que en este intervalo se las somete a un régimen de estabulación permanente.

La cubrición se realiza por inseminación artificial, desparasitandolas en el mismo acto y confirmando posteriormente el estado de gestación a los 45 días de la inseminación por palpación rectal.

Hemos tomado muestras de sangre con la siguiente pauta de recogida:

1.-La primera toma se realiza en el momento se la cubrición.
2.-Se prosigue la recogida mensualmente hasta el parto.
3.-Después del parto las extracciones se realizan semanalmente, correspondiendo a las cuatro primeras semanas del puerperio.

A partir de las muestras de sangre hemos determinado los siguientes parámetros: Hematocrito, Hemoglobina, Recuento Globular (eritrocitos y leucocitos) y Fórmula leucocitaria.

RESULTADOS Y DISCUSION

Hematocrito

El valor medio hallado a lo largo del muestreo fué de 32,23 ± 2,13 %, este resultado,si lo comparamos con los obtenidos en otras razas autóctonas españolas, es superior al descrito en la Pardo Alpina (9), inferior al de la Retinta Ibérica(2), Asturiana de los Valles (1) y muy similar al de la Blanca Cacereña (8).

El valor procentual inferior lo observamos en el tercer mes de gestación y por el contrario el más elevado lo hallamos en el noveno mes, continuando a partir de este momento un descenso progresivo durante el primer mes del puerperio (7). Gráfica nº1,Tabla 1.

Atribuimos el ascenso de los valores médios de hematocrito antes del parto al stress y como consecuencia del aumento de corticoesteroides en sangre, hecho que presumimos, es producido por el cambio de régimen de estabulación. Podemos asimismo justificar el aumento del hematocrito a lo largo de la gestación al correspondiente incremento de la progesterona, lo que proboca un aumento en la eliminación de sodio y agua.

Hemoglobina

La cifra media de hemoglobina es de 10,89 ± 0,91 g/dl, que resulta ser menor a la descrita en la mayoría de las razas autóctonas españolas, a excepción de la Pardo Alpina aclimatada a la provincia de León (9).

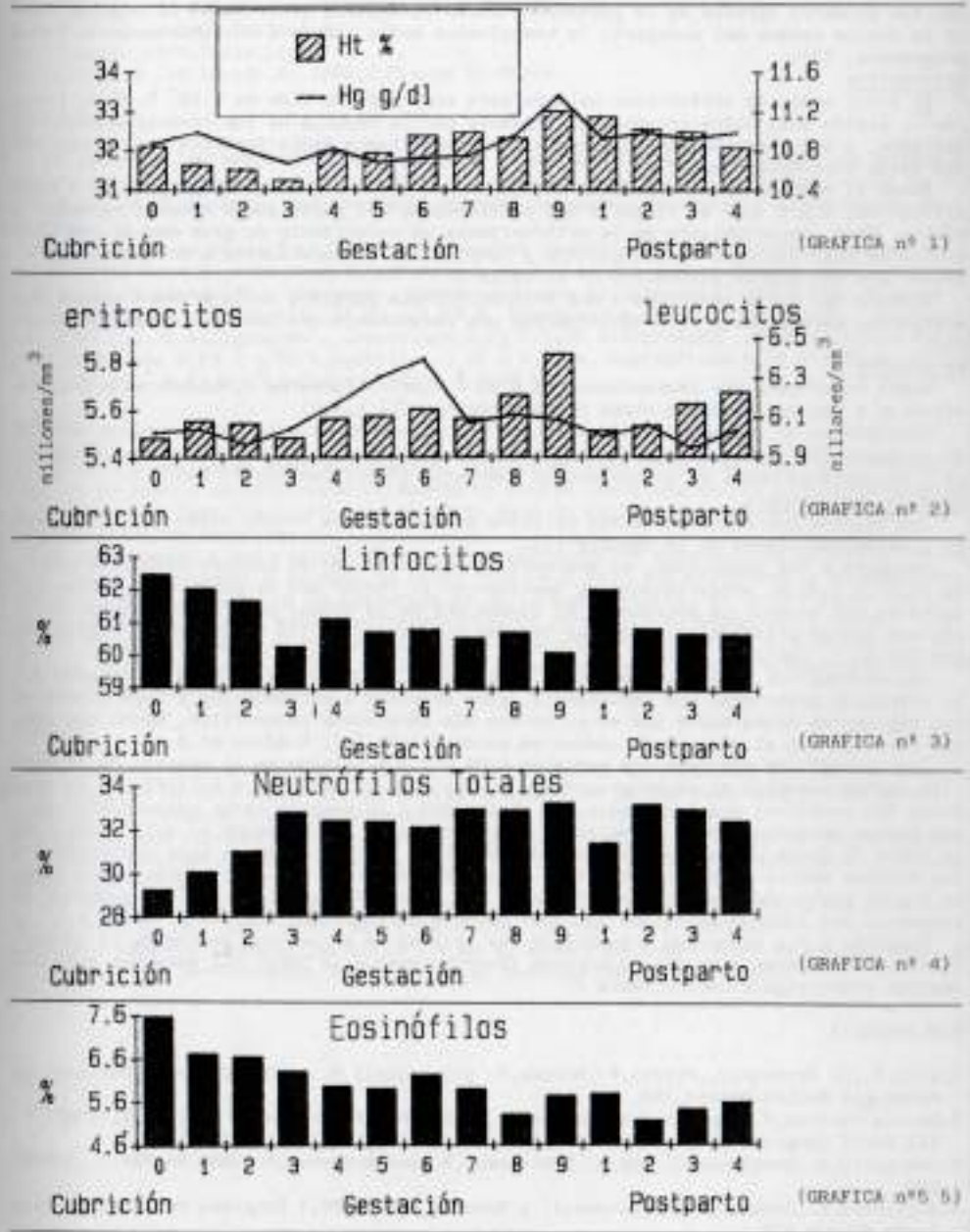
Durante la gestación la hemoglobina presenta valores muy similares, apreciando una elevación desde el septimo mes hasta el parto, donde alcanza los niveles máximos. Después del parto desciende a cifras muy semejantes a las que hemos encontrado durante --

TABLA I. Valores medios obtenidos durante el muestreo de los 102 animales.

		Hematocrito (%)	Hemoglobina (g/dl.)	Eritrocitos (10^6/mm^3)
Cubrición		32,16	10,87	5,49
Mes de gestación	1	31,68	10,99	5,56
	2	31,56	10,81	5,55
	3	31,29	10,67	5,49
	4	32,01	10,82	5,57
	5	31,98	10,68	5,56
	6	32,43	10,73	5,61
	7	32,53	10,75	5,57
	8	32,36	10,95	5,67
	9	33,06	10,36	5,84
Puerperio (semanas)	1	32,92	10,92	5,52
	2	32,61	10,99	5,54
	3	32,54	10,92	5,63
	4	32,13	10,99	5,68
Valor medio		32,23	10,89	5,59

TABLA II. Valores medios obtenidos durante el muestreo de los 102 animales.

		Leucocitos (10^3/mm^3.)	Linfocitos (%)	Monocitos (%)	N.Totales (%)	Eosinófilos (%)	Basófilos (%)
Cubrición		6,03	65,50	0,47	29,31	7,60	0,44
Mes de gestación	1	6,05	62,07	0,53	30,12	6,75	0,43
	2	5,97	61,71	0,50	31,06	6,67	0,43
	3	6,05	60,33	0,61	32,63	6,34	0,47
	4	6,17	61,19	0,60	32,42	5,99	0,27
	5	6,31	60,79	0,44	32,37	5,92	0,39
	6	6,40	60,88	0,52	32,11	6,24	0,38
	7	6,08	60,62	0,46	32,93	5,91	0,32
	8	6,12	60,81	0,52	32,87	5,33	0,28
	9	6,09	60,19	0,49	33,20	5,76	0,35
Puerperio (semanas)	1	6,01	62,11	0,57	31,32	5,80	0,39
	2	6,07	60,94	0,56	33,10	5,18	0,31
	3	5,95	60,78	0,50	32,84	5,44	0,40
	4	6,04	60,76	0,61	32,28	5,61	0,31
Valor media		6,10	61,12	0,53	32,05	6,04	0,37



GRAFICAS 1,2,3,4 y 5.- Evolución de los distintos parámetros hematológicos durante la cubrición (0),gestación (1 al 9) y las cuatro primeras semanas del puerperio.

los dos primeros tercios de la gestación, Tabla 1, Gráfica nº 1. Desde la segunda hasta la cuarta semana del puerperio la hemoglobina media tiende a incrementarse de forma progresiva. (3).

Eritrocitos

El valor medio de eritrocitos hallado para esta raza ha sido de 5,59 ± 0,38 millones /mm³., siendo esta cifra inferior al expuesto por la mayoría de los investigadores consultados, y a su vez también inferior a la eritrocitemia media descrita en las restantes razas autóctonas españolas.

Desde el momento de la cubrición detectamos un ascenso paulatino, progresivo y significativo, sobre todo al llegar a las proximidades del parto donde alcanza su valor máximo. Este comportamiento de la eritrocitemia es coincidente en gran medida con la evolución descrita para la hemoglobina y también con el hematocrito a excepción del primer mes del puerperio.Gráfica nº 2. Tabla 1.

Después del parto encontramos una eritrocitopenia pasajera en la primera semana del puerperio, para producirse a continuación una recuperación en las tres sucesivas semanas.

Leucocitos

Hemos encontrado una leucocitemia de 6,10 ± 1,26 millares/mm³., siendo esta cifra inferior a las que muestran otras razas autóctonas.(1,2,8,9).

Constatamos un incremento del número de leucocitos durante los dos primeros tercios de la gestación hasta alcanzar un máximo en el sexto mes; a partir de este mes disminuye y se mantiene hasta el primer mes de puerperio.(9,10).Gráfica 2. Tabla 2.

Fórmula leucocitaria

Los valores procentuales medios hallados para esta raza bovina están dentro del rango considerado normal en la especie (5).

Respecto a los linfocitos, el procentaje medio obtenido en nuestro estudio a sido de 61,12 ± 5,49 %, produciendose un descenso en el tercer mes de gestación, aunque el descenso más acusado se produce en el noveno mes de la misma, para incrementarse en la primera semana postparto y descender de modo progresivo en las tres semanas siguientes. Gráfica nº 3. Tabla 2.

Los neutrófilos totales alcanzan valores medios de 32,05 ± 6,51 %.En el momento de la cubrición detectamos una neutropenia, para ascender a continuación y ligeramente en los siguientes meses hasta que en el noveno mes apreciamos neutrofilia, hecho que además coincide con el regimen de cambio de estabulación (6). Gráfica nº 3.

Los eosinófilos muestran una media de 6,04 ± 3,3 %, siendo en el momento de la cubrición cuando muestran el mayor procentaje medio, que no volvemos a hallarlo en el transcurso del muestreo, y que por ello, lo atribuimos a la posible carga parasitaria que aun pueden presentar estos animales al no haber pasado posiblemente el suficiente tiempo entre la desparasitación y la toma de muestras. Posteriormente a esta eosinofilia los niveles medios se mantienen y tras descender, alcanzan el nivel mínimo en el octavo mes de gestación y haciendose patente en las proximidades del parto (6), pudiendo imputarlo a una intensa actividad muscular durante este periodo.Gráfica 4. Tabla 2.

Respecto a los monocitos y basófilos sus valores se encuentran dentro de la normalidad para la especie bovina, mostrando fluctuaciones a lo largo del muestreo pero sin mostrar clara significación.Tabla 2.

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RESUMEN

Se estudian 102 hembras bovinas sanas de raza Rubia Gallega, de diferentes edades y número de partos, todas ellas inscritas en el Libro Genealógico de la Asociación de Criadores de dicha raza, explotadas en régimen de semiestabulación y alimentación controlada.

El muestreo se realizó en la zona peninsular española donde se encuentra el mayor censo de hembras de esta raza.A cada animal se le recogió sangre durante distintos momentos de la gestación y puerperio, comprobando que el "standard" medio hematológico es el siguiente: Hematocrito 32,23 ± 2,13 %, Hemoglobina 10,89 ± 0,91 mg/dl., Eritrocitos 5,59 ± 0,38 millones/mm³.,Leucocitos 6,10 ± 1,26 millares/mm³.,Fórmula leucocitaria: Monocitos 0,53 ± 0,60 %,Basófilos 0,37 ± 0,52 %, Eosinófilos 6,04 ± 3,30 %, Neutrófilos 32,05 ± 6,51 % y Linfocitos 61,12 ± 5,49 %.

RÉSUMÉ

On a fait une étude avec 102 femelles bovines de race "Rubia Gallega" de différents ages et de nombre de différents vêlements et toutes inscrites sur le Livre Généalogique de la race.Ces femelles bournes se trouvent dans un régimen de semistabulation et avec une alimentation controlée.

Le prelévesent a été réalisé dans la zone peninsulaire espagnole où se trouve le plus grand recensement d'animaux de cette race.Ou a fait des prelévements de sang pendant différents moments de la gestation et de la puerpéralité, et au analysant les paramétres hématologiques suivants: Hématocrite 32,23 ± 2,13 %, Hémoglobine 10,89 ± 0,91 mg/dl.,Eritrocites 5,59 ± 0,38 millions/mm³.,Leucocites 6,10 ± 1,26 milliers/mm³., et formule leucocytarie: Monocites 0,53 ± 0,60 %,Basophiles0,37 ± 0,52 %,Eosinophiles 6,40 ± 3,30 %,Neurophiles 32,05 ± 6,51 et Lymphocites 61,12 ± 5,49 %.

SUMMARY

102 healthy bovine females of "Rubia Gallega" breed, of different age and deliveries were analyzed in this work. All of them were inscribed in the Hear Book of the Breed. These bovine females live semi-intensive and on controlled feeding.

Sampling was carried out in a spanish area where most of the breed is concentrated.

Several samples of blood were taken for each animal during different gestation and postpartum periods.

The haematic parameters were established as follows: Haematocrit 32,23 ± 2,13 % ---, Haemoglobin 10,89 ± 0,91 g/dl, RBC 5,59 ± 0,38 10^6 /mm³ , WBC 6,10 ± 1,26 10^3/mm, Leukocyte Formula: Lymphocytes 61,12 ± 5,49 %, Monocytes 0,53 ± 0,60 %, Basophyles 0,37 ± 0,52 %, Total Neutrophyles 32,05 ± 6,51 %, Eosinophiles 6,40 ± 5,49%.

PERFIL METABOLICO E IONOGRAMA SERICO EN HEMBRAS BOVINAS DE RAZA RUBIA GALLEGA.

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INTRODUCCION

Este estudio va encaminado al conocimiento de las variaciones fisiológicas-reproductivas de los diferentes parámetros séricos que hemos determinado durante el periodo de gestación y el primer mes de puerperio, conformando así el metabolismo energético, mineral y electrolítico de la raza bovina Rubia Gallega, y evaluando en lo posible la incidencia que representa la sobrecarga del feto en el metabolismo de la madre.

MATERIAL Y METODOS

Para este trabajo hemos elegido 102 hembras de raza Rubia Gallega de diferentes edades y número de partos, inscritas todas ellas en el Libro Genealógico de la raza, para así poder sunarlas en un standard de conformación y producción.

Estas hembras bovinas de producción cárnica son explotadas en regimen de semiestabulación, a excepción de las dos semanas anteriores y posteriores al parto, que pasan a estabulación permanente.La alimentación es a base de pasto suplementado con ensilado de maiz y/o hierba según la época.

Se seleccionó a los animales en periodo láctco nulo y que no presentaran ningun proceso patológico.La cubrición fué por inseminación artificial, confirmando la gestación por palpación rectal a los 45 días.

Hemos tomado muestras de sangre para la obtención se suero, en el momento de la cubrición, mensualmente hasta el parto y semanalmente durante el primer mes de puerperio.

A partir del suero de los animales muestreados hemos valorado los siguientes parámetros: Glucosa,lípidos totales, colesterol, bilirrubina total y directa, urea, actividad enzimática relativa a ASAT, ALAT y AP, y los niveles de cálcio,fósforo inorgánico, magnesio, sodio y potasio.

RESULTADOS Y DISCUSION

Glucosa

El valor medio obtenido es de 64,48 ± 10,28 mg/dl., cifra que se encuentra dentro de los rangos normales de la especie (13). La glucemia de esta raza es similar o ligeramente superior a la de las restantes razas autóctonas españolas (Avileña, Retinta Ibérica, Asturiana de los Valles, Blanca Cacereña).

Aunque sabemos que son muchos los factores que condicionan la glucemia, en nuestro caso, no es posible estudiar la influencia de estos al tratarse de un grupo homogéneo, por lo que nos hemos limitado a estudiar la posible diferencia que pueda acontecer en los periodos de gestación y primer mes de puerperio.Tabla I.

Como podemos observar en la gráfica nº 1, el comportamiento de la glucemia es estable a lo largo de la gestación, aunque hemos constatado un descenso no significativo en el noveno mes (7,10).

Lípidos totales

La lipedemia media para esta raza es de 681,01 ± 136,04 mg/dl., muy semejante a la que muestran el resto de las razas autóctonas del país.Durante la gestación la lipidemia se mantiene, decreciendo en las proximidades del parto, esta disminución y posterior recuperación al principio de la lactación ha sido descrita por la mayoria de los investigadores (2,21). Consideramos que la caida brusca de las concentraciones de lípidos totales en las proximidades del parto junto con la colesterolemia y a su vez con con la elevación de la glucemia es fisiológico, ya que el comportamiento de este parámetro está ligado al balance energético negativo y al aumento de glucocorticoides que se produce en el stress.Tabla I.

Colesterol

La media de colesterol alcanza los valores de 149,44 ± 31,28 mg/dl., los valores mínimos y máximos presentan una evolución parecida a la de la lipidemia (Tabla I), llegando a coincidir desde los dos últimos meses de gestación hasta la tercera semana posterior al parto. La evolución al final de la gestación y en periodo peripártico está justificado por la lipomovilización que se produce durante este periodo (2).El descenso que se produce en la primera semana tras el parto es muy significativo, coincidiendo esto con lo observado por NAGY (15), que lo considera consecuencia del incremento de los niveles de ácidos grasos no esterificados.

Bilirrubina total y directa

Hemos hallado valores de 0,449 ± 0,152 mg/dl. y de 0,239 ± 0,072 mg/dl. para la bilirrubina total y directa respectivamente, cifras inferiores a los obtenidos en la mayoria de nuestras razas autóctonas, aunque ligeramente superiores a los expuestos por otros investigadores (1,14).

Durante los seis primeros meses ambas bilirrubinas presentan oscilaciones en sus concentraciones, mientras que en el último mes de gestación se produce un incremento evidente. Tras el parto observamos un descenso muy significativo que se mantiene hasta la tercera semana, donde comienza su recuperación (2).Tabla I. Conjuntamente con la elevación de las bilirrubinas al final de la gestación, existe un claro incremento de ASAT y de colesterol, situación descrita por BENEDITO (2), pero sin observar a su vez la elevación de la fosfatasa alcalina como indica MCSHERRY (14).

Urea

El valor medio hallado para este parámetro es el de 23,09 ± 7,6 mg/dl..Su evolución refleja un ascenso progresivo desde la cubrición hasta el septimo mes de gestación, que atribuimos al incremento de la ingesta y metabolismo, ya que el mayor ingreso de proteinas conduce al incremento de amoniaco ruminal, lo que supone una elevación de la uremia sérica (17).Tabla I.

Hemos obtenido dos descensos, el primero desde el octavo al noveno mes y lo atribuimos al gran volumen fetal, el cual va a restar espacio abdominal a la madre, limitando así el volumen de ingesta; el segundo descenso se aprecia en la primera semana postparto debido posiblemente a la formación de proteinas de la leche (8).

Aspartato aminotransferasa (ASAT)

La actividad media en esta raza es de 43,67 ± 13,46 UI/l..Encontramos un incremento muy significativo de esta enzima durante la gestación, aunque más notoriamente desde el quinto mes hasta el final, donde alcanza sus valores máximos.Tabla I..En la Primera semana de puerperio desciende la actividad media hasta alcanzar el mínimo en la cuarta semana del mismo (8,14).

Alanina aminotransferasa (ALAT)

En su estudio hemos obtenido una media de 14,13 ± 5,06 UI/l., encontrando la media máxima en los dos primeros tercios de la gestación, produciendose las mínimas en el último tercio.En el postparto los niveles se mantienen para descender a partir de la tercera semana, estabilizandose a continuación(9).Tabla I.

Fosfatasa alcalina (AP)

Esta enzima muestra un valor medio de 25,24 ± 11,22 U/l., aunque hemos observado un incremento de su actividad durante la gestación, sin poder establecer una relación concreta con cada una de los meses de la misma.Su elevación la detectamos ya desde la cubrición hasta el octavo mes de gestación (6,7).Transcurrido el parto se produce un descenso que continua hasta el dia ventiocho del puerperio.Tabla I.

Ionograma sérico:Calcio

La calcemia que hemos obtenido es de 9,12 ± 0,91 mg/dl., comprobando un descenso de sus niveles a lo largo de la segunda mitad de la gestación,pudiendo atribuir este hecho a la demanda de este mineral por parte del feto (3).Tabla II.Aunque el máximo descenso que detectamos y que consideramos fisiológico se produce en los momentos peripartales.

Después del parto se produce un incremento de los niveles medios del calcio sérico, pasando de 8,95 mg/l. en la primera semana a 9,26 mg/l. en la segunda (7,9).

Fósforo inorgánico

Los animales muestran una fosfatemia media de 5,16 ± 0,74 mg/dl..Al estudiar su comportamiento detectamos dos momentos en los que la fosfatemia desciende de forma apreciable, así el primer descenso ocurre en el tercer mes de gestación y el segundo lo cons-

TABLA I. Valores medios de los diferentes parámetros séricos delos IC2 animales muestreados.

		Glucosa (mg/dl.)	Lípidos T. (mg/dl.)	Colesterol (mg/dl.)	BT (mg/dl.)	BD (mg/dl.)	Urea (mg/dl.)	ASAT (UI/l.)	ALAT (UI/l.)	AP (U/l.)
Cubrición		62,99	684,84	147,61	0,45	0,23	19,00	32,38	13,83	26,95
Mes de gestación	1	63,66	669,67	152,12	0,43	0,21	19,79	32,71	16,78	23,84
	2	64,23	689,13	162,88	0,44	0,24	22,20	30,68	15,70	27,53
	3	63,96	689,68	153,43	0,44	0,24	23,24	34,76	16,95	25,99
	4	65,99	666,58	142,01	0,43	0,22	23,09	38,68	16,36	27,43
	5	66,87	685,53	153,86	0,43	0,23	24,02	39,26	15,72	26,84
	6	64,31	701,89	156,00	0,43	0,21	23,73	43,20	14,41	25,75
	7	65,30	705,56	155,95	0,46	0,24	26,16	47,70	13,24	25,04
	8	64,97	706,16	154,16	0,46	0,26	25,43	56,63	11,48	26,79
	9	61,52	673,90	142,66	0,51	0,29	25,89	65,16	11,68	24,66
Puerperio (semanas)	1	65,60	627,24	134,15	0,44	0,24	22,53	51,37	11,44	23,61
	2	66,08	661,94	143,52	0,43	0,23	22,94	50,96	13,22	22,42
	3	63,84	671,81	149,02	0,41	0,20	22,37	46,00	13,82	23,77
	4	63,45	700,20	144,72	0,45	0,24	22,92	41,82	13,23	22,47
Valor medio		64,48	681,01	149,73	0,44	0,23	23,09	43,67	14,13	25,24

BT.-Bilirrubina total BD.-Bilirrubina directa ASAT.-Aspartato aminotransferasa ALAT.-Alanino aminotransferasa
AP.- Fosfatasa alcalina

TABLA II. Valor medio del lonograma sérico durante la gestación y primer mes de puerperio de las IC2 hembras bovinas muestreadas.

		Calcio (mg/dl.)	Fósforo inorgánico (mg/dl.)	Magnesio (mg/dl.)	Sodio (meq/l.)	Potasio (meq/l.)
Cubrición		9,24	5,17	2,77	142,96	4,72
Mes de gestación	1	9,48	5,47	2,86	141,49	4,70
	2	9,15	5,29	2,86	142,73	4,75
	3	9,14	5,20	2,61	143,53	4,66
	4	9,44	5,39	2,55	142,95	4,70
	5	9,78	5,40	2,50	143,47	4,78
	6	9,09	5,43	2,58	143,73	4,75
	7	9,12	5,25	2,69	142,54	4,62
	8	9,06	5,12	2,62	144,86	4,73
	9	8,72	4,62	2,58	144,35	4,79
Puerperio (semanas)	1	8,95	4,81	2,59	144,61	4,89
	2	8,94	4,86	2,60	144,80	4,76
	3	9,27	5,11	2,70	143,91	4,73
	4	9,28	5,16	2,82	145,76	4,76
Valor medio		9,12	5,16	2,67	143,71	4,74

tatamos al final de la misma, aunque debemos indicar que su descenso ya lo apreciamos a partir del sexto mes de gestación. Despues del parto y casi de forma inmediata se produce la recuperación de sus niveles, llegando a alcanzar valor similar al obtenido en el momento de la cubrición (5,13).Tabla II.

El hecho de que tanto los valores de calcio como de fósforo inorgánico sérico sean normales, nos explica el que en estas hembras no se hallan producido durante nuestra experiencia desequilibrios minerales.

Magnesio

El valor medio para el magnesio en estas hembras es de 2,67 ± 0,38 mg/dl..Durante su evolución alcanza el máximo nivel en los dos últimos meses de gestación, seguidamente sufre un descenso paulatino - salvo en el séptimo mes - hasta llegar al final de la gestación (11).

Tras el parto la magnesemia media manifiesta una tendencia a su recuperación de forma lenta a lo largo de las dos primeras semanas de lactación (16). Tabla II.

Sodio

Hemos encontrado un valor medio a lo largo de la experiencia de 143,71 ± 5,10 meq/l. observando los mínimos y máximos valores en el primer mes de gestación y a los veintiocho dias de la lactación respectivamente. Tabla II.

El comportamiento de la natremia durante la gestación es fluctuante, con oscilaciones muy significativas entre sus valores medios, si bien existe una cierta tendencia a incrementarse desde el octavo mes de gestación hasta el final del primer mes del puerperio, aunque se observó una ligera inflexión en la tercera semana del mismo.(12).

Potasio

La kalemia media es de 4,74 ± 0,5 meq/l..Tambien el comportamiento del potasio sérico es alternativo, el incremento de la kalemia se produce de forma constante y rápida a partir del séptimo mes de gestación, para alcanzar sus valores máximos en la primera semana postparto, descendiendo a continuación de forma brusca durante la segunda y tercera semana del mismo (12).Como sabemos el comportamiento del potasio a nivel sanguineo está controlado fundamentalmente por la corteza adrenal, lo que implica un incremento de este parámetro durante el stress y justifica,por lo tanto, nuestros resultados.Tabla II.

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RESUMEN

Se estudian 102 hembras bovinas sanas de raza Rubia Gallega, de diferentes edades y número de partos, todas ellas inscritas en el Libro Genealógico de la asociación de Criadores de dicha raza, explotadas en régimen de semiestabulación y alimentación controlada.

A cada animal se le recogió sangre durante distintos momentos de la gestación y puerperio, comprobando que el "standard" medio relativo al perfil metabólico y al ionograma es el siguiente:Glucosa 64,48 ± 16,28 mg/dl.,Lípidos totales 681,01 ± 136,04 mg/l., Colesterol 149,44 ± 31,28 mg/dl.,Bilirrubina total 0,44 ± 0,15 mg/dl., Bilirrubina directa 0,23 ± 0,07 mg/dl.,Urea 23,09 ± 7,60 mg/dl.,ASAT 43,67 ± 13,46 UI/l., ALAT 14,13 ± 5,06 UI/l., AP 25,24 ± 11,22 UI/l., Calcio 9,12 ± 0,91 mg/dl.,Fósforo 5,16 ± 0,74 mg/dl., Magnesio 2,67 ± 0,38 mg/dl., Sodio 143,71 ± 5,10 meq/ 1. y Potasio 4,74 ± 0,50 — meq/l.

RÉSUMÉ

On a fait un étude avec 102 femalles bovines de race "Rubia Gallega" de différents ages et le nombre de différents vêlesenta, et toutes inscrites sur le Livre Généalogique de la race.Ces femelles bovines se trouvent dans un régimen de semistabulation et avec une alimentation controlée.

On a fait des prelévesents de sang à chaque animal, à différents xoments de la gestation et pendant les quatre premières semaines après levèlement.Nous avons détereminé les paramètres suivants: Glucose 64 ± 16,28 mg/dl.,Lipides totaux 681,01 ± 136,04 mg/dl Cholestérol 149,44 ± 31,28 mg/dl.,Bilirrubine total 0,44 ± 0,15 mg/dl.,Bilirrubine directe 0,23 ± 0,07 mg/dl.,Urée 23,09 ± 7,60 mg/dl.,ASAT 43,67 ± 13,46 UL/l.,ALAT 14,13 ± 5,06 UL/l.,AP 25,24 ± 11,22 UI/l.,Calcio 9,12 ± 0,91 mg/dl.,Phosphore5,16 ± 0,74 mg/dl. Magnésium 2,67 ± 0,38 mg/dl. Sodium 143,71 ± 5,10 meq/l. et Potassium 4,74 ± 0,50 meq/l.

SUMMARY

102 healthy bovine females of "Rubia Gallega" breed of different age and deliveries were analyzed in this study. All of them were inscribed in the Hear Book of the Breed.

These bovine females live semi-intensive and on controlled feeding.

Samples of blood were taken from each animal in differents moments of both gestation and postpartum periods.

Several parameters have been studied: Glucose 64 ± 16,28 mg/dl, Total lipids 681,01 ± 136,04 mg/dl, Cholesterol 149,44 ± 31,28 mg/dl, Total Bilirrubin 0,44 ± 0,15 mg/dl, - Direct Bilirrubin 0,23 ± 0,07 mg/dl, Urea 23,09 ± 7,60 mg/dl, ASAT 43,67 ± 13,46 UI/l , ALAT 14,13 ± 5,06 UI/l, AP 25,24 ± 11,22 U/l, Calcium 9,12 ± 0,91 mg/dl, Inorganic Phosphorus 5,16 ± 0,74 mg/dl, Magnesium 2,67 ± 0,38 mg/dl, Sodium 143,71 ± 5,10 meq/l and— Potasium 4,74 ± 0,50 meq/l.

Utility of EEG Application in Calf

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INTRODUCTION

One of obstacles for EEG application in large domestic animals is due to hyperactive movement of animal. However, it is how shown that good EEG recording can be obtained from calf without anesthetization (1, 2).

The objects of the present investigation are to examine extensively the applying of EEG in calf. At first, using EEG, I have tried to detect the calves which are injured or sick in the central nervous system. On the other hand, attempts to evaluate the effect of new sedative (Mafoprazine mesylate, a new phenylpiperazine derivative, Tanabe) using EEG were carried out.

MATERIALS AND METHODS

Japanese black calves and F$_1$ hybrids, both females and males, aged one day to 4 months after birth were used. They were divided into two groups (A and B). Group A (Japanese black calf) includes appearently those which are injured or sick in the central nervous system. Group B (F$_1$ hybrids) is healthy animal. EEG was recorded by unipolar and bipolar leading methods as previously described (1).

Mafoprazine mesylate was to be given intramusclarly in a dose 0.1~0.5 mg/kg at a each animal (Group B).

Analysis of EEG recording concerning frequency was made using a computer program (Fast Fourier Transform). The flash light was delivered at a frequency of one flash per second. The evoked responses which had been stored on tape were subsequently averaged with signal processor (Nihon denki Sanei) using a 250 msec sweep duration.

RESULTS AND DISCUSSTION

Group A : EEG pattern was often, but not always, abnormal. In case of complete hydranencephaly, EEG pattern became flat.

Group B : If the administration of mafoprazine is less than 0.2 mg/kg, these compounds are shown to be ineffective on the sedative effect. However, administration of 0.3 mg/kg or above produced sedative effect. After administration of mafoprazine 0.3 mg or above, at about 10 minutes, animal was completely undisturbed for 40-50 minutes. EEG wave usually became high amplitude and slow wave (Fig. 1). Fig. 1 illustrates a result in which mafoprazine treatment resulted in an increase of low frequency component. There was no significant difference on the visual-evoked potential between before and after the administration of mafoprazine.

The presented results suggest that EEG is very useful for the diagnosis of abnormality in central nervous system and judgement of sedative effect in calf.

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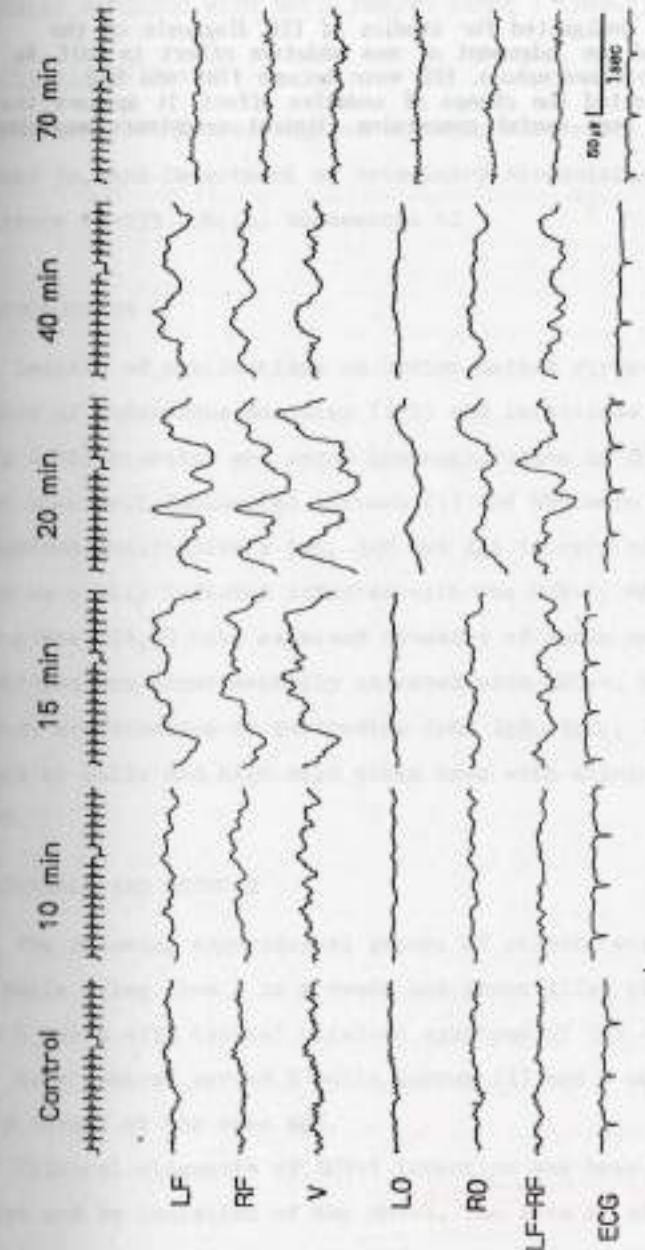

Figure 1 EEG recording after administration of mafoprazine in the calf. Administration of mafoprazine would elicit high amplitude activity.

SUMMARY

A series of experiment designated for studies of EEG diagnosis on the central nervous system and the judgement of new sedative effect in calf. As the results, in case of hydranencephaly, EEG wave became flat and EEG recording sufficietly reflected the change of sedative effect. It appears that EEG recording in calf is very useful concerning clinical veterinary medicine.

THE PATTERN OF CLASSES OF IMMUNOGLOBULINS IN SERA OF BULLS AND COWS NATURALLY INFECTED WITH BOVID HERPES VIRUS 1 (BHV-1)

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INTRODUCTION

Despite of publications on bovine herpes virus-1 (BHV-1) and pathogenesis of infectious pustular (IPV) and infectious pustulsr balanoposthitis (IPB) kinetics and serum immunoglobulins in BHV-1 infected cows has not been well documented. Straub (1) and Whitmore and Archbald (2) have examined qualitatively IgG, IgM and IgA in sera of cattle experimentally and naturally infected infected with the BHV-1. Moreover, Guy (3) and Potgieter (4,5) have examined dynamics of serum immunoglobulins in pregnant heifers experimentally infected with BHV-1. The objectives of the study are kinetics of antibodies from IgG, IgG_1, IgG_2, IgM and IgA in sera of bulls and high milk yield cows with clinical signs of IPV and IPB.

MATERIALS AND METHODS

The folowing experimental groups of animals were examined: group I - 5 bulls aging from 2 to 4 years and group III-5 cows at the age from 4 to 6 years with typical clinical symptoms of IPV - IPB.

As a control served 5 bulls (group II) and 5 cows (group IV) clinically normal at the same age.

Clinical diagnosis of BHV-1 infection has been confirmed at the SN test and by isolation of the BHV-1. The sera of sick animals reacted positively in the SN test with BHV-1 at a titre 1:2 or higher. Bulls and

cows of group II and IV were free of antibodies against VDMD virus, PI-3 virus, Adenovirus serotype 2, RSV and BLV. Microbiological investigations of preputial and vaginal swabs against infections caused by Chlamydia spp., Ureaplasma spp., Mycoplasma spp. and pathogenic aerobic bacteria were negative.

Clinical observations, virological examinations (isolation of BHV-1) and titre of serum specific antibodies and concentration of serum immunoglobulins by the use of standard plate method (Miles) were done in group I and III periodically from 1-84 days and in groups II and IV from the days 1 to 238 since the appearance of clinical symptoms of IPV and IPB. The results of immunological tests presented in Table 1 and 2 are statistically elaborated by the Student-t test at p-0,05.

RESULTS

It was found that clinical symptoms of IPB persisted for 84 days and IPV persisted for 182 days. The BHV-1 was isolated from preputial swabs of all bulls and from vaginal swabs of majority of cows only in the first period of the disease. In further stages of the disease, the BHV-1 was isolated only occasionally and mainly from cows. All sick bulls (group I) reacted positively in the SN test at the titre above 1:2 for BHV-1 between 1-84 days (a maximal titre 1:32 was noted in 2 bulls at the day 84 of examination). Almost all sick cows were positive for BHV-1 for 238 days of examination (a maximal titre 1:32 was noted in one cow on the day 154). Serum IgG, IgG_1 and IgA in sick bulls increased at 14-28, 1-84 and 1-56 days of examination, respectively, whereas the level of IgM lowered at the day 1-28, 56, 84 and IgG_2 at the day 14-84. In sick cows the level of serum IgG increased at the day 1-126, IgG_1 1-21 and IgG_2 98-128, whereas serum IgM and IgA lowered at the day 7-126 and 1-154, respectively.

Table 1. The level of classes of immunoglobulins in sera of bulls infected naturally with BHV-1 (group I) and normal bulls (group III)

Day			1			14			28			56			84		
Group			I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
Titre in the SN test			1:4 (4) 1:2 (1)		<1:2 (5)	1:4 (4) 1:2 (1)		<1:2 (5)	1:8 (3) 1:2 (2)		<1:2 (5)	1:8 (3) 1:2 (2)		<1:2 (5)	1:32 (1) 1:8 (3)		<1:2 (5)
G	$\bar{x}$		27,0		23,0	34,0*		24,0	32,0*		24,0	23,0		25,0	26,0		28,0
	min		23,0		21,0	32,0		22,5	30,0		22,5	20,0		23,0	24,0		27,0
	max		29,0		24,0	36,5		25,5	36,0		26,5	26,0		27,0	28,0		29,5
G_1	$\bar{x}$		15,5*		6,2	16,0*		6,8	15,9*		7,2	14,4*		8,1	16,4*		7,5
	min		13,2		4,8	14,2		5,2	14,1		6,5	12,4		6,8	13,8		6,5
	max		18,5		6,9	17,3		7,6	16,3		8,2	16,1		9,1	19,1		8,5
G_2	$\bar{x}$		6,1		6,6	5,4		8,5*	7,8		8,9*	7,2		8,9*	7,0		10,0*
	min		5,8		5,9	4,5		7,5	6,8		7,9	6,8		8,3	6,8		8,7
	max		6,8		7,2	6,8		9,1	8,1		9,3	7,8		9,1	7,4		12,4
M	$\bar{x}$		2,9		4,2*	3,4		3,5	3,0		6,2*	2,8		4,2*	3,8		5,6*
	min		1,9		3,8	2,0		3,0	2,8		4,6	2,8		3,8	2,9		4,9
	max		3,5		4,9	4,1		3,9	4,1		7,8	3,2		4,9	4,1		7,8
A	$\bar{x}$		0,51*		0,36	1,20*		0,20	0,88*		0,63	0,93*		0,61	0,86		0,68
	min		0,45		0,25	1,10		0,15	0,80		0,60	0,80		0,60	0,80		0,65
	max		0,55		0,45	1,30		0,25	0,90		0,80	1,11		0,70	0,95		0,85

Immunoglobulins (g/l)

* significantly numbers of animals in groups I and III

Table 2. The concentration of classes of immunoglobulins in sera of cows naturally infected with BHV-1 (group II) and healthy control cows (group IV)

Day		1		7		14		21		28		35		42		70		98		126		154		182		210		238	
Group		II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV	II	IV
Titre in the SN test		4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8	4.8	7.8
		(1)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(1)	(0)	(1)	(0)	(1)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)	(2)	(0)
		4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
		(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
		2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
		(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)	(3)	(2)
		1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
		(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)	(5)	(3)
		0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
		(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)
Immunoglobulins (g/l)																													
G	$\bar{x}$	210	1400	210	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400	335	1400
	min	23	20	25	18	25	18	23	18	22	18	25	18	24	18	25	18	24	18	24	18	24	18	20	18	20	18	20	18
	max	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25	30	25
G_1	$\bar{x}$	185	1150	180	1150	180	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150	96	1150
	min	42.5	86	60	86	60	86	45	86	45	86	45	86	45	86	45	86	45	86	45	86	45	86	45	86	45	86	45	86
	max	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215	215
G_2	$\bar{x}$	80	80	80	80	56	50	84	96	66	66	69	64	60	52	105	100	105	80	120	105	125	90	80	60	46	45	92	80
	min	60	7.8	9.2	9.5	6.4	6.4	7.4	9.6	6.2	5.6	5.9	5.3	5.5	6.3	6.3	4.3	6.9	9.8	7.3	6.0	6.9	4.3	5.4	4.5	5.4	4.7	3.5	
	max	9.2	22	10	11	6.2	6.2	9.8	8.9	8.9	8.9	7.3	6.4	3.6	6.3	11	9.2	11	11	12	11	11	9.2	9.8	7.4	8.9	7.2	9.8	8.5
M	$\bar{x}$	34	34	21	33	36	52	24	34	32	52	33	37	27	40	24	48	28	37	28	43	26	26	36	27	33	38	26	27
	min	26	23	14	18	26	18	19	26	18	18	18	22	18	22	20	19	26	33	22	41	22	21	33	21	32	31	33	23
	max	41	43	27	47	43	43	31	41	43	62	43	52	32	61	32	65	32	40	43	52	29	29	41	33	40	43	43	32
A	$\bar{x}$	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410	82	410
	min	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63
	max	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000	230	1000

x - significant differences, numbers in parentheses denot numbers of animals examined

DISCUSSION

Natural infection with the IBR/IPV induces in milking cows a profound quantitative changes in serum immunoglobulins. Their character is similar with those observed in heifers and cows for IgG and IgM by Straub(1) and Guy et al., (4) for IgG_1 and IgG_2 by Guy et al., (4) and for IgG and IgA by Whitemore and Archbald (2). It is worthy to note that the specific antibodies for BHV-1 active in the SN test irrespectively of primary and secondary immune response do not belong mainly to IgG, IgM and IgA as it has been suggested earlier (1,2,6,7). It was found later that they belong to IgG and IgG_1, IgG_2 or/and IgG_{2b}, and sporadically to IgA. Keutz(4) suggests that antibodies for BHV-1 active in the SN test belong to IgM whereas those active in the ELISA test belong to IgG. The observed decrease in IgM and IgA in sera of cows infected with BHV-1 is most probably the result of supressive action of the virus. Whitmore and Archbald (2) have noted a decrease of serum IgM and partly IgA in cows naturally infected with BHV-1.

The changes in immunoglobulins pattern in bulls in the course of IPB can not be interpeted in the same manner as in IPV cows. In sick bulls the concentration of serum IgA increased and serum IgM lowered in contrast to the behaviour of these two classes of immunoglobulins in sera of cows. One can assume that duration IBR/IPV infection or sex and age of animals and hence their physiologocal state affects such a specific bahaviour of IgA and IgM in sick bulls and in sick cows. It is well known that immune reactivity and secretion of immunocospetent cells is affected also by sex hormone especially by androgens and estrogens.

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SUMMARY

Natural course of IPB in bulls and IPV in cows induces a characteristic increase of the content of serum IgG and IgG_1 and decrease of serum IgM sugesting that specific antibodies for BHV-1 occur mainly in these classes of immunoglobulins. The IgA and IgM differ in their dynamics in sick bulls and cows.

RESUME

Les recherches prouvent que pendant l'infection pustuleuse vulvo-vaginale et balano-postithe des bovins (pendant la maladie des bovins nommee comme...), independant de sexe, apparait une grande croissance (caracteristique au fil du temps) de IgG, IgG_1 et L'abaissement de IgM. Cela indiquerait que les anticorps BHV-1 se trouvett surtout a l'interieur de ces albumines. Ig de la sous-classe G_2 et de la classe A montre une sutre dynamique des changements chez les taureaux et les vaches atteins de cette maladie.

ZUSAMMENFASSUNG

Die Untersuchungen ergaben, dass im Laufe der bei den Vieh bezeichneten Krankheit, als Krankheit der Geschlechtsorgenen, unabhängig vom Geschlecht, kommt es zum grossen, charakteristischen in der Zeit des Wachsens IgG, IgG_1 und ebenfalls eine Senkung IgM, was nachweist, dass dei Antikörper Anti BHV-I sich hauptsächlich im Bereich dieser Weisskörper befinden. Ig Unterklassen G_2 und Klassen A, erweisen bei den Geschlechtskranken Bullen und Kühen verschiedene Änderungen der Dynamik.

STUDIES ON T AND B LYMPHOCYTE MARKERS IN FOUR TYPES OF BOVINE LYMPHOSARCOMA

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INTRODUCTION

Bovine leukosis is classified as enzootic bovine leukosis (EBL) and sporadic bovine leukosis (SBL), which is subclassified as skin (SLS), calf (CLS) and thymic (TLS) forms (2). The authors have recently determined what percentage of tumor cells from EBL cattle have surface immunoglobulins (SIg) (3,5). Tumor cells from many animals were negative for SIg, and there were only a few SIg-positive cases (3). The authors produced two kinds of monoclonal antibody (MoAb) that recognize B lymphocytes (6). The presence or absence of reaction of SIg-negative tumor cells with these MoAb was determined. It is still unknown which marker, T or B lymphocytes, SLS, CLS and TLS tumor cells possess. Zwahlen et al. recently determined, with a MoAb, that tumor cells of SLS from one animal were derived from T-helper/inducer (8). The authors investigated which of these tumor cells are constructed of T and which of B lymphocytes.

MATERIALS AND METHODS

Leukosis cattle

Fifty-eight animals with EBL, one with SLA, 8 with CLS and 3 with TLS were used. These cattle consisted of Japanese Black, Japanese Shorthorn and Holstein-Friesian. Each case was diagnosed by clinical, macroscopic and histopathological examinations. All EBL cases were positive for the antibody for bovine leukemia virus (BLV) in immunodiffusion tests. On the other hand, all SBL cattle were negative for the BLV antibody.

Lymphocyte preparation

Single cells were obtained by teasing the tumor tissues. Viable cells were then separated by the Ficoll-Conray mixture technique. Lymphocytes forming a layer were collected and washed three times with Hanks' balanced salt solution (HBSS) and counted by the trypan blue exclusion method.

MoAbs

BLMo-4 react with a all SIg-positive cells and also recognized monocytes, but did not react with T lymphocytes. BLMo-10 recognized a majority, although not all, B lymphocytes, but did not react with either T lymphocytes or monocytes. MoAbs that recognize T lymphocyte were used. B26A4, CH128A and BLMo-12 recognize the sheep red blood cell receptor (SRBCr; CD2). BAQ82A, IL-A12 and BAQ111A recognize pan T cell, CD4 and CD8 markers, respectively (1,7).

Immunofluorescent assay

SIg staining was done according to established procedures using FITC-rabbit anti-bovine IgG (1 : 32 dilution, Cappel, U.S.A.) (3). T lymphocytes were identified by indirect immunofluorescence test with

MoAbs (4,6). MoAb staining was carried out by incubating 5 x 10^6 cells with 50 µl of MoAbs for 30 min at 4°C. The cells were washed three times with HBSS and then stained with 50 µl of FITC-goat anti-mouse Ig (1 : 128 dilution, Cappel, U.S.A.) for 30 min at 4°C. The percentage of positive cells was determined by counting 200 cells or more, using a fluorescence microscope.

RESULTS

The 58 cases of EBL were divided according to the percentage of cells with SIg into a high group ($\geq$ 47%), a middle group (17%-46%) and a low group (0-16%). Thirty-four of them were included in the low group, and the percentage of SIg averaged 2.5%. Nine of the remaining cases were included in the middle group, and the percentage of SIg averaged 24%. The remaining 15 cases were included in the high group, and the percentage of SIg averaged 73.6%. These results suggest that most animals with EBL have SIg-negative tumor cells. When reactivity of the tumor cells with BLMo-4 was studied, 52 of the 58 cases showed a high value that averaged 74.6%. Five of the remaining cases showed a value intermediate between high and low values, which averaged 32.8%. Only one case showed a low percentage (1.8%). As far as reactivity of the tumor cells with BLMo-10 is concerned, 52 of the 58 cases showed a high value that averaged 71.7%. The 6 remaining cases showed a low percentage that averaged 12.5%. These results suggest that most cases in the SIg-low group react with BLMo-4 and -10 MoAbs, which recognizes B lymphocytes.

When the reactivity of tumor cells from a case of SLS with several MoAbs was determined, a high percentage of reactivity with BAQ82A that recognizes a pan T cell marker was observed (Table 1).

TABLE 1. Percentage of cells (SLS) positive for B or T-lymphocyte marker.

Antibodies	PBL	Tumor-1	Tumor-2	Tumor-3
SIg	12.8%	0.4%	1.5%	1.8%
BLMo-4	0	ND	0	0.5
BAQ82A(pan T)	50.0	63.9	68.9	55.1
B26A(SRBCr)	9.3	7.2	9.8	11.9
BLMo-12(SRBCr)	0.8	0	0.8	10.0
BAQ111A(CD8)	1.3	0	1.3	3.7

This results suggests that the SLS tumor cell is derived from T lymphocyte. However, these tumor cells were negative for CD2 and CD8 markers.

Results in the 3 cases of TLS revealed that TLS tumor cells were negative for SIg and gave no reaction with BLMo-4 or -10, as shown in Table 2. On the other hand, TLS tumor cells were positive for CD2 and pan T cell marker, but negative for CD4 and CD8 markers.

TABLE 2. Percentage of cells (TLS) positive for B or T lymphocyte marker.

Antibodies	Case No.1 (tumor)	Case No.2 (tumor)	Case No.3 (tumor)
SIg	14.2%	4.3%	5.7%
BLMo-4	19.9	3.4	0
BLMo-10	29.0	3.6	3.1
BAQ82A(pan T)	49.7	20.4	85.1
B26A4(SRBCr)	69.7	37.0	ND
CH128A(SRBCr)	79.6	68.8	ND
BLMo-12(SRBCr)	76.3	66.7	79.6
BAQ111A(CD8)	10.8	2.7	25.3
IL-A12(CD4)	12.1	1.9	ND

All 8 cases of CLS were negative for SIg. One of them reacted strongly with both BLMo-4 and -10 MoAbs. Four of the remaining cases reacted strongly only with BLMo-4. The remaining 3 cases did not react with BLMo-4 or -10. T cell markers were determined in 3 of the 8 cases, but the cells were negative for CD2 and pan T cell marker.

DISCUSSION

The authors showed in early studies that the EBL tumor cell is SIg-positive (3,5), but in the present study the number of SIg-negative cases was increased. MoAbs (BLMo-4 and -10) recognizing B lymphocytes were used in this study. Therefore, it is clear that the SIg-negative tumor cell is a B lymphocyte. The reason for the increase in the number of SIg-negative cases is unknown.

SLS distinctly reacted with BAQ82A (pan T cell marker) although there was only one case. A tumor was transplanted into nude mice, and was

passaged in nude mice. The transplantable tumor cells reacted strongly with both BAQ82A and B26A4 (CD2), but they were negative for CD4 and CD8 markers.

All tumor cells from the 3 cases of TLS were derived from T lymphocytes, but they were negative for CD4 and CD8 markers. These results provided no answer to the question of whether T lymphocytes were transformed before they acquired CD4 or CD8 markers or had lost the CD4 or CD8 marker by transformation. Tumor cells from 5 of the 8 cases of CLS reacted with BLMo-4, while those from only one of the 8 cases reacted with BLMo-10. Since BLMo-4 recognizes B lymphocytes and monocytes, it is impossible to decide whether or not the tumor cells from the 5 of the 8 cases which reacted with BLMo-4 are really B lymphocytes. Some antigens reacting with BLMo-4 may have been expressed on the cell surface by transformation of lymphocytes. The T lymphocyte marker could be determined in only 3 of the 8 cases, but all tumor cells were negative. However, it still can not be stated that the CLS tumor cells are not derived from T lymphocytes. When a T lymphocyte is transformed, it may lose its T lymphocyte markers. Even these results do not indicate whether CLS tumor cells are derived from T or B lymphocytes.

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SUMMARY

Bovine leukosis is classified as enzootic bovine leukosis (EBL) and sporadic bovine leukosis (SBL), which is subclassified as skin, calf and thymic forms. The authors investigated which of these tumor cells are constructed of T and which of B lymphocytes. Monoclonal antibodies (MoAbs) which recognize B lymphocytes, and surface immunoglobulin (SIg) were used for identification of B lymphocytes. MoAbs which recognize marker of pan T lymphocytes, and CD2, CD4 and CD8 markers were used for identification of T lymphocytes. The results were as follows:

1. The most EBL cases were negative to SIg, with the few cases they were SIg positive. Lymphocytes of every case reacted well with the MoAb recognizing B lymphocytes. These results suggest that tumor cells of EBL are derived from B lymphocytes.

2. Lymphocytes of a skin form of SBL were derived from T lymphocytes positive for the pan T cell marker, but negative for CD2 and CD8 markers along with negative E rosette formation.

3. Lymphocytes of 3 thymic-form cases were derived from T lymphocytes positive for CD2 and pan T cell markers, but negative for CD4 and CD8 markers.

4. Seven of 8 calf-form cases were negative for all markers of T or B lymphocytes. No evidence of a T lymphocyte-derived tumor was given. Lymphocytes of 1 case reacted well with the MoAb recognizing B lymphocytes.

MODELO DE SIMULACION PARA LA EVALUACION ECONOMICA DE DISTINTAS ALTERNATIVAS DE LUCHA CONTRA LA FIEBRE AFTOSA EN CASTILLA Y LEON (ESPAÑA)

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INTRODUCCION

En los últimos años ha recibido una creciente atención, la evaluación económica de los programas de control de las enfermedades animales, fundamentalmente las de origen infeccioso.

Con el fin de asumir decisiones racionales, desde el punto de vista económico, en la lucha contra estos procesos, es necesario evaluar los costes de cada posible acción a ser considerada, y los beneficios que podrían ser esperados si esta acción es llevada a cabo. En este trabajo se pretende la construcción de un modelo de simulación que permita realizar evaluaciones de este tipo en la lucha contra la fiebre aftosa en una región española, la Comunidad Autónoma de Castilla y León.

La fiebre aftosa es una enfermedad altamente contagiosa que puede afectar a gran número de especies animales, tanto domésticas como silvestres. Aunque el hombre no es normalmente receptivo, se ha diagnosticado con frecuencia. En este trabajo nos vamos a limitar al ganado bovino, aunque la utilización de los modelos puede ser realizada con otras especies con escasas modificaciones.

Puede se causado por siete tipos de virus conocidos: A, C, O, Asia, SAT 1, SAT 2 y SAT 3. El virus de la fiebre aftosa, se transmite por contacto directo con animales infectados. También puede ser transmitido indirectamente por contacto con material contaminado, como heno, paja o pieles. Tiene un periodo de incubación normal entre tres y ocho días, pero su desarrollo puede reducirse o ampliarse hasta los veintiun días. La sintomatología es similar a la de otras enfermedades vesiculares, aunque su diagnóstico solo puede ser realizado por análisis de laboratorio.

La estrategia básica de control de esta enfermedad en la región ha sido la campaña sistemática de vacunación. Los animales mayores de 6 meses, son vacunados regularmente una vez al año. Cuando el proceso aparece, las campañas se realizan con mayor frecuencia, dos veces al año, con aislamiento de las zonas afectadas y reduciendo al máximo el movimiento de los animales.

METODOS

Modelos de simulación de epidemias.

La simulación de procesos epidémicos es un método útil para abordar estos sistemas complejos. Sus inicios y desarrollo corresponden a procesos infecciosos humanos. Una importante revisión de los inicios de esta metodología puede ser consultado en el trabajo de Serfling (18). En la actualidad existe un amplio desarrollo tanto de las bases teóricas matemáticas como de las posibles aplicaciones (2).

En el campo veterinario se ha producido en todo el mundo una explosión de trabajos en este línea, sobre todo desde el año 1979 correspondiendo con la constitución de la Asociación Internacional de Epidemiología y Economía Veterinarias. Este nuevo abordaje de la epidemiología ha permitido utilizar conjuntamente criterios técnicos

y económicos para resolver determinados problemas de adopción de decisiones en la lucha contra los procesos epizootiológicos

Entre los métodos utilizados, el análisis beneficio-coste es, sin duda, el más conocido. Se fundamenta en la evaluación de los costes de distintas medidas de control de los procesos epidémicos y de los beneficios obtenidos, contabilizados a traves de las pérdidas económicas que se evitan con la aplicación de estas medidas de control (9). Los resultados se suelen expresar en términos de Relación Beneficio/Coste, de Tasa Interna de Rentabilidad o de Beneficios Actualizados. De cualquier manera se hace necesario la proyección durante un periodo de tiempo de los resultados del proceso en función de las distintas soluciones consideradas. Esto se ha conseguido con la utilización de modelos de simulación que han sido construidos utilizando distintas técnicas, según los objetivos que se perseguían en cada caso.

La fiebre aftosa ha sido uno de los procesos, en los que los investigadores han centrado más su atención. El análisis Beneficio-Coste fue utilizado en el Reino Unido (20), en Brasil y Colombia (17), en Paraguay (16,17), en Kenia(13), así como diversas aportaciones teóricas, sobre construcción de modelos en U.S.A. (5,11,19), en Inglaterra (12) o en Australia (13)

No conocemos la existencia de ninguna aportación en este campo en España, a pesar de que la fiebre aftosa, que puede ser considerada como erradicada en la actualidad de nuestra geografía, continúa siendo objeto de regulares campañas de vacunación.

El modelo desarrollado en este trabajo se ha realizado utilizando la técnica conocida como Dinámica de Sistemas. Los modelos DS son un grupo particular de modelos matemáticos enunciados por primera vez por Forrester (8). Pretenden dar una respuesta a la variación a lo largo del tiempo de determinadas variables de estado y conocer (o simular) como se comportará el sistema en el futuro ante diversas circunstancias, hipótesis o "escenarios" alternativos(10).

Aunque varios modelos han tenido en cuenta las características dinámicas de las epidemias animales (7), no conocemos que esta técnica haya sido utilizada con este motivo en este campo, aunque es de amplia utilización para la simulación de sistemas económicos de muy distinto signo (1,6).

Los modelos de Dinámica de Sistemas

Aunque no existen diferencias radicales de concepto entre la técnica de modelización DS y otros modelos matemáticos, sí existen peculiaridades propias que les identifican y se refieren fundamentalmente a la forma. Forrester(8), introduce un símil hidrodinámico para explicar el comportamiento de un sistema y, a partir de ahí, alige unos símbolos determinados para representar las distintas características estructurales y formales de los sistemas. Los distintos elementos del modelo se representan por medio de variables que pueden ser: variables de nivel, variables de flujo y variables auxiliares.

Un símil hidrodinámico podría ser representado por tres depósitos conectados uno a continuación de otro en los que se acumulan tres niveles N_1, N_2 y N_3. Las variaciones de los niveles vienen determinadas por las actuaciones sobre unas válvulas que regulan los caudales que alimentan a cada uno de los depósitos. La decisión sobre la apertura de estas válvulas se toma teniendo en cuenta como única información los valores alcanzados por los niveles en cada uno de los depósitos, en el instante de tiempo considerado. A partir de aquí se definen los elementos de la DS: niveles, variables de flujo,

variables auxiliares, variables exógenas, tasas, canales de material, canales de información, fuentes o sumideros, retardos, bucles de retroalimentación etc.. Todas estas características se representan normalmente mediante un diagrama causal que suele ser denominado Diagrama Dynamo o Diagrama Forrester. Por último todas las relaciones que aparecen en el Diagrama pueden ser programadas utilizando el lenguaje Dynamo (10)

EL MODELO FIEBRE AFTOSA EN CASTILLA Y LEON

Con el fin de abordar los objetivos reseñados, fue elaborado un modelo de DS, con dos partes diferenciadas, aunque ensamblados para permitir la simulación conjunta de todas las variables. Una primera parte está constituida por un modelo de comportamiento epidemiológico del proceso, mientras que el segundo es un modelo económico que permite abordar los cálculos de beneficio-coste. El esquema general del modelo aparece en la fig.1.

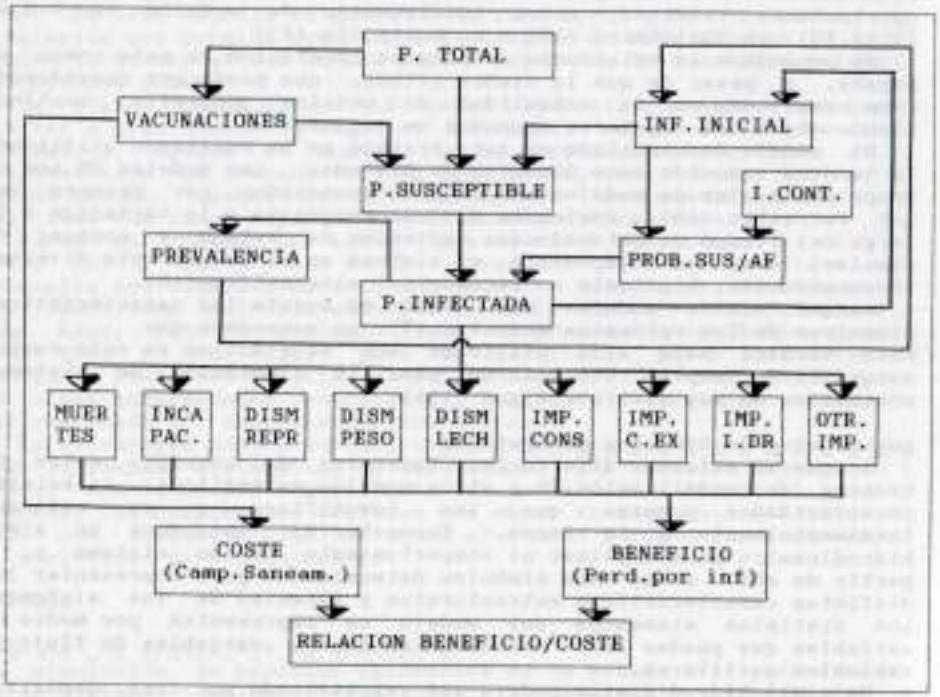

Figura 1 Diagrama Causal del Modelo Fiebre Aftosa en Castilla y León

Simulación de la epidemiología de la fiebre aftosa

El modelo epidemiológico se ha desarrollado adaptando el descrito por Thieme en 1982 (19). Este modelo se basa en el cálculo de la probabilidad estimada de infección de un animal susceptible. Esta probabilidad fue desarrollada por el grupo de fiebre aftosa de la Universidad de Minnesota en 1975 (5). Con este valor probable podemos

calcular el número absoluto de nuevos casos en un determinado periodo de tiempo, que hemos fijado en un año, con el fin de hacer coincidir este periodo base con el de referencia de los datos de que disponemos en Castilla y León.

Las principales variables que utilizamos en el modelo son las siguientes:

Clase	Simbolo	Definición
Nivel	PT	Población total
Tasa	EV	Tasa de evolución de PT
Auxiliar	PS	Población susceptible
Auxiliar	PA	Población infectada
Externa	VAC	Vacunacion en el año
Tasa	EFVAC	Eficiencia de la vacunación
Auxiliar	PREV	Prevalencia
Tasa	TPREV	Tasa de prevalencia
Auxiliar	IIN	Infección inicial
Tasa	INCON	Indice de contacto
Auxiliar	PSUIN	Probabilidad de infección de un animal susceptible

Las ecuaciones mas importantes que conforman el modelo son las siguientes:

```
PT(L)   = PT(L-1) + DT(EV)
PS(L)   = PT(L) - VAC(L)*EFVAC - IIN(L)
PSUIN   = 1 - exp(-IIN*INCON)
PA(L)   = PS(L)*PSUIN(L)
IIN(L)  = TPREV*PA(L-1)
```

Este sistema de ecuaciones permite la simulación mediante DS de la incidencia de la enfermedad en un año determinado en función de las tasas de vacunación y de la incidencia los años anteriores. Solamente se considera que se inicia un proceso cuando PSUIN llega a un valor determinado. En los casos en que PA=0 se grava con una probabilidad la posibilidad de que el año siguiente aparezca y se desarrolle el proceso.

Simulación de beneficios y costes

A cada estrategia de vacunación puede ser asociada una secuencia de costes y beneficios.

Los beneficios son calculados en términos de pérdidas evitadas mediante las distintas posibilidades de vacunación implícitas en el modelo. Para ello se realiza una simulación con valores de VAC=0 y se estiman las pérdidas anuales ocasionadas en el sector durante un determinado periodo de tiempo, en función de la incidencia del proceso. Posteriores simulaciones nos permitirán estimar la posible incidencia con un determinado valor de VAC. La diferencia entre ambos valores será el beneficio logrado por medio de la campaña.

Las pérdidas ocasionadas por el proceso son esquematizadas en el modelo causal del proyecto y se agrupan en los siguientes apartados:

1. Pérdidas por muertes.
2. Pérdidas por incapacidad permanente.
3. Pérdidas por disminución en la reproducción.
4. Pérdidas por disminución de peso.
5. Pérdidas por disminución de la leche.
6. Impacto en el consumo.
7. Impacto en el comercio exterior.
8. Impacto en las industrias derivadas.

4. Otros impactos.

Para la evaluación de las pérdidas en cada uno de estos apartados, fueron utilizados los diversos datos técnicos aportados por Aulaqi y Sundquist, en el trabajo de McCauley et al (11). Estos datos fueron adaptados a las circunstancias del censo de ganado vacuno de la Comunidad de Castilla y León. Las estimaciones fueron realizadas en base a los precios de mercado del año 1990 y en las proyecciones anuales no se tuvo en cuenta la posible inflacción.

Hemos asumido que los costes de las campañas son directamente proporcionales al numero de animales vacunados en cada alternativa. Por ello se realizaron estimaciones del coste de vacunación de un animal a precios de mercado de 1990, en las que han sido tenidos en cuenta, además del coste de la vacuna y del trabajo empleado para su aplicación, los costes administrativos de gestión de los programas de control

Las comparaciones entre beneficio y coste de las distintas estrategias de vacunación, y de otras posibles medias que pueden ser incluidas en el modelo, pueden realizarse en términos de relación beneficio-coste, valor actual neto o tasa interna de retorno. Cualquiera de estas posibilidades permite realizar comparaciones entre estas posibles estrategias.

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RESUMEN

En este trabajo se ha diseñado un análisis económico, usando técnicas beneficio-coste, para comparar estrategias alternativas de control de la fiebre aftosa en Castilla y León (España). Se ha construido un modelo de, por medio de la técnica de Dinámica de Sistemas, que permite la simulación temporal de las pérdidas económicas en una eventual situación epidémica de fiebre aftosa, con distintas estrategias de control. Estas pérdidas económicas esperadas son producidas por las consecuencias físicas de la enfermedad en los animales (muerte, pérdidas por incapacidad permanente, perdidas de reproducción, perdidas de carné, perdidas de leche...), pero también por los costes a los consumidores, los impactos en la industria derivada, los efectos en el comercio exterior, etc.. El coste de las diferentes estrategias de control se estimó en función de los recursos utilizados en cada caso.

SUMMARY

In this work an economic analysis was designed using benefit-cost techniques in order to compare alternative strategies for foot and mouth disease control in Castilla y León (Spain). We have built a simulation model, by means of a System Dynamic technique, which permits the temporal proyection simulation of the economical losses in an eventual foot and mouth epidemic situation, for different disease control strategies. These expected economic losses have been produced for the physical disease consequences to the animals (death, permanent disability, reproduction losses, weight loss, milk losses...), and also for the consumer costs, impacts on agribusiness, effects on foreign trade, etc.. The cost of the different control strategies was estimated as a function of the resources utilized in each case.

RESUME

Dans ce travail on a dessiné un analyse economique utilisant techniques bénéfice-coût, pour comparer alternatives de contrôle du fièvre aphteuse dans Castilla y León (Espagne). On a construit un modelé de simulation, pour moyen du technique de Dinamique de Systèmes, qui permet la simulation temporelle des pertes économiques dans une éventuel situation epidemique de fièvre aphteuse avec différentes stratégies de contrôle. Ces pertes economiques esperées se produit pour le consécuences phisiques des animaux (mort, incapacité permanent, pertes du reproduction, du poids, du lait...), et aussi pour les coûts des consommateurs, les impacts dans l'industrie de transformation, les effets sur le commerce estérieur, etc.. Le coût des differentes stratégies de contrôle sont estimées in fonction des resources utilisés in chaque cas.

VIDEO

BOTULISMUS BEI BRASILIANISCHEN RINDERN - KLINISCHE ERSCHEINUNGEN BEI ZWEI VERSCHIEDENEN AUSBRÜCHEN

K. Behrens

Institut für Pathologie, Tierärztliche Hochschule Hannover, Bünteweg, 3000 Hannover, West-Germany

Video-Kurzfilm in VHS/NTSC; 12,5 Min.

ZUSAMMENFASSUNG

In den letzten Jahren hat das Auftreten von Botulismus bei Rindern in Brasilien stark zugenommen. Die Diagnose der Erkrankung basiert neben dem Vorbericht insbesondere auch auf der klinischen Untersuchung erkrankter Tiere. Die Symptomatologie ist bei Rindern charakteristisch und gestattet in den meisten Fällen eine Diagnose mit ausreichender Sicherheit. Bei zwei unterschiedlichen Ausbrüchen von Botulismus wurden die typischen Symptome in ihrer Art und Abfolge bei mehreren Tieren und weitere Umstände filmisch dokumentiert. Die klinische Diagnose konnte dabei durch spätere Laboruntersuchungen (Toxinnachweis) bestätigt werden.

Fall I : Region mit phosphordefizienten Böden, ungenügende Supplementierung von Mineralien, Osteophagie, Verlust von 2000 Tieren - insbesondere Kühen - einer Herde innerhalb von zwei Jahren, in Zersetzung befindliche Kadaver auf der Weide und in Tränkestellen mit stehenden Wasser
= epizootische Form des Botulismus

Fall II: Verfütterung von vergifteter Geflügeleinstreu an Jungtiere, akutes Geschehen mit Verlust nahezu aller (ca. 60) Tiere innerhalb einer Woche
= sporadische Form des Botulismus

Dokumentierte Symptome: Bewegungsstörungen, Diarrhoe, generalisierte schlaffe Lähmung, ständiges Liegen in typischer Haltung, Unvermögen zu Kauen, zu Schlucken und Aufzustehen, doppelschlägige/biphasische Atmung (Phase 1: Erweiterung des Brustkorbes; kurze bewegungslose Pause; Phase 2: Vorwölben der schlaffen Bauchwand), schlaffe Lähmung der Zunge ("Zungentest") und des Schwanzes, unbeeinträchtigte Psyche, Evolution in Stunden bis Tagen.

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BOTULISMO EM BOVINOS BRASILEIROS -
ASPECTOS CLINICOS EM DOIS SURTOS DIFERENTES

K. Behrens

Curta-metragem em VHS/NTSC; 12,5 min.

RESUMO

Nos últimos anos agravaram-se os surtos de botulismo em bovinos no Brasil. O diagnóstico da doença está baseado nao somente na anamnese mas principalmente no exame clínico de animais doentes. A sintomatologia é característica em bovinos e permite na maioria dos casos um diagnóstico suficientemente seguro. Em dois diferentes surtos de botulismo foram relatados em documentário os sintomas típicos, e sua evolucao, no comportamento de vários animais e demais cirunstâncias. O diagnóstico clínico foi confirmado por exames laboratoriais (verificacao de toxina) efetuados posteriormente.

caso I : pasto com deficiência de fósforo, suplementacao insuficiente de minerais, osteofagia, perda de 2000 animais em dois anos, permanência de cadáveres em decomposicao no pasto e pocas de água estagnada ingerida pelo gado
= forma epizoótica de botulismo

caso II: alimentacao de novilhos com esterco de galinha intoxicado, doença aguda com perda de quase todos os animais (ca. 60) em uma semana
= forma esporádica de botulismo

Sintomatologia documentada: Distúrbio dos movimentos, diarrêia, paralisia flácida generalizada, permanencendo o animal deitado em postura típica, impossibilidade de mastigar, deglutir e levantar-se, respiracao bifasica (fase 1: expansao do tórax; curto intervalo sem movimento; fase 2: dilatacao da parede abdominal), paralisia flácida da língua ("teste de língua") e da cauda, psiquismo nao afetado, evolucao de horas a dias.

BOTULISM IN BRAZILIAN CATTLE -
CLINICAL ASPECTS IN TWO DIFFERENT OUTBREAKS

K. Behrens

Video-short-film in VHS/NTSC; 12,5 min.

SUMMARY

In the last few years the incidence of botulism in brazilian cattle has greatly increased. Diagnosis of the disease is based on the preliminary report and in particular on the clinical examination of affected animals. The bovine symptomatology is typical and therefore allows in most cases a diagnosis with sufficient certainty. The typical symptoms, development, and other facts of botulism were documented in two different outbreaks. The clinical diagnosis was later verified by laboratory examination (toxin detection).

case I : area with soil deficient in phosphorus, insufficient mineral-supplementation, osteophagia, loss of 2000 animals, especially cows, in one herd within two years, pasture with carcasses in decomposition and toxic drinking water
= epizootic form of botulism

case II: feeding young animals with toxic poultry litter, acute occurrence with loss of almost every animal (approx. 60) within one week
= sporadic form of botulism

Documented symptoms: locomotion with difficulties, diarrhea, generalized paralysis, constant lying in typical position, disturbance of mastication, deglutition, and standing up, biphasic respiration (phase 1: extension of the thorax; short interruption without movement; phase 2: dilatation of the relaxed abdominal wall), flaccid paralysis of the tongue ("tongue test") and the tail, unaffected psychic behaviour, evolution within hours or days.

L'EXAMEN CLINIQUE DE LA VACHE LAITIERE

(Videotape, 30 min.)

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Les besoins en lait de la population humaine, en perpétuelle augmentation, seront de plus en plus assurés, dans un proche avenir, par des vaches laitières à fort potentiel de production, provenant d'élevages toujours plus grands et plus évolués techniquement.

La clinique de la vache laitière doit donc d'adapter aux nouvelles situations rencontrées sur le terrain. Dans ce contexte, l'examen clinique individuel continue de représenter la base du diagnostic, tant individuel que d'élevage.
Ce film présente un protocole d'investigation particulièrement adapté à ces nouvelles situations.

1) ANAMNESE

La collecte des informations, permettant une anamnèse la plus complète possible, nous apparaît déterminante pour aboutir à un diagnostic correct.
L'étude des conditions d'élevage revêt une importance toute particulière, étant donné la place croissante des maladies d'embâcle.

* étude des conditions d'élevage

a) type d'exploitation agricole.
b) main d'oeuvre employée (présence ou non de salariés).
c) type de stabulation (ou de pâturage).
d) état des onglons.
e) niveau de fertilité du troupeau.
f) état de santé des veaux.
g) existence dans l'élevage d'infection virale ou bactérienne ou d'infestation parasitaire.
h) mamelles et qualité du lait.
i) type d'alimentation, rationnement et mode de distribution
k) taux de renouvellement et animaux concernés.

* anamnestiques

a) nombre d'individus atteints par la maladie et mode d'évolution épidémiologique.
b) date d'apparition des symptômes.
c) type et importance respective des symptômes rencontrés.
d) éventuelles visites vétérinaires antérieures, statut sanitaire du troupeau, enquêtes sérologiques, vaccinations ou traitements thérapeutiques déjà effectués par l'éleveur.

2) EXAMEN GENERAL

Muni du matériel indispensable et convenablement protégé (vêtement de travail, blouse, bottes, thermomètre, stéthoscope, marteau de percussion, sonde urinaire, gants de feuille à usage unique) le praticien peut effectuer un examen complet en quelques minutes (5 à 10), en procédant par régions anatomiques ainsi que par appareils.
L'examen général s'effectue en débutant par le côté gauche de l'animal au niveau de la tête: examen de la muqueuse oculaire et des vaisseaux épiscléraux, du mufle et des naseaux, de l'aspect de la muqueuse et de la présence éventuelle d'érosions ou d'ulcères et de mordillement, inspection de la bouche, extériorisation de la langue et examen rapide

de la cavité buccale, des ganglions mandibulaires, parotidiens, rétropharyngiens.

On poursuit alors par l'appréciation de l'état de la peau dont on prend un pli sur la face latérale de l'encolure, avec la recherche d'un éventuel poule veineux ou d'une tension à la jugulaire; on palpe ensuite les ganglions précapulaires, on ébranle la paroi thoracique, le type et les caractéristiques de la respiration, on examine à l'aide du stéthoscope le coeur et le poumon gauche, on inspecte et on palpe le flanc gauche, en passant ensuite directement à la double auscultation du rumen, on palpe le ganglion précrural, puis on se place derrière l'animal pour évaluer la tonicité de l'anus et de la queue. On prend la température rectale et on pratique une fouille rectale avec examen macroscopique des feces.

On passe alors à droite de l'animal: inspection-palpation du ganglion précrural droit, du flanc droit, contrôle de la tension de la paroi abdominale, palpation de la mamelle et examen des premiers jets de lait; on écoute le flanc droit et on réalise éventuellement une percussion: on effectue la percussion du poumon droit et de la région du foie et enfin on écoute le poumon droite.

Très souvent, à la lumière de ce rapide examen, il est possible d'obtenir des éléments diagnostiques intéressants voire déterminants.

Si des renseignements, intéressant un organe ou un appareil particulier, sont mis en évidence, celui-ci devra être examiné à l'aide des techniques classiques d'investigations clinique;

Si, à la fin, subsistent des doutes quant à l'entité pathologique, on s'orientera vers divers examens complémentaires, dans l'ordre suivant:

- prélèvement et examen des urines
- prélèvement et examen des feces
- prélèvement et examen du jus de rumen
- prélèvement et examen du sang
- prélèvement d'echantillons d'aliments

En fait, un grand nombre d'examens paracliniques est réalisable, ce qui permet si nécessaire, de compléter l'examen clinique initial des seuls organes et appareils. Il est important de remarquer que le prélèvement des échantillons doit survenir à la fin de l'examen clinique si celà est nécessaire (et pas avant!). Les echantillons prélevés doivent être correctement identifiés (propriétaire et animal) et, s'ils doivent être expédiés, bien conservés et accompagnés de la liste des examens demandés.

L'examen clinique a pour finalité l'obtention du diagnostic, aux niveaux successifs suivants.

- diagnostic de syndrome
- diagnostic anatomique
- diagnostic étiologique

L'objectif du praticien doit être de parvenir au diagnostic anatomo-étiologique.

PRONOSTIQUE ECONOMIQUE

En pratique rurale, en plus du diagnostic, il faut accorder un rôle particulier au pronostique économique, déterminé par la probabilité de récupération partielle ou totale de l'animal, ainsi que par le coût du traitement.

C'est pourquoi il est important de connaître les critères de pronostic défavorable. Signes-relefs de pronostic réservé ou défavorable (seuls ou diversement associés).

- Faciès: globes oculaire enfoncés, hipotrophie des muscatures, fosses supra-orbitales creusées.
- Système cardio-vasculaire: muqueuses infectées, violacées, distension jugulaire, pouls aortique abdominal, rythme cardiaque pendulaire.
- Respiration buccale.
- Polypnée et pâleur des muqueuses.
- Peau: déshidratation et perte d'élasticité.
- Pli cutané persistant.
- Adénomégalie généralisée.
- Hipothermie ou hypothermie persistante.
- Silence à l'auscultation des pré-estomacs.
- Météorisme ruminal avec ballonnement dorsal chronique.
- Appareil locomoteur: fasciculations musculaires, myélopathie diffuse, animal couché en position de "grenouille".
- Examens de laboratoire:
  - sang: hiperglycémie prononcée.
  - urine: pseudoprotéinurie, protéinurie.
  - fus de rumen complètement inactif.

En conclusion, rappelons, avec Stoeber, les erreurs les plus fréquentes que le praticien doit en toutes occasions chercher à éviter:

Erreurs les plus fréquentes de l'examen clinique:

- anamnèse incomplète (hâtive).
- anamnèse volontairement erronée (mauvaise foi du personnel)
- examen clinique superficiel.
- omissions lors de l'examen clinique.
- interprétation prématurée et fausse des données.
- ignorance et omission des méthodes d'investigation disponibles.
- mise en œuvre de traitement avant la détermination du diagnostique.

Ainsi, à l'avenir, le rôle principal du praticien rural devra être de poser un diagnostique à un niveau individuel.
Cet objectif ne pourra être atteint que par une remise à jour continuelle de ses connaissances, tant sur le plan clinique au sens strict que sur le plan zootechnique, par rapport à l'évolution technologique, souvent spectaculaire de l'élevage bovin.

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Résumé

L'examen clinique de la vache laitière

Après l'introduction sur l'importance social et économique de la production laitière dans le monde entier, le montage audiovisuel presente une schema simplifié mais complet d'investigation clinique, que est particulierement indique pour les modernes exploitation intensives des bovines laitieres. Tres important, selon l'opinion des Autore, est accordé à l'anamnese du milieu, à cause de la croissante diffusion de la pathologie environmental.

Zusammenfassung

Die klinische Untersuchung der Milchkuh

Nach einer Einleitung über die wirtschaftliche und soziale Bedeutung der Milchproduktion in der ganzen Welt, wird in Film ein praktisch brauchbarer Untersuchungsgang für das Einzeltier beschrieben, der besonders für die modernen intensiven Milchleistungsbetrieben von Betreung ist.
Nach Meinung der Verfasser, ist eine sorgfältige Aufnahme der Anamnese bei den Tieren der Herde, wegen der steigenden Bedeutung der umweltbedingten pathologischen Einflusse von Wichtigkeit.

Summary

The clinical examination of the dairy cow

After an introduction of economical and social importance of the milk production in the world, the vido tape show a practical way of clinical examination of single animal, wich seem to important in moderns and intensive dairy farming. The Editors are convinced that a precise anamnesis in the herd is significant the cause because of increasing pathological environmental influences.

DIE PARTIELLE TYPHLEKTOMIE BEI DER KUH MIT DEM LINEAREN KLAMMERGERÄT TA 90[1] (KOMMENTAR ZUM VHS-VIDEO-FILM)

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EINLEITUNG

Eine Zäkumamputation ist dann indiziert, wenn entweder ein Rezidiv nach Typhlotomie vorliegt, resp. die Rezidivgefahr reduziert werden soll (1,4,6,13), wenn die Zäkumwand aufgrund einer Torsion oder Abknickung devitalisiert ist (5,6,10,12,16) oder wenn eine Stenose des ostium ileocäcale vorliegt (7). Die Typhlektomie ist ein aufwendiger, und gegenüber der Typhlotomie mit deutlicher Verlängerung der Operationszeit und massiv erhöhter Kontaminationsgefahr einhergehender Eingriff (6).

In der Intestinalchirurgie beim Pferd wurden in neuerer Zeit zum Verschluss des Darmlumens immer häufiger Klammergeräte verwendet, mit dem hauptsächlichen Ziel die Operationszeit zu verkürzen (2,9,15). Es lag nahe, ein solches Gerät versuchsweise auch beim Rind einzusetzen. In der vorliegenden Arbeit wird eine mit Hilfe des Klammergerätes TA 90[1] stark vereinfachte Technik der partiellen Typhlektomie bei der Kuh beschrieben.

TIERE, MATERIAL UND METHODE

Kriterien für die Selektion der Fälle

Die vorliegenden Untersuchungen wurden an insgesamt 20 Kühen durchgeführt. Nicht berücksichtigt wurden Kälber und Rinder vor der ersten Kalbung. Im Zeitraum von Anfang September 1988 bis Ende April 1989 war bei 40 Kühen, welche zur Behandlung einer Blinddarmdilatation/-torsion an die Veterinär-Medizinische Klinik der Universität Zürich eingewiesen wurden, die Indikation zur Operation gegeben. Die Laparotomie war dann indiziert, wenn entweder das Allgemeinbefinden des Patienten stark gestört war, der Kotabsatz sistiert war, oder eine Torsion resp. Abknickung des Zäkums vorlag resp. diese nicht mit Sicherheit durch die rektale Palpation ausgeschlossen werden konnte (1). Abwechslungsweise wurde bei jeder zweiten Kuh eine Zäkumamputation mit dem linearen Klammergerät TA 90 durchgeführt, während die andere Hälfte lediglich typhlotomiert und in dieser Studie nicht berücksichtigt wurde. Hingegen miteinbezogen wurde 1 Patient bei dem ein Rezidiv nach Zäkotomie vorlag.

Patientengut

Von den 20 Kühen gehörten 13 (65%) der Rasse Braunvieh, 5 (25%) der Holstein-Friesian- und 2 (10%) der Simmental-Red-Holstein-Rasse an. Das Durchschnittsalter der Patienten betrug 5.2 Jahre. Die älteste Kuh war 10-jährig, die Jüngsten waren 3 Jahre alt.

Operationstechnik (siehe Videofilm)

Es wurde eine partielle Typhlektomie unmittelbar distal des ostium ileocäcale durchgeführt. Die Operation erfolgte am stehenden Tier von der rechten Flanke aus. Es wurde eine distale Paravertebralanästhesie (8) gesetzt, auf eine Sedation wurde verzichtet. Als Zugang wurde ein

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[1] Auto-Suture AG, Hori, Switzerland

ca. 20 cm langer, von kaudodorsal nach kranioventral verlaufender Schrägschnitt gewählt. Es erfolgte eine kurze Exploration der Bauchhöhle, um Veränderungen an den Organen, sowie Lage und Füllungszustand von Zäkum und Kolonscheibe zu ermitteln. Die Wundränder wurden mit einer Plastikmanschette abgedeckt, die Blinddarmspitze nach extraperitoneal verlagert und diese nach einer Schnittinzision am tiefstgelegenen Punkt eröffnet. Nach der Entleerung des Blinddarmes wurde versucht, auch den Inhalt der ansa proximalis coli durch massierende Bewegungen mit Arm und Hand abzuhebern. Nach der Entleerung wurde der vorgelagerte Darmteil mit physiologischer Kochsalzlösung abespült und mit einer Darmklemme provisorisch abgedichtet.

Da die Durchtrennung des ligamentum ileocäcale erfahrungsgemäss sehr schmerzhaft ist, wurde eine Lokalanästhesie gesetzt. Durch Infiltration von 20 ml Lidocain (2%-ig) mit Hyaluronidase wurden der dorsale und der ventrale Ast des Nervus cäcalis in der Nähe ihrer Kreuzungsstelle mit dem Ileum anästhesiert. Die Gekröseligatur erfolgte mit Einzelknopfheften, als Nahtmaterial kam ein Polyglycolsäurefaden der Stärke 2 zur Anwendung. Die Naht wurde nur 1 cm bis maximal 2 cm von der Zäkumwand entfernt gelegt, um die Blutversorgung des Ileums via Aeste der Arteria cäcalis (11) nicht zu gefährden. Das ligamentum ileocäcale wurde dann mit der Schere durchtrennt und blutende Gefässe zusätzlich ligiert.

Anschliessend wurde proximal und distal der vorgesehenen Resektionsstelle am Zäkum je eine Darmklemme gelegt. Mit dem linearen Klammergerät TA 90 wurde dann das Zäkumlumen verschlossen: Es waren dazu 2 Arbeitsschritte nötig, da ein Klammermagazin (90 mm) allein zu kurz war. Beginnend mit der mesenterialen Seite wurde das Gerät quer zum Darm gesetzt und durch Auslösen des Abzuges eine Doppelreihe 3,5 mm resp. 4,8 mm grosser Klammern aus rostfreiem Stahl appliziert. Danach wurde die Darmwand mit einen Skalpellschnitt entlang dem Klammermagazin abgesetzt und der Stumpf sofort mit Polyvinyl-Jod-Lösung desinfiziert. In der gleichen Weise wurde von der antimesenterialen Seite her vorgegangen. Es wurde darauf geachtet, dass die beiden Doppelklammerreihen sich in der Mitte des Zäkums überlappten, damit der Verschluss des Lumens sicher gewährleistet war. Alle Gefässe, die aus der Zäkumwand bluteten, wurden zusätzlich von Hand ligiert. Die Auswahl der Klammergrösse erfolgte in Abhängigkeit von der Dicke der Zäkumwand.

Die letzte Darmklemme wurde entfernt und der verbliebene Zäkumstumpf mit warmer physiologischer Kochsalzlösung abgespült und reponiert. Die Bauchhöhle wurde zur Prävention von Verklebungen und Infektionen mit Polyvinylpyrrolidon, Heparin und einer Neomycin-Penicillin-Suspension versorgt. Der Verschluss der Bauchdecke erfolgte in 5 Etagen.

Nachbehandlung

Via Dauerkatheter wurde jeden Pantienten in die Vena jugularis eine neostigminhaltige Kochsalz-Glucose-Infusion verabreicht. Parallel dazu wurden an den ersten 2 Tagen zusätzlich je 10 l der Kochsalz-Glucose-Lösung ohne Zusätze infundiert. Falls der Hämatokrit mehr als 36 Vol.% betrug, wurden unter Kontrolle desselben und der Plasmaproteinkonzentration entsprechend grössere Flüssigkeitsmengen intravenös appliziert. Lag eine Hypokalianie vor, wurde der Infusion Kaliumchlorat zugegeben. Auf die Verabreichung von Abführmitteln wurde in jedem Fall verzichtet. Die antibiotische Versorgung erfolgte bei allen Patienten in Form von Procain-Penicillin über 3 Tage. Zusätzlich wurden dreimal in Abstand von 24 Std. ein Antiphlogistikum (Flunixin Meglumin) und nach Bedarf ein Spasmoanalgetikum (Novaminsulfonsäure) verabreicht. Jedes Tier wurde während mindestens 72 Std. intensiv überwacht.

RESULTATE

Operationsbefunde

Das Zäkum war in 13 Fällen (65%) dilatiert, in 5 Fällen (25%) zusätzlich abgeknickt und in 2 Fällen (10%) dilatiert und 180° um die Längsachse torsiert. Die Dilatation war bei 11 Kühen (55%) hochgradig und bei 9 Kühen (45%) mittelgradig. Die Kolonscheibe lag in 18 Fällen (90%) in der normalen Position, in 2 Fällen (10%) war sie um 180° torsiert. In 9 Fällen (45%) lag eine hochgradige, in 11 Fällen (55%) eine mittelgradige Obstipation des colon ascendens vor.

Zusätlziche pathologische Veränderungen in der Bauchhöhle waren bei 2 Kühen (10%) vorhanden: Eine Kuh hatte umschriebene Verwachsungen zwischen omentum majus und parietalem Peritoneum im Pylorusbereich, die andere hatte diffuse Verwachsungen der Leber mit dem Zwerchfell. Nach der Entleerung kontrahierte sich das Zäkum als Folge der Manipulationen in 7 Fällen (35%) deutlich, in 10 Fällen (50%) blieb es schlaff. Angaben über die restlichen 3 Fälle (15%) fehlen.

Operationstechnik

Bei der Operation wurde so vorgegangen, wie im Kapitel "Tiere, Material und Methode" beschrieben. Bei 13 Kühen (65%) wurden 2 Magazine (blau) mit den 3,5 mm grossen Klammern und bei 7 Kühen (35%) 2 Magazine (grün) mit den 4,8 mm grossen Klammern verwendet. Zu pulsierenden arteriellen Blutungen aus den Mukosastumpf kam es in 17 von 19 Fällen (89%). Bei 12 Patienten (60%) mussten 1-3 Gefässe zusätzlich von Hand ligiert werden, bei 3 Patienten (15%) waren es 4-6 und bei 2 Patienten (10%) sogar mehr als 6 Arterien, welche abgebunden werden mussten. Bei einen Patienten liegen keine Angaben darüber vor.

Kurzfristige Behandlungsresultate

Siebzehn Kühe (85%) konnten gesund nach Hause entlassen werden. Die durchschnittliche Aufenthaltsdauer dieser Tiere an der Klinik betrug 5,5 Tage.

Bei 2 Kühen (10%) traten Komplikationen ein: Ein direkter Zusammenhang mit der Operation bestand bei einer Kuh, welche aufgrund einer umschriebenen, schwartigen Peritonitis im Bereich des Amputationsstumpfes geschlachtet werden musste. Bei der anderen Kuh wurde eine akute hintere funktionelle Stenose diagnostiziert. Der Blinddarmstumpf wies makroskopisch keine pathologischen Veränderungen auf.

Die dritte Kuh wurde 15 Tage nach der Operation nach Hause entlassen und dort wegen reduziertem Allgeneinbefinden und ungenügender Milchleistung geschlachtet. Die Untersuchung der Darmnaht ergab keine makroskopisch sichtbaren Veränderungen.

Langfristige Behandlungsresultate

Die Nachfrage beim Besitzer erfolgte telefonisch 8 bis 13 Monate nach der Operation. Es lebten noch 14 der 17 Kühe. Ein direkter Zusammenhang zwischen Blinddarmamputation und späterer Schlachtung bestand bei keiner der 3 Kühe: Eine Kuh wurde wegen eines Spätabortes, eine zweite wegen eines prolapsus uteri und eine dritte aus züchterischen Gründen geschlachtet.

Zusammenhang zwischen der Anzahl blutender Arterien und der Klammergrösse

Bei Verwendung der kleinen Klammern mussten durchschnittlich 2,3 und bei Verwendung der grossen Klammern durchschnittlich 4,0 Gefässe zusätzlich abgebunden werden.

DISKUSSION

In der vorliegenden Untersuchung wurde mit dem linearen Klammergerät TA 90 eine evertierende Naht am Zäkum erzeugt. Im Gegensatz zur Handnaht mit konventionellem Fadenmaterial, bei der die evertiernde Nahttechnik mit mehr Komplikationen behaftet ist als die Stoss-auf-Stoss-Naht resp. die invertierende Naht, ist dies bei der Klammernaht kein Nachteil (3). Die Gründe dafür liegen darin, dass die Klammern aufgrund ihrer Form eine Weiterdurchblutung des distal der Doppelklammerreihe gelegenen Stumpfes erlauben und ihn vor der Nekrose bewahren (3). In weiteren ist bei der Klammernaht die Gewebetraumatisierung sehr gering und die Naht von maximaler Präzision und dies gekoppelt mit einem minimalen Zeitaufwand (3,14).

Die Operationen der vorliegenden Studie wurden von 4 verschiedenen Chirurgen durchgeführt, deren Ausbildungsstand beim Umgang mit dem Klammerinstrumentarium sehr unterschiedlich war. Auf eine Auswertung der Operationszeiten wurde deshalb verzichtet. Bei dem mit der Klammertechnik vertrauten Operateur ist eine Zeiteinsparung gegenüber der konventionellen Nahttechnik mit Sicherheit vorhanden (3). Der Zeitgewinn ging jedoch teilweise wieder verloren durch den Aufwand der nötig war, um einzelne aus der Mukosa blutende Arterien zusätzlich abzubinden. Dies war bei 18 unserer Patienten der Fall. Bei 2 Kühen waren es sogar 7 Arterien, die so stark bluteten, dass sie ligiert werden mussten. Der zusätzliche Zeitaufwand war bei diesen Tieren entsprechend gross.

Wurden die kleineren Klammern (3,5 mm) verwendet, bluteten durchschnittlich weniger Gefässe als bei den grossen (4,8 mm) Klammern. Daraus kann gefolgert werden, dass bei der Zäkumamputation der Kuh zur Verkürzung der Operationszeit die blauen Magazine mit den kleineren, 3,5 mm Klammern verwendet werden sollten. Der Darm wird unter Verwendung der Magazine mit den grösseren Klammern auf eine Dicke von 2,0 mm und beim kleineren Magazin auf 1,5 mm komprimiert (3). Da dieser erste Schritt des Klammerns (=Fixation des Zäkums), manchmal übermässigen Kraftaufwand kostete, sollte die Wahl des Magazins resp. der Klammergrösse zusätzlich von der Dicke der Zäkumwand abhängig gemacht werden.

Die Resultate dieser Studie können an dieser Stelle noch nicht abschliessend bewertet werden. Es wird Ziel einer nächsten Arbeit sein, die Staplermethode mit der konventionellen Handmethode zu vergleichen und ein Urteil über die beiden Techniken abzugeben.

DANK

Wir danken der Auto-Suture AG, Höri, Switzerland für die kostenlose Bereitstellung des Klammergerätes und der Klammermagazine, sowie Herrn S. Tobler für die kompetente und liebenswürdige Unterstützung bei der Durchführung der Operationen.

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ZUSAMMENFASSUNG

In der Abdominalchirurgie beim Menschen und in der Intestinalchirurgie beim Pferd werden immer häufiger Klammergeräte eingesetzt, mit dem hauptsächlichen Ziel, die Operationszeit zu verkürzen. Es ist naheliegend, ein solches Gerät versuchsweise auch beim Rind einzusetzen. In der vorliegenden Arbeit wird die Technik der partiellen Typhlektomie mit dem linearen Klammergerät TA 90 bei der Kuh beschrieben. Die Untersuchungen wurden an 20 Kühen durchgeführt, die an einer Blinddarmdilatation/-torsion erkrankt waren.

Die Amputation erfolgte unmittelbar distal des Ostium ileocaecale. Bei 13 Kühen wurden 3,5 mm grosse Klammern und bei 7 Kühen 4,8 mm grosse Klammern verwendet. In allen Fällen waren zum Verschluss des Lumens 2 Magazine nötig, da ein 90 mm langes Magazin allein zu kurz war. Bei 17 Kühen mussten nach Anbringen der Klammernaht bis zu maximal 7 Arterien, die aus der Mukosa bluteten, zusätzlich von Hand ligiert werden. Ein Teil der durch den Einsatz des Klammergerätes gewonnenen Zeit ging dadurch wieder verloren. Aus den Nähten mit den kleinen Klammern (3,5 mm) bluteten durchschnittlich weniger Gefässe als aus den Nähten, bei welchen die grösseren Klammern (4,8 mm) verwendet wurden.

Die Resultate dieser Studie können an dieser Stelle noch nicht abschliessend bewertet werden. Es wird Ziel einer nächsten Arbeit sein, die Staplermethode mit der konventionellen Handmethode zu vergleichen und ein Urteil über die beiden Techniken abzugeben.

RESUME

L'utilisation d'agrafes en chirurgie abdominale chez l'homme et en chirurgie intestinale chez le cheval se pratique toujours plus fréquemment, ceci dans le but principal de raccourcir le temps d'opération. C'est entre autre dans cette optique que cette technique opératoire a également été expérimentée chez le bovin. On présente dans ce travail une technique de typhlectomie partielle au moyen d'une agrafeuse linéaire TA 90 chez 20 vaches souffrant d'une dilatation/torsion du caecum.

L'amputation a été effectuée distalement de l'ostium iléo-caecal. On a utilisé chez 13 vaches des agrafes de 3,5 mm, chez 7 autres vaches des agrafes de 4,8 mm. Un seul magazine de 90 mm ne suffisant pas, on a du utiliser dans les 20 cas deux magazines pour fermer la lumière intestinale. Chez 17 vaches, on a en plus effectué manuellement la ligature d'au maximum 7 artères de la muqueuse présentants une hémorragie. Le gain de temps obtenu par l'utilisation de l'agrafeuse a ainsi été partiellement perdu. Les sutures faites à l'aide des petites agrafes (3,5 mm) ont en moyenne présentés moins de vaisseaux hémorragiques que celles effectuées à l'aide des grandes agrafes (4,8 mm).

A ce jour, les résultats de cette étude ne peuvent pas encore être évalués définitivement. Le but d'une prochaine étude sera de comparer cette technique opératoire avec la technique manuelle conventionelle et de porter un jugement sur ces deux méthodes.

SUMMARY

In human abdominal surgery and also in equine intestinal surgery stapling devices are used more and more frequently to shorten the time of surgery. The present work describes the technique of a partial typhlectomy in cows with the linear stapling device TA 90. The study was performed in 20 cows suffering from dilatation and/or torsion of the caecum.

The amputation was performed just distal to the ostium ileocaecale. In 13 cows we used staples of 3.5 mm and in 7 cows the stapler size was 4.8 mm. In each case two cartridges containing a double row of stainless steel staples were necessary to close the lumen, as one cartridge of 90 mm was too short. The staple line was then inspected and in 17 cows it was necessary to manually ligate up to 7 arteries in the mucosa. This, of course, meant a loss of time won by using the stapling instrument. The stapling line with 3.5 mm staples showed less hemorrhage than the one using 4.8 mm staples.

The results cannot be discussed yet in full detail. It will be the purpose of a next study to compare and evaluate the stapling with the suture technique in typhlectomy in cows.

SIMPÓSIOS

SYMPOSIA

DANOFLOXACIN IN THE THERAPY OF RESPIRATORY DISEASE

THE EFFICACY OF DANOFLOXACIN IN THE THERAPY OF ACUTE BACTERIAL PNEUMONIA IN HOUSED BEEF CATTLE

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INTRODUCTION

Danofloxacin (CP-76,136) is a novel third generation fluoroquinolone antimicrobial with potent in vitro activity against a broad spectrum of Gram-negative and Gram-positive bacteria and mycoplasma including the principal pathogens involved in respiratory disease in cattle. Testing contemporary bovine field isolates of Pasteurella haemolytica and P.multocida from several European countries has demonstrated that danofloxacin has an MIC_{90} against P.haemolytica and P.multocida of 0.125 and 0.06 µg/ml respectively and also has potent in vitro activity against Haemophilus somnus and Mycoplasma bovis. Pharmacokinetic studies with danofloxacin in cattle have demonstrated an attractive plasma half-life (4 hours) following intramuscular or subcutaneous injection and a marked ability to concentrate in lung tissue, with peak levels in the lung exceeding those of plasma by a factor of 4. Danofloxacin is also highly active against experimental P.haemolytica infection at a dose of 1.25 mg/kg. The combination of spectrum, potency and pharmacokinetic properties indicate that this compound should be an efficacious treatment for naturally-occurring outbreaks of bacterial respiratory disease in cattle. A 2.5% aqueous formulation of danofloxacin was therefore evaluated in the therapy of typical field outbreaks of acute pneumonia in beef cattle.

MATERIALS AND METHODS

Animals and Management

Two hundred and seventy Charolais cross bull calves were studied on two farms (farms A and B) in northern France. Both farms had a recurring history of acute pneumonia associated with Pasteurella infection when cattle were brought inside for intensive fattening over the winter months. The cattle were spring-born and single suckled at pasture. In late autumn (November - December) when the cattle weighed approximately 300 kg they were transported to the farms and housed in a single large barn on each farm. They were kept in groups of 18 - 20, bedded on straw, and fed an ad libitum ration of beet pulp, oilseed cake, cereal waste and chopped straw (farm A) or maize silage (farm B) with the addition of a protein/mineral supplement. Water was supplied ad libitum.

Experimental procedure

All cattle were uniquely identified and, following housing, were inspected daily. Any animal showing signs of respiratory disease was restrained and examined clinically but treated only when it showed definite signs of acute bacterial pneumonia combined with a rectal temperature of 40°C or greater. Cattle fulfilling these criteria were weighed and a nasal swab collected. Cattle were then randomly allocated to one of two treatment groups, and given danofloxacin (1.25 mg/kg) or oxytetracycline (10 mg/kg) respectively by intramuscular injection. All cattle were treated once daily for three consecutive days. If, on clinical examination 24 hours after the third injection, the rectal temperature of an animal equalled or exceeded 39.5°C and, or pronounced clinical signs of pneumonia were still present, treatment was continued for a further two days. Rectal temperatures and clinical observations were recorded daily during the period of treatment and for a subsequent 5-day period of observation. At each clinical examination the rate and character of respiration were assessed, together with any other significant clinical signs and used to assign an overall clinical assessment score ranging from 0 (completely normal) to 4 (moribund).

Bacteriological procedures

Bacterial isolations from nasal swabs were performed by standard methods. Isolates of Pasteurella sp were retained lyophilised. Minimum inhibitory concentrations (MIC) were determined using a micro-adaptation of the broth dilution method (Sensititre susceptibility system, Sensititre Ltd.), in which 96-well microtitre plates were pre-dosed with appropriate serial dilutions of antibiotics and then dried and stabilised for subsequent use. A custom plate incorporating danofloxacin and several other antibiotics of veterinary importance was specially commissioned for this purpose.

Statistical procedures

Fisher's exact test (two-tailed) was used to examine differences between treatment groups in the duration of, and response to therapy. Differences in group mean rectal temperatures by time and by treatment were analysed using a split-plot ANOVA model.

RESULTS

Clinical observations

In all 132 pneumonic cattle were studied. Sixty-seven were treated with danofloxacin and 65 with oxytetracycline. On both farms outbreaks of disease commenced within one week of housing and cases developed within the subsequent two-week period. The clinical nature and severity of the disease was similar on the two farms and was typical of transit fever pneumonia of moderate severity. No cattle died during the study. Fifty-three cattle (82 per cent) treated with oxytetracycline required the full 5 days of therapy since their rectal temperature had not fallen to below 39.5°C by 24 hours after the third treatment. In contrast significantly ($p<0.05$) fewer cattle (38, 57 per cent) treated with danofloxacin required treatment for the longer period. Following treatment, the majority of cattle in both groups improved clinically so that by the fifth day of the post-treatment observation period 95 per cent of oxytetracycline-treated cattle and 77 per cent of danofloxacin-treated cattle were judged clinically normal. During the observation period a proportion of cattle in both groups re-met the original criteria for treatment (i.e. their temperature equalled or exceeded 40°C and they still showed clinical signs of pneumonia). These animals were judged to require additional therapy and were withdrawn from the study, 26 per cent of cattle treated with oxytetracycline were in this category compared to 15 per cent of cattle treated with danofloxacin.

Responses in Rectal Temperature

Group mean rectal temperatures on the day of commencement of treatment were approximately equal at about 40.5°C (Fig.1). Treatment with danofloxacin caused a rapid fall in group mean rectal temperature over the first 24 hours of medication (Fig.1) and this reduction in pyrexia was maintained so that the group mean temperature remained significantly ($p<0.05$) lower than its value before treatment. The reduction in pyrexia associated with oxytetracycline treatment was less marked so that on the three days following treatment the mean rectal temperature of danofloxacin-treated cattle was significantly ($p<0.05$) lower than the oxytetracycline group. This was also true for the subset of animals that received 5 days of medication (Fig.1) although the differences did not reach statistical significance on days 1 and 3.

Response to treatment

Twenty-four hours after treatment ceased, any animal whose temperature had dropped to below 39.5°C and had, in addition, demonstrated clear clinical improvement (i.e. whose clinical illness score was less than on its first day of treatment), was classified as having responded satisfactorily. Significantly more danofloxacin-treated cattle (81%) than oxytetracycline-treated cattle (38%) responded successfully by this criteria (Table 1).

Bacteriological observations

The MIC values for 13 isolates of Pasteurella haemolytica and 15 isolates of P.multocida indicated that all were highly sensitive to danofloxacin with an MIC_{90} to P.haemolytica and P.multocida of 0.06 µg/ml and 0.03 µg/ml respectively. A mixture of

both sensitive and resistance strains to oxytetracycline were recovered (Fig.2).

TABLE 1
Responses of pneumonic cattle to treatment with danofloxacin or oxytetracycline

Treatment	No. of Animals	No. requiring 5 Days Treatment	% Making a Successful Response*	% Clinically Normal 5 days post Post Treatment
Danofloxacin	67	38[b]	81[b]	77[b]
Oxytetracycline	65	53[a]	38[a]	55[a]

[ab] The treatment means differ significantly ($p<0.05$).
* A successful response is recorded for any animal which, 24 hours after the end of treatment, has a rectal temperature of below 39.5°C, together with a clinical illness score which is less than its value on the first day of treatment.

DISCUSSION AND CONCLUSIONS

Many infectious agents have been associated with outbreaks of acute respiratory disease in cattle including viruses, mycoplasma and bacteria (1) and the precise aetiology of any individual outbreak is often multifactorial. Nevertheless bacteria of the genus Pasteurella are the most important pathogens to invade the lung tissue in cases of acute bronchopneumonia and the clinical, epidemiological and bacteriological features of the present outbreaks were typical of the transit or shipping fever type of pneumonia commonly associated with Pasteurella infection which is frequently seen in Western Europe when single-suckled beef cattle are housed in the autumn.(1) The immediate reduction in pyrexia following treatment with danofloxacin, the high successful response rate and the improvements in clinical condition indicate that this novel fluoroquinolone antimicrobial agent was highly efficacious in the therapy of these outbreaks. In this study responses to treatment with danofloxacin were superior to oxytetracycline, this may be at least partly explicable by a degree of resistance to oxytetracycline observed among nasal isolates of Pasteurella (although this may not exactly reflect the characteristics of lung isolates) it is likely however that the superior efficacy of danofloxacin is probably also related to the intrinsically higher potency and superior pharmacokinetic properties of the new fluoroquinolones. These agents possess several advantages over traditional antibiotics in the therapy of bacterial respiratory diseases including bactericidal activity at low concentrations, wide spectrum, potent anti-mycoplasmal activity and the ability to distribute widely in body fluids and readily penetrate tissues, particularly those of the respiratory system. Although worldwide field evaluation is still in progress it would appear from this initial study that danofloxacin should prove a highly effective therapy for pneumonia in cattle.

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SUMMARY

Danofloxacin, a novel fluoroquinolone antimicrobial was evaluated in the treatment of acute bacterial pneumonia in recently-housed beef cattle of approximately 300 kg liveweight. The clinical responses of 67 pneumonic cattle treated with danofloxacin were compared with those of 65 cattle treated with oxytetracycline, both treatments being given by intramuscular injection for either three or five days depending on

clinical response. Both treatments resulted in a rapid fall in group mean rectal temperature and improved the clinical condition of the majority of cases. However, in comparison to oxytetracycline, danofloxacin therapy was characterised by significantly fewer treatment days, a significantly higher response rate, significantly better reduction of pyrexia and fewer cattle requiring re-treatment.

RESUME:

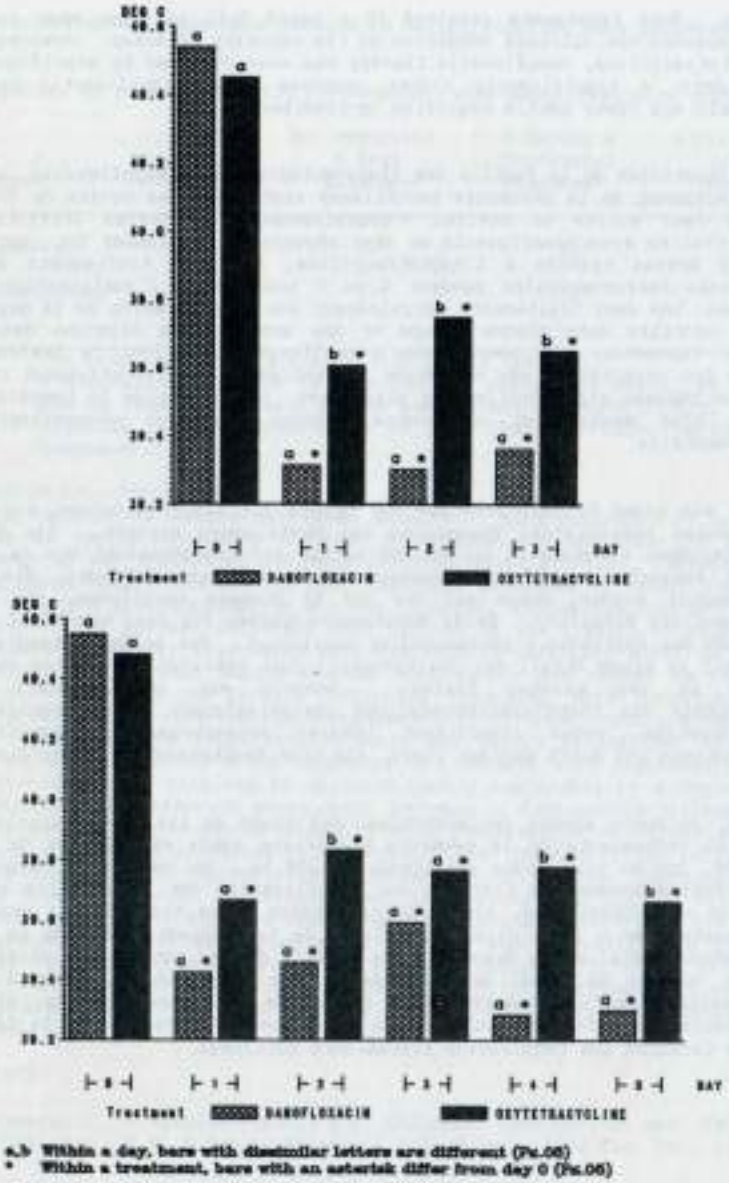
Un nouvel antibactérien de la famille des fluoroquinolones, la Danofloxacin a été évalué pour le traitement de la pneumonie bactérienne aigüe chez des bovins de 300 kg de poids vif, à leur entrée en atelier d'engraissement. 67 bovins atteints de pneumonie furent traités avec Danafloxacin et leur réponse au traitement fut comparée avec celle de 65 bovins traités à l'oxytétracycline, les deux traitements étant administrés par voie intramusculaire pendant 3 ou 5 jours selon l'amélioration des symptomes cliniques. Les deux traitements entrainèrent une baisse rapide de la moyenne des températures rectales dans chaque groupe et une amélioration clinique dans la majorité des cas. Cependant, par comparaison avec l'oxytétracycline, le traitement avec Danofloxacin fut caractérisé par une durée de traitement significativement moins longue, un taux de réponse significativement plus élevé, une baisse de la température significativement plus marquée et un nombre moindre d'animaux nécessitant un traitement supplémentaire.

ZUSAMMENFASSUNG:

Danofloxacin, ein neues Antibiotikum aus der Gruppe der Fluoroquinolone, wurde in der Behandlung akuter bakterieller Pneumonien bei Mastrindern erprobt. Die Tiere waren kürzlich zusammen aufgestallt worden und hatten ein Lebendgewicht von ca. 300 kg. Die klinische Antwort, von 67 am Lungenentzündung erkrankter Rinder, die mit Danofloxacin behandelt wurden, wurde mit der von 65 Rindern verglichen, die eine Oxytetracyclinbehandlung erhielten. Beide Medikaments wurden für drei oder fünf Tage - je nach Besserung des Zustandes - intramuskulär appliziert. Bei beiden Behandlungen kam es sehr schnell zu einem Abfall der durchschnittlichen rektalen Temperatur und zu einer Besserung in den meisten Fällen. Dennoch war im Gegensatz zur Oxytetracyclintherapie die Danofloxacinbehandlung charakterisiert durch signifikant weniger Behandlungstage, einer signifikant höheren Besserungsrate, signifikant besserem Fieberrückgang und durch weniger Tiere, die eine Nachbehandlung benötigten.

RESUMEN:

Danofloxacina, un nuevo agente antimicrobiano del grupo de las fluoroquinolonas, fué evaluado en el tratamiento de la neumonia bacteriana aguda en terneros de cebo recién estabulados, con un peso medio aproximado de 300 kg. Se comparó la respuesta clinica de 67 animales neumónicos tratados con danofloxacina con la obtenida en 65 casos tratados con oxitetraciclina, siendo administrados ambos tratamientos por via intramuscular durante tres o cinco dias, dependiendo de la respuesta obtenida en cada caso. La temperatura rectal media descendió una mejoria de las condiciones clinicas. Sin embargo, el número de dias de tratamiento que requirió la terapia con danofloxacina resultó significativamente menor que el de la oxiterraciclina, siendo asimismo significativa la diferencia en el grado de respuesta, reducción de la fiebre y en el número de terneros que requirieron tratamiento adicional.



a,b Within a day, bars with dissimilar letters are different (P≤.05)
* Within a treatment, bars with an asterisk differ from day 0 (P≤.05)

Fig.1. Mean group rectal temperatures of pneumonic cattle. Upper plot all cattle, lower plot cattle receiving 5 days of therapy.

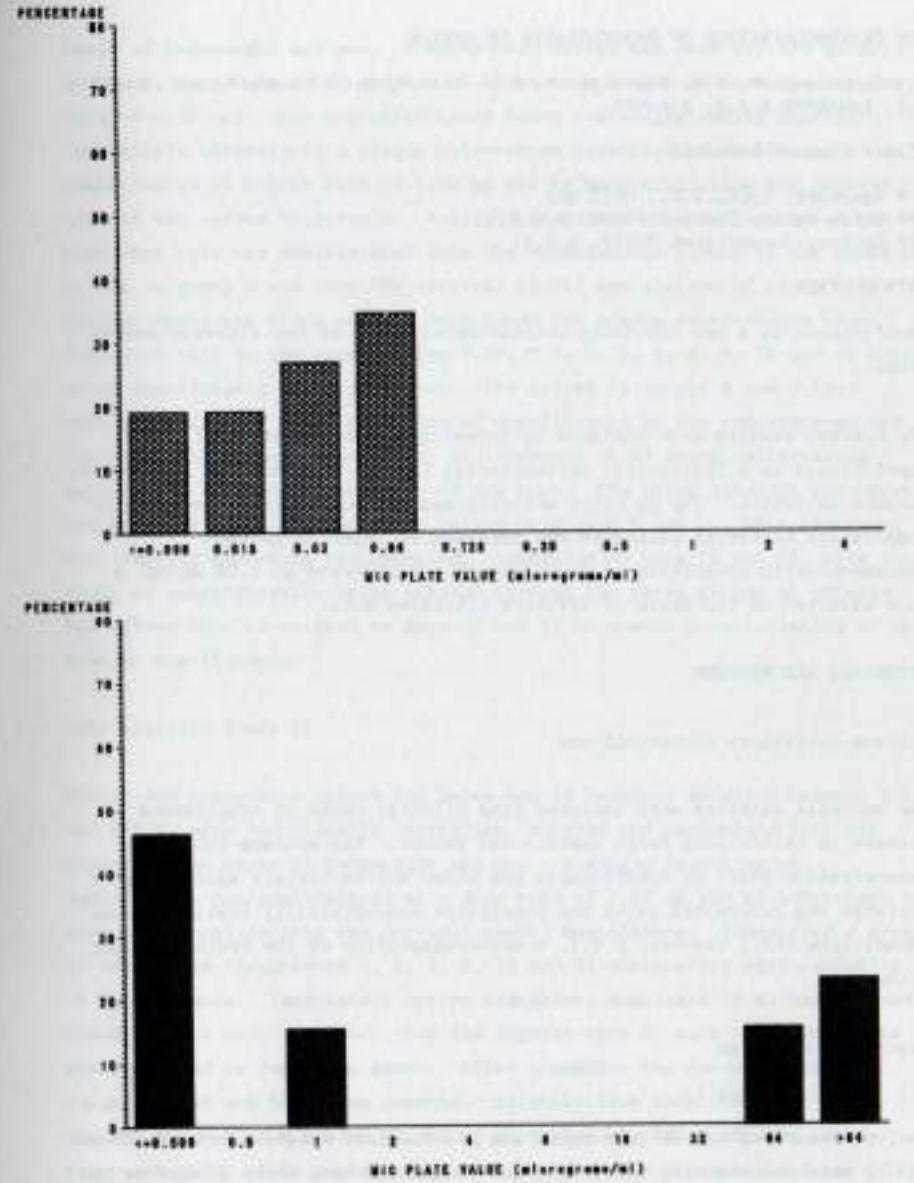


Fig.2. Distribution of MIC values (µg/ml) of danofloxacin (upper plot) and oxytetracycline (lower plot) for 20 isolates of Pasteurella sp.

THE PHARMACOKINETICS OF DANOFLOXACIN IN CATTLE

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INTRODUCTION

Danofloxacin is a new synthetic antibacterial agent of the fluoroquinolone class.

The current studies were designed to investigate the potential of danofloxacin as a therapeutic antibacterial for the treatment of respiratory disease in cattle. The in vitro activity against contemporary isolates of respiratory bacterial pathogens was assessed in relation to the pharmacokinetic properties of danofloxacin administered at 1.25 mg/kg, a dose selected on the basis of efficacy titration data.

MATERIALS AND METHODS

Minimum Inhibitory Concentrations

The bacteria examined were isolated from clinical cases of respiratory disease in cattle from North America and Europe. The minimum inhibitory concentration (MIC) of danofloxacin and other antibacterials against these isolates was determined using the Sensititre susceptibility testing system (Sensititre Ltd., Crawley, U.K.), a micro-adaptation of the broth dilution method.

Experimental Design

(i) Kinetics Study I

Twelve cross-bred calves (six males and six females) weighing between 90 and 133 kg were individually identified and randomised into three groups on the

basis of bodyweight and sex. A cross-over design was used for the study. On day 0, duplicate 10 ml samples of blood were collected from the jugular vein of each calf into evacuated glass tubes containing sodium heparin. Immediately thereafter, a single intravenous injection of danofloxacin was administered at a dose rate of 1.25 mg per kg bodyweight into the jugular vein of the calves in group A. A single injection of danofloxacin at the same dose rate was administered into the subcutaneous tissue of the necks of calves in group B and into the cervical (neck) musculature of group C. Further duplicate blood samples were taken for plasma danofloxacin assay from each calf in the three groups 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after danofloxacin administration. The calves in groups B and C then received a further four injections of danofloxacin by the subcutaneous and intramuscular routes respectively at intervals of 24 hours, alternating between the left and right sides of the neck. The blood sampling procedure was repeated on days 2 and 4 for calves in groups B and C. This treatment and sampling procedure was repeated, commencing on days 12 and 24, with the route of administration being rotated through the three groups of animals. The calves were re-weighed on days 12 and 24 to enable re-calculation of the dose of danofloxacin.

(ii) Kinetics Study II

Thirty-six cross-bred calves (18 males and 18 females) weighing between 223 and 291 kg were individually identified, weighed and randomised into six groups on the basis of bodyweight and sex. A single injection of danofloxacin was administered at a dose rate of 1.25 mg per kg bodyweight to each of the calves into the cervical (neck) musculature. Thereafter a group of calves was slaughtered 1, 2, 4, 8, 12 and 24 hours after administration of danofloxacin. Immediately before slaughter, duplicate 10 ml heparinised blood samples were collected from the jugular vein of each calf and plasma was harvested as described above. After slaughter the carcasses were exsanguinated and the lungs removed. Aliquots from each lung lobe were removed to provide a pooled sample of approximately 500 g of lung tissue from each calf. This pooled sample was blended in a processor and two 100 g aliquots of the lung homogenate were taken for analysis.

Danofloxacin assay

The plasma and lung tissue concentrations of danofloxacin were assayed by a liquid chromatography method. The limits of quantitation of the assay were 0.02-2.0 µg/ml for plasma and 0.1-2.0 µg/ml for lung tissue.

RESULTS

The MICs of danofloxacin was 0.06 µg/ml for North American isolates of both Pasteurella spp. and for European isolates of P.multocida, and 0.125 µg/ml for European isolates of P.haemolytica. The MIC$_{90}$ of danofloxacin against Haemophilus somnus was 0.06 µg/ml for North American and European isolates. Individual MIC values ranged between ≤0.008 µg/ml and 2 µg/ml.

In kinetics study I, plasma concentrations of danofloxacin following intravenous administration appeared to decline in a biexponential manner indicative of distribution and elimination phases. The major pharmacokinetic parameters for danofloxacin following intravenous administration are summarised in Table 1 and following the first, third and fifth daily administration of drug by the intramuscular and subcutaneous routes in Table 2.

TABLE 1

Pharmacokinetic parameters related to distribution and elimination of danofloxacin after intravenous administration to calves at 1.25 mg/kg.

Pharmacokinetic Parameter		Mean
V_{dz}	(l/kg)	2.72
V_{dss}	(l/kg)	2.46
Cl_B	(l/hr.kg)	0.47
B	(hr^{-1})	0.18
$t_{1/2}$	(hrs)	3.85
$AUC_{(0-\infty)}$	(µg.hr/ml)	2.73

TABLE 2

Plasma pharmacokinetic parameters for danofloxacin after administration of one, three and five daily doses of 1.25 mg/kg to calves by the intramuscular or subcutaneous routes.

Route of Administration	Number of Doses at 24 hour Intervals (hours^{-1})	C_{max} (µg/ml)	T_{max} (hours)	$AUC_{(0-\infty)}$ (µg.h/ml)	B (hours^{-1})	$t_{1/2}$ (hours)
Intramuscular	1	0.47	1.0	2.8	0.18	3.85
	3	0.48	1.3	3.0	0.17	4.07
	5	0.48	0.8	3.1	0.16	4.33
Subcutaneous	1	0.37	1.1	2.5	0.17	4.07
	3	0.39	1.1	2.8	0.16	4.33
	5	0.44	1.2	2.9	0.17	4.07

Peak plasma concentrations of danofloxacin in individual animals were observed between a quarter and two hours after administration by both routes. The mean peak plasma concentration achieved by the intramuscular route was higher than by the subcutaneous route and following the first and third administrations this difference was statistically significant. However mean AUCs for the two routes were similar and confidence limits well within the accepted 20% bounds for demonstrating bioequivalence. There were no significant differences in the values of T_{max} and B. Bioavailability of danofloxacin was virtually complete by both the intramuscular and subcutaneous routes (104 and 93 percent relative to intravenous route respectively).

The major pharmacokinetic parameters recorded in kinetics study II are summarised in Table 3.

TABLE 3

Comparative plasma and lung pharmacokinetic parameters for danofloxacin after administration of a single dose of 1.25 mg/kg to calves by the intramuscular route.

Tissue	C_{max} (µg/ml)	T_{max} (hours)	$AUC_{(0-\infty)}$ (µg.h/ml)	β^* ($hours^{-1}$)	$t_{1/2}$* (hours)
Plasma	0.35	1.0	2.0	0.21	3.3
Lung	1.44	1.0	7.4	0.20	3.5

*Calculated from peak to 12 hours

Peak mean concentrations in both plasma and lung tissue were observed in the first sample taken. The peak mean lung concentration was 4.1 times greater than that of plasma. Thereafter depletion from lung and plasma over the succeeding 12 hours followed a parallel course, the elimination half-life being very similar for the two tissues. Examination of the lung data to 24 hours indicates that there may be a slower, later elimination phase with a half-life of approximately 4.3 hours. The $AUC_{(0-\infty)}$ for lung tissue was 3.7 times greater than that for plasma.

CONCLUSIONS

The data generated in these studies demonstrate that (i) danofloxacin was rapidly absorbed after intramuscular and subcutaneous administration and bioavailability for both routes was virtually complete; (ii) the pharmacokinetics of danofloxacin were similar for the intramuscular and subcutaneous routes of administration; (iii) danofloxacin penetrated lung tissue to provide peak concentrations over four times those of plasma; (iv) the plasma and lung concentrations of danofloxacin achieved after parenteral administration of a dose of 1.25 mg/kg were substantially above the MIC_{90}s for contemporary isolates of the most important bacterial respiratory pathogens of cattle in North America and Europe. It is concluded that danofloxacin has attributes which are particularly appropriate to an antibacterial drug indicated for the treatment of respiratory disease in cattle and it is projected that 1.25 mg/kg should be a clinically efficacious dose.

SUMMARY

The pharmacokinetic properties of danofloxacin, a new synthetic fluoroquinolone antibacterial, were investigated in plasma and lung kinetic studies in cattle when administered at a dose rate of 1.25 mg/kg. The plasma kinetics of danofloxacin were similar for the intramuscular and subcutaneous routes of administration with rapid absorption and virtually complete bioavailability. Danofloxacin penetrated lung tissue to provide peak concentrations over four times those of plasma. Plasma and lung concentrations of danofloxacin were substantially above the MIC_{90}s for contemporary isolates of the most important bacterial respiratory pathogens of cattle in North America and Europe.

RESUME

Le comportement pharmacocinetique de danofloxacin, un nouvel anti-infectieux de synthese fluoroquinolone a ete evalue dans le plasma et dans les tissues pulmonaires des bovins quand elle est administree a la dose de 1.25 mg/kg. Les distributions plasmatiques par voie intramusculaire ou sous cutanee sont comparable. Il y avait aussie une absorption rapide et une biodisponibilite presque totale. La concentration tissulaire maximal de la danofloxacin dans les poumons etait quatre fois plus elevee que la concentration plasmatique. Les concentrations de la danofloxacin dans le plasma et dans les poumons etaient nettement plus elevees que les C.I.M.90 faites sur des souches des principaux germes pathogenes isolees de l'appareil pulmonaire des bovins aux Etats Unis et en Europe.

SUMARIO

Se investigaron las propiedades farmacocineticas del danofloxacin, nuevo antibacterico con fluoroquinolon, en estudios cineticos del plasma y de los pulmones del ganado cuando se habia administrado a la proporcion de 1,25 mg/kg. Las cineticas del danofloxacin en el plasma fueron semejantes en las vias de administracion intramuscular y subcutanea, con absorcion rapida y biocficacia virtualmente completa. El danofloxacin penetro por el tejido pulmonar produciendo concentraciones maximas de mas de cuatro veces que las del plasma. Las concestraciones del danofloxacin en el plasma y en los pulmones fueron substancialmente mayores que la C.I.M.90 tratandose de aislados contemporaneos del los mas importantes patogenos bacterianos de la respiracion del ganado en Norteamerica y Europa.

DANOFLOXACIN THERAPY OF PNEUMONIC PASTEURELLOSIS OF FEEDLOT CATTLE IN THE UNITED STATES AND CANADA

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INTRODUCTION

Respiratory disease remains a major cause of economic loss in the cattle industry and *Pasteurella* species continue to be the most commonly isolated pathogens (1,2). Danofloxacin is a new fluoroquinolone antimicrobial with potent activity against a broad spectrum of bacteria and mycoplasmas of veterinary importance, including *Pasteurella haemolytica* and *P. multocida* (3,4). In cattle danofloxacin has exhibited favorable plasma pharmacokinetics along with high, persistent lung tissue levels (5). Under typical U.S. and Canadian feedlot conditions, danofloxacin was evaluated for efficacy against naturally occurring bovine pneumonic pasteurellosis. Pooled data is presented from five field trials in which the same protocol was followed at all sites.

MATERIALS AND METHODS

Locations

Three feedlot sites in the U.S. (Colorado, Kansas and Texas) and two in Canada (Alberta and Saskatchewan) were selected. The test sites were either university test stations, private contract facilities or commercial feedlots.

Animals

Commercial beef calves were assembled at sale barns and transported from 300 to 2100 km to the feedlot facility. A total of 214 individuals were selected for the five studies from about 1200 candidates. The calves weighed approximately 200 kg each. No antibacterial treatments were administered during the calves' acquisition or handling.

Study design

Animals were randomly assigned to one of two treatment groups after individually meeting the criteria for selection: clinical signs consistent with acute pneumonia (depression and dyspnea) and a rectal temperature of ≥40°C. The treatment groups were a saline control group (n=60), given 10 ml saline SID for 5 days IM and a danofloxacin group (n=154) given 1.25 mg/kg danofloxacin SID for 3 or 5 days. Cattle in the danofloxacin group were administered 3 consecutive days of therapy, after which if their rectal temperature was ≥39.7°C or if they were judged to be moderately ill (illness score of 2 or greater), they were treated for an additional two consecutive days with danofloxacin. Each animal was clinically examined daily during its three- or five-day treatment period. Rectal temperature was recorded and an illness score assigned (0=normal and healthy, 1=slightly ill, 2=moderately ill, 3=severely ill and 4=moribund or dead) each day. Body weights were recorded on day 0 and at trial termination or death. A successful response to therapy was defined as a rectal temperature of <39.7°C and an illness score of ≤2 at 24 hours after the last treatment was given.

The variables rectal temperature, response rate and weight gain were analyzed with a linear model fit by the methods of least squares. The illness score variable was analyzed with a repeated measure weighted least squares linear model. Multiple comparison tests were used to compare treatment, or treatment by day on test, means depending on the results from the analysis of the model. The SAS (SAS Institute, Cary, N.C.) general linear model (GLM) and categorical (CATMOD) procedures were used in the analysis.

Bacteriology

Prior to initial treatment, at 24 hours after an animal's last injection and again at trial completion, nasopharyngeal swabs were taken for culture. Lung swabs were taken after necropsy from animals that died. Pathogens were identified and their sensitivity to danofloxacin was determined using a microtiter plate system (Sensititre Ltd., Imberhorne Lane, East Grinstead, Sussex, England) from which minimum inhibitory concentrations (MICs) were generated. The MIC level which inhibited the growth of 90% of the isolates of a particular species is reported as the MIC_{90}.

RESULTS

Prior to treatment (day 0), mean rectal temperature of all cattle selected was greater than 41°C. Twenty-four hours after the first injection, the mean temperature of cattle treated with danofloxacin had fallen to 39.5°C and remained below 39.7°C during the treatment period. Over the same time period mean temperatures of the saline group remained above 40.3°C. The mean temperature of the danofloxacin group was significantly ($P \leq 0.05$) lower than that of the saline group on each day and also significantly lower than its own pretreatment mean temperature. On the day after the last treatment, 69% of danofloxacin treated cattle had made a successful response to therapy, while by the same criteria 12% of the saline controls had responded. Following treatment, the clinical condition of danofloxacin treated cattle improved as shown by comparing pretreatment illness scores with post-treatment illness scores (figure 1). Overall, there was a 43% mortality rate in the saline controls and a 5% mortality rate in the

danofloxacin group. Necropsy of these animals revealed fibrinous bronchopneumonic lung lesions typical of pneumonic pasteurellosis. The average daily gain for danofloxacin treated cattle was significantly improved over that of the saline controls, which lost weight during the experimental period (Figure 2).

The MIC_{90} of danofloxacin for *P. haemolytica* isolates (n=144), *P. multocida* isolates (n=150) and *H. somnus* isolates (n=16) was 0.125, 0.125 and 0.06 mcg/ml, respectively.

DISCUSSION

The high mortality rate observed in the saline negative control group in these studies is indicative of the severity of the outbreaks encountered. The *P. haemolytica, P. multocida and H. somnus* isolates from nasal swabs and lung samples were highly sensitive to danofloxacin. The prompt temperature response and other indicators of clinical efficacy reported here are consistent with responses reported for other new antibacterial agents stanced for bovine respiratory disease: enrofloxacin (6), tilmicosin (7) and sulbactam-ampicillin (8). In this series of five short-term studies, the administration of danofloxacin to cattle severely affected with naturally acquired peracute to acute pneumonic pasteurellosis provided a high level of efficacy as measured by rapid reduction in pyrexia, improvement in clinical condition, maintenance of weight gain and reduction of mortality, all of which combined to confirm the utility of the described regimen under field conditions.

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SUMMARY

Danofloxacin, a new fluoroquinolone antibacterial, was evaluated in the therapy of naturally occurring pneumonic pasteurellosis which occurred in 200 kg beef calves shortly after arrival to five U.S. or Canadian feedlots. After random allotment, they were treated by intramuscular injection with either danofloxacin at 1.25 mg/kg for 3 or 5 days (n=154) or saline for 5 days (n=60). Danofloxacin produced a significant (P≤0.05) reduction in mean body temperature within 24 hours of initial administration and throughout the treatment period compared to saline.

Danofloxacin was also associated with marked improvement in clinical condition, with significantly less mortality and improved average daily gain over the 9 or 11 day experimental period.

RESUME

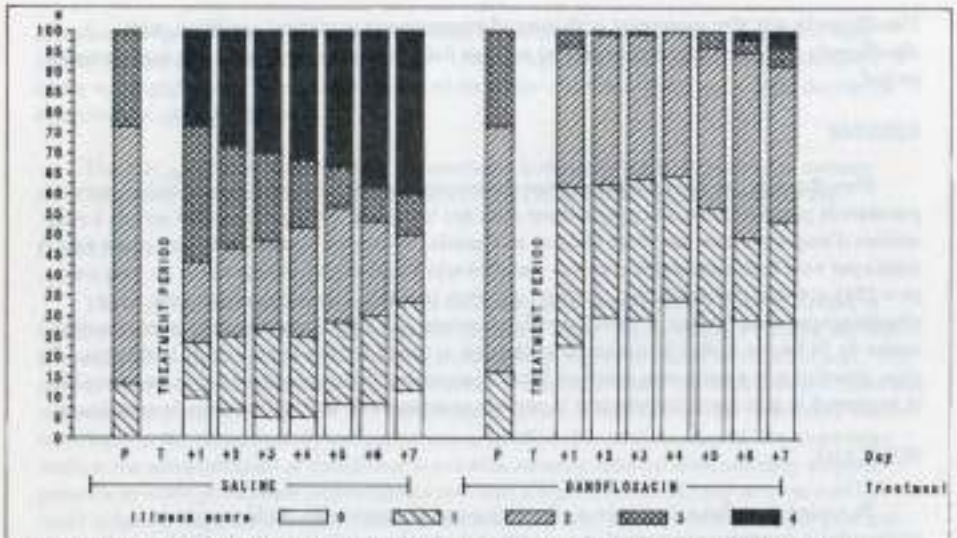
Danofloxacin, un nouvel antibactérien fluoroquinolone, a été évalué dans le traitement de pneumonie pasteurellique d'origine naturel chez des bovins de 200 kg récemment arrivés à cinq ateliers d'engraissement aux Etats Unis ou au Canada. Ils ont été répartis au hasard et ont été traités par voie intramusculaire avec soit danofloxacin à 1.25 mg/kg pendant trois ou cinq jours, (n = 154), soit eau physiologique pendant cinq jours (n = 60). Par comparaison avec l'eau physiologique, danofloxacin a baissé significativement ($p \leq 0.05$) la température moyenne dans moins de 24 heures après l'initiation du traitement, et pendant toute la période de la thérapie. En plus, danofloxacin a nettement amélioré l'état clinique des animaux, réduit la taux de mortalité, et augmenté le gain quotidien pendant la periode expérimentale de neuf jours ou de onze jours.

SUMARIO

Se evaluó danofloxacin, nuevo antibactérico con fluroquinolón, en la terapia de la pasteurolosis de ocurrencia natural que se presentó en temeros de engorde de 200 kg., poco después de su llegada a cinco lugares de engorde de los E.E. U.U. o del Canadá. Después del repartimiento al azar, se les trató con inyecciones intramusculares ya sea de danofloxacin a 1,25 mg /kg. durante 3 ó 5 días (a un número de 154) o de solución salina durante 5 días (a un número de 60). El danofloxacin produjo una baja signficativa (P≤0,05) de la temperatura media del cuerpo dantro de las 24 horas a partir de la administración inicial y durante todo el tiempo del tratamiento, en comparación con la solución salina. Asi mismo, se encontró relación del danofloxacin con la majoria destacada del estado clinico, con una mortalidad significativamente menor y un provecho medio diario mejorado durante el periodo experimental de 9 u 11 días.

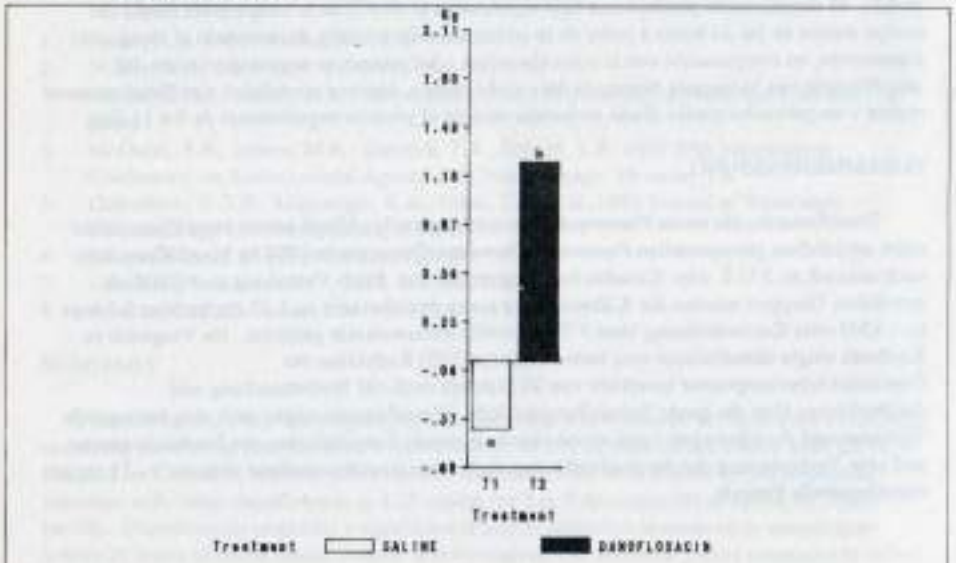
ZUSAMMENFASSUNG

Danofloxacin, ein neues Fluoroquinolon-antibakterielles Mittel wurde beim Theraparien einer natürlichen pneumonellen Pasterellose bewertet. Diese trat bei 200 kg Mastkälbern kurz nach ankunft an 5 U.S. oder Kanadischen Maststellen auf. Nach Verteilung in willkürlich gewählten Gruppen wurden die Kälber mit entweder danofloxacin zu 1.25 mg/kg über 3-5 tage (n = 154) oder Kochsalzlösung über 5 Tage (n=60) intramuskulär gespritzt. Im Vergleich zu Kochsalz zeigte danofloxacin eine bedeutende (p≤0.05) Reduktion der Durchnittkörpertemperatur innerhalb von 24 Stunden nach der Erstbehandlung und darüberhinaus über die ganze Behandlungsperiode. Danofloxacin zeigte auch eine bedeutende Verbesserung der klinischen Lage sowie eine bedeutende Verminderung der Sterblichkeitsrate und eine Verbesserung der durchschnittlichen täglichen Gewichtszunahme über die 9 - 11 tägige experimentelle Periode.



Illness scores: 0=Normal and healthy 1=Slightly ill 2=Moderately ill 3=Severely ill 4=Moribund or dead

FIGURE 1 DISTRIBUTION (%) OF ILLNESS SCORES FOR CATTLE PRIOR TO TREATMENT AND 7 DAYS POST TREATMENT



a,b Bars with dissimilar letters are different (P≤.05)

FIGURE 2 AVERAGE DAILY GAIN

A DOSE RESPONSE STUDY OF THE FLUOROQUINOLONE, DANOFLOXACIN AGAINST INDUCED BOVINE PNEUMONIC PASTEURELLOSIS

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INTRODUCTION

Danofloxacin is a novel synthetic third generation fluoroquinolone antimicrobial (1). It exhibits high potency *in vitro* against a broad range of Gram-positive and Gram-negative bacterial and mycoplasmal veterinary pathogens (2) and in common with other members of the fluoroquinolone class is bactericidal (3). The minimum inhibitory concentration of danofloxacin against 90 percent (MIC_{90}) of over 250 recently isolated field strains of *Pasteurella haemolytica*, *P. multocida* and *Haemophilus somnus* was found to be ≤ 0.125 µg/ml (4). In an evaluation of the pharmacokinetic profile of danofloxacin in cattle following intramuscular administration, it was shown that the drug achieved peak mean concentrations and AUC (area under the curve) in lung that were approximately 4-fold those found in plasma (4).

The development of disease models for reliably inducing pneumonic pasteurellosis in healthy calves has facilitated the evaluation and development of antibacterials (5) and vaccines (6). This approach has enabled researchers to determine the efficacy of new antimicrobials in a controlled environment before commencing field investigations. The purpose of the present study was to establish efficacy for danofloxacin and to determine the relationship between dose and efficacy against pneumonic pasteurellosis in calves by means of an experimentally induced respiratory infection of *P. haemolytica*.

MATERIALS AND METHODS

Animals/housing/ration

Sixty male Holstein calves, one to two days old, were purchased from a dairy farm normally free from endemic pasteurellosis. Upon arrival at the test facility calves were given a uniquely numbered eartag and placed into individual hutches with solid walls and 1.4 sq m floor space under a large roofed shelter without side walls. During the study, calves were offered two kg of grain daily, with monitoring of intake, and water ad libitum. Calves received no vaccinations or medication prior to study initiation. Calves were five weeks old at the beginning of the study and averaged 48 kg (range of 32 to 64 kg).

Experimental design

Prior to challenge calves were weighed for dose calculation, had respiratory rates taken (over a 60 second period) and were then challenged twice with a *P. haemolytica* culture, as described below. The animals were monitored for clinical signs of respiratory disease and were allocated to the experiment as they met the following selection criteria: increase in rectal temperature of $\geq 0.7^{0}$C, depression, and/or increase in resting respiratory rate of 10 or more counts per minute.

A total of 56 calves were selected within 42 hours after the second infective challenge and assigned to one of the five treatment groups in a preset random order. Four treatment groups, of eleven animals each, received danofloxacin at doses of 0.312, 0.625, 1.25, or 2.50 mg/kg/day, respectively, for three days; while the twelve calves in the fifth group each received 5 ml of saline per day for three days, by a single intramuscular injection.

Calves were examined individually each day for a period of 10 days commencing on the first day of treatment. The variables assessed daily were rectal temperature and illness score (based upon presence or absence of depression, character of respiration, nasal discharge, coughing, general appearance and overall clinical impression). Illness scores were assigned on a scale of 0 to 4, as follows: 0 = normal and healthy, 1 = slightly ill, 2 = moderately ill, 3 = severely ill, and 4 = moribund or dead.

At the end of the 10-day experiment, calves were humanely sacrificed and were necropsied for lung-lesion scoring and collection of lung swabs. Lung-lesion scores were calculated based on the percent pneumonic involvement of each of the eight lobes or segments of the bovine lung. Representative pneumonic areas were swabbed and streaked onto 5% bovine-blood agar plates for identification of pathogens.

The variables rectal temperature, response rate (see table 1 for definition) and lung lesion score were analyzed with a linear model fit by the methods of least squares. Multiple comparison tests were used to compare treatment, or treatment by day on test, means depending on the results from the analysis of the model. The SAS (SAS Institute, Cary, N.C.) general linear model (GLM) and categorical data (CATMOD) procedures were used in the analysis.

Inoculum/challenge

The *Pasteurella haemolytica* culture used in this experiment was derived from a field isolate obtained at the University of California and designated 86B0721. All calves were challenged twice intratracheally with cultures of this organism containing approximately 3×10^7 cfu/ml: the first, 7-8 ml challenge was acidified to pH 4.5±0.1 with acetic acid, while the second challenge of 10 ml, administered four hours later, was not acidified. The challenge culture was grown from a discrete colony of the organism which was inoculated from a blood agar plate into 200 ml of Brain Heart Infusion Broth (BHI) and incubated at 37°C for 18 hours. This broth was then used for inoculation. The number of challenge organisms used to infect the calves was verified using a standard plate count method.

RESULTS

Prior to challenge, all calves had a normal rectal temperature, respiration rate (38-41 per minute), and appearance (clinical illness score of 0). At the time of initial treatment, mean temperatures for each group of calves in the study had risen to 40.1°C or above, respiration rates had increased by more than 20 counts per minute (58-63), and clinical scores were in the slightly ill to moderately ill range (score 1 or 2). Danofloxacin treatment produced a prompt and dose-related reduction in temperature (Figure 1). After the second and third injections, the two higher doses of danofloxacin could not be differentiated from each other. Clinical illness scores also showed a dose-related response to danofloxacin, with all calves at the highest dose levels (1.25 and 2.5 mg/kg) being scored as normal and healthy (0) after three injections of danofloxacin. Calves were designated as responding successfully if, on the day after the third injection, their

rectal temperatures were less than 39.5°C and their illness score was less than that observed on the day of initial treatment. One hundred percent of calves at each of the two upper dose levels of danofloxacin showed such a response to therapy, whereas 82% responded at the 0.625 mg/kg dose, 27% at the 0.312 mg/kg dose, and none in the saline control group (Table 1).

Five of the twelve saline-treated calves died during the 10-day term of the experiment (one on day 4, two on day 5 and two on day 7); and two of eleven calves died in the group receiving the lowest dose of danofloxacin (0.312 mg/kg), both on day 7 (Table 1). There were no deaths in the groups receiving higher doses of danofloxacin. Lung lesion scores indicated (Table 1) a clear dose-related response to danofloxacin. Again, the two highest doses were not distinguishable from each other, but were statistically superior to lower doses and to the saline control group. The lung lesions were all identified as characteristic of fibrinous bronchopneumonia. *Pasteurella haemolytica* was cultured from the lung tissue of eleven saline-treated calves and from between four and seven calves in each of the danofloxacin groups.

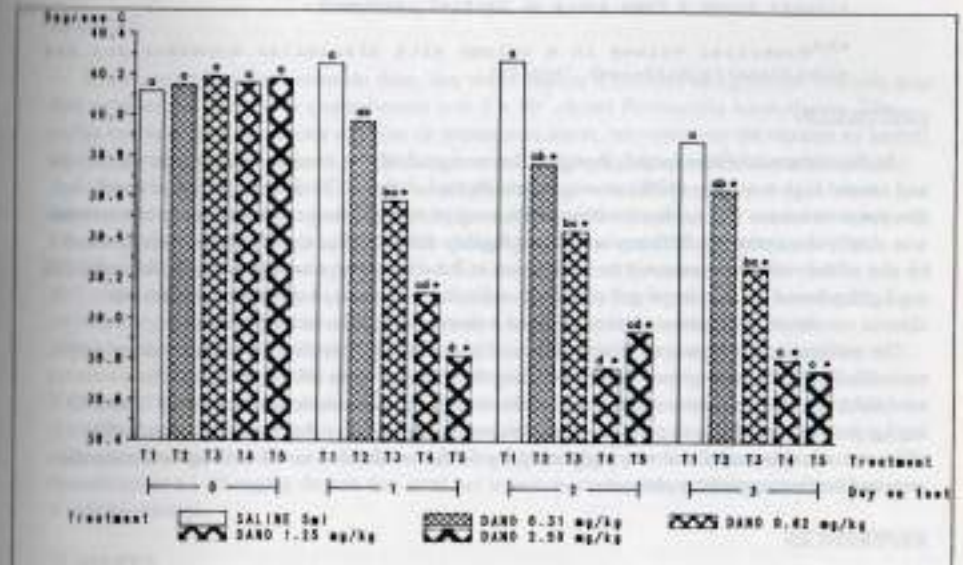

FIGURE 1 MEAN DAILY RECTAL TEMPERATURE OF PNEUMONIC CALVES DURING THERAPY

TABLE 1 - CLINICAL RESPONSE OF CALVES TO TREATMENT WITH DANOFLOXACIN FOLLOWING INFECTION WITH *P. HAEMOLYTICA*

TREATMENT	DOSE MG/KG	INITIAL NO. CALVES	% ANIMALS RESPONDING	% MORTALITY	MEAN LUNG LESION SCORE %AFFECTED
SALINE	-	12	0^{a}	41.7	29.7^{a}
DANOFLOXACIN	0.312	11	27^{ab}	18.2	21.5^{b}
DANOFLOXACIN	0.625	11	82^{bc}	0.0	19.0^{b}
DANOFLOXACIN	1.25	11	100^{c}	0.0	8.0^{c}
DANOFLOXACIN	2.50	11	100^{c}	0.0	4.2^{c}

*At 24 hours following last treatment: rectal temperature <39.5°C; illness score < than score at initial treatment.

^{abc}Numerical values in a column with dissimilar superscripts are significantly different ($P \leq 0.05$).

DISCUSSION

In this induced disease model, though onset was gradual, the resultant pneumonia was severe and caused high mortality (42%) among nonmedicated controls. Moreover, the severity of disease at treatment was uniform within and among treatment groups. The response to treatment was clearly dose related. Efficacy at 1.25 mg/kg/day did not differ significantly when assessed by any of the variables measured from that seen at 2.5 mg/kg but was superior to 0.625 and 0.312 mg/kg/day based upon prompt and sustained reduction in pyrexia, a rapid improvement in clinical condition, prevention of mortality and a marked reduction in lung lesions.

The uniformity of respiratory disease induced in the model described herein provides a well controlled experimental system for determining the efficacy of an antibacterial and for establishing a dose-response relationship in the therapy of pneumonic pasteurellosis. The 1.25 mg/kg dose of danofloxacin given daily by intramuscular injection for three days was highly efficacious in this model and was progressed for further evaluation in the field against naturally acquired bovine respiratory disease.

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SUMMARY

In a dose titration study dairy calves weighing approximately 48 kg were challenged twice over a four hour period with an inoculum containing *Pasteurella haemolytica* (3×10^{7} cfu/ml). Upon displaying clinical signs of acute pneumonia calves were randomly assigned to treatment groups receiving either saline (12 animals) or danofloxacin at 0.312, 0.625, 1.25 or 2.5 mg/kg (11 animals each dose) SID for 3 days by intramuscular injection. Daily during the ten day study, rectal temperature was recorded and a clinical assessment carried out for each calf. All calves were necropsied upon death or after euthanatization at the end of the study to determine lung pathology and to obtain lung culture samples. Mortality occurred in the saline and 0.312 mg/kg danofloxacin groups. Mean lung lesion scores were 29.7% for the saline group and 21.5%, 19.0%, 8.0% and 4.2% for the danofloxacin groups, respectively, all of which were statistically improved over the saline group. It was concluded that the 1.25 and 2.5 mg/kg doses were equally effective and the 1.25 mg/kg dose was progressed to further testing against naturally occurring pneumonic pasteurellosis under field conditions.

RESUME

Dans une étude d'évaluation de dose, des veaux laitiers d'environ 48 kg ont été inoculés deux fois pendant une période de quatre heures avec 3×10^{7} cfu/ml *Pasteurella haemolytica*. Dès qu'ils ont manifesté des signes clinique de pneumonie aigue, les veaux ont été répartis au hasard en cinq groupes expérimentaux, et traités soit avec l'eau physiologique (12 animaux) soit avec danofloxacin à 0.312, 0.625, 1.25 on 2.5 mg/kg (11 animaux en chaque groupe) par voie intramusculaire une fois par jour pendant 3 jours. Pendant les dix jours de l'essai, les veaux ont été examinés quotidiennement pour signes cliniques, et leurs températures rectales ont été enrégistrées. Les veaux morts pendant l'essai, ainsi que les survivants qui ont été abattus à la fin de l'essai, ont été necroscopiés pour détermination de la pathologie pulmonaire et examen microbiologique des poumons. Il y avait que des morts dans le groupe traité à l'eau physiologique et dans celui traité à 0.312 mg/kg danofloxacin. Les pourcentages moyens de poumons atteint étaient 29.7% dans le groupe traité à l'eau physiologique, et 21.5%, 19.0%, 8.0% et 4.2% respectivement dans les groupes traités à danofloxacin dont les résultats étaient statistiquement meilleurs que ceux du groupe traité à l'eau physiologique. On a conclu que les concentrations de danofloxacin à 1.25 et à 2.5 mg/kg sont également efficace et que la danofloxacin à 1.25 mg/kg devrait être testé sur le terrain contre les problèmes respiratoires d'origine naturel.

SUMARIO

En un estudio de valoración de dosis, se les practicó a temeros de lecheria que pesaban aproximadamente 48 kg., la prueba de inmunidad por exposición, dos veces durante el periodo de cuatro horas con material de inoculación que contenia *Pasteurella hemolytica* (3×10^{7}) unidades de formación de colonia/ml). Al dar señales de neumonia aguda se distribuyeron los temeros al azar en grupos de tratamiento que recibian, ya sea solución salina (12 animales) o danofloxacin a 0, 312, 0, 625, 1,25 o 2,5 mg./kg. (11 animales cada dosis) en una sola dosis

diaria durante 3 dias con inyección intramuscular. Diariamente durante el estudio de diez dias, se anotó la temperatura del recto y se efectuó la evaluación clínica de cada ternero. A todos los terneros se les hizo la necropsia una vez que estaban muertos o después de haberles practicado la eutanasia al fin del estudio, para determinar la patología pulmonar y para obtener muestras de cultivos pulmonares. La mortalidad occurió solamente en los grupos de la solución salina y del danofloxacin a 0,312 mg./kg. Los resultados finales medios de lesiones pulmonares fueron del 29, 7% en el grupo de solución salina y 21,5%, 19,0%, 8,0% y 4,2% en los grupos del danofloxacin respectivamente, todos los cuales mejoraron estadisticamente más que el grupo de la solución salina. Se concluyó que las dosis de 1,25 y 2,5 mg./kg. eran igualmente eficacces y se llevó adelante la dosis de 1,25 mg./kg. para fomentar las pruebas contra la pasteurelosis neumónica que ocurre naturalmente en situaciones del campo.

ZUSAMMENFASSUNG

In einem Dosistitrationsversuch wurden circa 48 kg-Milchkälber zweimal über eine 4-stündige Periode mit einem Inoculum mit Pasteurelle hemolytica (3×10^7 cfu/ml) behandelt. Beim Auftreten der klinischen Symptome einer akuten Lungenentzündung wurden die Kälber willkürlich in Behandlungsgruppen verteilt. 12 Tiere erhielten Kochsalzlösung und 11 Tiere danofloxacin zu 0.312, 0.625, 1,25 oder 2.5 mg/kg per intramuskuläre Spritze über 3 Tage. Während des 10 Tägigen Versuches wurde die Rektaltemperatur aufgenommen und bei jedem Kalb eine klinische Bewertung ausgeführt. Beim Abschluß des Versuches wurden alle Tiere nach Eintritt des Todes oder nach Euthanatizierung obduziert um die Lungenpathologie festzustellen sowie um Proben der Lüngenkultur zu erhalten. Nur in dem Kochsalzgruppe und in dem 0.312 mg/kg danofloxacin Gruppe giebte es Sterblichkeit. Durchschnittswerte für Veränderungen des Lungengewebes waren bei der Kochsalzgruppe 29.7% und 21.5, 19.0, 8.0 and 4.2% bei der danofloxacin Gruppe, war eine große statistische Besserung zeigte. Es wurde beschloßen, daß die 1.25 und 2.5 mg/kg Dosen gleichwertig sind und also die 1.25 mg/kg Dosis unter praktishen Bedingungen gegen natürlich auftretende Krankheiten der Luftwege zu untersuchen.

THE EFFICACY OF DANOFLOXACIN IN THE THERAPY OF ACUTE BACTERIAL PNEUMONIA OF YOUNG CALVES IN THE TROPICS

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INTRODUCTION

Danofloxacin, a new synthetic antibacterial is a novel third generation fluoroquinolone with a broad spectrum of activity against gram positive and gram negative bacteria and mycoplasmas, including the main etiological agents involved in the respiratory disease complex of cattle. The minimum inhibitory concentration of danofloxacin against 90 percent (MIC90) of over 250 recent field isolates of Pasteurella haemolytica, P. multocida and Haemophilus somnus was found to be ≤0.125 µg/ml. The pharmacokinetic profile of danofloxacin in cattle after intramuscular or subcutaneous injection indicates that the drug reaches peak mean concentration in lung tissues four times higher than the concentration achieved in plasma (1). The characteristics of potency and pharmacokinetics of danofloxacin combined with its spectrum of activity suggest that danofloxacin should be very effective for the treatment of bacterial respiratory disease. The objective of this study was to evaluate the efficacy of a 2.5% aqueous formulation of danofloxacin in the therapy of acute bacterial pneumonia of young calves in tropical conditions.

MATERIALS AND METHODS

Animals and management

Seventy five crossbreed Cebu calves were studied in a commercial dairy farm with a history of respiratory problems located in the state of Zulia, Venezuela. The experiment was conducted during the rainy season, from July to October 1989, in a tropical region characterized by the variability of daily temperature (from 22°C to 39°C) and high relative humidity (95%). The calves were 2 to 6 months-old, male and female, born in the same farm. They were housed in individual pens and fed pelleted feed according to their age and weight plus 3 liters of milk replacer. Water was supplied ad libitum.

Experimental procedure

Animals were identified by an individual ear tag and inspected daily. Calves showing signs of respiratory distress were individually examined, weighed and a nasopharyngeal swab collected for bacteriological examination. Calves that showed clinical signs of acute pneumonia combined with a rectal temperature of 39.5°C or greater, were randomly assigned to one of two treatment groups. They received daily intramuscular injections of either danofloxacin at 1.25 mg/kg or trimethoprim (0.026 mg/kg)-sulpha (0.13 mg/kg) for three consecutive days. On the fourth day calves that had a rectal temperature of 39.5°C or above and/or pronounced clinical signs of pneumonia were treated for two additional days. Daily clinical observations and rectal temperatures were recorded for each animal for the medication period and five days thereafter. At each clinical examination, the rate and character of respiration together with other significant clinical signs were assessed and used to assign an overall clinical score from 0 (normal) to 4 (moribund).

Bacteriological procedures

Bacterial isolations from nasopharyngeal swabs were performed by

standard methods. Minimum inhibitory concentrations (MIC) of the bacterial isolates were determined using a micro-adaptation of the broth dilution method (sensititre susceptibility system, Sensititre LTD). A custom plate incorporating serial dilutions of danofloxacin and several other antibiotics of veterinary importance was specially commissioned for this purpose.

Statistical procedures

A Fisher's Exact Test (two-tailed) was used to examine differences between treatment groups in the duration of, and response to therapy. Differences in group mean rectal temperatures by time and by treatment were analyzed using a split-plot ANOVA model. Differences in group average daily gain by treatment were analyzed using a one-way ANOVA model.

RESULTS

Clinical observations

A total of 75 pneumonic calves were studied. Thirty-eight were treated with danofloxacin (DFX) and thirty-seven with trimethoprim-sulpha (TS). All clinical cases occurred during a period of 27 days and the severity of the disease varied from moderate to severe. The number of calves that required therapy for 5 and 3 days was 36 and 2 in the DFX group, and 33 and 4 in the TS group, respectively. Following treatment the majority of cattle improved clinically but there were differences between treatments in the number of animals that responded to therapy and how fast they returned to normal. At 24 hours post three-day treatment, 58% of the DFX treated calves and 35% of the TS treated calves were found to have responded satisfactorily to treatment. That is, their rectal temperature had dropped to or below 39.2°C and their illness score was less than on the first day of treatment. At 24 hours after 5 day-treatment, 45% of the calves in the DFX group had an illness score of 0 (normal) and the other 55% had an illness score of 1 (slightly ill). In the TS group 25% of the calves had a score of 0, 65% had a score of 1, 7% had a score of 2 (moderately ill) and 3% re-met the original criteria for treatment. At the end of the post-treatment observation period of 5 days a successful response to treatment was recorded in 71% of the DFX treated animals and in 46% of the TS group. The 29% of animals left in the DFX group had a score of 1 while the TS group had 44% of calves with a score of 1 and 10% with a score of 2 or more. There were no mortalities in either group.

Response in rectal temperature

The average rectal temperature was similar in both groups (39.9°C for TS and 40.0°C for DFX) on the day therapy was started. Both treatments caused a significant fall ($P<0.05$) in group mean rectal temperature over the first 24 hours of medication which persisted to the end of the 3 or 5 days of treatment. However, at the end of 5 days of treatment calves of the DFX group had a significantly ($P<0.05$) lower mean rectal temperature than calves of the TS group.

Bacteriological observations

Bacteriological cultures from nasopharyngeal swabs taken before therapy was initiated were positive to three specific pathogens. Thirty samples (40%) were positive to *Pasteurella* sp.; 28 to *P. multocida* and 2 to *P. hemolytica*; and thirteen (17%) were positive to *Corynebacterium pyogenes*. These results would seem to indicate that *C. pyogenes* plays an important role in the pathogenesis of acute pneumonias of young calves in the tropics. The MIC values for 30 *Pasteurella sp.* isolates indicated that they were sensitive to danofloxacin with a MIC90 of 0.5 µg/ml. Over 70% of the isolates had a MIC value of 0.125 µg/ml or less.

TABLE 1. RESPONSES OF PNEUMONIC CATTLE TO TREATMENT WITH DANOFLOXACIN OR TRIMETHOPRIM-SULPHA

Treatment	No. of Animals	% Making a Successful Response* 3-day Treatment	Mean Group Rectal Temp. after 5-day Treatment (°C)	% Animals Clinically Normal 5 days Post-Treatment Observation Period
Danofloxacin	38	58[a]	38.6[a]	71
Trimet/Sulpha	37	35[a]	39.2[b]	46

a,b Treatment means with different superscipts are different ($P<0.05$).

* Successful response is recorded for any animal which, 24 hours after the end of treatment, has a rectal temperature of 39.2°C or below, together with a clinical illness score which is less than on the first day of treatment.

DISCUSSIONS AND CONCLUSIONS

Although in many cases respiratory problems in young calves appear as an integral part of the pneumo-enteric complex, in the tropical areas of Venezuela a greater incidence of pneumonia is seen during the rainy season. This has been associated with the stress of constant changes in the daily temperature and the high relative humidity seen at that time of the year. Under these conditions, treatment with danofloxacin at 1.25 mg/kg/day for 3 or 5 days produced an immediate reduction of pyrexia and a rapid improvement of clinical signs. Calves treated with danofloxacin returned to normal faster and had consistently lower illness scores than those treated with trimethoprim-sulpha. The improved efficacy of danofloxacin against respiratory disease found in this study is probably due to its high potency, characteristic of the new fluoroquinolones (2), to the ability of the drug to achieve high concentration in body fluids very rapidly, and to its concentrations in the lung, a target tissue for respiratory pathogens. In addition, danofloxacin has bacteriocidal activity even at low concentrations (3) and its MIC90 is well below the levels obtained in plasma and tissues. Results of this study indicate that danofloxacin is highly efficacious therapy for the treatment of acute bacterial pneumonia of young calves in tropical conditions.

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SUMMARY

Danofloxacin, a new synthetic fluoroquinolone antimicrobial was evaluated in the therapy of natural cases of acute bacterial pneumonia

of crossbreed cebu calves in a tropical region of Venezuela. The clinical responses of 38 pneumonic calves treated with danofloxacin were compared with those of 37 calves treated with trimethoprim-sulpha. Treatments were given by intramuscular injection for three consecutive days. On the fourth day, calves that had a rectal temperature of 39.5°C or above and/or pronounced clinical signs of pneumonia were treated for two additional days. Following treatment the majority of calves improved clinically but there were differences between treatments in the number of animals that responded to therapy and how fast they returned to normal. Danofloxacin treated calves returned to normal faster and in higher numbers, had consistently lower illness scores, and had significantly lower rectal temperatures than calves treated with trimethoprim-sulpha.

RESUMEN

Danofloxacina, un nuevo agente sintético antimicrobiano del grupo de las fluoroquinolonas, fue evaluado en el tratamiento de casos naturales de neumonias agudas bacterianas de terneros cebu cruzados, en una región tropical de Venezuela. Se comparó la respuesta clínica de 38 terneros neumonicos tratados con danofloxacina con la respuesta obtenida en 37 terneros tratados con trimethoprim-sulpha. Ambos tratamientos fueron administrados por vía intramuscular durante tres dias seguidos. En el cuarto dia terneros que tenian una temperature rectal de 39.5°C o más y/o marcados signos clinicos de neumonia fueron tratodos por dos dias adicionales. Despues del tratamiento la mayoria de los terneros mejorarón clinicamente, pero se encontrarón diferencias entre los tratamientos en el número de animales que respondieron a la terapia y en la rapidez con que retornaron a la normalidad. Los terneros tratados con danofloxacina retornaron a lo normal en mayor número y más rapido, tuvieron consistentemente menores indices de enfermedad, y mostraron temperaturas rectales significativamente menores que los terneros tratados con trimethoprim-sulpha.

THE EFFICACY OF DANOFLOXACIN IN THE THERAPY OF ACUTE BACTERIAL PNEUMONIA IN YOUNG CALVES.

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INTRODUCTION

The fluoroquinolone antimicrobial danofloxacin is a new synthetic antibacterial selected from a series of 6 fluoro-7 diazabicyclo-substituted quinolones(1). Danofloxacin exhibits potent in vitro activity against a broad range of Gram-negative and Gram-positive bacteria and mycoplasma including the major bacterial pathogens involved in respiratory disease in cattle(2). Results of minimal inhibitory concentration (MIC) determinations for field isolates obtained in North America and Europe revealed that danofloxacin has an MIC_{90} against Pasteurella haemolytica, Pasteurella multocida and Haemophilus somnus of ≤ 0.125 mcg/ml(3).

Danofloxacin has a plasma half-life of four hours following intramuscular or subcutaneous administration. Pharmacokinetic studies have also shown that danofloxacin concentrates in lung tissue, with peak levels in the lung exceeding those of plasma by a factor of four(3). Danofloxacin has also shown to be highly active against induced bovine pneumonic pasteurellosis at a dose of 1.25mg/kg(4).

The pharmacokinetic properties, the broad spectrum and the potency indicate that danofloxacin should be effective in the treatment of bacterial respiratory disease in cattle. The objective of this study was to evaluate the efficacy of a 2.5 percent aqueous formulation of danofloxacin in the therapy of acute bacterial pneumonia in young intensively reared calves.

MATERIAL AND METHODS

Animals and Management

Five hundred and twenty-three young calves were studied on two farms in France and one in Ireland. On one farm in France and on the Irish farm the animals were individually caged and fed reconstituted milk powder. On the second farm in France the calves were kept in pens of eight on permanent straw, and fed milk replacer but concentrate and hay were part of the ration also. The calves were of mixed dairy breed on all three farms and were approximately 10 days of age when received at the farms.

Experimental Procedure

All calves were uniquely identified on arrival. Animals in poor condition or exhibiting clinical signs of disease other than acute pneumonia were excluded from the trial. When calves showed clinical signs of acute pneumonia combined with a rectal temperature of 39.5°C or greater they were weighed and randomly assigned to one of two treatment groups. Calves in one group received danofloxacin at 1.25mg/kg and the calves in the second were administered oxytetracycline at 10mg/kg. Both drugs were administered by intramuscular injection once daily for three days. On the fourth day, 24 hours after the third injection, any animal that had a rectal temperature of 39.5°C or above and/or had pronounced clinical signs of pneumonia was treated for two further days. Rectal temperatures were recorded and clinical examinations made for the medication period and for five days thereafter. At each clinical examination the rate and character

of respiration were assessed and, together with other significant clinical signs, used to assign an overall clinical assessment score ranging from 0(normal) to 4(moribund).

Statistical Procedure

A Fisher's Exact Test (two-tailed) was used to examine differences between treatment groups in the duration of, and response to therapy. Differences in group mean rectal temperatures by time and by treatment were analysed using a split-plot ANOVA model. Differences in group average daily gain by treatment were analysed using a one-way ANOVA model.

RESULTS

Clinical observations

In total 187 calves met the criteria for disease and entered the study. On the farm in Ireland 79 calves were studied and the corresponding figures for the French farms were 68 and 40 respectively. In all 100 calves were treated with danofloxacin and 87 with oxytetracycline. On the farm in Ireland and on one farm in France the clinical nature and severity of the disease was similar. On the second farm in France the outbreak of respiratory disease was clinically assessed as more severe. In total three calves died during the trial, two in the danofloxacin treatment group and one in the oxytetracycline treatment group. Two deaths occurred on the French farm with severe disease, one in each treatment group, and in both cases the cause of death was respiratory disease. Post-mortem examination of the third calf confirmed the presence of pneumonia, but a severe pyelonephritis was probably a major contributor to death.

Thirty-nine calves (45 percent) treated with oxytetracycline required the full five days therapy. Only 29 calves (29 percent) required five days therapy in the danofloxacin treatment group and the difference between groups is significant ($p<0.05$).

Following treatment, the majority of cattle in both groups improved clinically. At the end of the post-treatment observation period 68 percent of the danofloxacin treated calves were judged clinically normal (Fig. 3). The corresponding figure for oxytetracycline treated calves was 62 percent. At the same time an illness score of one (slightly ill) was assigned to 18 percent of calves in the danofloxacin treated group compared to 10 percent in the oxytetracycline treated group.

During the observation period a number of calves in each group met the criteria established for re-treatment, i.e. the illness score assigned was three (severely ill) or four (moribund). Twenty- four per cent of calves treated with oxytetracycline required additional therapy compared to ten percent of calves treated with danofloxacin.

Observation of Rectal Temperature

On the day of initiation of treatment the group mean rectal temperatures were approximately equal at about 40.3°C (Fig. 1). A rapid fall in rectal temperatures over the first 24 hours of treatment was observed in both treatment groups. This reduction in pyrexia was maintained through the treatment period and the group mean rectal temperature remained significantly lower (p 0.05) than its value before treatment (Fig. 1). The reduction in pyrexia in the danofloxacin group was more pronounced than in the oxytetracycline group but the difference between groups was not statistically significant. The same pattern of reduction in pyrexia was observed for the subset of animals treated for five days.

Response to treatment

Any animal whose rectal temperature had dropped to below 39.2°C and, in addition, had improved clinically 24 hours after last treatment was classified as having responded successfully. The assessment of clinical improvement was determined by clinical illness score less than the value of the score before treatment. Eighty-two percent of the danofloxacin treated calves responded successfully by these criteria, compared to 55 percent in the oxytetracycline treated group of calves (Table 1). This difference is statistical significant ($p<0.05$).

TABLE 1

Response of pneumonic calves to treatment with danofloxacin or oxytetracycline

Treatment	No. of Animals	No. requiring 5 Days Treatment	% Making a Successful Response*
Danofloxacin	100	29[a]	82[a]
Oxytetracycline	87	39[b]	62[b]

a, b The treatment means differ significantly ($p<0.05$).

* A successful response is recorded for any animal which, 24 hours after the end of treatment, has a rectal temperature of below 39.2°C, together with an illness score which is less than its value on the first day of treatment.

DISCUSSION AND CONCLUSIONS

The aetiology of any individual outbreak of respiratory disease is often multifactorial. Many infectious agents have been associated with the disease including viruses, mycoplasma and bacteria(5). Pasteurella haemolytica and Pasteurella multocida are the most common and important bacterial pathogens to invade the lung tissue in cases of acute bronchopneumonia. The clinical, epidemiological and bacteriological features of the outbreaks on the farms included in this study were typical of the pneumonia which is often seen in Europe in intensively reared young calves, and commonly associated with Pasteurella infections. Danofloxacin was highly efficacious in the therapy of these outbreaks as shown by the immediate reduction in pyrexia, the high successful response rate and the improvement in clinical condition. In this study the responses to treatment with danofloxacin were superior to that of oxytetracycline. It is likely that the superior efficacy of danofloxacin is related to the inherent higher potency and superior pharmacokinetic properties of this new fluoroquinolone, although a degree of resistance to oxytetracycline could not be excluded.

Danofloxacin has several advantages compared to traditional antibacterials in the therapy of bacterial respiratory disease. The drug is bacteriocidal at low concentrations, has a broad spectrum including anti-mycoplasmal activity. In addition danofloxacin is distributed rapidly in body fluids and readily penetrates tissues, particularly those of the respiratory tract. Worldwide field evaluation is in progress but this study indicates that danofloxacin should prove to be highly effective in the treatment of bovine respiratory disease.

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SUMMARY

The novel fluoroquinolone antimicrobial, danofloxacin, was evaluated in the therapy of pneumonic pasteurellosis in intensively reared calves on two farms in France and one in Ireland. In France, 40 calves of mixed dairy breed were involved in the trial at one farm and 68 at the other, whereas in Ireland a total of 79 dairy calves were involved. The efficacy of danofloxacin was compared to that of oxytetracycline. Danofloxacin was administered to 100 calves and oxytetracycline to 87. Both drugs were administered by intramuscular injection for either three or five days depending on clinical response. Both treatments resulted in a rapid fall of mean rectal temperature and improved the clinical condition of the majority of calves. However, in comparison with oxytetracycline, treatment with danofloxacin resulted in significantly fewer treatment days, a significantly higher response rate, better reduction of pyrexia and fewer calves requiring re-treatment.

ZUSAMMENFASSUNG

Das neue Antibiotikum aus der Gruppe der Fluoroquinolone, Danofloxacin, wurde in der Behandlung von pasteurelen Pneumonien bei intensiv gehaltenen Kälbern auf zwei Betrieben in Frankreich und in einem Bestand in Irland erprobt. In Frankreich wurden 40 Kälber gemischter Milchrassen in einem, 68 auf dem anderen Bestand, und in Irland 79 Kälber in die Versuche einbezogen. Die Wirksamkeit von Danofloxacin wurde mit Oxytetracyclin verglichen. Hundert Kälber erhielten Danofloxacin und 87 Oxytetracyclin. Beide Medikamente wurden für drei oder fünf Tage - je nach Besserung des klinischen Zustandes - intramuskular appliziert. Bei beiden Behandlungen kam es sehr schnell zu einem Abfall der durchschnittlichen rektalen Temperatur und zu einer Besserung des klinischen Zustandes in den meisten Kälbern. Jedoch, im Vergleich zur Oxytetracyclintherapie, war die Behandlung mit Danofloxacin durch signifikant weniger Behandlungstage, signifikant höheren Besserungsrate, besserem Fieberrückgang und wenigern Kälbern, die eine Nachbehandlung benötigten, charakterisiert.

RESUMEN

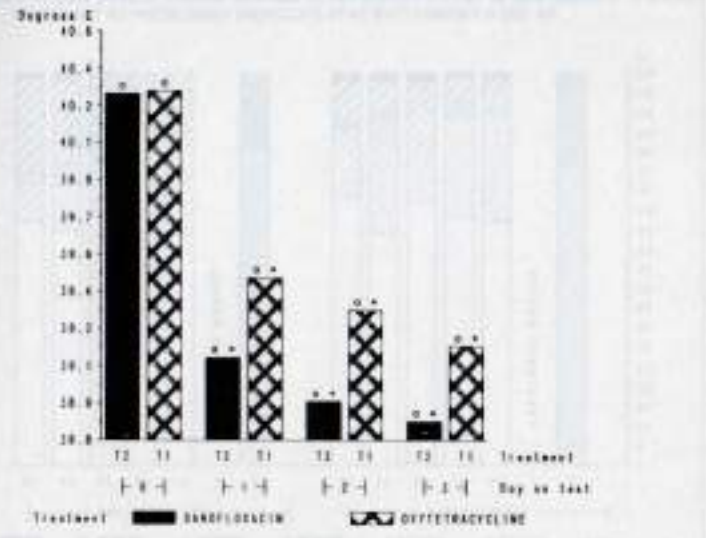
Danofloxacin, un nuevo agente antimicrobiano del grupo de las Fluoroquinolonas, fue evaluado en la terapia de neumonias debidas a Pasteurella spp., en terneros criados intensivamente en dos granjas en Francia y en una granja en Irlanda. En Francia se estudiaron 40 terneros de una raza lechera cruzada en una localidad y 60 en la otra, mientras que en Irlanda se estudiaron 70 terneros. Se comparo la respuesta clinica de 100 terneros tratados con danofloxacin con la respuesta obtenida en 87 terneros tratados con oxitetraciclina. Los dos medicamentos fueron

administrados por via intramuscular por tres o cinco dias, dependiendo de la respuesta clinica. Ambos tratamientos resultaron en una disminucion rapida de la temperatura rectal promedio y mejoraron la condicion clinica de la mayoria de los terneros. Sin embargo, en comparacion con los animales tratados con oxitetraciclina, los terneros que recibieron danofloxacina nesesitaron menos dias de tratamiento, tuvieron una mejor respuesta clinica, mostraron temperaturas rectales menores y un menor numero de animales requirio de ser tratados nuevamente.

RESUME

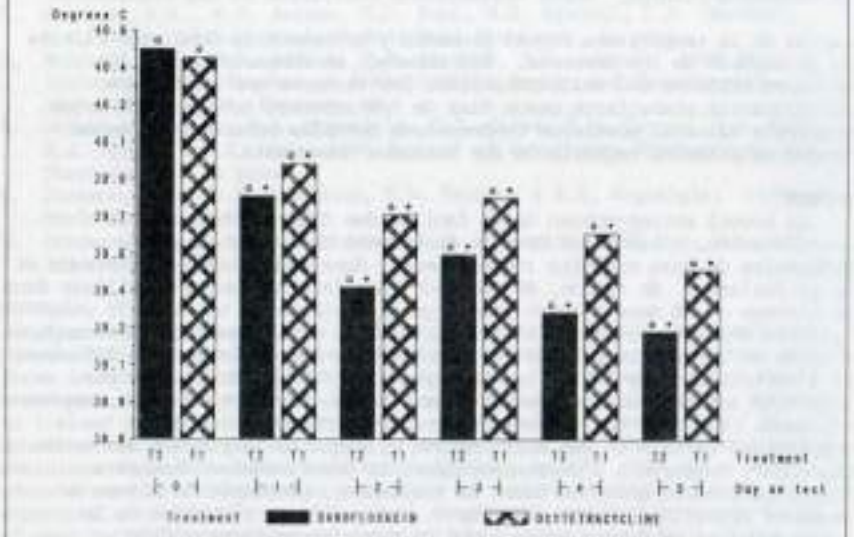
Le nouvel antimicrobien de la famille des fluoroquinolones, la danofloxacine, a été testé dans le traitement de la pasteurellose pulmonaire de veau conduits intensivement, dans deux élevages en France et un en Irelande. En France, 40 veaux de race laitiere ont été utilisés dans un élevage et 68 dans l'autre tandis qu'en Irelande un total de 79 veaux laitiers étaient utilisés. L'efficacité de la danofloxacine a été comparée a celle de l'oxytetracycline. La danofloxacin fut administrée a 100 veaux et l'oxytetracycline a 87. Les deux produits furent administrés par injection intramusculaire pendant 3 ou 5 jours, en fonction de la response clinique. Les deux traitements amenerent une chute rapide de la température rectale et une amélioration clinique de la plupart des veaux. Cependant, comparée a l'oxytetracycline, la danofloxacine nécessita significativement moins de jours de traitement, produisit un niveau de response significativement plus élevé, une meilleure réduction de la temperature et un nombre moins élevé de veaux necessitant d'être re-traités.

FIGURE 1 MEAN DAILY RECTAL TEMPERATURE OF PNEUMONIC CATTLE DURING THERAPY: ALL CATTLE



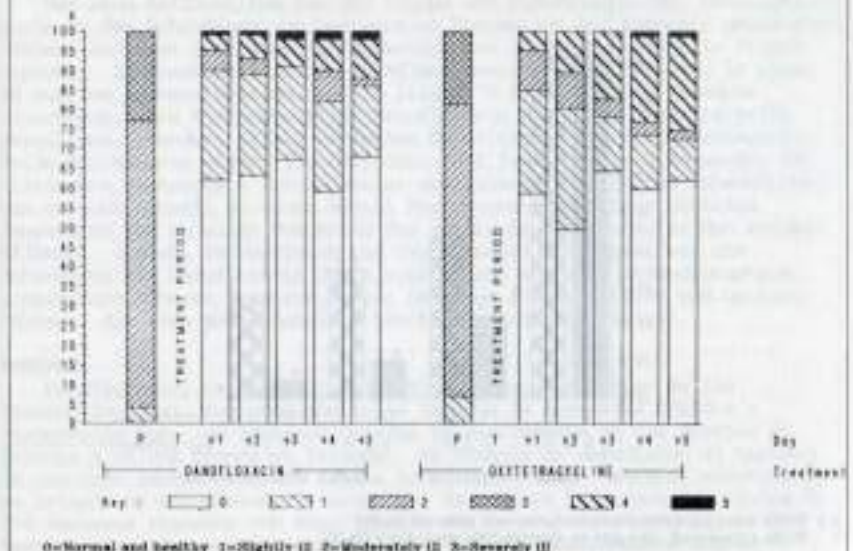
a,b Within a day, bars with dissimilar letters are different (P≤.05)
* Within a treatment, bars with an asterisk differ from day 0 (P≤.05)

FIGURE 2 MEAN DAILY RECTAL TEMPERATURE OF PNEUMONIC CATTLE DURING THERAPY: 5-DAY TREATED CATTLE



a,b Within a day, bars with dissimilar letters are different (Pc.05)
* Within a treatment, bars with an asterisk differ from day 0 (Pc.05)

FIGURE 3 PERCENTAGE OF CATTLE CLASSIFIED BY CLINICAL ILLNESS SCORE PRIOR TO TREATMENT AND ON THE 5 CONSECUTIVE DAYS FOLLOWING COMPLETION OF TREATMENT



0=Normal and healthy 1=Slightly ill 2=Moderately ill 3=Severely ill
4=Animal meeting the criteria for re-treatment 5=Morribund or dead

MOSCA DOS CHIFRES
HORN FLIES

A MOSCA DO CHIFRE NA AMERICA LATINA: DISTRIBUIÇAO, ECOLOGIA E METODOS ALTERNATIVOS DE COMBATE.

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DISTRIBUIÇÃO

A mosca do chifre, Haematobia irritans (L) entrou na América Latina através do México no final do século XIX e atualmente é encontrada em todos os países da América Central. A invasão da mosca do chifre na América do Sul deu-se através da Colombia em 1937. No momento está atacando aos bovinos na Venezuela, Equador, Perú, Guiana e Brasil. Neste último país a mosca entrou em 1983 (13) e se dispersou nos estados de Roraima, Amazonas, Tocantins, Pará, Maranhão, Piauí, Ceará, Rio Grande do Norte, Goiás, Mato Grosso do Sul, Minas Gerais, São Paulo e Paraná. Provavelmente esta peste tomará conta do Uruguai, Paraguai, Bolivia e Argentina no final do ano de 1991. Esta mosca veio da Europa e adaptou-se muito bem nos Estados Unidos e Canadá o que significa que sua distribuição atingiu o hemisferio norte até o paralelo 50. Existe uma grande possibilidade que esta peste chegue a distribuir-se no hemisferio sul até a mesma latitude, neste caso todo o gado da Argentina e Chile sofrerão com o ataque de mais um ectoparasito.

ECOLOGIA

Observações preliminares realizadas na região amazônica do Brasil indicam a existência de dois picos populacionais da H. irritans os quais ocorrem no inicio e no final da estação chuvosa. Em certas áreas onde os periodos de chuva ou seca são prolongados, as populações da mosca são reduzidas drasticamente. Um efeito similar tem sido observado por Butler (2) durante o verão da Florida, E.U.A.. Nos E.U.A. e Canadá a mosca do chifre assegura sua sobrevivência entrando em diapausa durante o inverno (6); e o mesmo poderá acontecer nos países do Cone Sul, durante os meses frios.

Nos países montanhosos da América Latina, tais como Colombia e Equador este inseto foi observado atacando aos bovinos em altitudes que vão do nível do mar até os dois mil metros.

A H. irritans é uma mosca que ataca, preferencialmente aos bovinos. Os cavalos são atacados com menor intensidade. Em uma fazenda de São Paulo a média de moscas por cavalo foi de 11,2 comparada com 92,2 moscas por bovino. Vários autores (2, 6, 14) indicam que esta mosca pode alimentar-se, esporadicamente, sobre outros animais, tais como búfalos, veados, ovelhas, porcos e eventualmente sobre o homem. Os animais selvagens do trópico úmido da América do Sul parecem não serem atacados pela mosca do chifre, tendo em vista que nenhum dos animais capturados (aproximadamente 10.000) na Usina Hidrelétrica Samuel de Rondônia, Brasil, estiveram infestados por esta peste (11).

PREJUIZOS

A mosca do chifre provoca prejuizos de 730 milhões de dólares por ano nos E.U.A. (4); e, é possível que este inseto chegue a causar perdas maiores no Brasil, devido ao manejo deficiente do gado bovino. Os níveis populacionais (50 moscas por animal) que começam a provocar perdas na produção de leite e carne no gado europeio, não são aplicáveis nos trópicos úmidos; tendo em vista que o gado zebuino que prevalece

nestas áreas parece estressar-se mais que o gado europeio com um mesmo número de moscas por animal. Ao consumo de sangue vicioso deste parasito, deve acrescentar-se a possibilidade de transmissão do agente causal da Anaplasmose nos bovinos (5). Ataques maciços desta mosca provocam feridas nos bovinos que podem permitir o estabelecimento de miíases causadas pela Cochliomyia hominivorax. Aproximadamente 20 por cento destas miíases foram estabelecidas em feridas previamente causadas pela mosca do chifre, nos E.U.A. (6).

Outro problema que poderá surgir com a presência da mosca do chifre na América Latina é um incremento dos casos da Dermatobiose nos bovinos. As seguintes caracteristicas bionômicas da H. irritans podem transformar-lo num excelente vetor dos ovos da Dermatobia hominis: 1. Altas populações da mosca do chifre estão constantemente associadas com o gado. 2. As larvas da mosca do chifre desenvolvem-se, exclussivamente, nas fezes dos bovinos. 3. As moscas do chifre são menores que as moscas da Dermatobia e mais fáceis de serem capturadas. 4. As moscas do chifre são de hábitos diurnos.

CONTROLE

O controle da H. irritans na América Latina vem-se realizando, principalmente com piretroides e em menor escala com inseticidas organosforados aplicados com bombas de aspersão. De um modo geral as doses dos inseticidas utilizadas no combate aos carrapatos proporcionam um bom controle da mosca dos chifres. Ultimamente,vem-se aplicando nos bovinos brincos impregnados com inseticidas com excelentes resultados. Brincos com cipermetrina protegeram aos bovinos durante três meses, na região amazónica (9). Problemas serios de resistência desta mosca aos inseticidas tem sido observados nos E.U.A. em lugares onde foram utilizados estes brincos de forma intensa (4). Em menor escala vem-se aplicando piretroides por "pour-on". A decametrina aplicada por este método tem mostrado alta eficácia no controle desta peste até por cinco semanas na Amazônia brasileira.

O controle químico das larvas da H. irritans nas fezes dos bovinos tem sido realizado nos E.U.A. O metopreno e a ivermectina são as substâncias mais promissoras neste combate. Formulações em bolus para liberação lenta do metopreno, por exemplo, vem dando excelente controle desta mosca durante sete a oito meses (7). Em relação com a ivermectina uma única dose subcutânea de 200 microgramos por kilograma de peso vivo controla as larvas da H. irritans durante quatro semanas (8). No entanto, pesquisa serias deveriam ser direcionadas no sentido de conhecer a toxides destas substâncias sobre todos os insetos associados com o bolo fecal dos bovinos, especialmente no trópico úmido, onde existe uma grande variedade de organismos que usam este meio alimenticio.

O controle biológico da mosca do chifre tem-se se iniciado no Brasil com a importação dos coleópteros "rola-bostas" da espécie Onthophagus gazella dos E.U.A.. O Centro Nacional de Pesquisa Agropecuária (CNPA-Embrapa), em Campo Grande, Mato Grosso do Sul, vem criando em forma maciça este coleóptero e esperam liberar no campo, brevemente, com resultados imprevisíveis. A introdução do O. gazella nos E.U.A., faz 16 anos, visando um controle eficaz da mosca do chifre, não teve o êxito esperado (6). Formigas da espécie Solenopsis invicta tem sido observadas nos E.U.A., predando pupas da H. irritans (12). Espera-se que estas formigas que são originarias do Brasil possam ajudar no bio-controle da mosca do chifre. Estudos sobre os parasitoides do gênero Spalangia devem ser intensificados.

Sumarizando, na América Latina, o combate a mosca do chifre vem-se realizando exclusivamente com inseticidas; mas o uso intensivo e indiscriminado destas substâncias pode provocar problemas tais como: 1. Resistência das moscas aos inseticidas. 2. Residuos tóxicos

indesejáveis no leite e carne. 3. Eliminação de agentes do bio-controle da H. irritans. Devido a estos problemas, estudos sobre métodos alternativos de combate devem ser iniciados, enfatizando o bio-controle, tendo em vista que Blume e seus colaboradores (1), nos E.U.A. mostraram uma relação inversa entre o número de moscas do chifre emergidas do bolo fecal e o número de outras especies de insetos do mesmo meio nutritivo.

Um projeto de cooperação internacional entre a Universidade da Florida, E.U.A. e a Universidade Federal Rural do Rio de Janeiro acha-se em estudo visando desenvolver sistemas de bio-controle da mosca do chifre.

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RESUMO

A mosca do chifre, Haematobia irritans (L) invadiu a América Latina através do México no final do século XIX e atualmente encontra-se distribuida em todo América Central. Esta peste foi introduzida na América do Sul através da Colombia em 1937 e na atualidade acha-se distribuida na Venezuela, Equador, Perú, Guiana e Brasil. Sua invasão ao Brasil ocorreu em 1983. Nos países montanhosos a mosca do chifre tem sido observada desde o nivel do mar até os dois mil metros. Altas populações da H. irritans ocorrem na região amazônica no inicio e no final da estação chuvosa. O controle químico da H. irritans , na América Latina, é realizada, basicamente, com inseticidas piretroides ou organofosforados;e, problemas de resistência poderão surgir brevemente. Agentes do bio-controle presentes na América Latina devem ser identificados visando seu uso no manejo integrado da mosca do chifre. Um programa cooperativo internacional sobre o bio-controle da H. irritans entre a Universidade da Florida, E.U.A, e a U.F.R.R.J., Brasil, está em estudo.

RESUMEN

La mosca de los cuernos, Haematobia irritans (L) invadió la América Latina a través de México al final del siglo XIX y actualmente se encuentra distribuida en toda la América Central. La América del Sur fue invadida a través de Colombia em 1937 y en este momento se encuentra distribuida en Venezuela, Ecuador, Perú, Guayana y Brasil. La invasión al Brasil ocurrió em 1983. En los países montañosos, la mosca de los cuernos ha sido observada desde el nivel del mar hasta los dos mil metros. Altas poblaciones de H. irritans ocurrem en la región amazónica al comienzo y al final de la estación lluviosa. El control químico de esta peste en América Latina se realiza, basicamente, com insecticidas piretroides y organofosforados y problemas de resistencia pueden surgir brevesente. Agentes del biocontrol, presentes en América Latina, deben ser identificados procurando incluirlos en un futuro manejo integrado de la mosca de los cuernos. Un programa cooperativo internacional sobre el biocontrol de la H. irritans entre la Universidad de Florida, E.U.A. y la U.F.R.R.J., Brasil, esta en estudio.

SUMMARY

The horn fly, Haematobia irritans (L) invaded Latin America through Mexico at the end of the nineteenth century and currently has spread to all Central American Countries. This pest was introduced into South America through Colombia in 1937 and nov is found in Venezuela, Ecuador, Peru, Guyana and Brazil. The Extension into Brazil occurred in 1983. In mountainous countries, horn fly infestations have been reported from sea level to 2,000 m altitude. High populations occur in the amazon region at the beginning and end of the rainy season. Horn fly chemical control in Latin America is basically carried out using pyrethroids or organophosphate insecticides and resistance problems are projected to appear soon. Horn fly biocontrol agents present in Latin America must be identified to integrate these methods into a pest management program. An international cooperative program on horn fly biocontrol between the University of Florida, U.S.A. and the U.F.R.R.J, Brazil is under study.

Haematobia irritans: ECONOMIC IMPORTANCE AND BIONOMICAL CHARACTERISTICS

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INTRODUCTION

The horn fly, Haematobia irritans (L), is an Old World species and an obligate blood-feeding ectoparasite of cattle. Although horn fly populations on animals in Europe are usually low at 200 per animal or less (24), this fly may reach extreme numbers on animals in the tropics and semitropics with numbers of 1,100/animal for beef cows to as high as 20,000 for bulls (39).

DISTRIBUTION

The horn fly was introduced into North America at Philadelphia from southern Europe in 1885-6 (37,47) and rapidly spread to Florida in 1891 (45), Michigan in 1892, California by 1893 (29), and Hawaii by 1897. By 1900, they had been reported in most of the United States, Canada, and Puerto Rico (25). New World distribution in 1977 was from Venezuela to Canada (22). The extension into Brazil in 1983-4 is a relatively recent but critical event.

BASIC BIONOMICS

Haematobia irritans is a parasite of cattle which will also feed on other animals such as sheep and horses. Both sexes feed almost continuously with as many as 12 blood meals per day (4,27). The horn fly ecological niche is split between larval and adult stages. Larvae develop in individual cow pats and adult flies locate on animals.

The life cycle of the horn fly is summarized as follows: adult longevity lasts from 28 days (39) to 8 weeks (4). The minimum developmental period from egg to adult takes 10 (15) to more than 50 days (52). Single mating for females and multiple for males was noted (26). Three day postemergence to egg production is required (49) with 15-24 eggs per batch and up to 15 batches or 225-375 eggs per female (4,49). Eggs are deposited day or night only on fresh cow dung pats (3,39,48). Egg hatching requires 11 to 20 hours (40) at 90 to 97% hatching rate (4,52). Larvae pupate in and under the aged manure pat (8). First instar larvae require 10 hrs, 2nd 18 hrs, 3rd 2.7 days (4,52). Three to 5 days are required for the pupal stage (4,35,52).

FLY MOVEMENT

Migrations of 12 km have been demonstrated under field conditions for horn flies. Flies have been shown to live for 18 to 26 hours without feeding and would have about 1 day in nature to locate or relocate a new host. In migration studies, we have seen up to 50% of the gravid females leaving the cow per day. In field control studies, 100% control of developing larvae-pupae have not suppressed adult fly populations (7,20,28,31,52). Adult mortality seems dependent on migration and is apparently population dependent with an average number of 1,000/animal in Florida.

BIOCONTROL

Natural biocontrol of horn flies observed in Florida has identified pupal parasites as the most important factor in the manure pat. Maximum rates during the fly season were 16.5% for Spalangia cameroni (June), 6.7% for Spalangia nigra (July), 4.7% for Spalangia endius (June), 4.5% for

Spalangia haematobiae (August), and 3.4% for Spalangia nigroaenea (August) (8). Seasonal variation seen with these pupal parasites demonstrates the interaction between time of year, parasite levels, parasite species, and the change in developmental rates of horn fly pupae. Pupal parasite growth rates vary with species and season, taking as little as 21 days for S. nigroaenea in July to 59 days for S. nigra in February. This makes parasite control of the fast developing horn fly host difficult to achieve, because of the time lag of as little 3 days for horn fly pupal eclosion and at least 21 days for parasitized pupae to produce parasitic wasps.

The literature implies the adverse effects on parasites presented by insecticide treatment to larval and pupal sites, as it more easily kills the susceptible parasite while not affecting the more resistant host fly. In effect, ill-timed and/or ill-selected insecticide treatment could kill all the natural parasites and not the fly, thus increasing fly levels (34).

Other biocontrol factors such as the Gamasina mites, staphylinid beetles, and the habitat-disturbing dung beetles (1,13,17,35,43), while important natural control factors, have similar problems in suppressing fly populations. This is primarily because the horn fly is moving through the dung habitat so rapidly that it cannot be attacked and controlled. Exceptions to this rule would be potential egg and larval parasites transported by the adult fly, such as the parasitic nematode seen for the face fly Musca autumnalis (51). We have observed a potential nematode parasite in horn fly adults, larvae, and pupae which may fit this model and are initiating research on this biocontrol method.

CHEMICAL CONTROL

Primary control of the horn fly throughout the United States has been through the use of insecticides. In the past this pest has always been **"the easiest fly to kill, but the hardest to control."** This is because of the initial insecticide susceptibility of the fly (38), the reproductive potential of the fly, its herd parasite behavior, and major dispersal tendencies (52).

Adult horn flies remain on the host except when laying eggs or migrating to or between hosts. Their close association with cattle makes them susceptible to on-animal chemical control measures. Forced-use dust bags and eartags have given the best control prior to the development of pesticide resistance (10,12,33,50). The pesticide elution rate from tags tends to "tail out" over long periods of time (41) and is thought to be the major cause of horn fly pesticide resistance development in the USA.

Backrubbers can also give control, but are usually less successful on horn flies. Sprays, dips, and fogging of animals give excellent short-term 1 to 2 week control (19,33).

Feed additives or boluses of insecticides or insect juvenile hormones are utilized in controlling larval and pupal stages in the manure pats. A number of formulations have been found which if present at high enough rates in manure will give effective control (19). The adult migration rates exhibited by this fly may maintain flies on animals and thus damage can occur even with near 100% control of the immature stages.

ECONOMIC DAMAGE

The horn fly is probably one of the more serious pests of cattle in Florida. It causes pain and annoyance and interferes with host feeding, resting, and other normal activities. With high summertime populations (May-November in Florida) horn flies cause reduced weight gains and lowered milk production (7).

Horn fly populations on untreated animals in Florida reach 1,000 to 1,200 flies per animal with bimodal peaks occuring in May-June and

again in August-September (7,22). Winter populations are as low as 5 flies per animal with no apparent diapause noted. Overwintering in Florida is thought to be continued slow development in the manure pat (52). Elsewhere fly populations are found from spring to late fall (53). Diapausing pupae are thought to occur in these areas (16). Bulls in Florida may exceed 10,000 horn flies, but 20,000 flies per animal have been reported (39).

Horn flies may cause open sores on the head and underline, which can predispose the animals to secondary infections of both diseases and parasites. In the tropics, this skin damage may increase screwworm and "Torsalo" infestations. Because of their piercing-sucking mouth parts, they are listed as mechanically transmitting Anaplasmosis and other diseases within the herd (23). Regurgitative transmission is of lesser risk from this fly than the stable fly (9).

ECONOMIC EFFECTS

The horn fly causes direct damage to the host as the result of blood loss and fly worry, which produces parasitic stress. Under optimum pasture and feed conditions, this loss is apparently minimized by increased intake of energy. Under average to low intake maintenance conditions, fly damage can be seen as a reduction of 10% or more in meat production (7,11,19,30,46) and milk production (5).

Observations in Florida for various cattle breeds on improved pasture demonstrated differences in weight gains at 24.5% for animals with fly levels reduced with Rabon dust bags (7). Table 1 summarizes these observations.

Table 1. Summary of production data compiled at the University of Florida on pastured beef cattle as affected by horn flies (Haematobia irritans) [Adapted from Rabon dust bag trials (7)].

Breed evaluated	Days in test	Number animals	Treated-Check Lb/day	Kg/day	% change
Charolais	52	14	0.4038*	0.1835*	33*
Brahman	52	10	0.6538*	0.2972*	49*
Santa Gertrudis	52	17	0.0577	0.0262	6
Cross-bred F	52	13	0.4039*	0.1836*	44*
Cross-bred M	52	14	0.1731	0.1036	21
Average	52	68	0.2308*	0.0787*	25*

* significant differences at P=0.5 or less

It should be noted that in these trials the average fly numbers on treated animals were 14 flies per animal and 600 for untreated animals. Other trials rank fly attractiveness to different breeds, with Charolais usually carrying the greatest fly loads, and Brahman the least. These results imply that the few flies on Brahman animals may produce damage at higher rates than higher numbers on other breeds. Santa Gertrudis, as well as Cross-bred Males, showed no significant differences due to fly control here and may be seen as fly tolerant. The majority of published weight differential tests run elsewhere are usually conducted with lower numbers of flies than seen in Florida. Losses seen on animals in the tropics, with high fly numbers and potentially poor forage conditions, can be expected to be higher than in the temperate areas.

NATURAL POPULATION REGULATION

Natural horn fly numbers on animals are initially regulated by host-finding characteristics of the fly. Flies find the host by animal color (4,6,21,24,25), radiating temperatures (14,32,44), CO2 levels (32), and possibly animal odors regulated in part by testosterone levels (18).

Fly-produced pheromones and semiochemical materials are also important in marking and regulating fly populations on animals. Horn fly sex, mating, and aggregation chemicals have been identified (2,36,42) and evaluated on cattle as well as horses. Blends of four olefins, identified as attractants for horn flies when applied to cows and horses, demonstrated attraction for horn flies and house flies. An olefin blend at high levels poured on animals reduced fly numbers on cattle, but increased levels on horses (42). Research utilizing these and other semiochemicals for regulating fly numbers on animals is continuing at the University of Florida.

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SUMMARY

The horn fly, Haematobia irritans (L), is an Old World species and an obligate blood-feeding ectoparasite of cattle. Although horn fly populations on animals in Europe are usually low at 200 per animal or less, this fly may reach extreme numbers on animals in the tropics and semitropics with numbers of 1,100/animal for beef cows to as high as 20,000 for bulls. The horn fly was introduced into North America at Philadelphia from Europe in 1885-6 and rapidly spread to Michigan in 1892, California by 1893 and Hawaii by 1897. By 1900, they had been reported in most of the United States, Canada, and Puerto Rico. New world distribution in 1977 was from Venezuela to Canada. The extension into Brazil in 1983-4 is a relatively recent but critical event. The presentation for this Congress will be a summary of research performed at the University of Florida over the past 20 years on Haematobia irritans which includes: basic bionomics; biocontrol; control methods; movement and distribution; semiochemical and pheromonal regulation; and the potential economic effects of this parasite on meat and milk production.

ZUSAMMENFASSUNG

Die Fliege der Hoerner, H. irritans (L) ist ein Spezimen der alten Welt, und ein ectoparasit der sich von Rinderblut ernaert wenn die Fliege ausgewachsen ist. In Europa ist die Zahl der Fliegen H. irritans per Rind um die 200 oder weniger, doch in den Tropen und Subtropen ist die Population der Fliegen per Rind in der Groessenordnumg 1.100 Fliegen per Kuh bis zu 20.000 Fliegen per Ochse. Die Fliege der Hoerner wurde von Europa nach Philadelphia in die Vereinigten Staaten eingefuehrt im Jahre 1885-6 und sie verbreitete sich schnellstens bis nach Michigan (1892), Californien (1893) und Hawaii (1897). Im Jahre 1900 fand man die Fliege der Hoerner im groessten Teil der Vereinigten Staaten, Canada und Puerto Rico. Im Jahre 1977 hatte sich die Fluge der Hoerner von Venezuela bis nach Canada schon verbreitet, und nach Brasilien kam sie in Jahre 1983-4. Dies ist ein ziemlich neues Geschaehniss doch ein sehr kritisches Ereigniss. Die University of Florida wird zum Kongress eine Zusammenfassung der 20 Jaehrigen Forschung der H. irritans ausstellen, welsche die folgende Aspecte beschreibt: Basis der Bionomics; biologische-Kontrolle und Verteilung; semiochemical und pheromonal regulierung; und die oekonomische Auswirkung der Angerichteten Schaden der Fliege der Hoener in der Milch-und Fleischproduktion.

RESUMO

A mosca do chifre, Haematobia irritans (L) é um hemoparasito do gado bovino originaria do Velho Mundo. Embora as populações desta mosca sobre os animais sejam comumente menores que 200 por animal, nas áreas tropicais e subtropicais podem chegar a observar-se 1.100 por vaca e até 20.000 por touro. A mosca do chifre foi introduzida na América do Norte pela Filadelfia vinda do Europa em 1885-6 e rapidamente dispersou-se a Michigan em 1892, California em 1893 e Havai em 1897. Em 1900 esta peste tem sido encontrada na maioria dos Estados Unidos, Canadá e Porto Rico. Sua distribuição no Novo Mundo, em 1977 foi de Canadá a Venezuela. Sua recente invassão ao Brasil em 1983-4 é um evento crítico. Neste Congresso será apresentado um resumo da pesquisa realizada na Universidade da Florida nos últimos 20 anos sobre a H. irritans, a qual inclue; bionomia; biocontrole; métodos de combate; movimento e distribuição; regularização feromônica e semioquímica; e, os prejuízos causados por esta peste na produção de leite e carne.

INSECTICIDE RESISTANCE IN HORN FLIES IN THE USA

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INTRODUCTION

Insecticide resistance in horn flies, Haematobia irritans (Diptera: Muscidae) was not considered a major problem previous to the 1980's. Organochlorine and organophosphate insecticides were used as sprays or in self-application devices such as backrubbers (oilers) or dust bags. These techniques delivered high, but usually discontinuous doses either inherently (sprays) or because of human forgetfulness (backrubbers and dust bags). The first documented case of organophosphate resistance is a report of ronnel (fenchlorphos) resistance selected with well maintained backrubbers (2) in 1962. The backrubbers had been treated weekly for 3 years and were on a two day schedule when the authors first visited the ranch. This treatment was continuous, selecting from every generation. Thus, this earliest documented case of insecticide resistance in horn flies had much in common with the cattle ear tag-selected resistance which was to follow almost 20 years later. Other early cases of resistance are given in Table 1, along with more recent ones.

EAR TAG SELECTED RESISTANCE

Recent cases of resistance in horn flies have all been associated with insecticide impregnated cattle ear tags. Stirofos tags began to fail after two years of use in GA (12), and later in Kansas (7). More cases would probably have been reported, but the more effective pyrethroid tags came into use and the stirofos tags received less attention. Pyrethroid resistance was widely recognized in the S.E. USA 2-3 years after these tags were introduced (Table 1) and continues to spread. Resistance selected with one pyrethroid has conferred cross-resistance to all other pyrethroids.

The continuous low dose of pyrethroid release from tags kills susceptible horn flies for months, but lets many or all resistant horn flies live, depending on the age of the tag (9,11). The horn flies habit of spending practically its entire adult life on the cow makes this selection virtually complete. Furthermore no susceptible immigrants can survive on a tag-treated herd to reduce resistance once it develops. With sprays resistance selection is much less. Sheppard (13) in a two year study with fenvalerate formulations found that 2 tags promoted a maximum resistance factor (RF; LC_{50} resistant population divided by LC_{50} of susceptible population) of 183, one tag selected for a RF of 63 and sprays selected for a maximum RF of 31. Cattle in this study were either ear tagged twice each year, or sprayed 3 times. Horn fly control and amount of pyrethroid used were very similar among the three treatments. Resistance in the spray treated pastures would probably have been even less if there had not been tag treated cattle nearby. Each spray treatment initially killed all horn flies, even resistant ones. And at the end of a spray period more susceptible flies could survive to mate and lower the RF. With tags the resistant flies advantage is continuous (11,9).

The same factors favoring rapid resistance selection are present with the use of organophosphate tags, just as they are with pyrethroid

tags. Stirofos tags rapidly developed resistance problems (7,12) but the more recently introduced diazinon and pirimiphosmethyl cattle ear tags have been in widespread use for 3-4 years with no resistance reported. Something in this organophosphate "tag-fly" selection system is different than in the pyrethroid "tag-fly" system. The organophosphate tags are rather similar to the pyrethroid tags and, if anything, are less potent, judging from LC_{50}'s of susceptible horn flies (14). It seems that this lowered potency might even enhance resistance selection by more nearly assuring the survival of heterozygous resistant individuals. But resistance has been very slow to develop, if at all. Since this is so, the "fly" part of this "tag-fly" system may be different. Horn flies with a mechanism to defeat these organophosphates may be very rare, or absent altogether. Other explainations are that this organophosphate resistance carries deleterious traits with it, or selection pressure has not been as widespread as it was when the pyrethroid tags were introduced. In any case, the pyrethroid tag-horn fly resistance selection system operates much differently than the recent organophosphate tag-horn fly system.

Table 1. Insecticide resistance in horn flies in the USA (after Sparks et al. 1985, as revised by Sheppard 1990).

Insecticide Group	Insecticide	Year	State	Reference or Workers
Chlorinated hydrocarbons	Toxaphene	1960	TX	McDuffie, 1960
		1961	TX	Harris, 1964
	DDT	1961	TX	Harris, 1964
		1984	LA	Byford et al, 1985
	Methoxychlor	1965	LA	Burns and Wilson, 1967
Organophos-phates	Fenchlorphos	1962	LA	Burns and Wilson, 1963
	Stirofos	1978	GA	Sheppard, 1983
		1984	KS	Harvey et al.
Pyrethroids	Cypermethrin	1984	LA	Byford et al, 1985
	Cyhalothrin (with high metabolic activity)	1988	GA	Sheppard (unpublished)
	Deltamethrin	1984	LA	Byford et al, 1985
	Fenvalerate	1982	GA	Sheppard, 1984
		1983	LA	Quisenberry et al, 1984
		1984	KS	Harvey et al, 1984
		1984	CA	Dunning et al, 1986
		1986	ND	Meyer and Kopp, 1987
	Permethrin	1984	KS	Harvey et al, 1984
		1984	CA	Dunning et al, 1986

MECHANISMS OF RESISTANCE

The major mechanism of pyrethroid resistance in horn flies appears to be nerve insensitivity, also known as kdr (knock down resistance). Kdr confers resistance to all pyrethroids and DDT; does not confer resistance to insecticides with other sites of action (such as organophosphates and carbamates); and does not respond to metabolic synergists such as PBO and DEF. This has been the pattern of pyrethroid resistance in horn flies and confirms kdr as the major mechanism. Besides this indirect evidence Crosby et al (5) using a hot probe-larval bioassay has detected direct evidence that kdr is the

major mechanism of pyrethroid resistance in horn flies. Other resistance mechanisms are metabolic detoxification (1) and behavioral resistance (8). Behavioral resistance may be most important in helping horn flies avoid highly treated areas of the host until other mechanisms are well established in the population. With ear tag treatments a gradient of insecticide occurs over the hosts body that makes this mechanism feasible.

While studying metabolic resistance Bull et al (1) used DEF (a hydrolase inhibitor), and piperonyl butoxide (PBO, a microsomal oxidase inhibitor) to synergize permethrin and found synergistic ratios of 4 and 10 respectively. These values were achieved with horn flies with RF = 50 to 70. Synergists were less active on horn flies with RF = 25. Bull (1) wrote "metabolism can become an important secondary factor that might facilitate accelerated development of resistance", when horn flies are continuously selected with pyrethroids. Indeed, this may have happened in at least one horn fly population at the University of Georgia Central Branch Station. Cyhalothrin cattle ear tags, after three years of use, have selected for fenvalerate RF to 100,000 and greater. The major component of this resistance must be metabolic, since PBO reduces this RF to ca. 35. This is the approximate level of resistance previously produced by kdr in other studies (including this area in GA), when metabolic resistance was judged to be a minor factor.

RESISTANCE MANAGEMENT AND CONCLUSION

Pyrethroid resistance in horn flies has developed very rapidly in the warmer parts of the USA wherever pyrethroid cattle ear tags have been used. The release characteristics of these tags and the habits of horn flies produce an almost perfect system for resistance selection. Space doesn't allow discussion of the several resistance management plans discussed in the literature, but generally, important assumptions of these plans cannot be met with these tags. The readers is referred to Sparks et al (14) and Byford et al (3,4) for further reading on insecticide resistance management in horn flies. These authorities, and the author of this paper agree that currently available pyrethroid cattle ear tags have little or no potential for inclusion in a system to retard resistance in horn flies.

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Summary

Prior to the 1980's insecticide resistance in horn flies occurred sporadically. Horn flies were usually easy to control with the available organochlorine and organophosphate insecticides. Application methods usually fostered sporadic exposure and resistance selection was rarely continuous for long periods. But about 1980 pyrethroid impregnated cattle ear tags became very popular. These tags released small doses of insecticide continuously. This dose was at a level that was highly selective for resistant horn flies. After 2-3 years use of these tags resistance was widespread over the warmer parts of the USA and continued to spread. Failure to achieve control was very common. The major mechanism of resistance is target site insensitivity (kdr) with components of behavior and metabolic resistance. More recently introduced diazinon and pirimiphosmethyl cattle ear tags continue to perform well, far past the time where pyrethroid tags were showing distinct control failures. Although subject to the same operational and biological factors favoring resistance, these organophosphate tags are not selecting rapidly for horn fly resistance. Resistance management in horn flies with currently available pyrethroid cattle ear tags is probably not achievable due to inherent characteristics of the tags and horn flies. In an area with susceptible horn flies the best resistance management is probably to avoid pyrethroid tag use to maximize the useful life of other pyrethroid formulations.

Résumé

Avant les années 1980 la résistance aux insecticides était sporadique chez les mouches Haematobia irritans (L.). Les mouches H. irritans sont ordinairement facile à controller avec les insecticides organochlorés et organophosphorés disponibles. Les méthodes habituelles d'application favorisaient une exposition sporadique et la selection pour la résistance aux insecticides était rarement continue pour de longues périodes. Mais vers les années 1980 les étiquettes d'oreille imprégnées de pyréthroides sont devenues trés populaires. Ces étiquettes relâchaient de petites doses d'insecticide de facon continue. Ce dosage était à un niveau hautement selectif pour les mouches H. irritans resistantes. Après 2 à 3 années, l'utilisation de ces étiquettes était très répandue dans les endroits chauds des Etats Unis et continuaient à se répandre. Le manque de control était très commun. Le mécanisme majeur de la résistance est l'insensibilité du point de cible (kdr) avec des constituents de conduite et de résistance métabolique. Des étiquettes d'oreille à base de diazinone et de pirimiphosmethyl récemment introduites continuent de bien performer, bien après que les étiquettes à base de pyréthroides aient echoué. Ces étiquettes à base d'organophosphorés ne selectionnent pas rapidement pour des mouches resistantes bien que sujettes aux mêmes facteurs opérationnels et biologiques favorisant le developpement de la résistance. Le control de la résistance aux étiquettes à base de pyréthoides chez H. irritans n'est probablement pas accompli à cause de caractéristiques qui sont inhérentes aux étiquettes et aux mouches H. irritans. Dans un endroit avec des mouches qui sont susceptibles, la meilleure facon de controller la résistance est probablement d'éviter l'utilisation d'étiquette à base de pyréthroide pour porter au maximum la durée utile des autres formulations de pyréthroides.

ZUSAMMENFASSUNG

Bevor 1980, die Widerstandsfaehigkeit des Haematobia irritans (L.) gegen Insektvernichtungsmittel trat nur vereinzelt auf und H. irritans war meist leicht knotrollbar mit den verfuegbaren organochlorine und organophosphate Insektvernichtungsmitteln. Die Anwendungs Methoden begunstigten sporadische Aussetzung und die Widerstand Selektion war selten fortdauernd fuer lange Zeit Perioden. Aber gegen 1980 wurde es populaer Ohren Klammern die pyrethroid enthielten an Grossvieh (Rindvieh) anzuwenden. Diese Klammern liessen stetig kleine Dosierungen von Insektvernichtungsmittel frei. Das Grad der Dosierung war hoechst selektiv fuer widerstandsfaehige H. irritans. Nach 2-3 Jahren der Benuetzung dieser Klammern wurde jedoch die Widerstandsfaehigkeit weitverbreitet ueber die waermeren Gegenden der USA, und sie verbreitete sich sogar stetig weiter. Es war sehr schwierig Kontrolle zu gewinnen. Der Haupt Mechanismus des Widerstandes ist die Unempfindlichkeit des Nervensystems (kdr) mit Bestandteilen des Verhaltens und des Metabolischen Widerstandes. Die vor kurzem eingefuehrten diazinon und pirimiphosmethyl Grossvieh Ohren Klammern erfuellen ihre Pflicht wesentlich besser. Lange nachdem die pyrethroid Klammern deutliche Kontrollenmangel zeigen, findet mit den neuen Klammern noch kein Nachlass statt. Obwohl sie abhaengig sind von denselben operationalen und biologischen Faktoren die Widerstandguenstig sind, sind diese neue organophosphate Klammern nicht so schnell selektiv fuer H. irritans Widerstandfaehigkeit. Widerstand Regulierung in H. irritans mit gegenwaertig verfuegbaren pyrethroid Grossvieh Ohren Klammern ist wahrscheinlich nicht ausfuehrbar. Der Grund liegt in den eigenen und angeborenen Charakterisierungen sowohl der Klammern als auch des H. irritans. Die beste Widerstand Verwaltung fuer Bereiche die H. irritans empfaenglich sind ist wahrscheinlich die Vermeidung von pyrethroid Klammern. Statt deren sollten andere pyrethroid Formulierungen benuetzt werden die ein viel laengeres Nutzleben haben.

"BÚFALOS: OPÇÃO ECOLÓGICA PARA A AMAZÔNIA."

"BUFFALOS: ECOLOGICAL AMAZON OPTION."

ASPECTOS SANITÁRIOS DA BUBALINOCULTURA NA AMAZÔNIA

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Indiscutivelmente a bubalinocultura constitue hoje uma importante atividade agropecuária de triplo propósito, com índices bastante satisfatórios no tocante a produção de carne, leite e trabalho.

A criação de búfalos, que até há bem pouco tempo não despertava maiores atenções por parte dos pecuaristas, passou de ser uma simples curiosidade para transformar-se en uma nova opção de exploração agropecuária, em progressiva e expressiva evolução, especialmente em áreas onde os bovinos e a exploração agrícola não apresentam resultados promissores.

Um dos principais motivos desse novo panorama reside, sem dúvida, no melhor conhecimento das muitas vantagens oferecidas pela espécie, entre elas a notável resistência orgânica frente às enfermidades.

Os bubalinos, no entanto, apesar de mostraren-se bastante saudáveis, também estão sujeitos a sofreren diversas enfermidades. Esses animais são susceptíveis às mesmas doenças dos bovinos, com algumas diferenças na prevalência, patogenia e sintomatologia.

Uma das etapas que merece especial atenção por parte dos pecuaristas, é a fase de cria dos bezerros, em vista da reduzida performance vital desses aninais quando mal conduzidos.

Dentre os problemas sanitários que interferem na saúde dos bubalinos, nas suas primeiras idades, é a ascaríase que ocupa lugar de destaque. Essa doença constitue, sem sombra de dúvida, um dos mais sérios e graves entraves com que se defrontam nossos criadores, especialmente aqueles desprovidos de infra-estrutura básica. .

Ocasionada pelo *Neoascaris vitulorum*, a ascaríase é responsável por cerca de 47% das mortes desses animais, nos primeiros três meses de vida. É considerada também o principal fator predisponente para outros transtornos gastrintestinais, tais como enterites e pneumoenterites. A infestação pré-natal parece ser a regra, uma vez que animais com onze dias de nascido apresentan altas infestações de vermes adultos.

A patogenia do *N. vitulorum* cursa com lesões nas paredes intestinais, digestão incompleta dos alimentos, diminuição da absorção de nutrientes e, consequente baixa conversão alimentar. As lesões são responsáveis ainda por hemorragias e infecções que desencadeian anemias e enterites, respectivamente. A digestão alimentar incompleta dos alimentos, contribue para a diniuição da absorção da água, com conseqüente diarréia e perda acentuada de nutrientes. Todos esses fatores resultan na síndrome parasitária, que manifesta-se principalmente por emagrecimento progressivo, crescimento retardado, pêlos arrepiados e sem brilho, ventre flácido e abaulado, desidratação, diarréia e morte por síncope cardíaca. Redução do número de eritrócitos, taxa de hemoglobina, volume globular, além de leucocitose, linfocitose e eosinofilia, con anemia do tipo normocítico normocrômico, são os efeitos nalêficos ocasionados por essa helmintose nos elementos sanguíneos dos animais parasitados.

Além do *N. vitulorum*, os bezerros búfalos poden ser parasitados por outros tipos de vermes, tais como o *Strongyloides papillosus* e os tricostrongilídeos *(Haemonchus contortus, Trichostrongylus axei, Cooperia curticei)*. O *S. papillosus* acompanha praticamente a curva epidemiológi-

ca do *N. vitulorum*. Ambos marcam presença nos recém-nascidos, alcançando altas infestações aos 30 e 60 dias, após parto. Os tricostrangilídeos, por sua vez, iniciam o parasitismo quando os bezerros atingem a idade em torno de 90 dias e tendem a mostrar piques críticos quando estes chegam aos 180 dias de nascidos.

Resultados de pesquisa mostram que em bezerros criados extensivamente em áreas alagadiças, que sofrem inundações periódicas, a infestação parasitária é inexpressiva, em vista das inadequadas condições de sobrevivência das larvas infestantes nesses locais, devido o excesso de umidade. Nessas áreas os animais sofrem mais com a falta de alimentação nas épocas das inundações, do que com a verminose.

Como segunda preocupação básica dos criadores destaca-se a pediculose ocasionada pelo piolho *Haematopinus tuberculatus*. Praticamente exclusiva dos bubalinos, essa ectoparasitose, apesar de não ser mortal, tende a assumir proporções graves nos períodos mais chuvosos do ano, quando a insolação e a temperatura encontram-se diminuidas e os animais permanecem com maior quantidade de pêlos e procuram menos o banho. A patogenia desse piolho caracteriza-se não somente pela ação espoliativa, mas também pelo estresse de suas picadas e pela sua constante presença no corpo do animal. A transmissão de doenças é outro fator a ser considerado nesse tipo de parasitismo.

Favorecidas pelas condições climáticas da região e pelo grande número de vetores, destacam-se ainda parasitoses como a tripanossomíase e filariíase. Essas, apesar de ocorrem esporadicamente e em regiões definidas, especialmente aquelas com alta incidência de insetos hemotófagos, são de difícil controle e muitas vezes mortais.

Até a presente data diagnosticou-se nos meios criatórios da região, a presença do *Trypanosoma vivax* que, dependendo do grau de infestação, tende a cursar assintomaticamente. Os animais quando recuperados da tripanossomíase, geralmente apresentam pouca parasitemia nas infestações subsequentes, originando assim formas subclínicas da enfermidade.

Quanto às filaríases, dois são os agentes etiológicos conhecidos: *Parafilaria bovicola* e *Onchocerca eebei*, ambas também transmitidas por insetos hematófagos. A *P. bovicola* ocasiona lesões cutâneas hemorrágicas, principalmente na região das axilas, virilha, face interna da coxa e prepúcio dos animais. Essas tendem a cura espontânea, com fibrose no local, podendo haver formação de abcessos em decorrência de infecções secundárias. A *O. eebei* por sua vez, manifesta-se através de nódulos subcutâneos arredondados de conteúdo purulento e consistência pastosa, que surgem, inicialmente, na região do peito, flancos e entrepernas dos animais.

As infestações por sarna (*Psoroptis equi var. bovis*) apesar de ocorrerem praticamente em animais estabulados, é um problema que merece ser considerado, em vista da depreciação do couro do animal parasitado.

Um problema que começa a causar inquietação nos meios criatórios da região é a presença da mosca *Haematobia irritans*. Suas picadas dolorosas causam irritação e estresse nos animais, com conseqüente interferência na produção de carne e leite.

Quanto as doenças de origem infecciosa, estas são bastante similares às dos bovinos e ocorren com maior ou menor freqüência, de acordo com o estado higiênico-sanitário dos animais e com as zonas endêmicas. No rol dessas doenças, as mais prevalentes e problemáticas são a brucelose e tuberculose, pelas dificuldades de tratamento e facilidades de disseminação; a febre aftosa, pelo descuido dos pecuaristas na vacinação; a raiva

pelo seu efeito devastador e o carbúnculo sintomático pelas conseqüências maléficas nos animais jovens. Essa é uma área bastante carente de conhecimentos. Em vista das características fisiológicas dos bubalinos, diferentes das dos bovinos, pelo menos dois estudos urgem a ser realizados. Um visando a adequação da vacinação contra a brucelose, uma vez que o período de imunidade dessa vacina mostra-se bastante curto em relação a vida produtiva das vacas búfalas. O outro, sobre o diagnóstico alérgico da tuberculose, considerando que as reações cutâneas nos bubalinos são bem mais acentuadas que nos bovinos, fato que tem originado diagnósticos errôneos.

Comunente freqüentes e igualmente maléficas as doenças carenciais representan uma constante ameaça aos bubalinocultores. De uma maneira geral, os animais possuem deficiência crônica de cálcio e fósforo.

As intoxicações por plantas, que apesar de existirem em grande quantidades na região, parecem não representar um problema de proporções graves para os bubalinos. Esses animais tem demonstrado pouca vulnerabilidade a esse tipo de afecção, uma vez que dificilmente perdem o instinto seletivo dessas plantas. A maior ocorrência de casos de intoxicação está relacionada com o fungo *Pithomyces chartarum* que encontra-se presente na matéria morta das gramíneas. As ocorrências tendem a ser mais intensas nos períodos de transição do ano, isto é, no final do período sêco e início do chuvoso e vice-versa.

Vale salientar ainda a ocorrência de casos de mal formação congênitas (atresia anal, anoftalmia, artrogripose, hérnia inguinal, braquiocefalia) que, apesar de constarem atualmente poucos casos, causam preocupação, tendo em vista representarem um futuro risco a qualidade zootécnica do rebanho. Essas, carecem ainda totalmente de estudos.

Finalmente, pode-se dizer que o conhecimento científico nacional relacionado com a saúde dos bovinos, são ainda bastante restritos. Se considerarmos entretanto que, a pouco mais de uma década, praticamente nada havia no Brasil sobre o assunto, são bastante significativos os resultados já conseguidos pela pesquisa. Obviamente que substanciais conhecimentos devem ser ainda gerados e colocados a disposição dos bubalinocultores que ocupan um grande espaço no setor agropecuário, produzindo alimentos nobres a baixo custo.

SUMMARY

Buffalo deseases are the same as those of cattle with few differences in their prevalence, pathogenicity and symptomatology. Parasitic diseases are the most prevalent. *Neoascaris vitulorum* is the most common helmintosis of young buffalo calves. Lice infestation, psoroptic mange, trypanosomiasis, haemorrhagic cutaneous filariasis have been diagnosed in the Amazon Region. Among the infectious and contagious deseases that buffaloes are subjected, the following were detected: foot and mouth disease, brucellosis, tuberculosis, rabies and blackleg. In the Amazon Region non - infectious diseases, like hipophosphataemia, hipocalcaemia and plant poisoning were also observed.

A EXPLORAÇÃO ECOLÓGICA E O MELHORAMENTO DOS BÚFALOS (*Bubalus bubalis* L.) NA AMAZÔNIA

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INTRODUÇÃO

A região amazônica é uma área ainda carente de tecnologia agropecuária em função das suas características muito peculiares, destacando-se as grandes extensões , ora de campos naturais e várzeas ora de cerrados e florestas. Os sistemas de produção animal com base em pastagens cultivadas devem respeitar tais características sob pena de não se aproveitar bem os solos, em geral distróficos, ou não se obter os índices de produtividade que a região tem potencial para expressar.

Observa-se, de um modo geral, uma exploração pecuária desordenada através da implantação de pastagens em áreas de mata, iniciando-se um processo, muitas vezes, irreversível de destruição de ecossistemas. Hoje na amazônia existem 17 milhões de hectares de pastagens cultivadas em áreas de florestas dos quais, aproximadamente, 10 milhões estão degradados ou em vias de degradação (16), que necessitam ser recuperados e incorporados ao processo produtivo, se manejados adequadamente.

A região tem vocação natural para a exploração agropecuária desde que no seu planejamento sejam respeitados os mais variados ecossistemas naturais, visto que, os órgãos responsáveis pela fiscalização do meio ambiente não possuem, ainda, condições materiais para isso, impossibilitados em grande parte pelas dificuldades naturais da região.

Este trabalho objetiva mostrar o potencial do ambiente amazônico como suporte para a produção de proteínas nobres, com base no búfalo, que será analisado como um componente ecológico a mais, podendo produzir, através de técnicas de manejo e melhoramento, carne e leite, e desenvolver trabalhos de tração, sem interferir negativamente no complexo solo - vegetação - clima, o que não invalida o aproveitamento consciente da amazônia como um todo, para produção vegetal e/ou animal e exploração de seus recursos naturais.

A AMAZÔNIA BRASILEIRA

A amazônia confunde-se com o trópico úmido brasileiro, ocupando cerca de 5,1 milhões de km^2 o que representa, aproximadamente, 60,44% do território nacional, englobando toda a região Norte e parte dos estados do Mato Grosso, Goiás e Maranhão (7).

Os principais tipos climáticos pela classificação de Köppen são o *Af*, *Am* e o *Aw*, caracterizando-se por uma precipitação pluviométrica média de 2.700mm, distribuídos em apenas dois períodos do ano. O clima quente e úmido é o predominante com uma pequena variação na temperatura média anual de 26°C. A umidade relativa do ar durante todo o ano é, em média, 81% (2, 13).

Somada à diversidade climática a amazônia apresenta uma grande variação de solos de terras firme, inundável e/ou semi-inundável, além de uma cobertura vegetal que vai desde a floresta densa até os campos naturais, o que representa um grande suporte à produção de alimentos

por meio da agropecuária.

Segundo Serrão & Dantas (15) existem na região cerca de 50 milhões de hectares de pastagens nativas. Para Nascimento & Homma (13) as áreas de pastagens nativas localizadas em terras inundáveis são estimadas em torno de 11 milhões de hectares, havendo, ainda, aproximadamente, 100 milhões de hectares de cerrados e campos naturais de solos pobres de terra firme com vegetação arbustiva e arbórea e pastagens de baixa qualidade. Por outro lado, Costa *et al.* (5) afirmaram que a região amazônica brasileira possui cerca de 67 milhões de hectares de área inundável, representando 13 % da superfície regional.

As principais espécies gramíneas forrageiras nativas da amazônia são: *Echinochloa polystachia, Hymenachne amplexicaulis, Paspalum repens, Leersia hexandra, Luziola spruceana, Oriza spp., Paspalum fasciculatum, Paratheria prostata, Brachiaria mutica* e *Paspalum fizanioides*, em áreas de terras inundáveis; os gêneros de gramíneas predominantes nos campos naturais e/ou cerrados são: *Andropogon, Trachypogon, Axonopus, Paspalum* e *Panicum*. As leguminosas mais importantes são dos gêneros *Cassia, Desmodium, Stylosanthes, Zornia, Galactia* e *Centrosema*(6, 9 e 17).

Pelo exposto pode-se ressaltar que o trópico úmido se constitui no habitat natural para os bubalinos, principalmente, pela característica que possuem de transformar em proteínas as pastagens mais grosseiros, em ambientes completamente adversos, o que não se consegue com outras espécies *Bovidae*.

Deve-se acrescentar que a amazônia não produz o que a sua população necessita consumir. Em 1987 o déficit de proteína de origem animal por habitante/ano, na região Norte, girou em torno de 120 milhões de toneladas (11).

A bubalinocultura no contexto amazônico

Desde a sua introdução na amazônia o búfalo vem demonstrando grande adapatabilidade aos vários criatórios naturais, principalmente, os campos da ilha de Marajó e áreas de várzeas da calha do rio Amazonas. A população bubalina da região Norte representa 50% do total nacional que já atinge mais de 2 milhões de cabeças (14).

A grande demonstração da adaptação dos búfalos à amazônia é a taxa de crescimento verificada no período de 1973 a 1987, ou seja, 484%, equivalendo a 13,57% ao ano. No mesmo período a pecuária bovina cresceu, na região, 250,02%, representando uma taxa anual de 9,78% (TABELA 1).

TABELA 1 - Crescimento dos efetivos bovino e bubalino na região Norte- 1973 / 1987 (1).

Anos	Bovinos			Bubalinos		
	Efetivo (1000 cab.)	Crescimento (%) Período	Anual	Efetivo (1000 cab.)	Crescimento (%) Período	Anual
1973	1.971	-	-	100	-	-
1974	2.211	12,18	12,18	118	18,00	18,00
:	:	:	:	:	:	:
1987	6.899	250,02	13,19	584	484,00	7,35
Tot./$\bar{x}$		250,02	9,78		484,00	13,57

A mesma fonte (1) mostra um crescimento para a bovinocultura, em todo o Brasil, no mesmo período, de apenas 3,54% contra 17,06% dos bubalinos (TABELA 2).

TABELA 2 - Crescimento dos efetivos bovino e bubalino no Brasil no período 1973 - 1987.

Anos	Bovinos			Bubalinos		
	Efetivo (1000 cab.)	Crescimento (%) Período	Anual	Efetivo (1000 cab.)	Crescimento (%) Período	Anual
1973	90.830	-	-	157	-	-
1974	92.495	1,83	1,83	203	29,30	29,30
:	:	:	:	:	:	:
1987	135.726	49,43	2,65	1.082	589,17	9,85
Tot./$\bar{X}$	-	35,96	3,54	-	297,45	17,06

É óbvio que em alguns casos os dados não mostram o crescimento real dos rebanhos e sim um aprimoramento das estatísticas realizadas periodicamente, ambos os casos, contudo, para os bubalinos, mostra a tendência do grande crescimento do efetivo em todo o país, não obstante, beneficiado, também, por uma menor taxa de abate.

Carvalho (3) relatou o crescimento da bubalinocultura nacional em torno de 12,7% ao ano, mostrando, de toda forma, um incremento muito grande e, apesar de algumas citações desencontradas com relação ao efetivo total, os dados revelam que o búfalo emerge com muita força na pecuária nacional e persistindo tal situação, a médio prazo, o país terá um grandioso rebanho, sendo a amazônia o seu maior reduto.

Deve-se ressaltar que a quase totalidade da criação de búfalos é desenvolvida nas áreas de campos nativos pobres e de várzeas, sem incentivos dos órgãos de desenvolvimento.

Os bubalinos têm apresentado resultados muito bons na performance produtiva, mesmo em regime exclusivo de pastagens naturais, onde as fêmeas produzem, em média, 5,0 litros de leite durante quase oito meses do ano, em áreas de várzeas, caindo para 4,0 litros nos campos baixos do Marajó. O percentual de gordura pode atingir até 8% em ambas as condições e, somada a uma maior concentração de sólidos totais, apresenta um excelente rendimento quando da transformação em subprodutos (8). Em pastagens cultivadas (*Echynochloa pyramidalis*), do estuário do rio Amazonas, observou-se (12) uma produção média de leite, em treze anos de estudos, de 1.655,60 ± 306,60 kg, com 7,1 ± 0,8 % de gordura, num período de lactação de 274,20 ± 64,6 dias, em animais da raça Mediterrâneo e mestiços Murrah - Mediterrâneo (TABELA 3), o que mostra o grande potencial de produção naquelas condições.

TABELA 3 - Produção de leite (kg) e período de lactação (dias) de animais Mediterrâneo (Me), Murrah (Mu) e seus mestiços, no estuário do rio Amazonas, em pastagens cultivadas de *Echinochloa pyramidalis*.

Raça / GS	No. obs.	Produção de leite
Mediterrâneo	231	1.526,00 ± 29,30
1/2 Mu - Me	345	1.715,90 ± 29,90
3/4 Mu - Me	122	1.627,00 ± 40,50
≥ 7/8 Mu	47	1.623,90 ± 59,10
Média		1.655,60 ± 306,60

GS = Grau de sangue

Nessas mesmas áreas os machos atingem um peso vivo de 400 a 500 kg com menos de dois anos de idade e podem ser criados para esse fim somente nas áreas inundáveis ou em sistemas integrados de pastagens nativas de várzeas com as cultivadas de terra firme (4, 5 e 10).

Estudos realizados (12) na amazônia sobre eficiência reprodutiva de bubalinos apresentaram excelentes índices (TABELA 4).

TABELA 4 - Índices de eficiência reprodutiva de bubalinos na amazônia.

Raça / GS	Idade à primeira cria ($\bar{X}$/dias)	Intervalo entre partos ($\bar{X}$/dias)	Eficiência reprodutiva (%)
Mediterrâneo	1.248	491	74,5
1/2 Mu - Me	1.191	450	81,1
3/4 Mu - Me	1.296	472	77,5
≥ 7/8 Mu	1.317	484	75,6
Médias	1.263	467	78,2

São utilizados, também, como animais de tração, apresentando ótimos rendimentos e, no momento, é grande o interesse de instituições e produtores para utilização dos búfalos com essa finalidade.

O melhoramento dos bubalinos na amazônia

As quatro raças oficialmente reconhecidas pela Associação Brasileira de Criadores de Búfalos (ABCB) existem na amazônia,(Jafarabadi, Murrah, Mediterrâneo e Carabao). Há uma predominância numérica da Mediterrâneo, porém, os mestiços entre esta raça e a Murrah são bastante numerosos.

A adaptabilidade dos búfalos aos mais diversificados ambientes deve ser resultante da grande variabilidade genética da espécie, oriunda dos vários grupos raciais existentes na Ásia e, até mesmo, da raça Mediterrâneo formada a partir de variados "pools" gênicos. Esta diversidade genética é de grande importância para o melhoramento dos níveis produtivos hoje observados na região. Deduz-se que devido atais fatos os efeitos da consanguinidade ainda não são tão evidentes.

Há várias maneiras de se promover o melhoramento genético do rebanho bubalino da amazônia, contudo, seria importante a elaboração de um "Programa de Melhoramento Genético do Rebanho Bubalino da Amazônia", enfatizando-se os seguintes aspectos :

Rebanhos de Corte:

- Identificação de indivíduos com eficiência de ganho em peso em várias idades;
- Características de carcaça;

Rebanhos Leiteiros:

- Produção de leite e de gordura;

Outras características como: eficiência reprodutiva, vida útil produtiva, habilidade materna, ausência de defeitos hereditários, tipo e conformação seriam comuns para ambas as finalidades. O programa teria apoio, de uma maneira geral, nas informações dos indivíduos e de seus parentes, ou seja:

a) Desempenho individual;
b) Genealogia;
c) Teste de progênie ou de desempenho dos descencentes;
d) Desempenho dos colaterais e,
e) Combinação do desempenho individual e familiar.

Antes disso, porém, há importantes procedimentos a nível de rebanho que devem ser considerados:

- Seleção fenotípica dentro de cada raça, principalmente, pela habilidade materna (vacas), fertilidade (vacas e novilhas) e pelos pesos nas idades padrões de 365 e 550 dias (touros), e destiná-los a centros de avaliação implantados regionalmente;
- Acasalamentos entre raças distintas , principalmente, Murrah e Mediterrâneo;
- Introdução de genes de outras regiões e, até mesmo, de outros países criadores;
- Implantação de um sistema de acompanhamento zootécnico a nível de fazenda;
- Implantação de controles leiteiros nas propriedades;

Sob qualquer condição é imprescindível a utilização da inseminação artificial e dos recursos biotecnológicos, principalmente, a transferência de embriões.

As técnicas de manejo dos rebanhos e das pastagens bem como os recursos básicos de nutrição animal e controle sanitário, são fundamentais para o sucesso de um programa de melhoramento genético.

CONCLUSÕES

Conclui-se que a amazônia brasileira reune excelentes condições de clima, solo, topografia, disponibilidade de água, pastagens naturais, além de grandes vazios improdutivos, evidenciando a sua vocação para a produção animal com base, principalmente, na criação do búfalo, que desponta como verdadeiro animal ecológico, que, por outro lado, através de recursos do melhoramento genético, pode evoluir para tipos amazônicos cada vez mais adaptados e produtivos sob as condições naturais. Tais fatos são da maior importância no momento em que está sendo debatida a ocupação e/ou exploração daquela região de maneira mais racional e criativa.

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SUMÁRIO

A amazônia brasileira é uma vasta região onde há grandes extensões de campos altos e inundáveis, cerrados e várzeas, com abundância de pastagens naturais, constituindo-se num perfeito habitat para o búfalo (*Bubalus bubalis* L.) que, sob técnicas de manejo e de melhoramento genético, pode desenvolver tipos amazônicos bem adaptados e mais produtivos, minimizando-se os efeitos danosos que a pecuária praticada desordenadamente em área de florestas, vem causando aos ecossistemas naturais.

PASTAGENS DA AMAZÔNIA UTILIZADAS NA CRIAÇÃO DE BUBALINOS

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INTRODUÇÃO

As pastagens da região Amazônica ocupam uma área estimada superior a 80 milhões de hectares, 80% dos quais constituídos por pastagens nativas e o restante por pastagens cultivadas. Nessa região está a metade do efetivo bubalino do Brasil, representando atualmente uma população em torno de 1,4 milhões de cabeças, avaliada com base em projeções a partir de 1985. A maior parte desse rebanho é criada basicamente em dois tipos de pastagens: nativas da Ilha de Marajó e nativas de várzeas inundáveis do Baixo e Médio Amazonas. Até o final dos anos 60 esses dois ecossistemas se constituiram quase que exclusivamente na única fonte de alimentos para a pecuária bovina e bubalina da região. A partir de então, com a formação acelerada de pastagens em áreas de floresta, incentivada pelo governo, para onde se deslocou grande parte a bovinocultura, e com o crescente fortalecimento da bubalinocultura, aquelas áreas adquiriram grande importância, por suas peculiaridades favoráveis à criação mais eficiente de búfalos. Além desses dois ecossistemas, outros dois também são utilizados na bubalinocultura, embora en menor escala: pastagens nativas de terra firme (em áreas de cerrado) e pastagens cultivadas de terra firme (em áreas de floresta). Tem-se verificado nos últimos anos, entretanto, uma tendência ao aumento no uso dessas áreas para a criação de bubalinos. Considerando-se o rebanho atual e a disponibilidade de pastagens nativas, verifica-se que há um enorme potencial para expansão da bubalinocultura na Amazônia. Mesmo estabelecendo-se uma lotação baixa, de um animal para cada cinco hectares, por exemplo, é possível aumentar a população atual em pelo menos dez vezes.

Neste trabalho são descritas as características das pastagens da Amazônia utilizadas na criação de bubalinos, discutindo seu potencial e suas limitações, e são apresentados resultados de pesquisa de produção de carne e leite desses animais.

PASTAGENS NATIVAS DA ILHA DE MARAJÓ

A ilha de Marajó possui, na sua parte oriental, cerca de 23 milhões de hectares de pastagens nativas (1) e uma população bubalina estimada em pouco mais de 600 mil cabeças. O tipo climático da região é o tropical chuvoso com pequeno período seco, com precipitação pluviométrica variando de 2.000 a mais de 3.000 mm anuais. A temperatura média anual é de 27 °C, tendo como médias das máximas 30,5 °C e das mínimas 23,8 °C. A umidade relativa do ar oscila en torno de 90% e raramente é inferior a 70%. Os solos são Hidromórficos, mal drenados, ácidos, de baixa fertilidade natural e com teores elevados de alumínio. A topografia geral da ilha é muito plana, com algumas formações mais elevadas (tesos). A vegetação predominante é do tipo cerrado, onde ocorrem principalmente gramíneas, ciperáceas e leguminosas (2).

A região, denominada de "campos", está sujeita a sérios problemas de ordem climática, com períodos extremos e alternados de excessos de água e de seca. Na época das chuvas, extensas áreas de campos são inundadas, e na época de seca, às vezes intensa, há uma sensível diminuição na disponibilidade de forragem. Esse regime de águas permite a divisão

dos campos em três gradientes: 1 - não alagado ou alagado por pouco tempo; 2- inundado por três a seis meses; e 3- alagado quase todo o ano (3).

As gramíneas mais frequentes nos gradientes 1 e 2 pertencem aos gêneros *Axonopus*, *Trachypogon*, *Paspalum* e *Eragrostis*. No gradiente 3 aparecem as gramíneas dos gêneros *Hymenachne*, *Leersia*, *Luziola*, *Oriza*, *Paspalum* e *Eriochloa*. Além das gramíneas aparecem com frequência nos gradientes 1 e 2 as ciperáceas, principalmente as dos gêneros *Rhynchospora*, *Cyperus*, *Fimbristylis* e *Eleocharis* (1, 4); e em menor escala algumas leguminosas, destacando-se as dos gêneros *Aeschynomene*, *Centrosema*, *Desmodium*, *Macroptilium*, *Stylosanthes*, *Zornia*, *Calopogonium*, *Phaseolus*, *Diocklea*, *Cassia*, *Clitoria*, *Eriosema*, *Galactia* e *Mimosa* (5). Os bubalinos utilizam as pastagens dos três gradientes o ano inteiro, ao contrário dos bovinos.

O potencial produtivo e o valor nutritivo das plantas forrageiras dos campos da Ilha de Marajó são baixos. A disponibilidade média de forragem nos períodos de chuva e seco é de 1.100, e 600 kg/ha de matéria seca, respectivamente, e os teores de proteína bruta são inferiores a 7% no estrato herbáceo (6). O potencial de resposta do estrato herbáceo à adubação é muito baixo (4). A capacidade de suporte é de uma unidade animal por três hectares (1). Os bubalinos, recebebdo suplementação mineral, conseguem atingir peso vivo de 370 kg aos dois anos de idade, com ganhos de peso diários de 0,450 kg, superiores aos ganhos dos bovinos (7).

A pesquisa de melhoramento de pastagens na Ilha de Marajó evidenciou, entre todas as gramíneas testadas até o presente, a espécie *Brachiaria humidicola* como a forrageira de maior potencial para formação de pastagens cultivadas nos gradientes 1 e 2. Essa gramíneas já foi introduzida em escala comercial em algumas fazendas, com um mínimo de fertilização. Nesse ecossistema, a *B. humidicola* não tem sofrido danos aparentes por cigarrinha quando formadas nos gradientes 1 e 2 dos campos da ilha (3).

Resultados de pesquisa en andamento na Ilha, desenvolvidas com a colaboração do autor, em pastagens cultivadas de *B. humidicola* sob três taxas de lotações, 1, 1,5 e 2 animais/ha, evidenciaram ganhos diários por animal de 330, 383 e 294 g, respectivamente, em 364 dias. Na época chuvosa (janeiro a maio) os ganhos foram de 574, 517 e 527 g e na época seca (setembro a dezembro), 85, 235 e 83 g, respectivamente. No segundo ano, os ganhos obtidos até os 231 dias foram de 420, 364 e 385 g respectivamente para as taxas de lotação baixa, média e alta.

Em outro trabalho, os pesos de machos bubalinos ao nascer e aos 24 meses foram respectivamente de 39,3 e 356,9 kg (Carabao), 37,9 e 324,1 kg (Jafarabadi), e 37,5 e 394,6 (Mediterrâneo). Nas mesmas condições, os pesos para bovinos Nelore foram de 24,4 e 264,6 kg respectivamente (8, 9 e 10). Bubalinos machos da raça Murrah, apresentaram pesos médios de 36,7 ao nascer e 186,0 kg aos 12 meses (540 g/animal/dia) (11). Não há dados registrados para produção de leite nesse ecossistema.

As pastagens nativas da Ilha de Marajó são ainda muito pouco estudadas no que diz respeito ao seu melhor aproveitamento, e conseqüentemente na diminuição da pressão da pecuária sobre as áreas de floresta. São necessárias pesquisas sobre a caracterização dos recursos das pastagens, efeitos fisiológicos (fogo, estresse hídrico, inundação), biológicos (simbioses, pragas, doenças) e ciclagem de nutrientes no sistema solo-pastagem-animal (3).

PASTAGEM NATIVA DE TERRA INUNDÁVEL

As pastagens nativas de terra inundável ocorrem nas áreas de várzea do rio Amazonas e seus afluentes de águas barrentas. Anualmente, elas são inundadas, em maior ou menor escala em função do regime climático, e durante o refluxo das águas, as partículas orgânicas e minerais contidas em suspensão nas águas são depositadas no solo, tornando-o de alta fertilidade, quando comparado aos solos de terra firme da região. As áreas mais representativas dessas pastagens estão localizadas nas regiões do Baixo e Médio Amazonas, embora haja ocorrência no estuário do rio Amazonas e, em menor escala em outras regiões da Amazônia. Essas pastagens têm representado um papel fundamental no desenvolvimento da criação de búfalos, por possuirem elevado potencial de produção de forragem de bom valor nutritivo e um habitat apropriado a esse tipo de animais. Estima-se que 20% dos búfalos do Estado do Pará são criados nessas pastagens.

O clima na região do Baixo Amazonas é caracterizado por um período de estiagem de dois a três meses, temperatura média anual de 27 °C com pequena oscilação, precipitação pluviométrica anual de 2.100 mm e umidade relativa do ar de 84%. Os principais tipos de solo são os hidromórficos (Gléi Pouco Húmico e Gléi Húmico) resultantes do acúmulo de sedimentos depositados durante a vazante dos rios de água barrenta (12).

A água é um componente de alta importância nesse ecossistema na composição da vegetação herbácea, formada principalmente por gramíneas anfíbias (sobrevivem flutuando, submersas nas águas ou em solos relativamente secos), cuja produtividade depende da concentração de partículas em suspensão.

As gramíneas mais importantes sob o ponto de vista da alimentação animal nas pastagens nativas de terra inundável são *Echinochloa polystachya*, *Hymenachne amplexicaulis*, *Paspalum fasciculatum*, *P. repens*, *Leersia hexandra*, *Luziola spruceana*, *Oryza alta* e *O. grandiglumis*. A produção de matéria seca dessas gramíneas varia de 2.500 a 6.000 kg/ha/ano, sendo muito influenciada pelas condições climáticas, edáficas e hidrológicas (11). Os teores de proteína bruta da forragem variam de 6.4 a 14.3% e a digestibilidade de 31 a 70% (11, 14). Esses valores são superiores àqueles encontrados na Ilha de Marajó e em pastagens cultivadas de terra firme da Amazônia (6). Os teores de minerais satisfazem as necessidades mínimas para nutrição do gado de corte, exceto cálcio e fósforo (13). A superioridade do valor nutritivo das gramíneas de pastagens de terra inundável, sobre aquelas de outros ecossistemas da Amazônia, decorre dos solos de boa fertilidade e de fatores morfofisiológicos das plantas (1, 15) *Leersia hexandra* e *Hymenachne amplexicaulis* possuem mecanismo fotossintético semelhante aos das gramíneas típicas de climas temperados (15), e é possível que outras gramíneas façam parte de grupo.

Não há dados na literatura sobre a performance animal nas pastagens nativas de terra inundável. Em experimentos conduzidos pela EMBRAPA,com bubalinos, obteve-se ganhos de peso diários de até 735 g/aninal, resultados superiores dos normalmente reportados sobre pastagens cultivadas tropicais (16). As limitações para a criação de búfalos nessas pastagens são mínimas, ao contrário do que ocorre na criação de bovinos, a qual é bastante prejudicada no período das enchentes. Essas pastagens possuem alto potencial para a criação de bubalinos, em função da grande produção de forragem de elevado valor nutritivo e da boa adaptação desses animais a áreas alagadas.

PASTAGEM NATIVA DE TERRA FIRME

As pastagens nativas de terra firme são representadas principalmente pela vegetação de savana tipo cerrado, caracterizadas pela predominância de gramíneas nativas de porte baixo, com ocorrência variável de arbustos e árvores tortuosas de pouca altura. Estas formações ocorrem, em grande extensão, nos Estados do Amapá e Roraima, e em áreas menores onde a floresta é interrompida.

As principais gramíneas são dos gêneros *Andropogem*, *Axonopus*, *Eragrostis*, *Paspalum*, *Mesosetum* e *Trachipogon*. Essas gramíneas são perenes e podem ficar em dormência no período seco. As principais ciperáceas estão incluídas nos gêneros *Cyperus*, *Bulbostylis*, *Fimbristylias*, *Rhynchospora*, *Diehromena* e *Scleria* (3). Na estação chuvosa, o crescimento dessas gramíneas e ciperáceas é satisfatório, ficando o solo praticamente coberto pelo estrato herbáceo por elas formado, sendo bastante suscetível à queima no período seco. Essas forrageiras são adaptadas à condições de elevada acidez e baixa fertilidade dos solos e suportan bem os efeitos das queimadas (6).

Nas pastagens nativas de terra firme existem também algumas espécies de leguminosas dos gêneros *Desmodium*, *Stilosanthes*, *Zornia*, *Cassia*, *Galactia* e *Centrosema*. De um modo geral, essas leguminosas são mais frequentes nas savanas com vegetação arbórea esparsa ou nas áreas de transição entre o cerrado e a mata (6).

Resultados obtidos em pastagens nativas de tipo "campo coberto" (um ecossistema intermediário - en relação a drenagem - entre savanas bem e mal drenadas, e com algum potencial para alimentação de bubalinos) em Monte Alegre-PA, revelaram que a disponibilidade de forragem e teores de proteína bruta nas épocas chuvosa e seca foran, respectivamente, de 1210 kg/ha de MS e 4,4%, e 899 kg/ha de MS e 3,7%. Os capins *Axonopus purpusii* e *Mesosetum altum* predominantes no extrato herbáceo apresentaram 24,7% de digestibilidade "in vitro" da matéria seca, 0,15% de cálcio e 0,05% de fósforo na matéria seca, mostrando que essas pastagens são de baixa produtividade e de baixo valor nutritivo (13).

PASTAGEM CULTIVADA DE TERRA FIRME

As pastagens cultivadas na Amazônia são ainda muito pouco utilizadas por bubalinos, encontrando-se no entanto, en fase de expansão. Atualmente, os capins mais plantados são *Panicum maximum*, *Brachiaria humidicola*, *B. brisantha*, *Andropogon gayanus* ou *Planaltina* e *Pennisetum purpureum* em menor escala.

O capim *Brachiaria humidicola* é uma das gramíneas mais importantes para a formação de pastagens en solos de terra firme de baixa fertilidade na Amazônia. Esta gramínea é capaz de produzir de 3 t de matéria seca MS/ha/ano (em solo de cerrado bem drenado, sem fertilização, Roraima) até 28 t de MS/ha/ano (en solo de áreas florestadas fertilizado com 50 kg de P_2O_5/ha, Porto Velho - RO) (17).

Foi avaliada uma pastagem de *B. humidicola* sob pastejo rotativo de bubalinos leiteiros na taxa de lotação média de 1,6 unidade animal (U.A.)/ha, com período de ocupação de 5 dias e descanso de 36 dias em Belém, PA. A quantidade de folhas obtidas nas épocas mais e menos chuvosa foram, respectivamente, 6,92 e 4,01 kg de MS/100 kg de peso vivo/dia, ficando um pouco acima do mínimo necessário para o consumo em pastejo, que é de 4 - 6 kg de MS/100 kg de peso vivo/dia. Na composição botânica

da dieta selecionada por bubalinos fistulados no esôfago predominaram as folhas (acima de 80%).

O teor de proteína bruta (PB) da dieta da época mais chuvosa (9,5%) foi superior ao da época menos chuvosa (6,81%). A digestibilidade "in vitro" da matéria orgânica (DIVMO) na época mais chuvosa (56,12%) foi também superior a da menos chuvosa (46,96%).

O consumo de forragem foi superior ao obtido com bovinos em pastagem de *B. humidicola*. A diminuição do consumo de MS na época menos chuvosa, ocorreu devido principalmente à diminuição nos teores de PB e na redução da DIVMO, haja visto que não houve deficit de forragem. Os ganhos de peso nas épocas mais e menos chuvosa respectivamente de 516 e 370 g/animal/dia foram diferentes da mesma forma como ocorreu com os teores dos parâmetros do valor nutritivo (18).

Bubalinos engordados em pastagens de quicuio-da-amazônia, durante 364 dias em Belém, Pará, com suplementação mineral à vontade em pastejo contínuo tiveram ganho diário de 636, 627 e 579 g/animal/ha, respectivamente, nas taxas de lotação de 2,0, 1,5 e 1,0 animal/ha. Nesse sistema, os bubalinos atingiram 440 kg de peso vivo com dois anos de idade(19). Bubalinos da raça Mediterrâneo, engordados em sistemas integrado de pastagens nativas de terra inundável com pastagem cultivada de quicuio-da-amazônia em terra firme, com suplementação mineral, no município de Monte Alegre, Pará, tiveram ganhos de 669, 615 e 551 g/animal/dia, respectivamente, nas taxas de lotação de 1,2 e 3 animais/ha, em pastejo contínio, durante 196 dias. Não houve diferenças significativas entre os ganhos diários, indicando que as taxas de lotações estavam subestimadas. Este sistema integrado revelou excelente comportamento produtivo e econômico na terminação de búfalos, permitindo aos animais atingiren aproximadamente 450 kg de peso vivo, com idade inferior a dois anos, superior à média regional de 350 kg aos dois anos e meio de idade (14). Vacas bubalinas de primeira cria, com produção de leite e idade semelhantes, em pastejo rotativo em piquetes de capim quicuio-da-amazônia, com período de descanso médio de 39 dias e suplementação mineral, produziram 6,34 kg de leite por dia, com 7,58% de gordura (20).

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RESUMO

As pastagens da região Amazônica ocupan uma área estimada em mais de 80 milhões de hectares, 80% dos quais constituídos por pastagens nativas. A população bubalina dessa região é estimada en cerca de 1,4 milhões de cabeças e a maior parte desse rebanho é criada em dois tipos de pastagens nativas: as da Ilha de Marajó e as inundáveis do Baixo e Médio Amazonas. Essas áreas são de grande importância, em função de suas peculiaridades favoráveis à criação mais eficiente de búfalos. As pastagens da Ilha de Marajó possuem uma limitação de ordem nutricional, tendo em vista o baixo valor nutritivo das forrageiras e a baixa disponibilidade de forragem no período seco. As pastagens do Baixo e Médio Amazonas se constituem no habitat ideal para a criação de búfalos, considerando o alto valor nutritivo das forragens, a disponibilidade de forragem e o regime de águas. Além dessas pastagens, em escala muito menor, são utilizadas as nativas de terra firme (cerrado, de baixo potencial) e cultivadas em área de floresta (de bom potencial). São apresentadas uma descrição das pastagens, seu potencial, suas limitações e resultados de pesquisa.

ABSTRACT

The Amazon Region has an area of about 80 million hectares covered by native (80%) and cultivated (20%) pastures. The water buffalo population is estinated in 1.4 million head, and most of the herds is raised in two main pasture ecossystems: Native pastures of the Marajo Island and native pastures of the low and mid Amazon River (floodplain

grassland). Those areas are of great significance because of their suitable characterists for raising buffaloes more efficiently. The Marajo Island pastures are limited by the low nutritive value of the grasses and low availability of forege during the dry period. The pastures of the low and mid Amazon River are the ideal habitat for water buffaloes, considering the high nutritive and availability of the forage. Besides those ecossystems, two ofhers are used, in a lower scale, for buffalo raising: wel drained savannas and cultivated pastures in forest areas. A description of the pastures, their limits and potential and research results are presented.

ASPECTOS BÁSICOS DA FISIOPATOLOGIA DA REPRODUÇÃO DE BÚFALAS CRIADAS NA REGIÃO AMAZÔNICA.

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INTRODUÇÃO

O búfalo tem demonstrado ser de fundamental importância para a pecuária da Amazônia, em virtude de, dentro das suas inúmeras qualidades zootécnicas, o mesmo ter a capacidade de produzir e se reproduzir em áreas alagadiças e de pastagens naturais, tornando essas áreas, outrora improdutivas para pecuária bovina, em áreas potencialmente produtoras de proteína animal, a custos reduzidos.

Em função desta sua característica, ou seja, de se reproduzir em perfeita harmonia com a natureza, sem agredir o meio ambiente, o mesmo tem despertado atualmente interesse para o aproveitamento desta potencialidade da espécie, para a exploração pecuária na Amazônia, sem prejudicar o ecossistema.

No presente trabalho apresentamos de modo resumido os estudos sobre os aspectos básicos da reprodução do búfalo na Amazônia, culminando com o uso da técnica de Inseminação Artificial, com resultados altamente promissores, demonstrando que este animal pode ser utilizado como uma das grandes opções para a exploração racional e econômica da Amazônia.

SISTEMA GENITAL FEMININO

O sistema genital da búfala se assemelha ao da vaca bovina, havendo entretanto diferenças quanto ao seu tamanho, especialmente com relação aos ovários , os quais são menores que os da vaca. Em búfalas da Amazônia, a média do comprimento do ovário é cerca de 2,54 ± 0,67 e 2,47 ± 0,68 cm, largura de 1,45 ± 0,45 e 1,41 ± 0,45 cm e o peso de 4,80 ±2,87 e 4,66 ± 2,92 g para o ovário direito e esquerdo respectivamente (VALE et al., 1982). Os cornos uterinos são de tamanho similar aos do bovino, porém, com parede uterina mais espessa, promovendo uma contratilidade dos cornos uterinos, durante o cio, mais acentuada que na vaca. A cérvice é bem menor, apresentando anéis menos proeminentes, especialmente em novilhas.

CICLO ESTRAL

A duração do ciclo estral da búfala em condições amazônicas, varia entre 18 a 32 dias, com média de 23 dias, sendo que a duração do cio varia de 12 a 36 horas, com média de 21 horas, com a ovulação ocorrendo em torno de 19 horas após o término do cio (VALE et al., 1984).

Os sintomas de cio na búfala são menos pronunciados que na vaca. O quadro 1 apresenta os principais sinais que caracterizam a búfala em cio, nas condições amazônicas.

O período de aparecimento dos sintomas de cio na búfala esta relacionado com o hora do dia, havendo predominância de hábitos

sexuais noturnos. VALE et al. (1988) observaram que de 150 cios, 126 (84%) ocorreram a noite, entre 17 e 7 horas, 8 (5,3%) pela parte da tarde (12 a 17 horas) e 16 (10,6%) pela manhã (7 e 12 horas). Apesar dos sintomas acima descritos, a detecção da búfala em cio é difícil por observação visual, mesmo por um vaqueiro treinado, sendo o uso do rufião com burçal marcador o melhor método de identificar a fêmea da referida espécie em cio.

Durante o ciclo estral é possível a palpação dos folículos e do corpo lúteo, entretanto, sendo o folículo de menor tamanho que na vaca bovina e o corpo lúteo ser na maioria dos casos mais aprofundado em direção a medular do ovário (incluso), a sua palpação se torna mais difícil que na vaca bovina.

QUADRO 1. Principais sintomas de cio observadas em 70 búfalas nas condições amazônicas.

Sintomas	Nº de casos	%
Mugido frequente	40	57,1
Muco vaginal	53	75,7
Edema de vulva	47	67,1
Levantamento da cauda	67	95,7
Hiperemia vulvar	46	65,7
Montar em outra búfala	12	17,1
Deixa-se montar	23	32,8
Micção frequente	47	67,1

CIO PÓS-PARTO (CPP)

São inúmeros os fatores que interferem no aparecimento do 1º cio pós-parto (CPP), sendo que o manejo nutricional adequado, durante e após a gestação, de fundamental importância para o encurtamento deste período, contribuindo desta forma para a melhoria da eficiência reprodutiva do rebanho.

MARQUES et al. (1966) estudando o intervalo entre parto de búfalas em condições de trópico úmido, observaram que a média em 533 intervalos estudados foi de 462,9 ± 104,2 dias. Concluiram que este intervalo entre partos pode ser diminuido consideravelvemente desde que, associado ao manejo, seja implementada uma boa seleção, visando aos aspectos reprodutivos, bem como um manejo pós-parto adequado.

VALE et al. (1988) estudando o 1º cio pós-parto através de dosagem de progeterona no leite de búfalas da Amazônia, observaram CPP em média de 30,2 ± 14 dias, em 10 búfalas mantidas em boas condições de manejo e alimentação (pasto + sal mineral + concentrado + amamentação 2 vezes/dia) , enquanto que em 10 animais mantidos em manejo standard (pasto + sal mineral + bezerro ao pé) apresentaram o CPP em 102,4 ± 42 dias. A diferença do período do CPP entre os dois grupos deve-se não somente a diferença nutricional, mas também ao manejo do bezerro, uma vez

que o estímulo constante da amamentação pelo bezerro parece deprimir a liberação de gonadotrofinas pela hipófise.

PATOLOGIA DO SISTEMA GENITAL FEMININO

Apesar da boa eficiência reprodutiva que este animal apresenta nas condições de trópico úmido amazônico, os mesmos apresentam anomalias no sistema reprodutivo que comprometem a fertilidade do animal e a eficiência reprodutiva do rebanho.

Os quadros 2, 3 e 4 apresentam, respectivamente, as principais anomalias do ovário, útero e tuba uterina em animais oriundos da Ilha do Marajó (OHASHI, 1984ab) e o quadro 5 dos animais da Região do Baixo Amazonas (RIBEIRO, 1986).

As anomalias observadas foram tanto de natureza adquirida como de natureza genética. Dentro das anomalias de origem genética podemos citar a hipoplasia ovariana, dupla cérvice, aplasia segmentar de útero e tuba uterina, intersexo , etc.. Nas condições atuais do rebanho bubalino nacional, essas anomalias de carácter hereditário apresentam um significado especial, tendo em vista que, com o aumento da consanguinidade no rebanho, essas anomalias tenderão a se disseminar, podendo provocar sérios problemas na fertilidade do rebanho bubalino.

Salienta-se também o problema da inflamação uterina, observado como uma das mais frequentes alterações reprodutivas, o qual parece ser provocado principalmente devido ao fato do animal banhar-se em lama com água estagnada, em função de manejo inadequaddo, principalmente no pós-parto, local que facilita a proliferação e disseminação de agentes patogênicos.

INSEMINAÇÃO ARTIFICIAL (IA)

Após o conhecimento dos aspectos básicos da fisiologia reprodutiva da búfala nas condições amazônicas, foi desenvolvido um programa experimental para o uso da técnica de Inseminação Artificial em búfalas, utilizando semen congelado, de touros regionais, em diluidores a base de tris e tes, segundo técnica descrita por GUENZEL et al. (1979) e HEUER (1980).

O primeiro experimento foi desenvolvido no ano de 1986-87, utilizando-se 42 búfalas, inseminadas com semen congelado a base de tris, as quais foram inseminadas 24 horas após o início do cio, ou seja búfalas observdas em cio pela manhã foram inseminadas na manhã do dia seguinte, o mesmo acontecendo para os animais detectados em cio pela parte da tarde. O segundo experimento foi desenvolvido no ano de 1987-88, com semen congelado a base de tris, utilizando-se 64 búfalas, as quais foram inseminadas seguindo o esquema de inseminação utilizado em bovinos. O terceiro experimento foi desenvolvido no ano de 1988-89, com semen congelado a base de tes, utilizando-se 70 búfalas, as quais foram inseminadas somente após a mesma não mais aceitar ser montada pelo rufião, ou seja no final do cio do animal.

Os resultados destes experimentos, usando-se a técnica de I.A na búfala, estão sumarizados no quadro 5 (VALE et al., 1990).

Resaltamos que, para a detecção da búfala em cio, é muito importante o uso de um rufião, em função das suas características de cio serem menos intenso que nas vacas. Além disso deve-se evitar o uso de novilhas em programas de Inseminação Artificial, em virtude da dificuldade da passagem da pipeta pelo canal cervical, o qual em novilhas búfalas são de diâmetro reduzido, comprometendo com isso a taxa de concepção.

QUADRO 2. Principais alterações do ovário e tuba uterina observadas em 590 sistemas genitais examinados,de búfalas provenientes da Ilha do Marajó, Estado do Pará, Brasil.

Alterações	Localização Dir.	Esq.	Bil.	Gestação Sim	Não	Frequência nº	%
OVÁRIO							
- Hipoplasia	1	2	1	1	3	4	0,67
- Cisto folicular	6	3	1	0	10	10	1,69
- Cisto luteinizado	3	1	0	0	4	4	0,67
- Cisto parovárico	12	12	1	11	14	24	4,23
TUBA UTERINA							
- Adesão difusa	8	1	10	0	19	19	3,22
- Adesão focal	9	5	21	10	25	35	5,93
- Cisto tubo-ovárico	4	0	7	0	11	11	1,86
- Hidrossalpinge	6	6	9	1	20	21	3,55
- Aplasia	1	3	3	0	7	7	1,18

QUADRO 3. Principais alterações do útero observadas em 590 sistemas genitais examinados, de búfalas provenientes da Ilha do Marajó, Estado do Pará, Brasil.

Alterações	Frequência nº	%
Inflamação	44	7,45
Cisto das Glândulas endometriais	2	0,33
Cisto cervical	4	0,67
Cisto da serosa uterina	14	2,37
Septo dorso-ventral da cérvice	1	0,16
Aplasia segmentar	2	0,33
Dupla cérvice	1	0,16
Intersexo	1	0,16

QUADRO 4. Principais alterações do ovário, útero e tuba uterina de 629 búfalas examinadas, provenientes do Baixo Amazonas, Estado do Pará, Brasil.

Alteração	Frequência nº	%
OVÁRIO		
- Afuncionais	102	16,22
- Cisto folicular	1	0,16
- Teratoma	1	0,16
- Cisto parováricos	4	0,64
TUBA UTERINA		
- Cisto tubo-ovárico	1	0,16
- Aderência focal	20	3,18
- Aderência difusa	5	0,79
- Hidrossalpinge	2	0,32
ÚTERO		
- Inflamação	37	5,88
VULVA		
- Vulva dilacerada	12	1,91
- Vaginite	4	0,64

QUADRO 5 - Ano, número de búfalas, repetições da I.A, total de doses utilizadas no experimento, número de doses por búfala gestante e número e percentagem de animais nascidos por experimento.

Ano	Nº Animais	Inseminação 1a.	2a.	3a.	Total	Dose/gestação	Nascimento Nº	%
86-87	42	42	27	10	79	1,88	25	59,0
87-88	64	64	47	37	148	2,31	29	43,3
88-89	70	70	21	6	97	1,38	47	67,1
TOTAL	176	176	95	53	324	1,85	101	x=57,1

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ASPECTOS BÁSICOS DA FISIOPATOLOGIA DA REPRODUÇÃO DE BÚFALAS CRIADAS NA REGIÃO AMAZÔNICA.

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RESUMO

São descritas as características reprodutivas básicas, bem como o uso da I.A na búfala da Amazônia, tais como, a biometria do sistema genital, o perído médio do ciclo estral, o qual foi de 23-24 dias, com duração do cio de 12-36 horas, com a ovulação ocorrendo 19 horas após o término do cio. O 1º cio pós-parto em animais mantidos com bom manejo e alimentação foi de 30,2 ± 14 dias e de 102,4 ± 42 dias, em animais mantidos com manejo standard, demonstrando a importância da nutrição no aparecimento do 1º cio pós-parto. Foram detectadas várias patologias do sistema genital, sendo as de natureza genética de grande importância, em virtude de seu caráter hereditário, enquanto que dentre as adquiridas, destaca-se a infecção uterina , pela sua alta incidência. A técnica de Inseminação Artificial tem demonstrado resultados promissores com uma taxa média de nascimento de 57,1%, com 1,85 dose/gestação.

SUMMARY

The basic reproductive caracteristics of buffalo cows raised in the Amazon Region was studied. The morphology of genital system was described, as well as, the estral cycle length, which was 23-24 days, with the heat period of 12-36 hours and the ovulation ocurring about 19 hours after the end of heat. The first post partum ovulation ocurred 30,2 ± 14 days in animals kept under improved management and 102,4 ± 42 days for animals kept under standard management. Such fact stress the importance of nutrition to reduce the period of the first post partum ovulation. Several pathology of genital system was observed, where the genetic anomaly play a important rule due to its hereditary caracteristics while among the acquired anomalies the uterine inflamation is very important due its high incidence. The artificial insemination tecnhic has presented satisfactory results with a birth rate of 57.1% and the 1.85 dose/pregnant animal.

TRACE ELEMENT DEFICIENCIES: DIAGNOSIS, TREATMENT AND PREVENTION

Copper Metabolism and Status in Cattle

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Introduction

The essentiality of copper (Cu) for ruminants was first established in 1931 when a Cu deficiency in grazing cattle was demonstrated in Florida.[1] Cattle had exhibited a wasting disease ("salt-sick") which eventually was found to be deficiencies of cobalt (Co) and iron (Fe), in addition to Cu. In 1953, investigators in Northern Europe[2] discovered that a wasting disease ("Lechsucht") characterized by diarrhoea, loss of appetite and anaemia was caused by a Cu deficiency. These researchers established that this was a Cu deficiency by finding marked differences in the Cu content of the forage in "healthy" and "sick" areas and by curing the trouble with Cu therapy. Since then, a large quantity of scientific information has been accumulated concerning Cu nutrition, physiology and metabolism in animals, particularly regarding the diagnosis, treatment and prevention of Cu-related diseases. The present review concentrates on our knowledge of Cu metabolism, interaction with other nutrients, deficiency, requirements and assessment of Cu status in livestock.

Copper Absorption, Transport, Storage and Excretion

In most animal species Cu is poorly absorbed and the extent of absorption is influenced by the chemical form of the Cu.[3] Only 1-3% of Cu is absorbed in ruminants. Although sites in the upper section of the small intestine appear to play the major role of Cu absorption, a substantial absorptive activity has been demonstrated in the large intestine.[4,5] The preruminant animal absorbs Cu more efficiently than the mature ruminant.

Homeostasis is effected by controlling the rate of absorption, which in turn is regulated by the intestinal mucosa. There is good evidence that the intestinal absorption of Cu is regulated by the need of the organism, and that metallothionein in the epithelial cells of the intestine plays a key role in that regulation. Absorption of Cu is higher in the presence of a Cu deficiency than it is at adequate nutritional status.[6] The breed[7] and status of the animal also influence absorption, which may double during the terminal stages of pregnancy to meet increased maternal demand.[7] Intestinal absorption of Cu is influenced by the chemical form in which the element is present and by a substantial number of interactions with other dietary factors that affect bioavailability. Dietary proteins, high levels of calcium (Ca) carbonate, Fe, sulfur (S), also Zn, cadmium (Cd), or molybdenum (Mo) reduce absorption. In general, Cu carbonate and the water soluble Cu sulfate, nitrate and chloride, are absorbed to a greater extent than Cu oxide. Metallic Cu is very poorly absorbed.

Copper appears to be absorbed by two mechanisms, one saturable and the other unsaturable, suggesting active transport for the former and simple diffusion for the latter.[8] As is also true for other transport systems, low concentrations of dietary Cu are predominantly transported via the saturable, active pathway, whereas the diffusion process comes into play at higher concentrations. Dawson[9] has reviewed the potential role of metallothionein as a regulator of Cu absorption and as the site off intestinal interaction between Cu and Zn. He postulated that excesses of intracellular Cu or Zn induce the synthesis of thionenine, the latter would bind the intracellular ions and remove them from further transport into the portal circulation until the epithelial cell is eventually sloughed off and the minerals become part of the intestinal contents again.

Absorbed Cu becomes loosely bound to serum albumin and amino acids and thereby transported throughout the body, with the liver being the major storage organ. From the cellular and subcellular fractions of the liver, Cu is released primarily for hepatic synthesis of ceruloplasmin, or synthesis of erythrocuprein by normoblasts of the bone marrow and for incorporation into many enzymes.[10] Nearly 90% of the Cu in mammalian plasma is tightly bound in the form of the Cu metalloprotein, ceruloplasmin. Ceruloplasmin is the carrier for the tissue-specific export of Cu from the liver to the target organs. It is, together with metallothionein, the dominant component of intermediary Cu metabolism, mediator of the many physiological and pathological influences on Cu metabolism.

The Cu content of body tissues is dependent upon animal species, age, stage of reproduction and Cu status.[11] Liver and brain tissue contains the highest concentration of Cu. The liver is the central organ of Cu metabolism; its concentrations reflect intake and the Cu status of the organism.

In all species studied, a high proportion of ingested Cu appears in the feces. Most of this is unabsorbed Cu, but active excretion also occurs via the bile.[3] The major pathway for excretion of endogenous Cu is via the bile. Intermediate quantities are excreted through the urine, milk and intestine and small amounts are excreted via perspiration.

Copper-Molybdenum-Sulfur Interrelationships

A three-way interaction among Cu, Mo, and S in ruminant animals has been recognized for over 35 years. The interaction

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between Cu, Mo and S is complex and not fully understood. Ignoring possible animal variations, interrelationships may be summarized as follows:

1. Molybdenum, and especially Mo in the presence of S, reduces the deposition of Cu in organs and the synthesis of ceruloplasmin; as a result, the excretion of Cu with bile decreases, but Cu excretion in urine increases.
2. An increase in the Cu content of the diet reduces deposition of Mo in the liver.
3. When S level is increased, excretion of Mo with urine increases substantially, while its deposition in the tissues decreases correspondingly.

Originally the antagonism mechanism were thought to involve dietary inorganic sulfate, but more recently, it was established that total S is the more meaningful interacting factor in the Cu/Mo/S complex. This was demonstrated by Suttle[12] in an experiment with hypocuprosmic ewes in which there was an equally marked interaction between Mo and organic S (methionine) and between Mo and inorganic S (sodium sulfate) in depressing the recovery of plasma Cu.

Some of the Cu/Mo/S interactions take place at the level of the digestive tract, while others are transferred to the metabolic area. The primary site of the three-way interaction controlling Cu storage is believed to be in the gut. The following is the three-step sequence: reduction of sulfate to sulfide in the rumen; reaction of this sulfide with Mo to form thiomolybdate; reaction of thiomolybdate with Cu to form Cu thiomolybdate.[13] Thiomolybdate is more readily absorbed than is

the oxygen analog, molybdate, and forms the highly insoluble and non-utilizable Cu thiomolybdate. The concept of the formation of a thiomolybdate ion and the tight complexing of this ion with Cu has similarly been proposed by Suttle.[14] Plasma Cu increases because of the formation of the Cu thiomolybdate complex, and clinical signs of Cu deficiency result. A study with rats confirmed this model by showing that 500 ppm of dietary Mo increased plasma Cu levels without relieving Cu deficiency pathology.[15]

Recently, it has been suggested that the thiomolybdate hypothesis for explanation of the Cu/Mo/S interaction in ruminants should be modified by including the association of tetrathiomolybdate (TTM) and Cu with proteins, and de-emphasizing the formation of insoluble Cu thiomolybdates.[16] Experiments using S^{35}-labelled metallothionein (MT) revealed that changes in the Cu profile were due to removal of Cu from MT by stronger chelators produced by the association of TTM with high molecular weight proteins.

Sulfur, in the absence of Mo, may also cause a Cu deficiency due to the formation of insoluble, unabsorbed Cu sulfide in the rumen. Ruminal protozoa may increase such formation by digesting protein particles and releasing extra S into the S pool.[17,18] The formation of both Cu sulfide and Cu thiomolybdate would greatly reduce Cu absorption, since both render Cu unavailable.[19] There are also effects of Fe-rich soils on Cu availability in S-rich diets. This may be a carryover of sulfide as FeS from the rumen to the abomasum, where it forms insoluble CuS.[20] Ruminant animals, especially sheep, are much more susceptible to Mo/Cu imbalance than are non-ruminant animals. The primary effect probably occurs in the rumen through the involvement of sulfide-generating bacteria and the consequent formation of unavailable compounds such as CuS and cupric thiomolybdate.

Evidence suggests that the yield of higher thiomolybdates (e.g., MoS_4^{2-} and $MoOS_3^{2-}$) is related, directly, to the intake of dietary S sources potentially degradable to sulfide in the rumen. While the effects of thiomolybdates in inhibiting Cu absorption are likely to be due to the tri- and tetrathio species, post-absorptive effects on Cu metabolism are probably due to di- or trithiomolybdate.[21]

The rates of absorption, retention, and excretion of Mo are inversely related to the level of dietary S. In sheep, for instance, increasing the dietary S from 0.1 to 0.3% in a diet supplemented with 10 mg Mo per day decreased the Mo retention from 37 to 4%. A working hypothesis for the effect of S on Mo retention is that S inhibits membrane transport of Mo, thus decreasing reabsorption of Mo by the renal tubules.[22]

Formation of thiomolybdates also affects the kinetics of S metabolism by affecting sulfide formation and absorption. Thiomolybdates rapidly react with particulate matter and proteins to form complexes that bind Cu strongly, reducing its solubility and decreasing the H_2S concentration and thereby the rate of sulfide absorption.[23]

Interactions Among Copper, Zinc, and Iron

Several inorganic dietary factors markedly affect Cu absorption and metabolism within the body. Close structural similarities between Cu and trace elements such as zinc, iron, etc. have suggested that isomorphous substitution within carrier systems may be the basis of their antagonism.[24] Cu, and some of its antagonistic elements such as Zn and Cd have been shown to have one 4s and three 4p orbitals vacant for the formation of coordinate bonds in a tetrahedral (SP^3) array. The conception that competitive trace element antagonism may be exhibited where cations or anions have similar electron distribution in their outer orbitals, and when ionic radii do not differ greatly, has been validated by dozen experimentations.[25] Zinc antagonizes Cu absorption by inducing the synthesis of a copper-binding ligand, probably a thionein, in the mucosal cells which sequesters copper from the nutritional medium.[26] It was reported that in housed sheep increased dietary zinc acts as a metabolic antagonistic of Cu.[27] Campbell and Mills (1979) observed in pregnant ewes symptoms of Cu deficiency following dietary zinc supplementation. In the housed pregnant sheep the transport of Cu to liver decreases by increasing the dietary ratio of zinc to copper.[27] By decreasing Cu absorption and thus causing a functional Cu deficiency, high levels of zinc have shown to result in poor utilization of dietary iron.

A few years ago, Davis (1960) suggested that copper and iron share a common gastrointestinal transport mechanism. There has been some indication that Cu and Fe compete for binding sites on transferrin, with Cu being preferentially bound.[28] Researchers were able to depress Cu absorption in calves by supplementation of daily dietary doses of Fe equivalent to 1.4 g per kilograms of dry matter.[29]

One study showed that with an increased dietary intake of Fe by calves, liver, and plasma were rapidly depleted.[30] Another study demonstrated that a supplement of only 250 mg Fe/kg diet per calf causes a major depletion of liver reserves.[31]

In general, the interrelationship between two nutrients is more critical, and there is less tolerance with regard to increased levels of one nutrient compared with the other nutrient when this nutrient is deficient.

Deficiency

A wide variety of disorders in ruminants are associated with a simple or induced Cu deficiency including anaemia, severe diarrhoea, depressed growth, change of hair color, neonatal ataxia, temporary infertility, heart failure, and weak, fragile long bones that break easily.[34] Not all of these signs necessarily occur in every Cu-deficient animal, and some may be due to a combination of causes. In cattle, dietary Mo (3 ppm) significantly delayed the occurrence of first ovulation (26 to >40 weeks) compared with controls.[35] Anaemia is a general clinical sign for most species, while other signs may be observed in one or more species.

Another Cu deficiency sign is the development of fragile bones, particularly the long bones, which break easily, sometimes without apparent cause. Lameness in animals may also be a result of the deficiency. In extremely Cu-deficient cattle, there is a swelling or enlargement of the ends of the leg bones, especially above the pasterns (fetlocks); this condition, which may involve muscles and nerves, is easily corrected with adequate Cu.

A condition often known as "falling disease" sometimes occurs in Cu-deficient cattle.[34,36] Deficient cattle may die suddenly when exerted, and postmortem examination may reveal small lesions of the heart. Evidence indicates that the essential lesion is a slow and progressive degeneration of the myocardium with replacement fibrosis.[3]

Low fertility in cattle grazing Cu-deficient pastures, associated with delayed or depressed estrus, occurs in several widely separated areas, and infertility, associated in some cases with aborted small dead fetuses, has been reported in experimental Cu-deficiency in ewes.[34] Estrus in cattle may be delayed or depressed and conception rate reduced.[3,37] Likewise, effects such as calving difficulties, retained placenta, and calves born with congenital rickets have been described when cows are Cu-deficient.

A Cu-responsive diarrhoea has been observed in cattle in a number of world regions. Often the diarrhoea is more prevalent when excess Mo is a major cause of the Cu deficiency.[38,39] This is especially true when the diarrhoea develops very rapidly and is quite severe, as low Cu does not cause this type of diarrhoea. Histochemical and ultrastructural changes have been observed in the small intestinal epithelium in animals with the diarrhoea. The clinical sign of the severe diarrhoea is different in sheep and cattle, including much less diarrhoea in sheep. However, scouring has been observed in goats maintained on the pastures that induce scouring in cattle.[34]

Subclinical Cu deficiencies are thought to be very widespread and are likely to be of more economic significance than are easily recognized cases, with inadequate Cu, the animals may be unthrifty and have lower milk production, growth and

reproduction efficiency, without readily recognizable signs such as described.[39] Thornton et al[40] those previously reported Cu supplementation during a 6-month period increased liveweight gains in cattle by 10-70% over controls, even though, with few exceptions, control stock showed no clinical signs of hypocuprosis.

Of the numerous world reports of Cu deficiency in ruminants, only a few are concerned with a deficiency induced by the presence of unusually low dietary concentrations of Cu (<3 ppm). The majority of world reports are concerned with a "conditioned" Cu deficiency, where normal amounts of Cu (6-10 ppm) are inadequate dine to other forage constituents such as rumen degradable protein, Mo, S, Fe, and other factors that block utilization of Cu.[41-42] Copper deficiencies usually occur when forage Mo exceeds 3 ppm and the Cu level is below 5 ppm.[43] Some studies indicate that ingestion of soils containing substantial amounts of Mo can be an important contributing factor in Cu deficiency, especially of sheep in certain situations such as those grazing winter pasture.[44]

Ward[45] categorized Cu deficiencies into four groups where the feed contained (1) high levels of Mo (more than 20 ppm), (2) low Cu but significant amounts of Mo, (3) deficient Cu (<5 ppm) and (4) normal Cu and low Mo, with high levels of soluble protein. It is suggested that the last situation is the result of high intakes of rumen degradable protein from fresh forage, which increases the amounts of sulfide produced in the rumen, thus resulting in Cu sulfide[42] which is unavailable.

Studies with cattle[45] and sheep[27] have suggested that the high Fe intake may depress Cu status of ruminants. An elevated Fe intake could result from consumption of groundwater, from soil ingestion, or from an increase in plant Fe concentrations due to waterlogging of the soil. A large number of studies from tropical regions have indicated that forages grown on acid soils are extremely high in Fe, which may be aggravating the low Cu status of grazing livestock in many regions.

Copper Deficiency and Immune Responsiveness

In recent years, great interest has been generated regarding the influence of nutrition on host immunocompetence. Studies with laboratory animals investigating the role of copper metabolism and immune function have demonstrated effects on T and B cells, neutrophils and macrophages. Prohaska and Lukasewycz observed in mice with hypocuprosis an impaired humoral-immune response (decreased numbers of antibody-producing cells).[46] The magnitude of this impairment was highly correlated with the degree of its functional deficiency. Cu deficiency in cattle decreased neutrophil microbicidal activity, and there is evidence for impaired production of superoxide (O_2^-).[47] In cattle affected from copper deficiency induced by molybdenum, it was reported[48] that the neutrophils were impaired in their ability to kill ingested Candida albicans. It was reported recently[49] that in sheep with low copper status the ability of polymorphonuclear leukocytes to phagocytose C. albicans is comparatively lower than those from sheep on a normal Cu diet. Olkowski et al. suspect that in hypocuprosis cattle raised in the Canadian prairies their immune function may be impaired. In sheep affected by Cu deficiency a decrease of their resistance to infection was observed.[50]

From these few studies on immune destruction in cattle by using in vitro tests, it is quite clear that there is a need of further studies on the immunomodulation of hypocuprosis cattle.

Requirements

In view of the many dietary factors which may influence Cu requirement including Fe, Mo, S, Zn, Pb, Cd, and the protein source, and the limited number of conclusive studies in this area, it is difficult to state a precise Cu requirement for livestock. Since Cu requirements are so strongly influenced by other mineral elements and dietary components a series of minimum requirements are required, depending on the extent to which these influencing factors are present or absent from the diet, and on the criteria of adequacy employed. The following estimates of Cu requirements, established with a minimum level of common dietary antagonists, are suggested by the National Research Council:[51] 8
to 10 mg/kg for dairy and beef cattle.

General recommendations for the minimum Cu requirement of grazing livestock can not reasonably be made without reference to pasture Cu, Mo, and S concentrations. The intake of Mo is a most important variable affecting the minimum Cu needs of ruminants.

Molybdenum and S can either increase or decrease the Cu status of an animal. When the Cu:Mo ratio of forages is less than 2.8:1, in the presence of adequate S, the incidence of hypocuprosis in the animals is evident.[52] The critical Cu:Mo ratio in feeds appears to be 2.0:1, and feeds or pastures with lower ratios result in a "conditioned" Cu deficiency.[53] A Cu:Mo ratio of no less than 4:1 has been proposed[54] to ensure that the Cu requirement will be met.

In sheep, a diet high in Zn reduces Cu toxicity and liver stores.[55] Steers grazing a pasture sprayed with Fe, Zn, and Pb developed a marked Cu deficiency, whereas controls remained normal.[56] For goats a Cd induced secondary Cu deficiency has been reported.

Limited data also indicate that genetic differences have a major influence on Cu metabolism of cattle.[57] Cu deficiency in Simmental cattle from Canada[58] was more frequent than in other breeds. Feeding high levels and combinations of Cu, Mo and/or S resulted in greatly enhanced biliary Cu excretion in Simmental versus Angus cattle.

Physical form of feeds appears to be an important factor affecting the requirement for Cu. For instance, more Cu is required from pasture than when dry forage or concentrates are fed. The Cu content of forage declines with increased maturity. However, the Cu status of cattle grazing more mature forage is better than in those grazing immature forage.[59] This suggests low availability of Cu in immature forage or a change in the relationship of Cu to interfering factors such as ruminal degradability of protein.[58]

Injectable Cu Complexes to Cattle

Parenteral injections of organic compounds of copper are administered to cattle to prevent nutritional copper deficiency and its accompanying clinical signs.[34] The effectiveness of parenteral administration was demonstrated first by Kitsoff (1947) and reported by Green (1949)[60]. An intravenous injection of 500 mg of copper, as a solution of copper sulphate in physiological saline, was shown to maintain normal blood copper levels and to control scouring in cattle grazing "[illegible]" pasture in Somerset (UK). Considerable research has been undertaken on parenteral administration of Cu complexes with glycine, ethylenediamine tetracetic acid (EDTA) and methionine. Ideally, parenteral treatment should given an increment of Cu in the blood that will remain for a period of time. However, to date the parenteral route provided nothing more than a quick route by which a known dose of Cu would be introduced in case of severe and acute deficiencies.

In subsequent response of cattle to injected Cu has usually depended on the initial deficit of tissue Cu and on the content and availability of Cu in the diet.[61] In single and conditioned Cu deficiency in cattle repeated injection of Cu complexes has been shown to be necessary to maintain normal serum Cu levels adequate gain in growing calves and to permit moderate liver storage in mature cows.[34,62] According to a study in western Canada following subcutaneous injection of copper ethylenediamine tetracetate to calves there was improved growth comparatively to the untreated calves.[63] A field experiment with beef cattle was undertaken in western Manitoba, Canada in order to estimate the effects upon body weight, serum Cu and severity of reaction at the site of injection of three commercial Cu preparations. These preparations of copper (Cu) salts, were CuEa EDTA, Cu glycinate and Cu methionate. There were no significant differences ($P>0.05$) in final body weight among cattle injected with Cu preparations, or between treated and untreated cattle. Serum Cu was higher in treated than untreated cattle, but there was no difference between Cu preparations. Reaction at the site of injection was: CuEa EDTA moderate, Cu

glycinate moderate, Cu methionate moderate to severe. Where Cu supply is continuously inadequate, treatment was shown to be repeated every three months.[67] In beef cattle a subcutaneous injection of the Cu complex into the brisket area is preferable to intramuscular.[68] The possible effect upon carcass is less important at this subcutaneous site.

Assessment of Copper Status

Clinical signs, growth response, and analysis of forages and tissues have all been used to determine the various Cu deficiency areas of the world. The criterion most widely used for Cu deficiency is concentration of Cu in the liver. The liver is the main storage organ of the body for Cu, so liver Cu concentrations would be expected to provide a useful index of the Cu status of the animal.[69] Among ruminant livestock, liver Cu values in healthy sheep and cattle have a normal range of 100-400 ppm on a dry matter basis. In sheep and cattle, liver concentrations vary slightly from birth to maturity. Liver Cu concentrations reflect the dietary status,[70] but they are influenced by the dietary proportions of Mo and S and by high intakes of Zn and Ca carbonate and other dietary compounds. They must, therefore, be used with caution as diagnostic aids. Evidence suggests that the Cu values below 25-75 ppm of liver dry matter in ruminants should be used to differentiate deficient from normal animals.

Changes in the activities of a number of Cu metalloenzymes in the blood and tissues occur during the development of Cu deficiency and offer diagnostic possibilities. It has been shown by Todd[71] that ceruloplasmin estimations on blood serum provide advantages over whole-blood or plasma Cu determinations because of the relative stability of the enzyme, the small size of the serum samples required, and the technical convenience of the assay. One advantage of using the enzyme rather than plasma Cu is that contamination of samples are much less serious problems.

The erythrocyte superoxide dismutase (ESOD) may be an even superior reflection of Cu status. Activity of ESOD is severely depressed by Cu deficiency in rats, pigs and chicks.[72] For lambs ESOD was better than plasma Cu as a prediction of Cu status.[73] Also, plasma diethyldithiocarbamate cuproxidase activity was rapidly elevated following Cu dosing.[74] In human studies ceruloplasmin and cytochrome C oxidase in platelets and mononucleated white cells were more sensitive indicators of Cu status than plasma Cu or ESOD.[75]

From a review of the tropical world literature[76] on minerals, Cu has been suggested as the second most severe mineral deficiency for grazing ruminants next to phosphorus.[76] Many tropical forages are very low in Cu and/or contain excess Mo. There are some indications that bioavailability of Cu differs among forages with a higher absorptive efficiency from dried forages than fresh forages.[5] The soluble protein of the pasture is readily hydrolyzed, resulting in the formation of insoluble Cu sulfide, thus producing Cu deficiency. Drying of the forage as hay reduces the protein solubility so sulfide production is reduced.[50]

Copper deficiencies caused by low dietary Cu and/or excess Mo are prevented by a number of different methods of providing supplemental Cu. Both Mo toxicity and Cu deficiency are generally corrected by providing additional Cu to the animal diets. If Mo content of the diet is less than 1 ppm (dry basis), the optimal level of Cu would be between 6 and 8 ppm. Copper at 6 ppm is inadequate when Mo is between 1 and 3 ppm. With sheep, great care has to be taken to avoid over-supply of Cu, due to the extreme sensitivity of this species for Cu toxicity. When ruminants are pen-fed or in dry-lot feeding, such as finishing beef cattle and lambs or milking cows, supplementary Cu is best supplied by incorporation of the element into a concentrate mixture. When this option is available, this is the preferred supplemental method as each animal would likely receive the desired dose. However, this method is usually economically prohibitive under grazing conditions.

Copper can be added to the drinking waterer at the rate of 2-3 mg Cu/L to prevent deficiencies.[77] Supplying Cu in the water probably has more problems and may be less satisfactory than other methods of supplementation.[78]

Under range conditions, Cu deficiency can be prevented by the provision of Cu-containing supplements, by dosing or drenching animals at intervals with Cu compounds, or by injection of organic complexes of Cu. Mineral supplements containing 0.1-0.2% Cu sulfate are generally consumed voluntarily by grazing animals in amounts sufficient to maintain adequate and safe total Cu intakes. Free-choice consumption of a complete mineral supplement, which includes Cu, is often the method of choice for grazing livestock, since forages are often deficient in a number of minerals which would not be included in supplementation methods which provide for only Cu and/or several other trace elements. Ruminant animals have the ability to store Cu in their livers during periods of excess intake and to draw on those stores when intakes are inadequate. Continuous ingestion of licks or frequent oral dosing is, therefore, less important than it is with Co. Drenching at monthly or long intervals has been found satisfactory in Cu-deficient areas, except where Mo contents in the forages are sufficiently high to induce scouring (5 ppm or higher). In these circumstances the Cu supplementation must be regular and more frequent.[34]

Subcutaneous or intramuscular injection[79] of some safe and slowly absorbed forms of Cu (i.e., glycinate and EDTA) constitute satisfactory means of treating animals in Cu-deficient areas where the pasture Mo contents are moderate, even at intervals as long as 4-7 months.[80] For severe Mo-toxic areas, injections of Cu compounds are often the preferred method of administration, since the primary site for Cu and Mo interaction is the gut.[81] Copper oxide needles[82] and Cu-containing controlled release glass[83] are forms of supplemental Cu that have been used successfully.

The application of Cu-containing fertilizers can be an effective means of raising the Cu content of pasture to levels adequate for grazing livestock and increasing pasture yields. The amounts required vary with the soil type and climatic conditions. Australian experience indicates that a single dressing of 5-7 kg/ha of $CuSO_4$ or its Cu equivalent in the form of cheaper Cu ores, is usually sufficient for 3 or 4 years, except on calcareous soils. Often fertilization is not a dependable supplement method because problem pastures are sometimes flooded part of the year. Also, a main cause of Cu deficiency in grazing livestock is poor uptake of Cu by the plant, not necessarily low Cu in soil.[76]

Supplemental sources of Cu, from highest to lowest biological availability, include $CuCl_2$, $CuSO_4$, $CuCO_3$, and CuO. Copper chloride appeared to give responses similar to Cu sulfate.[84] Amino acid chelates or organically bound forms apparently have a higher bioavailability than inorganic Cu sources.[37] Bioavailability of dietary Cu from Cu proteinate was greater than from Cu sulfate for calves fed diets containing Mo.[85]

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Selenium Deficiency in Cattle

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Biochemical Role of Selenium

In 1957, it was found that the erythrocyte enzyme glutathione peroxidase (EC 1.11.1.9), in the presence of reduced glutathione, would protect erythrocytes against hemoglobin oxidation and subsequent hemolysis caused by H_2O_2 (organic hydroperoxides) and ascorbate (vitamin C).[1] Later, it was found that selenium (Se) was a component of glutathione peroxidase (GSH-Px).[2,3] Rats affected by selenium deficiency had low GSH-Px activity in blood and signs of selenium deficiencies.[4] Glutathione peroxidase has been purified from the tissues of cattle, humans, swine, sheep, and rats.[5] It has been found to have a molecular weight of approximately 80,000 daltons (consisting of four subunits[6]) and to contain 4-gram atoms of selenium per mole.[5] GSH-Px is presently the only verified biochemical role for selenium in mammals, although a number of nonheme proteins containing selenium have been discovered. The role of these other molecules is not clear.

Selenium-dependent GSH-Px appears to play a central role in cellular oxidation-reduction reactions. GSH-Px catalyzes reactions that aid the destruction of hydrogen peroxide and fatty acid hydroperoxides. The probable cellular reactions involving GSH-Px are outlined (Figure 1). Peroxides are reduced via the generalized GSH-Px-catalyzed reaction, for which reduced glutathione is a hydrogen donor. Glutathione is reduced (i.e., regenerated for subsequent reactions) by a reaction involving glutathione reductase and a hydrogen donor ($NADPH+H^+$). High levels of peroxides in the cell tend to oxidize proteins and lipids and thus alter the normal function of these molecules. The reactions catalyzed by GSH-Px aid the control of peroxide levels in mammalian cells.

Selenium-dependent GSH-Px, along with superoxide dismutase (SOD); catalase; ascorbate; vitamin E (α-tocopherol); β-carotene; and other compounds, is important for protecting mammalian cells from oxidative damage. While GSH-Px acts to reduce peroxides, vitamin E acts as a free radical scavenger. Vitamin E can react directly with superoxide (O_2^-), peroxy (ROO·), singlet oxygen ($^1O_2$), and hydroxyl (OH·) free radicals. Vitamin E represents the major lipid-soluble antioxidant of cell membranes[8] and has been shown to protect against myopathies associated with diets high in polyunsaturated fats.[7] The activities of vitamin E and GSH-Px are similar; this aids in explaining the observations concerning many selenium/vitamin E-responsive diseases. These similarities may also explain the "sparing effect" of selenium and vitamin E on one another in various diets. A severe deficiency of one nutrient can not always be compensated for with an abundance of another; this is illustrated in cases of selenium-normal, vitamin E-deficient ruminants that experience severe myodegeneration.

In cattle, some GSH-Px activity is expressed by glutathione S-transferase (GSH-Tr), an enzyme that does not contain selenium.[9] Under appropriate laboratory conditions, GSH-Px utilizes H_2O_2 as a substrate but GSH-Tr does not.[9,10] The levels of activity of these enzymes have been measured in calves.[11] Tissues found to contain only GSH-Px include spleen, cardiac muscle, striated muscle, erythrocytes, brain, thymus, and adipose tissue.[11] Tissues containing both GSH-Px and GSH-Tr are liver, lungs, adrenal glands, testes, and kidney; hepatic tissue was found to contain the highest percentage of GSH-Tr.[11]

Pathogenesis of Selenium Deficiency Conditions

The effects of selenium deficiency may lead to nutritional myodegeneration (NMD); this may result from the destruction of cell membranes and proteins, which leads to losses of cell function and membrane integrity. During normal cellular metabolism, highly reactive forms of oxygen (e.g., free radicals, hydrogen peroxides and lipoperoxides) are produced. GSH-Px catalyzes the conversion of hydrogen peroxide and lipoperoxides to water and alcohols, respectively. Vitamin E and SOD act to make free radicals (e.g., singlet oxygen, superoxide, and hydroxyl ions) less toxic.

Increased metabolic rate, inflammation, and other stressors may increase demands for GSH-Px activity and the activity of other antioxidants. Additionally, the level of polyunsaturated fatty acids (PUFA) in the diet may be important in the pathogenesis of NMD in ruminants.[7] Dietary PUFA can undergo peroxidation to lipoperoxides, which, in turn, can form toxic free radicals. During periods of active pasture growth, grasses can contain high levels of linolenic acid, a PUFA. Under normal conditions, the rumen is active in saturating dietary PUFA. In calves recently pastured, however, the plasma PUFA levels often increase.[12] This occurrence suggests that dietary PUFA may increase the risk of oxidative damage in tissues. Other causes of oxidative stress are also very important because a high percentage of calves with NMD are on a milk diet. Although the exact mechanisms that result in NMD are not fully understood, the importance of selenium and/or vitamin E deficiency in precipitating this syndrome cannot be overemphasized.

In a number of mammalian species, selenium deficiency has been associated with decreased immune system function. Decreased GSH-Px activity in phagocytic cells has been reported in selenium-deficient rats and heifers.[13,14] In bovine neutrophils, the bactericidal capacity for *Candida albicans* and *Staphylococcus aureus* is lowered in selenium-deficient cattle.[14,15] Inefficient immune cell function may predispose cattle to infectious diseases. An increased incidence of mastitis is associated with selenium deficiency and/or vitamin E deficiency.[16,17] Recently, it was reported that duration of experimental coliform mastitis in selenium-deficient Holstein heifers was significantly ($P < 0.05$) greater than in selenium-normal heifers.[18] The economic significance of this apparent increased susceptibility to mastitis is not currently known. Although reduced GSH-Px activity in immune cells is probably important, the precise pathogenic mechanisms by which immune function may be depressed are not known.

Reproductive diseases in cattle have been associated with selenium deficiency. The incidence of retained placenta in selenium-deficient cows has decreased markedly with prepartum injections (i.e., 3 weeks prepartum) with selenium and vitamin E.[19] Selenium injections appear necessary for this favorable response,[19] which correlates with maximal GSH-Px levels at parturition. The incidence of cystic ovaries has been associated with selenium deficiency[20]; selenium and vitamin E, in conjunction with gonadotropin-releasing hormone, have helped treat cystic ovaries in dairy cattle.[21] Prevention of retained placenta by any means should decrease the subsequent occurrence of metritis or cystic ovaries. Selenium's positive effects of decreasing reproductive disease in cattle have been associated with increased GSH-Px activity in blood and tissue. The precise pathophysiology of these conditions and the role of selenium, however, have not been characterized.

A number of clinical conditions are associated with selenium deficiency (Table 1).[22] These diseases conditions are multifactorial; selenium deficiency may be a central factor, but the pathogeneses of these conditions are complex.

Diagnosis

Selenium deficiency is frequently common in certain geographic areas such as North America (Figure 2), Europe, Australia, and New Zealand, where soils and feeds are selenium-deficient. This information can be used to anticipate areas or regions where selenium deficiency might occur.

WMD is the most severe selenium-deficiency disease in calves or other young ruminants. There are two WMD syndromes: a cardiac (congenital) form and a skeletal (juvenile) form. The cardiac form occurs in calves a few days of age; signs are due to peracute to acute myocardial failure. The animal may be found dead or in cardiopulmonary collapse. Calves found alive will be in depression and respiratory distress. A foamy nasal discharge, possibly blood stained, is often seen and results from pulmonary edema and dyspnea. Cardiac murmurs can occasionally be heard; tachycardia is present, and arrhythmias are common. Rectal temperature is usually increased because of extraordinary respiratory efforts. Affected calves may respond to therapy, however, recovering calves often fail to thrive and death can occur within 24 hours of the onset of clinical signs. At necropsy, pale streaks are seen in the heart muscle, diaphragm, and intercostal muscles. Hyaline degeneration and calcification are common histopathologic findings. Skeletal muscles may or may not be involved.

The skeletal form of WMD is characterized by stiffness, weakness, or both in calves 1 to 6 weeks of age, although it can occur in animals much older. These animals are recumbent and unable or reluctant to rise. Commonly affected muscle groups include the gastrocnemius, semitendinosus, semimembranosus, biceps femoris, gluteals, and muscles of the lumbar and neck regions. Animals may be dysphagic because of tongue muscle involvement. Cardiomyopathy may occur, but is less severe than that seen with the cardiac form. Affected muscles are firm, swollen and painful and can usually be easily palpated. At necropsy, affected muscles are pale and streaked. Histopathologic examination reveals hyaline degeneration, fibrosis, and calcification.

WMD must be differentiated from other diseases that cause peracute recumbency or sudden death. Conditions with signs similar to the cardiac form include septicemia, pneumonia, meningitis/myelitis, and neurotoxins (organophosphates). Acute heart failure may be due to cardiac anomalies, plant cardiotoxins (e.g., oleander, cassia, white snakeroot, and gossypol from cottonseeds), and polyether ionophore antibiotics. Diseases causing stiffness, weakness, and recumbency include tetanus, spinal cord compression, polyarthritis, fractures and other trauma, clostridial myositis, and cerebellar disease.

Treatment of WMD requires the use of injectable selenium; commonly, 2.5 mg selenium and 50 mg dl a-tocopheryl per 45 kg body weight is given to affected calves in one or more doses.

Animals affected with WMD have elevated serum levels of enzymes such as aspartate aminotransferase (AST) (EC 2.6.1.1), creatinine kinase (CK) (EC 2.7.3.2), and L-lactate dehydrogenase (LDH) (EC 1.1.1.27). AST and CK are the more specific enzymes for myodegeneration. Calves with WMD will have CK levels of 1000 to 50,000 U/L or higher while calves recumbent because of other diseases will typically have serum CK values from 100 to 2000 U/L. Elevated serum AST occurs with WMD and the peak concentration follows the CK peak. AST values of 300 to 500 U/L are not uncommon with WMD.

In addition to WMD, there are a number of clinical syndromes (Table 1) that have been associated with selenium deficiency. Each of these conditions has a number of potential causes in addition to selenium deficiency. However, if selenium deficiency is suspected, laboratory confirmation is indicated and relatively straightforward.

Blood or tissue samples from cattle can be analyzed for selenium content using atomic absorption spectroscopy using hydride generation, neutron activation analysis, or fluorometric measurement of selenium complexes. The method of choice of the American Association of Official Analytical Chemists is a fluorometric procedure.[22] Samples are digested with nitric and perchloric acids followed by reaction with 2,3-diaminonaphthalene. Upon extraction with dechydronaphthalene or cyclohexane, the piazselenol (2,3 DAN-selenium complex) is measured fluorometrically.[23] The detection limit for the fluorometric method is 0.003 mg/ml.[23]

Atomic absorption spectroscopy is a less sensitive method of selenium analysis.[23] We analyze for selenium by inductively coupled argon plasma (ICP) with atomic emission detection and mass spectrometric detection.[24] Biologic specimens are digested in nitric, sulfuric, and perchloric acids, which first reduce all selenium to selenate, which is then reduced to selenite with hydrochloric acid.[25] The sample is measured after ICP nebulization and vapor hydride generation.[24] The detection limit for blood and tissue samples is 0.004 mg/ml or mg/gm.[24]

The selenium-containing enzyme, GSH-Px, can also be measured and used to quantitate selenium status in cattle. The activity of GSH-Px strongly correlates with selenium concentration in blood and tissue and with dietary selenium status. The generalized laboratory reactions in the GSH-Px assay are shown (Figure 3). As is the case with any enzyme assay, the blood or tissue sample used for GSH-Px must be refrigerated (at 4°C) from the time of sampling until analysis. Blood samples held more than five days begin to lose activity. Analysis of GSH-Px is accomplished by many methods and modifications; values generated by one laboratory are not easily extrapolated or interpreted by another. Laboratories reporting GSH-Px values should correlate these to selenium concentrations for blood, tissue, or both. We use a modified method of Agergaard and Jensen.[26] Our results are reported in mU GSH-Px activity per milligram hemoglobin for blood samples. Reference ranges and interpretation of blood GSH-Px and blood selenium are listed (Table 2). For routine diagnostic procedures for GSH-Px and selenium analysis, blood samples in ethylenediaminetetraacetic acid (EDTA) vials are the preferred specimens. Interpretation of values obtained from either of these procedures is straightforward.

Treatment and Prevention of Selenium Deficiency

There are several methods of treating selenium-deficient cattle once a diagnosis has been made. The most commonly used methods of treatment in the U.S. involve the use of (1) injectable selenium products, (2) salt mix formulations with supplemental selenium, (3) a total ration formulation with supplemental selenium, or (4) intraruminal sustained-release boluses or pellets. Commercial injectable selenium products available in the U.S. for cattle contain 1 mg selenium and 50 mg dl a-tocopheryl per milliliter or 5 mg selenium and 50 mg dl a-tocopheryl per milliliter. The usual recommendation for supplementation by injection is 2.5 to 3 mg selenium/45 kg body weight, administered intramuscularly or subcutaneously. Additional injections may be required every 30 to 90 days to provide adequate supplementation. The injectable products are valuable in preventing overt disease (e.g., white muscle disease) in initial herd treatment and in treating herds where manipulation of the ration or salt mix is not possible. The use of these products is relatively more expensive than ration manipulations and requires a 30-day withdrawal period for treated slaughter cattle.

The current federal regulations govern nonprescription use in cattle, including selenium in food products (Table 3). Licensed veterinarians can prescribe selenium at the level deemed necessary for any particular case, but when prescribing levels that exceed nonprescription regulations, veterinarians must assume responsibility for untoward effects of selenium toxicity and problems that may arise as a result of adulterated meat or milk.

In many cases, salt mixes are a good vehicle for selenium supplementation. Assuming salt consumption of 1 oz per

head per day, a 20-parts per million (ppm) selenium-salt mix provides approximately 20% of the daily requirement of selenium for an adult cow; a 90-ppm selenium salt mix provides almost the entire daily requirement for an adult cow. Certain problems exist with salt mix supplementation, however, such as (1) sporadic intake dependent on palatability, location, pasture conditions, and time of year; (2) variability of individuals within a herd; (3) the need to keep fresh salt mix available at all times; and (4) mixing and solubility problems. Salt mixes tend to be inexpensive and intake is usually limited because of the salt itself; therefore, the occurrence of toxicosis is unlikely.

An excellent method for treating selenium-deficient cattle is supplementation of the total ration or a large portion of it with selenium. This requires rations in which feed additives are easily dispersed (e.g., corn silage, grain mixes with supplemented pellets, or haylage). This type of supplementation helps lessen the variability of intake among animals.

Sustained-release selenium boluses or pellets can also be an excellent means of supplementation for cattle. This is particularly effective for cattle on pasture or in less intensive management programs. Efficacy of selenium supplementation in beef cattle is compared between the selenium bolus, which releases 3 mg selenium/day, and the selenium pellet (Figure 4).[28] The selenium bolus is an osmotically activated pump that releases 3 mg selenium/day (i.e., as sodium selenite) and can provide at least seven months of supplementation for severely selenium-deficient cattle. The selenium pellets are widely used and can provide up to 18 months of selenium supplementation.[27,28]

Once an initial supplementation program has been created, the role of the veterinarian changes from that of a diagnostician to that of the medical advisor who monitors progress. The veterinarian should set up a routine for recording disease incidence. Analysis of this data allows the clinician to examine the effect of the recommended program on the clinical disease. This analysis also is very important psychologically to clients, who need to see hard facts that illustrate a decrease in their problems.

The clinician should check GSH-Px and/or whole blood levels periodically to ensure that the animals are responding physiologically. This will help define problems in (1) feed or salt consumption; (2) quality of premixes; (3) adequate dispersion and mixing of the micronutrient, selenium; and (4) client compliance with the recommendations.

Animal productivity should be monitored also, including milk production, daily weight gains, feed efficiency, and breeding performance. Economically, these are by far the most important areas. With good records and continuous monitoring, the veterinarian can give the client concise information regarding the cost-benefit relationships of diagnosis and supplementation as they relate to the herd. The conclusion that lack of clinical disease equals optimum health and productivity is erroneous; monitoring of the animal's productivity is needed for client education and evaluation of the supplement.

Summary

The veterinarian must first suspect a selenium deficiency or selenium-responsive disease and arrive at a definitive diagnosis. Selenium supplementation can be provided and a monitoring program can be established to determine progress. These steps will help reduce animal death and disease and help increase animal productivity and efficiency, both physiologically and economically.

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Figure Legends
Figure 1. Probable cellular reactions involving GSH-Px.[22]
Figure 2. Selenium concentrations in grains and forages in North America.[25]
Figure 3. Generalized laboratory reaction in the GSH-Px assay.[22]
Figure 4. Mean whole blood selenium (WBSe) concentration of cows.[26] Bar graph expressed as mean WBSe concentration ± standard deviation (SD).
Group A, selenium-deficient cows receiving no supplemental selenium (control group); Group B, 1 selenium bolus at Day 0, and a second selenium bolus at Day 119; Group C, 1 selenium bolus at Day 0; Group D, 2 selenium pellets at Day 0. ug/ml = ppm (parts per million).

Table 1.

Selenium-Responsive Disease of Cattle

Syndrome	Major Clinical Features
White muscle disease (nutritional myodegeneration)	Acute onset, respiratory distress, shiftiness, skeletal muscle swelling and stiffness, stunned; signs vary from acute death to chronic lameness; pale, cachectic state of muscle, Zenker's necrosis present histopathologically
Retained placenta	Retained placenta
Abortion, stillbirths	Late uterine tolerance abortions and stillbirths
Neonatal weakness	Calves born weak with or without gross lesions of white muscle disease
Diarrhea	Diarrhea, usually profuse, and weight loss in calves and adult cattle
"Ill thrift" appearance	Decreased feed efficiency, decreased weight gains, and unthrifty appearance
Immune system effects	Cell-mediated immune-response suppression, mastitis
Myodegeneration in adult cattle	Weakness, myodegeneration, myocardial fibrosis, myoglobinuria
Infertility	Decreased conception rate, irregular estrous cycles, early embryonic death, cystic ovaries

Table 2.

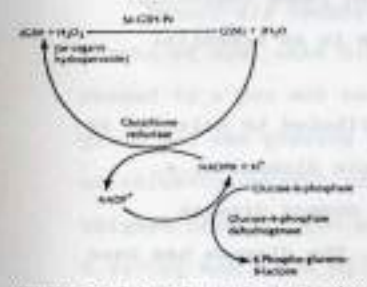
Bovine Blood Selenium Analysis
Interpretative Guide

WBSE (ppm)	GSH-Px (units/mg/dcm/minute)	Category	Interpretation
0.01-0.04	0-25	Deficient	Se supplementation is almost always beneficial
0.05-0.06	25-35	Marginal	Se supplementation is often beneficial
0.07-0.25	>35	Normal	Se supplementation is almost never beneficial

Table 3.

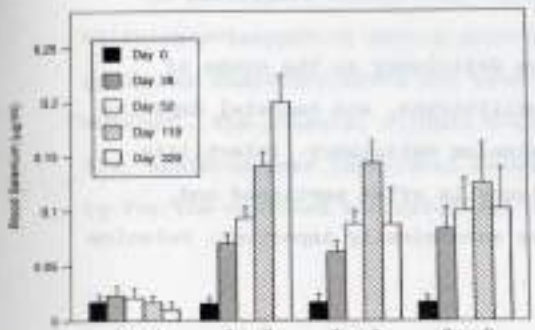
Current Federal Regulations Regarding Selenium in Cattle Feeds

Feed/Supplement Type	Maximum Level (Responsible Class)
Beef Cattle	
Total ration	0.1 ppm DMB 110 ppm to provide no more than 3 mg Se/head/day
Feed additives	Limited feed additive not to exceed 1 mg Se/head/day
Dairy cattle	
Total ration	0.3 ppm DMB



GSH—Reduced glutathione. Glutathione is a tripeptide (γ-glutamylcysteinylglycine).
NADP—Nicotinamide adenine dinucleotide phosphate. One of the pyridine nucleotides that act as hydrogen acceptors in reactions catalyzed by the "pyridine-linked dehydrogenases."
GSSG—Oxidized glutathione.

Figure 1—Probable cellular reactions involving GSH-Px.



USES AND INFLUENCE OF SELENIUM-a-TOCOPHEROL

C. Santiago

Presently, little is known and even less is understood about the effects of selenium and vitamin E on animals. For many years selenium was considered a toxic element in livestock nutrition. In 1957, the classic study by Schwarz and Foltz proved that selenium is a critical element in the prevention of nutritional hepatic necrosis. Subsequent researchers reported that selenium and vitamin E prevent white muscle disease in young ruminants. Further studies have confirmed that selenium is an essential nutrient for cattle and other species.

Various bovine syndromes have been attributed to selenium or vitamin E deficiency in the diet. White muscle disease (i.e, nutritional muscular dystrophy) is the most common disease attributed to selenium deficiency in calves. The disease has been seen throughout the United States, England, Europe, Australia, and New Zealand, among other parts of the world. Growth deficiency syndrome has been described as probably the most important problem associated with marginal selenium status from an economic standpoint.

Some studies fault selenium deficiency as the cause of retained placenta. Abortions, stillbirths, and neonatal debility also have been attributed to selenium deficiency. Infertility associated with selenium deficiency is often mentioned and, therefore, must be considered as economically important. Selenium

deficiency has also been held responsible for disturbances of the immune system. From a clinical standpoint, depending on the severity of the selenium or vitamin E deficiency, symptoms vary from a reduction of performance without clinical signs to sudden death of the affected animals. Because of the obvious importance of selenium-a-tocopherol, experiments were performed in Rio Grande Do Sul, Brazil to determine the drug's effects on feedlot calves and cattle.

Effect of Selenium-a-Tocopherol on the Growth of Feedlot Calves

Twenty-six Charolais breed animals, ranging from 7 to 11 months of age, were divided into four groups. Two groups were housed in a pen and two were kept in a pasture. One confined group and one grazing group were treated with 1 ml/100 kg selenium-tocopherol administered subcutaneously. The calves were weighed biweekly and achieved mean individual weight gains of 0.637 kg and 0.960 kg for the penned untreated and treated groups, respectively. The pastured calves achieved weight gains of 0.599 kg and 0.924 kg for the untreated and treated groups, respectively.

An additional twenty-four calves born of dams treated with selenium-a-tocopherol during gestation, were divided into four groups as described above and treated on Day 60 postpartum (i.e., weaning). The animals, weighed biweekly from weaning until Day 212, achieved mean individual weight gains of 1.293 kg and 1.551 kg for the confined untreated and treated animals, respectively,

while the pastured untreated and treated animals achieved weight gains of 1.345 and 1.407 kg, respectively.

Pure pedigreed animals from noble lines were used; each had a high capacity for feed conversion and had been previously examined and found free of infectious diseases.

Study of the weight development of the calves was based on individual biweekly weighings and simultaneous individual monitoring for the occurrence of symptoms that could be part of the selenium-tocopherol deficiency syndrome (i.e., diarrhea, tympanites, skin itching, hair loss, and laminitis). The data indicate that the selenium-α-tocopherol treatment reduced the stress of weaning and permitted the treated animals to show a larger weight gain.

Discussion

By selective mating, feedlot cattle with large muscle masses have been produced. These animals require abundant feed that is rich in proteins for early development of their physical potential and rapid growth of the muscles that will provide meat.

In certain situations, however, even when given a nutritionally balanced feed, these animals do not develop to their full production capacity. Consumption of legumes and alfalfa hay hinders selenium intake and stored grains and oil meal are often depleted of their α-tocopherol content. Cattle frequently suffer from selenium and/or vitamin E nutritional deficiency because of these factors.

Deficient growth syndrome and subclinical nutritional muscular dystrophy are probably responsible for the majority of economic losses from animals deficient in selenium and/or vitamin E. The losses caused by these subclinical forms of muscular dystrophy are greater and more difficult to detect than those produced by the other forms of the diseases and are attributed to marginal levels of selenium, vitamin E, or both in the diet.

Effect of Selenium-α-Tocopherol on Gestation and Fertility of Feedlot Cattle

Gestation, birth, puerperium, fetal viability, and fetal weight were observed in forty Charolais breed cattle, ranging from 3 1/2 to 10 years of age and weighing 650 to 1000 kg. On Day 240 (of 260) of gestation, they were divided into two groups: 15 untreated control cattle and 25 cattle treated with 1 ml/100 kg selenium-tocopherol administered subcutaneously. Treatment reduced the mean length of gestation, the number of assisted births and placenta retentions, and length of clinical puerperium. Cattle from the treated group also had calves with heavier birth weights and experienced their first postpartum estrus at date earlier than those of the untreated group.

Postpartum fertility was studied in thirty-six Charolais breed cattle, 3 1/2 to 10 years of age and weighing 650 to 1000 kg. The cattle were divided into three groups: 13 control cattle, 11 cattle treated with 1 ml/100 kg selenium-tocopherol 30 days

prepartum, and 12 cattle treated with 1 ml/100 kg selenium-tocopherol both 30 days prepartum and 60 days postpartum. Upon observation of the uteri, sexual cycles were regular and normal, no ovarian cysts were found, and the animals were inseminated sixty days after giving birth. Forty-five days after insemination, a pregnancy diagnosis was made by rectal palpation of the uterus. Use of the selenium-a-tocopherol increased the conception rate and reduced both the number of inseminations required for fertilization and the period between birth and fertilization.

Of all the mineral trace elements discovered to be essential, selenium has proved to be the most important for reproduction. Feedlot cattle give birth to calves with high birth weights; they are nourished after birth in order to promote growth and weight gain. These functions of procreating and nourishing the meat weight of a great milk producer cause exhaustion of the uterus, which is manifested by emaciation postpartum and during lactation (because of the stress imposed by the calf).

Conventional meat cattle management techniques include abundant feeding to overcome nutrient depletion and weight loss. In such a situation, pastures rich in legumes and alfalfa hay, oil concentrates, and protein supplements from oil meal are the approaches most indicated for recovery.

Grazing legumes and alfalfa hay hinder intake of selenium from feed, and concentrates of stored grains and oil meal are

deficient in vitamin E. This may result in an insufficient supply of selenium or vitamin E, thus causing a delay in appearance of the new reproductive cycle. Postpartum anestrus has been suggested to be the most costly of the infertility problems associated with selenium and/or vitamin E deficiency.

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TOMO II