



# Journal of the South African Veterinary Association

## Tydskrif van die Suid-Afrikaanse Veterinêre Vereniging

ISSN 0038-2809

March/Maart 1990

Volume • Jaargang 61 No. 1





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Maart/March 1990

Volume • Jaargang 61 No. 1

SA ISSN 0038-2809  
Dewey Cat No. 636.089

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Beria Printers/Drukkery,  
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## ARTERIAL BLOOD SUPPLY TO THE ILEOCAECAL JUNCTION IN THE HORSE

SANET H KOTZÉ\*

## ABSTRACT

The arteries supplying the ileocaecal junctions of 7 horses were injected with latex via the *A. ileocolica* and dissected macroscopically. The same basic pattern emerged whereby a single papillary artery arising from the *R. ilei mesenterialis*, supplied all aspects of the ileocaecal papilla. In one instance, the papillary artery arose directly from the ileocolic artery. No collateral arterial supply to the ileocaecal junction was present in any of the specimens dissected.

**Key words:** Equine, horse, ileocaecal junction, arterial supply, anatomy

Kotzé Sanet H. **Arterial blood supply to the ileocaecal junction in the horse.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 2-4 (En.) Department of Anatomy, Faculty of Veterinary Science, University of Pretoria, P/Bag X04, 0110 Onderstepoort, Republic of South Africa.

## INTRODUCTION

The anatomy of the ileocaecal junction in the horse has not received much attention in the literature. Schummer described the ileal orifice at the equine ileocaecal junction as centrally situated on the ileocaecal papilla which is a slight elevation formed by an annular fold of mucous membrane<sup>10</sup>. He briefly described a network of veins contained in this fold in the ileocaecal area of the horse<sup>9</sup>, which was also noticed by Dyce & Hartman<sup>1</sup> on endoscopic examination of the caecal base in the live horse. The presence and extent of this plexus was later confirmed<sup>4</sup>. Owing to a lack of the classical arrangement of the *M. sphincter ilei* in the horse<sup>3</sup>, it was postulated that engorgement of this plexus may aid in closure of the ileocaecal papilla of the horse<sup>4</sup>.

Although a detailed microscopic study describing the vascular architecture of the equine small intestine has been published<sup>12</sup>, no studies concerning the macroscopic arterial blood supply to the ileocaecal junction in the horse could be traced.

The purpose of this study was to examine the macroscopic arterial blood supply to the ileocaecal papilla in the

horse and to determine whether a collateral arterial supply to this area exists.

## MATERIALS AND METHODS

Samples for the present study were obtained from clinically healthy horses (n=7) of varying breeds, of both sexes and ages varying from 4 to 6 years. The horses were anaesthetised using barbiturate and exsanguinated by

catheterising the left common carotid artery.

The ileocaecal junction of each horse was removed soon after death, to include the terminal ileum, the ileocaecal papilla and the surrounding caecum and colon, together with the associated blood vessels. The specimens were stored at a temperature of -12°C for periods ranging from 4 to 8 months, after which they were thawed at room temperature. Red latex was injected via the *A. ileocolica* of each specimen, while clamping all severed arteries in the area to allow maximal filling of arteries in the immediate vicinity of the ileocaecal junction. After the specimens were fixed by immersion in 10% formalin for a period of 10 d, the ileocolic artery and the proximal parts of its main branches were dissected in each case. Special attention was given to arteries supplying the ileocaecal junction. They were dissected and traced by removing mesenteric fat, serosa and smooth muscle layers as the vessels penetrated the ileocaecal papilla. After dissection, photographs were taken, and semi-schematic diagrams of the arteries supplying the ileocaecal papilla were made.

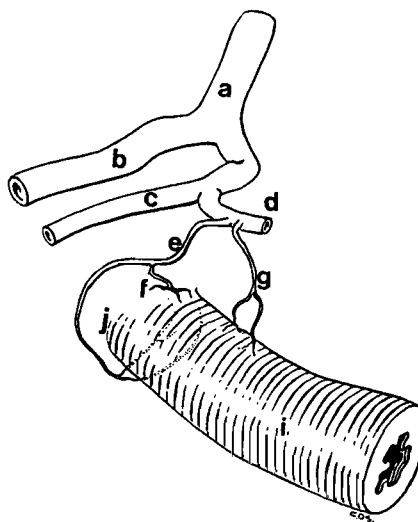


Fig. 1: A semi-schematic diagram of the ileocaecal junction and its associated blood vessels as seen from the serosal surface.

a. *A. ileocolica*, b. *R. colicus*, c. *A. caecalis lateralis*, d. *R. ilei mesenterialis*, e. papillary artery, supplying the cranial and caudo-ventral aspects of the papilla, f. dorsal branch of papillary artery, supplying the caudal-dorsal aspect of the papilla, g. a branch from the *R. ilei mesenterialis* supplying i, the distal ileum. The junction of distal ileum to the serosal surface of the caecum at the ileocaecal junction is marked j

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Received: June 1989 Accepted: September 1989



## RESULTS

In 6 of the specimens, the same basic pattern of arterial supply to the ileocaecal junction emerged. An artery arising directly from the *R. ilei mesenterialis* was the only arterial supply to the ileocaecal papilla, and will be referred to as the papillary artery in this study (Fig. 1e, 2e & 3e). After its origin from the *R. ilei mesenterialis* (Fig. 1d, 2d & 3d), the papillary artery ran for a short distance in the mesentery of the distal part of the ileum and then curved around the cranial edge of the base of the papilla, where it disappeared underneath the serosa and the smooth muscle layers at the base of the ileocaecal junction.

At the level of its origin, the diameter of the latex-filled papillary artery, measured 2 mm and that of the *R. ilei mesenterialis*, measured 5 mm in all the specimens examined. The length of the papillary artery from its origin to where it disappeared into the serosa and muscle layers, varied between 42 and 60 mm. The papillary artery continued its course embedded in the smooth muscle layers, while constantly giving off small branches that penetrated the muscle layers and extended into the submucosa of the ileocaecal papilla. As the artery became smaller in diameter, it curved around the ventral aspect of the papilla, where it ultimately reached and supplied the caudo-ventral aspect of the papilla (Fig. 1 & 2).

An additional dorsal branch (Fig. 1f, 2f & 3f), which varied between 18 and 21 mm in length, arose from the papillary artery at a distance of between 20 and 38 mm from the origin of the latter from the *R. ilei mesenterialis*. This small dorsal branch dipped below the serosa and in between the muscle layers to supply the caudo-dorsal aspects of the ileocaecal papilla.

In one of the horse specimens, the papillary artery (Fig. 3f) arose directly from the *A. ileocolica* (Fig. 3a) and not from the *R. ilei mesenterialis* (Fig. 3e). Apart from this difference, the artery supplied the papilla in an identical way as described in the other specimens.

Although the lateral caecal artery passed cranially over the distal ileum, close to the ileocaecal papilla, no branches supplying the papilla, were macroscopically visible in any of the specimens.

## DISCUSSION

The outstanding feature in this study was that in all the specimens examined, the ileocaecal papilla in the horse received its arterial blood supply from a single papillary artery. In all cases but one, the papillary artery was a branch of the *R. ilei mesenterialis*. Even though the latter

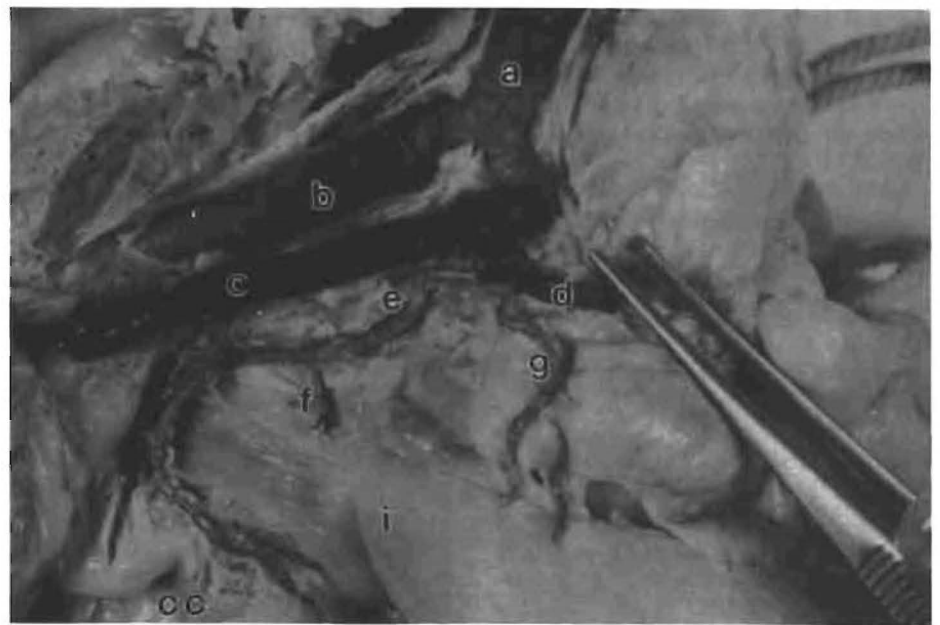


Fig. 2: A photograph of a dissection of the arteries supplying the ileocaecal junction from the serosal surface.

a. *A. ileocolica*, b. *R. colicus*, c. *A. caecalis lateralis*, d. *R. ilei mesenterialis*, e. papillary artery (serosa and smooth muscle removed at the base of the junction), f. the small dorsal branch of the papillary artery, g. branch of the *R. ilei mesenterialis* supplying the distal ileum, i. ileum, and cc. serosal surface of the caecum

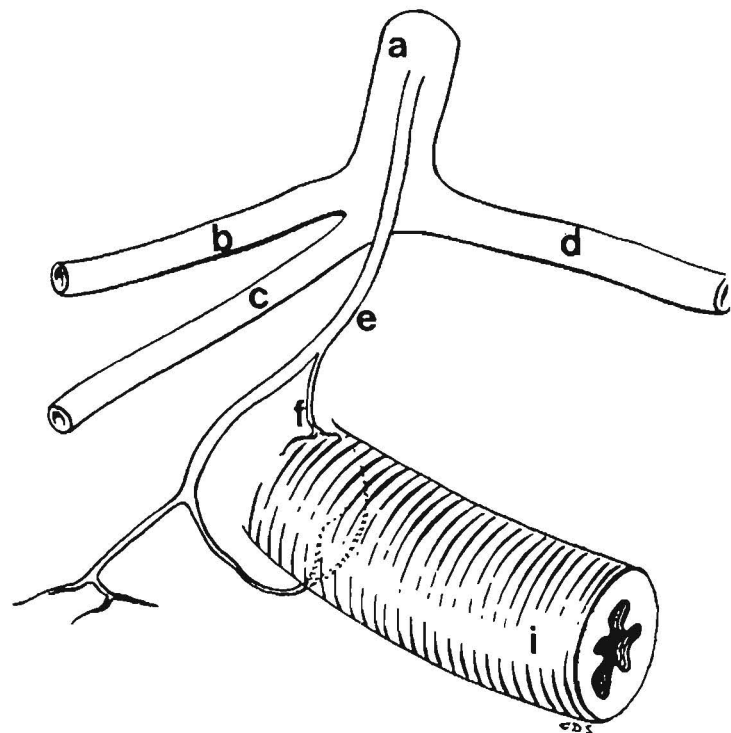


Fig. 3: A semi-schematic diagram of the atypical arterial supply to the ileocaecal junction observed in one of the specimens.

a. *A. ileocolica*, b. *R. colicus*, c. *A. caecalis lateralis*, d. *R. ilei mesenterialis*, e. papillary artery, arising directly from the *A. ileocolica* and not from the *R. ilei mesenterialis* as in the other specimens dissected, f. short dorsal branch of the papillary artery, i. ileum. The junction of the distal ileum to the serosal surface of the caecum is marked j



formed arch-like connections with the *Aa. ilei*, which are direct branches of the *A. mesenterica cranialis*, the distal ileum has no collateral blood supply<sup>11</sup>. In this study no macroscopically visible collateral blood supply to the ileocaecal papilla could be found. The distal ileum is therefore susceptible to vascular interference<sup>2</sup>, which may be even more so in the case of the ileocaecal papilla. Arterial lesions, such as thrombosis and verminous arteritis, associated with *Strongylus vulgaris* infestation, are most commonly reported in the *A. ileocolica* of the horse<sup>3</sup>. Even though these space-occupying lesions may not always be occlusive<sup>2</sup>, they impair normal blood flow to associated gut segments. In view of the absence of a collateral blood supply to the ileocaecal junction and the relatively small diameter of the papillary artery, even a slight occlusion may affect the filling of the submucosal venous plexus in the papilla, which may affect its function as an additional closing mechanism of the ileocaecal opening.

One of the most common forms of intussusception in the horse is ileocaecal intussusception, which has been attributed to segmental atony of the bowel, caused by reduced blood flow

through the ileocolic artery<sup>7</sup>.

The ileocaecal junction in the horse has often been associated with equine colic. Muscular hypertrophy of the ileum has been postulated to be secondary to stenosis caused by a neurogenic disturbance of the ileocaecal junction in the pig<sup>6</sup> and in the horse<sup>8</sup>.

The role of the ileocaecal junction in equine colic is still uncertain. The effect of impaired function of the submucosal venous plexus in the papilla has not been documented. However, impaired arterial blood supply to this area could affect the normal regulatory function of the equine ileocaecal junction, which may play a key role in colic originating in this and surrounding areas.

#### ACKNOWLEDGMENTS

The author wishes to thank Mr. D.J. Coetzer for technical assistance during the study, Mrs. H. Smit for photography and Mrs. C. Jansen van Vuren for the illustrations.

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# INTERACTIONS OF DIETARY CATION-ANION BALANCE AND PHOSPHORUS: EFFECTS ON BLOOD, BONE AND FAECAL PHOSPHORUS CONCENTRATION IN DAIRY CALVES<sup>1</sup>

D.E. BEIGHLE<sup>2</sup>, W.B. TUCKER<sup>3</sup> and R.W. HEMKEN<sup>4</sup>

## ABSTRACT

Dietary levels of cations and anions are shown to have an effect on concentrations of phosphorus (P) in blood, bone and faeces of dairy calves between 9 and 19 weeks of age. Calves fed diets high in anions, demonstrated higher concentrations of P in blood and faeces than calves whose diets were high in cations, and at the same time demonstrated lower concentrations of P in bone. Compared to diets with high cation levels, diets high in anions produced significantly ( $p < 0,05$ ) higher concentrations of total P in the faeces at different stages in the experiment, higher serum inorganic phosphorus (SiP) concentrations and lower P concentrations in the bone, which were significant at the end of the trial ( $P < 0,06$ ). When the diet was low in P (0,22%), the cation-anion balance of the diet seemed to have the greatest effect, so that the interaction of dietary P and dietary cation-anion balance (DCAB) was responsible for changes in blood, bone and faeces.

**Key words:** Phosphorus, cation-anion balance, dairy calves

Beighle, D.E.; Tucker, W.B.; Hemken, R.W. **Interactions of dietary cation-anion balance and phosphorus: effects on blood, bone and faecal phosphorus concentration in dairy calves.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 5-8 (En.) Department of Animal Sciences, College of Agriculture, University of Kentucky, Lexington, Kentucky, 40546 United States of America.

## INTRODUCTION

Research on the effect of dietary cation-anion balance (DCAB) in cattle has centered around the quest for a way to prevent milk fever in dairy cattle by increasing the availability of calcium during the periparturient period<sup>7</sup>. Block<sup>4</sup> manipulated dietary cations and anions in prepartum cows and found that the cows on high anionic diets did not become paretic because the level of

calcium was maintained during the time of calcium stress. Tucker<sup>19</sup> demonstrated that both acid-base status and feed intake in lactating dairy cattle are closely and directly correlated with DCAB within the range of -10 to +20 mmol 100 g<sup>-1</sup> diet dry matter. More recently research has shown that DCAB can have a significant effect on growth and blood concentrations of phosphorus (P) in dairy calves<sup>1</sup>. The objective of this research was to evaluate the effects of DCAB and dietary P on P homeostasis in blood, bone and faeces in dairy calves.

## MATERIALS AND METHODS

Holstein Friesland heifers (n=18), Holstein Friesland bulls (n=12) and Jersey bulls (n=6) were blocked according to breed and sex, then randomly assigned at approximately 9 weeks of age to 6 diets containing 2 levels of DCAB and 3 levels of dietary P for a 10 week period. DCAB was calculated ac-

cording to the formula  $(Na+K)-(Cl+S)^1$ . Anionic diets contained an excess of anions (ANEX) -14 mmol 100 g<sup>-1</sup> diet dry matter and were fed in conjunction with dietary P levels of 0,22% (ANEX -0,22), 0,29% (ANEX -0,29) and 0,37% (ANEX -0,37). Cationic diets contained an excess of cations (CATEX) + 39 mmol 100 g<sup>-1</sup> diet dry matter and were fed in conjunction with dietary P levels of 0,22% (CATEX -0,22), 0,29% (CATEX -0,29) and 0,37% (CATEX -0,37). Anionic balances were achieved by means of the addition of  $NH_4Cl$  and  $MgSO_4$ , cationic balances by the addition of  $NaHCO_3$ ,  $KHCO_3$  and urea, and dietary P was adjusted with dicalcium phosphate as previously reported<sup>1</sup>. All diets were formulated to meet or exceed established nutrient requirements of dairy calves<sup>16</sup>. Diets were fed once daily ad lib with weigh backs recorded each day. Calves were housed in hutches measuring 2 x 1,1 x 1,1 m with a 1,65 x 1,25 m fence surrounding the opening to allow freedom of movement. Hutches were bedded with sawdust when necessary.

Blood samples were collected via jugular venipuncture at 0, 3, 6 and 10 weeks, and were obtained immediately upon restraint to prevent variation of serum inorganic P (SiP) due to handling. Samples were allowed to clot, centrifuged within 3 h, and serum harvested into clean, plastic tubes and frozen at -20°C for subsequent SiP analysis<sup>8</sup>. Non-fasting body mass was determined thereafter, followed by rib bone biopsy as described by Little<sup>12</sup>. Bone samples were rinsed with a jet of distilled water, blotted dry, weighed and stored at -20°C before drying and ashing. Faeces were collected as fresh samples on the same day as blood and bone, were dried at 50°C for 48 h and ground through a 2 mm screen before weighing and ashing. Measurement of P in the serum, faeces and bone was performed by the method of Fiske & Subbarow<sup>8</sup> modified for use with an auto-analyzer (Technicon Instruments Corporation, 1967). Phosphorus in bone was measured as mg P per g fresh bone and as % dry weight. Faecal P was measured as mg P per g ash, and SiP

<sup>1</sup>This manuscript (89-5-194) is published with the approval of the director of the Kentucky Agricultural Experiment Station.

<sup>2</sup>Under appointment by the Foreign Mission Board Southern Baptist Convention on loan to the Department of Animal Health, School of Agriculture, University of Bophuthatswana, Private Bag X2046 Mmabatho 8681, Bophuthatswana

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Received: February 1989 Accepted: November 1989

was measured as mg dl<sup>-1</sup>.

Data were analysed according to SAS<sup>18</sup> as a randomised complete block design, variation attributable to block (breed and sex), DCAB, dietary P concentration, and DCAB x dietary P concentration interaction was included in the model.

## RESULTS

The anionic diets led to a higher level of total P in the faeces as compared to the cationic diets throughout the trial though this was significant only at Week 3 ( $p=0,001$ ) and Week 6 ( $p<0,05$ ) (Table 1). The level of P in the diet did not exert a significant effect on the total faecal P. The interaction of DCAB and dietary P concentration was responsible for significant differences in total faecal P at Week 3 (Table 1).

Concentrations of P in bone (Fig. 3) produced a different pattern compared to that established for the levels of SiP (Fig. 1) and faecal P (Fig. 2). In the serum (Fig. 1) and in the faeces (Fig. 2) the P concentrations from the anionic diets were found to be consistently above those of the cationic diets, but in the bone the results were reversed, so that the cationic diets were responsible for higher concentrations of P in the bone compared to the anionic diets (Fig. 3). In the beginning, the cationic diets produced higher concentrations of bone P, 59,6 mg/g in Week 3 as compared to 52,8 mg/g for the anionic diets (Table 2). However, at Week 10, the anionic diets resulted in higher concentrations of bone P; 53,1 mg/g compared to 52,3 mg/g from the cationic diets (Fig. 3). While these results are not significant they do point to a trend of lower bone P as the result of an anionic diet.

When bone P was measured as % dry weight the P concentration of the diet was responsible for significant differences ( $P<0,06$ ) in the concentration of P in the bone (Table 3).

## DISCUSSION

Effects of DCAB on growth and levels of SiP in this trial have been reported<sup>1</sup>. Anionic diets were responsible for higher average daily gains, and higher concentration of serum P when compared to cationic diets.

In the past, attempts have been made to use faecal P concentrations to determine P status, but with contradictory results. Belonje<sup>2</sup> found faecal P to be the best method for assessing dietary P intake in sheep while Lomba et al.<sup>15</sup> could find no significant correlation between faecal P and P intake in cattle. The difficulties inherent in using faecal P to estimate P status are countless, not the least of which is the presence of endogenous P from salivary gland secretion<sup>5</sup>. In this study, total faecal P was

**Table 1: Least squares mean, faecal phosphorus per ash weight (mg g<sup>-1</sup>) by diet and week during the trial**

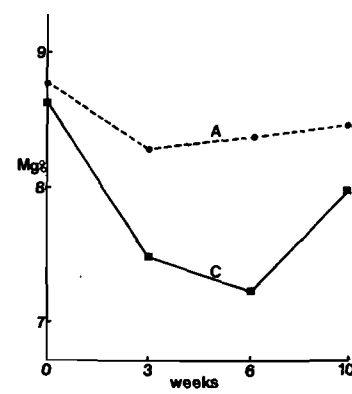
	Week			
	0	3	6	10
Diet <sup>1</sup>				
ANEX-0,22	75,6	54,9	64,5	67,0
ANEX-0,29	82,4	63,7	63,9	57,8
ANEX-0,37	76,6	86,9	69,5	69,9
CATEX-0,22	79,9	54,5	57,5	58,2
CATEX-0,29	68,7	60,0	58,4	58,3
CATEX-0,37	93,8	54,2	55,8	63,8
SEM	10,7	5,2	4,1	6,8
Dietary phosphorus %				
0,22	77,8	59,7	61,0	62,6
0,29	75,5	61,9	61,1	58,0
0,37	85,2	70,5	62,7	66,9
SEM	7,6	3,7	2,9	4,8
Dietary cation-anion balance				
ANEX	78,2	71,8	65,9	64,9
CATEX	80,8	56,2	57,2	60,1
SEM	6,2	3,0	2,4	4,0
Probability values:				
Catan <sup>2</sup>		0,001	0,018	
Catan x P concentration <sup>3</sup>		0,027		

<sup>1</sup>ANEX = Anion excess, CATEX = cation excess;

P concentration = 0,22 0,29 or 0,37% of diet DM

<sup>2</sup>Comparison of response to the cationic vs. anionic diets

<sup>3</sup>Interaction of cation-anion balance with dietary P concentration



**Fig. 1: Serum inorganic phosphorus concentrations with anionic (A) and cationic (C) diets**

measured and in general reflected the P content of the diet especially at 3 and 6 weeks (Table 1). This is in agreement with the findings of Belonje<sup>2</sup>.

It has been suggested that skeletal levels of P can be a reliable guide to P

nutrition in animals<sup>3</sup>. Hill<sup>9</sup> found rib bone to be more sensitive to resorption than long bones and therefore more suitable for mineral studies while Cohen<sup>6</sup> showed that the P content of rib bone was related to that of the pasture. Little<sup>13</sup> used rib biopsies to determine if unsupplemented animals were mobilising skeletal P to offset a dietary inadequacy. All of these reports emphasise the dynamic nature of bone, and results reported here concur. These results indicate that in the beginning of this trial, the anionic diets were removing P from the bone but later in the trial, the animals seemed to adjust to the ANEX so that P was replaced in bone (Table 2) and the blood concentrations of P were maintained (Fig. 1) by other means, perhaps better absorption.

Levels of P from bone analysis have been expressed in a number of ways. Little & McMeniman<sup>14</sup> expressed bone composition per unit of volume, Poppi & Ternouth<sup>17</sup> used fresh weight and



**Table 2: Least squares mean bone phosphorus per fresh weight (mg g<sup>-1</sup>), by diet and week throughout the trial**

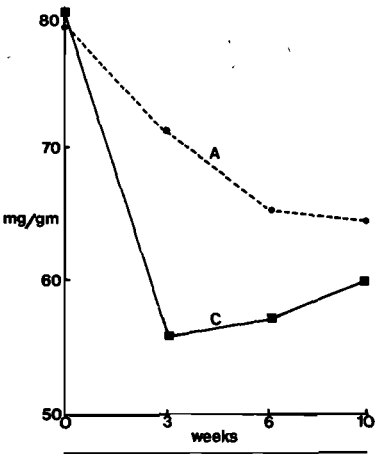
	Week		
	3	6	10
Diet <sup>1</sup>			
ANEX-0,22	57,1	46,6	51,9
ANEX-0,29	50,7	48,6	54,3
ANEX-0,37	50,5	40,9	53,1
CATEX-0,22	46,5	51,0	49,4
CATEX-0,29	69,5	39,5	49,9
CATEX-0,37	62,7	49,7	57,6
SEM	11,0	5,4	4,4
Dietary phosphorus %			
0,22	51,8	48,8	50,6
0,29	60,1	44,0	52,1
0,37	56,6	45,3	55,4
SEM	7,8	3,8	3,1
Dietary cation-anion balance			
ANEX	52,8	45,4	53,1
CATEX	59,6	46,7	52,3
SEM	6,4	3,1	2,5

<sup>1</sup>ANEX = Anion excess, CATEX = cation excess;  
P concentration = 0,22 0,29 or 0,37% of diet DM

**Table 3: Least squares mean bone phosphorus (% dry weight), by diet and week throughout the trial**

	Week		
	3	6	10
Diet <sup>1</sup>			
ANEX-0,22	6,14	5,89	6,90
ANEX-0,29	6,94	6,57	9,62
ANEX-0,37	6,59	6,49	9,52
CATEX-0,22	6,38	6,52	7,29
CATEX-0,29	8,69	4,36	9,36
CATEX-0,37	6,20	6,80	10,68
SEM	1,42	0,98	1,23
Dietary phosphorus %			
0,22	6,26	6,20	7,09
0,29	7,82	5,46	9,49
0,37	6,40	6,65	10,10
SEM	1,00	0,69	0,87
Dietary cation-anion balance			
ANEX	6,56	6,31	8,68
CATEX	7,09	5,89	9,11
SEM	0,82	0,56	0,71
Probability values:			
P Concentration <sup>2</sup>			0,053

<sup>1</sup>ANEX = Anion excess, CATEX = cation excess;  
P concentration = 0,22 0,29 or 0,37% of diet DM.  
<sup>2</sup>Comparison of response to the different concentrations of dietary P.



**Fig. 2: Faecal phosphorus ash weight with anionic (A) and cationic (C) diets**

Judkins et al<sup>11</sup> expressed bone P as a percentage of dry fat-free bone. In the current work, bone P was measured against fresh weight, dry non-fat-free weight, ash weight, and volume. The measurements which proved to be the most sensitive were mg per g fresh weight and % P dry weight. Contrary to the results of Little & McMeniman<sup>14</sup>, bone P per unit of volume (mg ml<sup>-1</sup>) did not show significant differences in P content.

A major problem was the acquisition of a biopsy sample of rib bone which was consistently free of trabecular bone, and red blood cells. In order to secure repeatable results in measuring P in rib bone biopsy samples, the trabecular bone and blood may need to be removed to prevent inconsistent contamination of P-containing red blood cells.

It has been shown that acid producing (anionic) diets can be responsible for the removal of minerals from bone in rats<sup>20</sup> and in goats<sup>10</sup>. The results reported here are suggestive that in this experiment P was removed from the bone as a result of ANEX (Fig. 3, Table 2) and resulted in higher concentrations of serum P and faecal P due to the anionic diets (Fig. 1 & 2) as compared to the cationic diets. In this case the differentiation between endogenous and total faecal P is not so critical. What is important is the increased P content of the faeces.

The anionic diets increased faecal P at 3, 6 and 10 weeks. The serum P was increased at the same time which should indicate that at least a part of this increase in faecal P was due to endogenous P. The decrease in bone P at Weeks 3 and 6, agree with the results obtained by Block<sup>4</sup> who suggested that the anionic diet allowed for easier bone mobilisation during calcium stress.

When calves were subjected to a low level of P in the diet, those on the

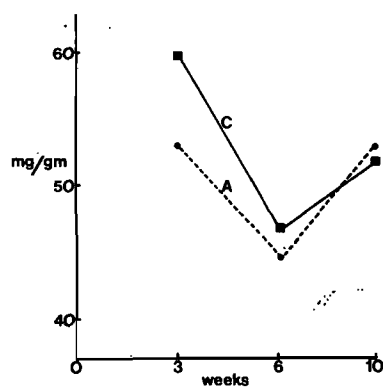


Fig. 3: Bone phosphorus fresh weight with anionic (A) and cationic (C) diets

anionic diets had higher levels of P in the blood<sup>1</sup>, and lower levels of P in the bones (Table 3) than those on the cationic diets. This indicates an important interaction of dietary P and DCAB, especially when the diet is low in P, and suggests a need for further research into this interaction.

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# DNA SCREENING FOR HEREDITARY GOITRE IN AFRIKANDER CATTLE

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## ABSTRACT

DNA isolated from blood samples of Afrikaner cattle (n=66) were screened by blot-hybridisation analysis to determine the frequency of carriers of the goitre mutation. No carriers were found, suggesting that the frequency of the goitre mutation is less than 0.01.

**Key words:** Hereditary goitre, thyroglobulin, DNA screening.

Ricketts M.H.; Vandenplas S. **DNA screening for hereditary goitre in Afrikaner cattle.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 9-10 (En.) MRC Centre for Molecular and Cellular Biology, Department of Medical Physiology and Biochemistry, University of Stellenbosch, P.O. Box 63, 7505 Tygerberg, Republic of South Africa.

## INTRODUCTION

Goitre in both man and animals is an enlargement of the thyroid gland, often as a compensatory response to defective thyroid hormone production<sup>2</sup>. Insufficient production of thyroid hormone could be due to environmental factors (such as a dietary iodine deficiency) or a hereditary defect in thyroid hormone synthesis.

In the first published description of goitre in Afrikaner cattle, the condition was thought to be hereditary<sup>3</sup>. In the 1950's, congenital goitre occurred in more than 30 herds of Afrikaner cattle in many areas of southern Africa<sup>6</sup>. In order to investigate the inheritance and molecular basis of the disease, primarily as an animal model of human disease, affected animals were collected and maintained as an inbred herd. Breeding studies showed that the congenital goitre was inherited as an autosomal recessive disease<sup>4</sup>. Affected animals have a higher perinatal mortality rate, due to asphyxiation by the goitre.

With the advent of recombinant DNA technology, the cause of the disease was identified as a point mutation in the thyroglobulin gene<sup>5</sup>. Thyroglobulin is the protein precursor of thyroid hormone. The cytosine (C) to Thymidine

(T) point mutation changes the triplet codon at position 697 from CGA (specifying the amino-acid Arginine) to TGA (a stop codon). The mutation also alters the DNA sequence recognised by the DNA restriction endonuclease *TaqI* from TCGA to TTGA. When DNA is cut with the restriction enzyme *TaqI*, a 1 500 base pair fragment is detected in normal animals. The goitre mutation results in loss of a *TaqI* cutting site and a 1 900 base pair fragment is generated. The presence of both a 1 500 and a 1 900 base pair band in the DNA of an individual indicates that the animal has one affected allele and is therefore a heterozygous carrier of the disease.

Because of the alteration in the *TaqI* recognition site, it is possible to screen animals for the presence of this mutation by Southern Blot analysis of isolated DNA<sup>7</sup>. As the disease is autosomal recessive, asymptomatic carriers of the disease can also be detected.

In this article we describe the method employed and the results of screening Afrikaner cattle to estimate the frequency of the mutation-causing goitre.

## MATERIALS AND METHODS

Blood samples for DNA isolation were obtained from Afrikaner cattle (n=66). Thirty-two animals were from a herd with no inbreeding in the Potchefstroom area, and represented the progeny of 7 bulls from 4 different breeders. A further 34 animals represented stock from 34 different breeders in the Pretoria area. Control

DNA was isolated from an inbred animal with hereditary goitre (homozygote) and a carrier of the goitre mutation (obtained by crossing a goitre animal with a normal bull) which were housed at the University of Stellenbosch experimental farm, Mariendal.

## Isolation of white blood cells:

Blood samples were collected in EDTA tubes. The red blood cells were lysed by addition of an equal volume of ice-cold water. After 1 min, isotonicity was restored with sodium chloride (final concentration of 0.9%). After mixing well, the white blood cells were pelleted by centrifugation at 3 500 rpm in a Beckman benchtop centrifuge. The pellet was washed twice with phosphate-buffered saline and used for isolation of DNA.

## Isolation of DNA:

The white blood cell pellet was suspended in 2 ml of digestion buffer (100 mM NaCl; 25 mM EDTA; 0.5% SDS; 10 mM Tris-HCl, pH 8.0) containing 0.1 mg ml<sup>-1</sup> proteinase K. This was incubated for 12 to 14 h at 50°C with gentle shaking in tightly-capped tubes. The samples were then extracted twice with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1) and then with ether. The DNA was precipitated by addition of 2 volumes ethanol and the precipitate was washed with 70% ethanol and dissolved in TE buffer (10 mM Tris-HCl, pH 7.5; 1 mM EDTA). DNA concentration was determined from its absorbance at 260 nm.

## Southern blot-hybridisation analysis of DNA:

Five µg of each DNA sample was digested with 25 units of the restriction enzyme *TaqI* at 65°C for 4 to 6 h. The DNA fragments were separated by electrophoresis in 1% agarose gels and transferred to nitrocellulose paper essentially as described<sup>7</sup>. Pre-hybridisation and hybridisation solution consisted of 50% Formamide, 6x SSPE (1.08 M NaCl, 0.06 M NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 0.6 mM Na<sub>2</sub>EDTA) 5x Denhardt's (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA) and 100 µg ml<sup>-1</sup> Herring sperm DNA. Pre-hybridisation was undertaken for 4 h at 42°C with gentle shaking. The

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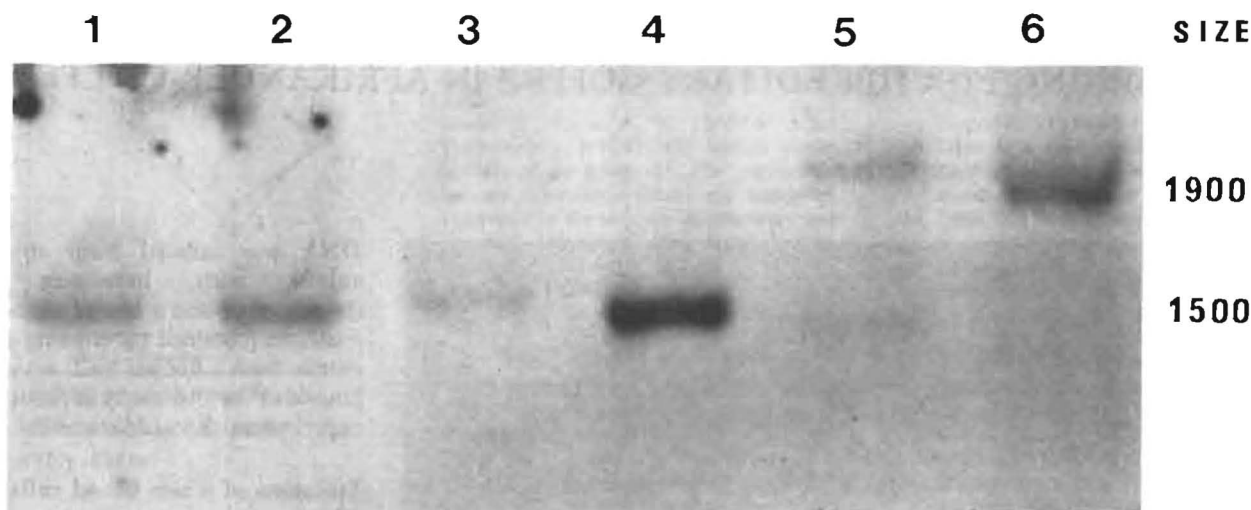


Fig. 1: Autoradiograph showing the thyroglobulin gene fragments hybridising with the cDNA probe in 4 Afrikaner cattle (lanes 1-4), as well as a heterozygote carrier of the disease (lane 5) and an animal with goitre (lane 6). The size of the hybridising DNA fragments are shown in base pairs

$^{32}$ P-labelled probe (500 000 cpm/ml) was added to 12 ml of solution and filters were hybridised for about 16 h at 42°C with gentle shaking. The filters were then washed 5 times in 3 x SSPE, 0.1% SDS at room temperature with gentle agitation, then at 42°C in 1 x SSPE, 0.1% SDS and autoradiographed for 2 to 5 d at -70°C.

#### NA probe:

A 148 base-pair (bp) DNA fragment was isolated from a low melting point agarose gel after digestion of the cloned thyroglobulin cDNA probe pbTg2.8 (formerly named pbTg2.6)<sup>1</sup> with EcoRI. The DNA probe was radioactively labelled using  $^{32}$ P-dCTP and a random primed DNA labelling kit (Boehringer Mannheim).

#### RESULTS

No carriers of the mutation were found in the 66 animals screened. A representative Southern Blot result is presented in Fig. 1. Lanes 1-4 have DNA from some of the animals which were screened. All have a single 1 500 base pair band hybridising with the radioactive probe. Lane 5 has DNA from a known heterozygote (with both 1 500 and 1 900 base pair hybridising bands), and lane 6 contains the DNA from an animal with goitre (a single 1 900 base pair band hybridising).

#### DISCUSSION

Pedigree analysis of animals in affected

herds indicated that the goitre mutation was transmitted by a bull that had been extensively used for breeding. Although no specific data concerning the frequency of the goitre mutation in the past is available, the earlier goitre outbreaks indicate that a significant number of carriers were then present. The 66 animals screened in this study represent 132 thyroglobulin alleles, none of which had the goitre mutation. While this suggests that breeding practices over the past 2 or 3 decades have significantly reduced the frequency of the mutation, it cannot be concluded that the goitre mutation has been eliminated. According to the Hardy-Weinberg law, the sample is unlikely to contain more than one heterozygote if the frequency of the gene with the goitre mutation in the population is less than 0.01. It is therefore concluded that the thyroglobulin allele with goitre mutation occurs in less than 1% of the Afrikaner cattle population.

The availability of techniques to detect carriers of the disease could encourage breeders, who may suspect the presence of the goitre mutation, to have animals tested. With this knowledge, transmission of the mutation can be avoided. It is perhaps noteworthy that techniques exist to detect mutations, such as the one responsible for hereditary goitre, from very small tissue samples, which can be obtained from in vitro-fertilised embryos before implantation.

#### ACKNOWLEDGEMENT

We thank Dr M.M. Scholz and Mr R. Visser for their help in collecting and sending blood samples, Prof H. Heydenrych for advice and critical reading of the manuscript, Mr F. Theron for assistance regarding the cattle at Mariendal and Prof A.J. Bester for advice and encouragement.

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# THE PERSISTENCE OF SEROLOGICAL REACTIONS FOLLOWING HEIFER VACCINATION WITH *BRUCELLA ABORTUS* STRAIN 19 ON BRUCELLOSIS-FREE FARMS

S HERR\*, W J EHRET\*\*, L M M RIBEIRO\* and F CHAPARRO\*

## ABSTRACT

Of 392 heifers vaccinated between 4 and 9,5 months of age and tested at 22 months or older, 3 (0,77%) had titres in the complement fixation test of between 30 and 98 international units per millilitre. The results confirm that persistent serological reactions can be a problem even in brucellosis-free herds. To reduce the incidence of these problem reactors, it is suggested that: in clean herds the age of inoculation be reduced to 3 months of age; the age at first testing be delayed as long as possible, but be done before the heifers are 4 months pregnant, c) consideration be given to reducing the vaccine dose for all heifers from the present 4 to 12 x 10<sup>10</sup> to between 3 x 10<sup>8</sup> and 3 x 10<sup>9</sup> and syringes used for strain 19 vaccination be exclusively reserved for this purpose to prevent the possibility of antigenic residues acting as a booster when used for other vaccinations.

Key words: Bovine brucellosis, vaccination

Herr S.; Ehret W.J.; Ribeiro L.M.M.; Chaparro F. The persistence of serological reactions following heifer vaccination with *Brucella abortus* strain 19 on brucellosis-free farms. *Journal of the South African Veterinary Association* (1990) 61 No. 1, 11-13 (En.), Section of Reproduction, Veterinary Research Institute, 0110 Onderstepoort, Republic of South Africa.

## INTRODUCTION

The use of *Brucella abortus* strain 19 (S19) vaccination has helped to control brucellosis in many countries<sup>13 14</sup>. The standard dose (4 to 12 x 10<sup>10</sup> viable organisms)<sup>21</sup> has been advised for use in heifers from 4 to 8 months of age<sup>7 13</sup>, followed by the use of the complement fixation test (CFT) to limit the numbers of false positive reactors<sup>2 4 6 16 19 20</sup>. This policy is followed until the overall prevalence of the disease has been reduced to below 0.2% in an area or country<sup>1 13</sup>. As false positive reactions following S19 vaccination may be affected by the presence of infection<sup>22</sup>, an investigation into the persistence of vac-

cinational reactions can only be carried out reliably in a known brucellosis-free environment. False positive reactors found by various workers in this field are reported, according to their definition of serologically positive reactors and to the sensitivity of the tests used. The reported results cannot therefore be applied directly to fit the CFT as standardised in South Africa, but may be used as a rough guide. In South Africa the CFT is used currently as the definitive test. Where heifer vaccination is practised, a titre of 18 to 24 IU ml<sup>-1</sup> is regarded as suspect and 30 IU ml<sup>-1</sup> or higher as positive. Heifers are tested at 18 months of age or older (Krige J, Department of Agriculture and Fisheries, Memorandum 12/1/8/6/B of 1981/07/30).

Investigations into the persistence of CFT titres following S19 vaccination under South African conditions, have been reported for adult cattle<sup>10 23</sup> and heifers<sup>9</sup>. The current work was undertaken to determine the number of reactors that could be expected in a clean environment, following S19 vaccination at 4 to 9,5 months of age and testing at 15 to 17,5 and 22 to 26 months of age.

Observations were done over 5 years to allow for possible variations in vaccine batches.

## MATERIALS AND METHODS

Bovelder heifers (a mixture of Simmental, Charolais, British beef breeds and various *Bos indicus* breeds) kept on 2 brucellosis-free farms of the Johannesburg City Council, north and south of the city, were vaccinated with various batches of S19 (*Brucella* strain 19 vaccine) manufactured by the Veterinary Research Institute (VRI), Onderstepoort. They were 4 - 9,5 months of age at the time of vaccination. The numbers included in the trial run during 1982, 1984, 1985, 1986 and 1987 were 125, 115, 115, 84 and 78, respectively. The 1982 heifers were tested at 15 to 17,5 months of age. All other heifers were tested at 22 to 26 months of age. Special syringes were not provided for the vaccination of S19, despite recommendations to this effect.

The serological tests were done at the VRI, using methods previously described<sup>8 11</sup>. Sera were screened, using rose bengal (RBT) and microtitration serum agglutination (M-SAT)<sup>11</sup> tests. All RBT positive reactors were subjected to the CFT, as were all sera with titres of 84 IU ml<sup>-1</sup> or higher in the M-SAT. The results in the M-SAT were based on a scale where 1000 IU ml<sup>-1</sup> is equivalent to 50% agglutination in a final dilution of 1:500<sup>11</sup>. The results in the CFT were also reported in IU ml<sup>-1</sup>, on a scale where 1 000 IU ml<sup>-1</sup> is equivalent to 50% haemolysis in a 1:220 serum dilution<sup>10 11</sup>. Since 1986, all reactors with CFT titres of 18 IU ml<sup>-1</sup> or higher, were subjected to retesting at 1 to 2 month intervals to determine whether the titres fluctuated. In 2 cases, where titres of 49 and 392 IU ml<sup>-1</sup> in the CFT were experienced on retesting, the animals were kept in isolation until calving and attempts were made to isolate *Brucella abortus* from colostrum, placenta and uterine exudate.

## RESULTS

The reactions of all the heifers to the RBT and CFT are given in Table 1 and to the M-SAT in Table 2. The serological results of the subsequent

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Received: July 1989 Accepted: October 1989

Table 1: Reactions to the CFT and RBT in heifers inoculated with *B. abortus* S19

Test year	Vaccination age (months)	Test age (months)	Total number tested	Number with reactions in the CFT (IU ml <sup>-1</sup> )					Number RBT Positive (%)	
				0-15	18	21	24	49	98	
1983	5 - 7,5	15 - 17,5	125	111	-	1	5	4	4	68 (54%)
1985	5 - 8	22 - 25	115	114	1	-	-	-	-	37 (32%)
1986	5,5 - 9,5	22 - 25	115	113	1	-	-	-	1	19 (17%)
1987	4 - 7	22 - 25	84	82	1	-	-	1	-	8 (10%)
1988	5 - 8	22 - 26	78	75	1	-	1	-	1	14 (18%)
Total of test age > 22 months			392	384	4	-	1	1	2	78
% of total with test age > 22 months				98%	1%	-	0,26%	0,26%	0,5-0,51%	(20%)

Table 2: M-SAT results in heifers inoculated with *B. abortus* S19

Test year	Vaccination age (months)	Test age (months)	No.	Number with reactions in the M-SAT (IU ml <sup>-1</sup> )		
				0 - 53	54 - 74	84 - 214
1983	5 - 7,5	15 - 17,5	125	100	25	-
1985	5 - 8	22 - 25	115	81	34	-
1986	5,5 - 9,5	22 - 25	115	89	26	-
1987	4 - 7	22 - 25	84	78	5	1
1988	5 - 8	22 - 26	78	66	9	3
Total %			517	414 80,1%	99 19,1%	4 0,8%

Table 3: M-SAT and CFT reactions of 2 heifers with persistent titres tested subsequent to 22 months of age and at calving

Month/year	Heifer No.			
	H 209		H 270	
	IU ml <sup>-1</sup>		IU ml <sup>-1</sup>	
	M-SAT	CFT	M-SAT	CFT
T <sub>1</sub> 08/88	84	24	84	98
T <sub>1</sub> 10/88	54	21	215	120
T <sub>2</sub> 12/88	84	49	256	392
C <sub>3</sub> 03/89	74	24	215	86

testing of the 2 animals kept till after calving, are given in Table 3. No isolations of *Brucella abortus* were made from these 2 animals.

## DISCUSSION

The high percentage of RBT positive

reactors and those with 49 and 98 IU ml<sup>-1</sup> in the CFT seen in the heifers tested at under 18 months of age, supports the official policy of not testing vaccinated heifers before 18 months of age. As abortions due to *Brucella abortus* occur mainly in the last 3 months of

pregnancy<sup>5</sup>, the test could be delayed until 4 months after breeding, with little danger of abortion and consequent spread of infection occurring.

The occurrence of a mean of 20% reactors to the RBT in the heifers tested after 22 months of age, causes concern to the laboratories which are then forced to do the technically more exacting CFT on these samples. A high rate of reactors in the RBT may be caused by antigenic residues in syringes used for S19 vaccination and subsequently used for other vaccinations<sup>9</sup>.

Of the heifers tested after 22 months of age, 98% passed the screening and CF tests while only 0,77% had positive titres in the CFT ( $\geq 30$  IU ml<sup>-1</sup>). These results compare favourably with those reported in the literature of between 0,5% and 2% reactors in the CFT<sup>1 3 4 6 12 14</sup>. Positive CFT reactors present an obvious problem in the certification of a herd and of the individual animals as brucellosis-free. The following measures could help to reduce this problem: a) heifers in clean herds could be vaccinated as soon after the officially-determined 4 months of age as possible, as the serological response is less prolonged in younger animals<sup>13</sup>, b) the minimum age for inoculation could officially be reduced to 3 months for clean herds as was the case in the UK<sup>2</sup> and elsewhere<sup>15</sup>, c) the age of first testing could be postponed as long as possible (4 months after breeding) without incurring the possibility of *Brucella* abortions so that the heifers have sufficient time to lose the positive titres<sup>13</sup>, d) the reduction of the vaccine dose to between  $3 \times 10^8$  to  $3 \times 10^9$  as has been done in the USA<sup>13</sup> should also be considered and e) the use of syringes exclusively reserved for S19 vaccination<sup>9</sup>. These measures would hopefully also reduce the numbers of heifers reacting to the screening tests (RBT and MSAT).



In the absence of any indication of either vaccine or field strains of *Brucella* in the heifers H209 and H270 or on the properties, the high titres found in the case of H270 may be due to a booster effect from S19 residues in syringes or to cross-reactions with other organisms<sup>13</sup>. The drop in the CFT titre that occurred at calving in H270, may be explained by the absence of active infection and the transfer of immunoglobulin G<sub>1</sub> (IgG<sub>1</sub>) from the bloodstream to the colostrum<sup>18</sup>. Mainly IgG<sub>1</sub> is measured in the CFT, but is less important in the agglutination tests<sup>17</sup> and this is supported by the failure of the M-SAT to drop (Table 3).

#### ACKNOWLEDGEMENTS

The authors wish to thank the City Engineer and the Medical Officer of Health of Johannesburg for permission to publish the results, the staff of Johannesburg Council Farms and of the Reproduction Section of the VRI and of the Directorate of Animal Health for their contributions which made this study possible.

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## HUMAN COMPANION-ANIMAL RELATIONSHIPS IN THE VETERINARY CONSULTING ROOM

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### ABSTRACT

A questionnaire, based on 14 years' private practice experience and psychological principles, was compiled to establish the reasons why veterinary clients are involved with their pets. Questionnaires were sent to South African veterinarians country-wide and only clients visiting veterinarians were requested to complete the questionnaires anonymously. Questionnaires (n=612) were returned and the data was processed by computer. The results indicated that clients are involved with their companion animals for emotional, social and relaxational reasons, which all have psychological implications. They are, however, also involved with their animals by caring for them, and they keep them for utility or economical reasons and also due to their general interest in nature. The latter reasons could be seen as natural reasons for keeping pets. The same relationship between clients and their pets continue during veterinary consultations. Because of this continuing involvement, some clients may consult veterinarians mainly for psychological reasons and others mainly for clinical reasons. There is a constant interaction between these motivations for veterinary consultations.

Client consultations of veterinarians could thus be categorised into psychological consultations which are predominantly human/client orientated and clinical consultations which are predominantly animal/patient orientated.

**Key words:** Client-patient relationship, veterinary consultations

Odendaal J.S.J.; Weyers A. **Human-companion animal relationships in the veterinary consulting room.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 14-23 (Eng.) Department of Veterinary Ethology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

### INTRODUCTION

Various human to animal relationships are particularly evident in an intensive contact situation such as in a veterinarian's consulting room. Contact of this nature can extend from the animal's birth or from its entrance into the owner's home until the bond between the owner and the animal is broken, mostly as a result of the animal's death. When one refers to the bond between humans and animals it is clear that there have to be 2 parties involved in this

emotional bond. The veterinarian will thus inevitably be involved with both parties in the intimate atmosphere of the consulting room. A bond does not only imply the presence of 2 parties, but also that they form a unit.

Even though the study of human and animal interaction has received a great deal of attention during the past decade or two, the implications for the veterinary consulting room, have not yet been fully investigated<sup>18</sup>. It also appears, that even though an informal awareness of the emotional bond between client and patient exists among companion animal veterinarians, no scientific recognition has been awarded to this field. This lack of recognition is inter alia reflected by the fact that in most veterinary curricula<sup>28</sup> this subject is not

dealt with.

The aim of this investigation was to establish the reasons why veterinary clients are involved with their animals. Such involvement was then considered with reference to the motivations which cause veterinary clients to consult veterinarians and these consultations were consequently categorised. The possible advantages of such a study for the veterinarian, is that if the various reasons for consultation could be interpreted correctly, he could improve and broaden his professional services. The identification of the relationship between client and patient may also lead to higher productivity, as more attention could be given to the true needs of the client.

### MATERIALS AND METHOD

In order to establish why veterinary clients keep companion animals, questionnaires (n=1200) were sent to veterinary practices (n=120). The contents of the questionnaires were founded on experience of companion animal practice and on the principles of the behavioural sciences. Questions were formulated in both Afrikaans and English.

The members of the research group were identified as clients who keep companion animals and who consult veterinarians about their companion animals. The questionnaires were therefore sent to most practices in South Africa which serve a significant percentage of companion animal owners. Clients were requested to complete the questionnaires anonymously and voluntarily.

All questionnaires were returned to the Unit for Professional Training and Service in the Behavioural Sciences (UPTB) at the University of the Orange Free State. Although most of the questionnaires were completed in the consulting rooms, some clients completed their questionnaires at home and posted them directly to UPTB. The data was processed by computer at the Bureau for Computer Services, University of the Orange Free State. This comprised the codifying of questionnaires as well as the numerical arrangement of each response as indicated in the tables.

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Received: January 1989 Accepted: August 1989

**Table 1: Emotional reasons for keeping companion animals (n = 612)**

	Emotional reasons for keeping companion animals	Scale 1 Neg %	Scale 2 Neg %	Scale 3 Pos %	Scale 4 Pos %	Scale 5 Pos %	No answer %
1	For the attractive appearance of the animal	21	13	22	20	21	4
2	To forget about other troubles	36	18	18	11	12	5
3	For the sake of friendship	9	10	26	26	25	4
4	Because the animal understands and accepts man as he is	12	11	21	23	29	4
5	To reduce frustrations	49	14	16	9	8	4
6	For the sake of greater personal appreciation	19	13	18	22	23	5
7	Due to childhood memories	62	14	10	5	4	5
8	Out of mere pity	55	11	12	6	10	6
9	For the tranquility which the animal provides	21	16	26	17	17	3
10	To gain more meaningfulness for oneself	28	14	25	18	12	3
11	Due to a feeling that the animal needs someone to take care of it	19	12	22	22	21	4
12	For the opportunity of showering the animal with love	10	9	23	22	33	3
13	To compensate for other problems	63	14	10	4	4	5

**Table 2: Social reasons for keeping companion animals (n = 612)**

	Social reasons for keeping companion animals	Scale 1 Neg %	Scale 2 Neg %	Scale 3 Pos %	Scale 4 Pos %	Scale 5 Pos %	No answer %
1	For the sake of friendship	5	8	23	26	34	4
2	To bring about contact with other people	52	20	12	7	4	5
3	For the particular status afforded by the animal	57	10	13	7	8	5
4	To compensate for loneliness	43	16	17	9	10	5
5	Because others also keep animals	78	9	3	3	2	5
6	Due to a love of animals	2	1	6	22	67	2

**Composition of the questionnaires**  
The questionnaires consist of 38 ques-

tions, which are grouped into sub-divisions. Clients' answers were evaluated

according to a five-point sliding scale and clients' choices were indicated by means of crosses. In two sections the clients' attitudes towards their animals were tested by a YES or NO response. Feelings and attitudes towards animals were given by clients as reasons why they keep their animals or why they are involved with their animals.

The five-point sliding scale evaluated the feelings and attitudes of clients as follows:

Scale 1 - A feeling which is not at all applicable to the client and therefore very negative.

Scale 2 - A feeling which is of little significance to the client and therefore mainly negative.

Scale 3 - A feeling which is applicable and therefore fairly positive.

Scale 4 - A feeling which is highly applicable and therefore mainly positive.

Scale 5 - A feeling which is exceptionally applicable and therefore very positive.

Respondents were not required to answer all questions. If they did not answer certain questions it was ascribed to an inability to express their own feelings, or because they did not experience a clear or applicable feeling regarding the question or because their feelings regarding the question were neutral.

The questionnaires were compiled as follows:

#### Category 1

These questions were aimed at establishing the psychological reasons why veterinary clients keep companion animals. This category comprised 3 sections:

#### Section 1:

Emotional reasons for keeping companion animals. Thirteen questions were compiled aimed at feelings which were directly related to the client's emotional experiences with his companion animal.

#### Section 2:

Social reasons for keeping companion animals. The 6 questions tested feelings about the social role of the animal which implicates other persons, as well as the actual social interactions between clients and their animals. Interaction of this kind includes keeping the animal as a companion and a general love (affection towards) of animals.

#### Section 3:

Relaxation as a reason for keeping companion animals. This category on relaxation was aimed at evaluating the psychological advantages of play, the utilisation of leisure time, exercise and entertainment for the client.



**Table 3: Relaxational reasons for keeping companion animals (n = 612)**

	Relaxational reasons for keeping companion animals	Scale 1 Neg %	Scale 2 Neg %	Scale 3 Pos %	Scale 4 Pos %	Scale 5 Pos %	No answer %
1	To play with	5	8	36	27	21	3
2	Use of leisure time	15	15	32	18	12	8
3	For physical exercise	44	19	17	7	5	8
4	Entertainment	26	15	21	15	16	7

**Table 4: Regular and personal care of companion animals by veterinary clients (n = 612)**

	Care of companion animals	Positive %	Negative %	No answer %
1	Food and water	94	4	2
2	Adequate shelter	95	2	3
3	Preventative veterinary medicine	90	5	5
4	Balanced diet	4	92	4

**Table 5: Natural and economical reasons why veterinary clients keep companion animals (n = 612)**

	Economical reasons for keeping companion animals	Scale 1 Neg %	Scale 2 Neg %	Scale 3 Pos %	Scale 4 Pos %	Scale 5 Pos %	No answer %
1	Personal protection	18	9	24	18	27	4
2	For pest control	67	11	8	4	3	7
3	For the sake of financial benefits	82	5	4	2	1	6

**Table 6: Natural scientific reasons why veterinary clients keep companion animals (n = 612)**

	Scientific reasons for keeping companion animals	Scale 1 Neg %	Scale 2 Neg %	Scale 3 Pos %	Scale 4 Pos %	Scale 5 Pos %	No answer %
1	Educational value	34	10	20	16	15	5
2	Due to an interest in animals	3	2	13	30	47	5
3	Due to its link with nature	18	13	25	19	21	4

**Category two:**

These questions were aimed at judging clients' attitudes towards the natural and basic care of their animals. At the same time it was established whether clients keep their animals for practical, utility or economical reasons.

**Section 1:**

Personal care of animals by clients with reference to the basic, as well as the special needs of the animal. The special needs include veterinary services and a balanced diet.

**Section 2:**

Protection and practical reasons why clients keep companion animals. These 3 questions were directly related to the needs of clients to keep companion animals as a link with nature.

The total, mainly positive feeling about each aspect, was expressed as a percentage. In order to calculate these percentages, the number of unanswered questions were subtracted from the questions answered in each section. The percentages for each aspect were indicated in tables in the same order as the sections of the results. The positive feeling with regard to the questions of each section was indicated from the highest to the lowest by means of a percentage.

**RESULTS**

Six hundred and twelve completed questionnaires were returned.

The results were divided into 2 categories, namely the psychological and the natural reasons for keeping companion animals. The sub-divisions of the categories are represented in Tables 1-12.

**DISCUSSION**

Consulting clients were asked how they were involved with their animals, without being directly asked their reasons for visiting the veterinarian. The involvement with their animals also included, amongst others, their consultations with the veterinarian. The veterinary consultation therefore forms part of the client's psychological involvement with the animal. The same reasons why clients keep animals can serve as motivation for visits to the veterinary surgeon, both directly and indirectly. This study therefore only assembled information specifically from consulting veterinary clients and not only from animal owners in general. This indirect method was employed due to the following considerations:

**Client**

If the client should be directly confronted with a view to classifying his visit to the veterinarian, he would most probably stress his natural care of the companion animal, either consciously or subconsciously. The reasons are the following:

- 1) Society still expects veterinary clients to visit the veterinarian only for reasons pertaining to illnesses of and injuries to their animals.
- 2) Social prejudices against classifying a visit to a veterinarian as a psychological visit, could motivate clients to deny such reasons.
- 3) Clients who visit a veterinarian for psychological reasons may not be capable of actually giving

- psychological reasons for the visit.
- 4) Clients can rationalise psychological reasons for visiting the veterinarian to non-psychological reasons.

### Veterinarian

If the veterinarian's view of his clients were elicited, his view might be too subjective.

- 1) A veterinarian is not qualified to judge his clients' emotions scientifically. Certain cases may possibly be easily identifiable, but the nuances in clients' feelings could only be supplied by the clients themselves.
- 2) There are veterinarians who are totally unaware of and uninformed about the psychological aspects of their practice, or who attempt to deny their existence.
- 3) The views of veterinarians, who are not psychological experts, cannot be compared, whereas the emotions of clients can be repeatedly evaluated by means of random samples and are therefore comparable.
- 4) If the information should be supplied by the veterinarian, it would be even more indirect than if received from clients themselves by means of indirect questions.

The method which was employed thus holds the following advantages:

- 1) the questions were put directly to the clients;
- 2) the information was gathered in the consulting room which proves the veterinary involvement of the clients;
- 3) the study is controllable and can be repeated if the random sample is large enough and if the same questions are used to the same investigative end;
- 4) objective results could be obtained as the test was conducted country-wide, voluntarily, anonymously and at random.

### Psychological considerations

The positive feelings of veterinary clients towards their animals include the friendship of the animal, the utilisation of the animal as an object of love and the understanding and the acceptance of man by the animal. Other researchers also referred to the importance of friendship between humans and their companion animals<sup>27 32 33</sup>. Katcher<sup>33</sup> similarly indicated how companion animals can be used as objects to be touched and pampered. The positive value of companion animals with regard to understanding and acceptance of man, was also highlighted by other studies<sup>11 12 22</sup>.

Other predominantly positive feelings regarding the clients' involvement with their animals, are the satisfaction of

**Table 7: Emotional involvement of veterinary clients with their companion animals (n = 584)**

Reasons for keeping companion animals		Mainly positive feelings for keeping companion animals &
1	For the sake of friendship	80
2	For the purpose of love	80
3	Because the animal understands and accepts man	76
4	Because the animal needs a caretaker	68
5	For greater personal appreciation	66
6	Because the animal is so attractive	65
7	For the tranquility the animal provides	62
8	To gain greater personal significance	57
9	To forget about other troubles	43
10	To relieve frustrations	34
11	Out of mere pity	30
12	Due to childhood memories	20
13	To compensate for other problems	19

needs related to the nurturing, ego-stimulation and acceptance, aesthetic appreciation and relief of tension<sup>10 19 20 21 45</sup>. Katcher<sup>33</sup> is also of the opinion that man's need to take care of others is satisfied by keeping companion animals, as is the experience of the aesthetic value of the animal.

Even though 62% of the clients were positive about the fact that animals contribute to relief of tension, it should be borne in mind that satisfying all emotional needs may lead to a reduction in stress. The relatively low percentages should therefore rather be viewed in the light of clients' recognition of animals as objects which can relieve tension. In reality, all the psychological advantages of keeping companion animals can lead to tension reduction<sup>8 9 24 32 34 37 44 48 51 56</sup>.

Fewer than 50% of all clients felt positive about keeping companion animals for purposes of relieving frustrations, or about keeping animals for reasons of compassion, or because of childhood memories or to compensate for problem situations. Serpell<sup>53</sup> indicated that primitive cultures kept companion animals to relieve their frustrations, by using dogs as "scapegoats". This type of tension-reduction should not be viewed in a positive light, as it does indeed indicate negative behaviour. It is interesting to note how many veterinary clients provide this as a reason for keeping companion animals. One should take note of the fact that the question was not whether the animal frustrated the client. This means that the animal is used to relieve frustrations other than those brought about by the animal itself. The use of animals for relieving frustrations can also be described in terms of a triangular effect, which implies that the aggressive behaviour or frustration is

displaced to a third party, or that communication which is motivated by the frustration, takes place via a third party. Cain<sup>15</sup> indicated in her study that 48% of companion animal owners are able to name examples in which their animals were used in a triangular situation to relieve tension. Salmon et al<sup>50</sup> also referred to the triangular effect of which an animal is part and which led to tension-reduction amongst the personnel of an institution by the utilisation of a dog.

Messent<sup>46</sup> indicated that animals and children have inborn reaction mechanism which draw universal attention. The so-called "cute response" and "oh shame response" probably underlie this fact. Only 30% of all clients, however, indicated a positive "oh shame" feeling as a reason for keeping animals.

Levinson<sup>43</sup> indicated that companion animals exert an important influence during a person's youth. Nevertheless, memories of youth are not regarded as an important reason for keeping animals during adulthood. The number of clients who do actually subconsciously keep animals due to positive memories of youth has however, not yet, been established. Similarly, companion animals are not kept by the majority of clients to consciously compensate for other problems or in order to forget about troubles.

Veterinary clients have an overwhelmingly positive feeling as regards a general love of animals. It should be noted that this statement does not necessarily pertain to the client's own animal. The 3% who are negative about this fact, could for instance possibly include a client who brings an animal belonging to another member of the family in for a consultation, or the client may be so attached to his animal that other animals are not important to him,

**Table 8: Social involvement of veterinary clients with their companion animals (n = 584)**

Reasons for keeping companion animals	Mainly positive feelings as reason for keeping companion animals %
1 Love for animals	97
2 For the sake of friendship	86
3 To compensate for loneliness	38
4 Due to the status the animal affords	29
5 In order to make contact with other people	24
6 Because other people keep animals	9

or a cat lover may for instance be a hater of dogs which may cause the client to feel that he does not experience a love of animals in general. The positive feelings regarding animals in general may be an identification of the need to include companion animals in man's social system.

The company provided by animals is also very important to veterinary clients. The company of animals is an important aspect of man's social intercommunication as is confirmed by various researchers<sup>12 19 26 32 34 38 44 45 50</sup>. It can therefore be understood that the animals which nowadays are most closely associated with man, are known as companion animals.

One would expect that there would be some relationship between the reasons for keeping animals to compensate for loneliness and for those for reasons of friendship and companionship. This is, however, not the case, since people who experience loneliness should perhaps rather be regarded as true "loners". Clients who have other friendships and companionships would, in any case, not have answered the question on loneliness positively, since animals only provide supplementary friendship and companionship on occasion. One can therefore rather believe that 38% of the veterinarian's clients really experience loneliness. The 10% of clients who feel extraordinarily strongly about this

aspect (scale 5 of reason 4 in Table 2), are presumably introverted solitary people. In the light of this interpretation, it appears that the clients who answered predominantly positively, represent a relatively high number of respondents. Keeping companion animals to relieve loneliness, as indicated by this investigation, is in line with studies<sup>1 22 44 45</sup> undertaken in other countries.

Even though clients do not generally acquire animals for the purpose of status symbols, the approximately 30% of clients who expressed this opinion with regard to their animals, represent a significant group.

Other researchers indicated that companion animals contribute towards communication and contact between people<sup>11 19 20 32 42 46 56</sup>. Almost a quarter of the veterinary clients in this investigation also indicated that they keep companion animals with the express purpose of establishing contact with other people. Clients who belong to social clubs where animals are involved might well have supplied this as a reason for keeping animals.

It appears that clients seldom keep animals in order to be popular or to be in fashion. Fewer than 10% of clients regard the fact that others keep animals as a reason why they should do so too. The influence of animal heroes, or human heroes who keep certain animals and appear in the media, most probably

form part of this percentage. People who like keeping exotic animals could be the clients who follow animal fashions. The large number of different breeds of animals which are available nowadays makes it more difficult to identify specific fashions.

Relaxed play and utilisation of leisure time are not necessarily the same concepts. Relaxed play with an animal is regarded as an unplanned activity which usually forms part of normal, friendly interaction. It is logical that the humour which is experienced with companion animals and which is highly regarded by some researchers, would also fit into this category. Other studies have also confirmed these advantages of keeping companion animals<sup>20 33 37 43 45</sup>.

Utilisation of leisure time is regarded as planned relaxation which is consciously decided upon beforehand. The animal is kept to occupy a person's leisure time and that can include participation in training, shows and obedience tests. Even a planned walk with a dog every day after work can be regarded as utilisation of leisure time. The positive use of leisure time with companion animals is also confirmed by various studies<sup>10 20 27 33 35 38 44 47</sup>.

Humour is also under consideration when the entertainment value of companion animals is studied. Nearly half of the clients in this study were positive about entertainment as a reason for keeping their animals. If entertainment is related to clients' attitudes towards play, the percentage is relatively low. This may possibly be attributed to the fact that clients might have interpreted "entertainment" as tricks which the animals should be able to perform. If this deduction is correct, the 44% who felt strongly about it, could be regarded as a relatively high percentage.

It should be borne in mind that the questionnaires were completed by clients who keep all types of companion animals. Only dog owners usually use their animals to accompany them during physical activity. In the light of this statement, the 31% who responded positively would represent a significant figure. Other studies have also stressed the physical exercise which animals can provide<sup>1 13 33 37 45</sup>.

#### Natural considerations

If what is natural is regarded as that aspect of life which is not created or conceived of by man, the natural considerations for keeping companion animals would concern the basic care of the animal in order to keep it healthy and alive (basic needs) and also utilisation of the natural characteristics and instincts of animals. The economical aspect also refers to the value of animals as

**Table 9: The contribution of companion animals to the relaxation of veterinary clients (n = 569)**

Reasons for keeping companion animals	Mainly positive feelings for keeping companion animals %
1 To play with	87
2 For utilisation of leisure time	67
3 Merely for entertainment	44
4 For physical exercise	31

**Table 10: Regular and basic personal care of companion animals by veterinary clients (n = 590)**

Means of caretaking		Yes answers in %
1	Adequate shelter	98
2	Food and water	96
3	Regular basic veterinary services	95
4	Balanced diet	4

natural products for utilisation by man. Natural considerations for keeping animals can also be related to man's wider interest in nature.

The high percentage of clients (between 95% and 98%) who indicated that they apply basic care, was to be expected amongst veterinary clients. One should therefore rather attempt to explain the negative answers. The clients who were of the opinion that they did not provide adequate shelter, most probably had watchdogs in the backyard who did not have special kennels or similar places of shelter. Clients who answered the question on the provision of food in the negative, were probably not the primary owners who provide food and water themselves. It might also be a case of clients assigning other people to provide food and in yet other cases, clients may anthropomorphistically think that they do not provide food regularly enough. Five percent of all clients also felt that they did not provide their animals with regular veterinary services, even though they were actually at the consulting room at the time of completing the questionnaire. This may be an indication of guilt feelings amongst clients, as they may feel that they are not doing for their animals what is expected by the veterinarian. It may also point to the fact that the client is not necessarily the primary owner, a fact which the veterinarian should always bear in mind.

The high percentage of negative answers by clients with regard to sup-

plying a balanced diet for their animals, was interesting. Notwithstanding the fact that a variety of balanced commercial animal foods was used and notwithstanding the fact that the greater majority of animal patients which were examined by veterinarians were in an excellent condition, the clients seemed to be of the opinion that their animals' diets were not balanced. A possible explanation for this fact may be found in the perception regarding the importance of balanced diets for humans, which is also stressed in the media. Since veterinary clients generally have insufficient knowledge about animal feeding, they may be of the opinion that they are falling short in this area. Guilt feelings about "forbidden" foods in-between meals which are shared with the client, may also play a role. This answer therefore represents a perception rather than a fact.

The 27% of clients who felt very strongly indeed (scale 5 of reason 1 in Table 5) about the personal protection which their animals provided them, may possibly be the owners of watch or alarm dogs. The remaining 44% (scale 3 of reason 1 in Table 5) who did not feel as strongly about this aspect, possibly live with the expectation that the presence of the companion animal could possibly contribute towards protection, even if it only meant that the person involved was not completely alone. Even cage birds are able to create an alarm.

It appears that clients do not regard the control of pests as an important

reason for keeping companion animals. The fact of the matter is that most of the clients in the investigation were from residential areas and pests are less of a problem in cities than in the country. Clients did feel positive about this aspect of companion animals which was a perk, since it was not the primary reason for their keeping the animals. Protection and pest control are 2 of the most ancient natural reasons for keeping companion animals. In both of these cases, the animals' inborn instincts are utilised to be of service to man.

The overwhelmingly negative feeling amongst modern veterinary clients regarding the keeping of companion animals for reasons of making monetary profit, should be taken cognisance of. This fact is possibly an indication of the emotional bonding between client and pet and the psychological value of the animal for the client which is thus indicated here in a different way. Serpell<sup>52</sup> discovered a negative correlation between the affection between humans and their companion animals and the economical utilisation of animals on the other hand. Low economical utilisation therefore indicates great affection. This implies that the veterinary client may sometimes be prepared to spend large amounts of money on veterinary services without the possibility of deriving financial benefits from it at some later stage. These attitudes can be contrasted to those of veterinary clients who keep production animals which are almost totally geared towards the making of financial profit from the animals. The 8% of clients who do keep companion animals for financial reasons are most probably breeders of companion animals.

Veterinary clients display a particularly positive interest in animals per se. As regards the 6% of clients who felt negative about this aspect, the reasons could for instance be that the primary owners were not necessarily the veterinary clients. Clients who keep animals merely for economical reasons or for protection purposes, may possibly also be negative with regard to a general interest in animals.

Sixty eight percent of the clients were positive that their companion animals brought them into closer contact with nature. Only 18% felt decidedly negative about this aspect (scale 1 of reason 3 in Table 6.) Some companion animals, however, live such sophisticated lives, so far removed from nature, that in fact the owners experience the animals as part of their technological urban surroundings. The actions of man and his companion animal, the intensive domestication and breeding of exotic species,

**Table 11: Practical usefulness of companion animals to veterinary clients (n = 574)**

Reasons for keeping companion animals		Mainly positive feelings for keeping companion animals %
1	Personal protection	71
2	For pest control	16
3	Financial gain	8

**Table 12: Interest in biology as a reason for veterinary clients to keep companion animals (n = 578)**

Reasons for keeping companion animals		Mainly positive feelings for keeping companion animals %
1	General interest in animals	94
2	Due to their link with nature	68
3	Educational value	54

and the psychosocial bonding between a person and his pet could cause clients to start believing that the companion animals of today represent culture rather than nature. Even though humans may regard animals as cultural objects and treat them accordingly, animals do however, remain biological products, representative of that which is natural.

The educational value to which the question in this section refers, has a bearing on the biological-educational value of keeping companion animals. An interest in animals should be regarded in the same light as an interest in the broader field of biology (as part of nature). The educational value of animals only motivates approximately half of all clients to keep companion animals. The educational value should be regarded as being educational in terms of a knowledge of biology. The relatively low percentage may possibly be explained in the light of the fact that only clients with young children appear to regard the education value of keeping companion animals in this light. Other studies have also indicated the advantages for children of keeping companion animals with regard to biology in general<sup>10 16 26 43 44 49 54</sup>.

On the basis of the findings of this study, it appears that veterinary clients are positively motivated to become involved with companion animals for psychological as well as for natural reasons. The conclusion can be drawn that such involvement of veterinary clients with their animals is necessarily present in the consulting room of the veterinarian.

Two categories of veterinary consultations can therefore be distinguished.

#### **Psychological consultations**

The first category of veterinary consultations is concerned with the human aspects of a veterinarian's consultations. The veterinary client's emotions are as much of a reality as the problems of the animal which is presented for examination. The fact that the client's interests are served primarily, is mirrored in the

fact that it is the client who decides to make use of veterinary services, whether the treatment is satisfactory, whether the client will again make use of the services or whether the services of the veterinarian will be recommended to others. The client's reasons for keeping a companion animal form part of the reason why he consults the veterinarian. The value which the client attributes to his animal will determine to him whether veterinary services are justified. The animal's value is not only economical, practical or utilitarian, but also emotional and sentimental. The emotional involvement of veterinary clients with their animals, as indicated in this study, can therefore serve as a motivation for clients to consult the veterinarian. This means that, if a client should keep an animal for psychological reasons, his involvement with his pet would automatically include a visit to the veterinarian. In other words, clients may visit the veterinarian for their own psychological reasons.

It can thus be deduced from the study that people often keep companion animals primarily to satisfy their own psychological needs and consequently not necessarily for the well-being of the animal. Furthermore a veterinarian delivers a professional service which is aimed at serving fellow-men and society<sup>30 31</sup>. If these facts are considered, it becomes clear that the animal patient can become of lesser importance during a consultation. The focus shifts to the human needs whereas the animal forms part of the background to the consultation. Psychological consultations can therefore be described as predominantly human-psychological in nature, rather than animal-clinical in nature. Modern society which generates tension due to the fast tempo and competitive element, has different ways of dealing with and reducing tension. The foregoing study indicates that, due to their emotional support and significance, companion animals are used by modern man for stress reduction or satisfaction of needs. Veterinarians should categorise their clients' consultations in the light of this

role played by companion animals. This means that the veterinarian will seriously have to re-evaluate his manner of consultation in order to be able to provide a more meaningful service to his clients on a psychological level. The veterinarian will also have to provide his clients with an opportunity to use their animals for purposes of tension reduction in the consulting room. He will have to provide his clients with an opportunity to explain their problems in order to establish the nature of their specific problems.

According to Case<sup>17</sup>, human and companion animal relationships are particularly complex and veterinarians should be aware of problems in handling both the client and the companion animal in such a relationship. Effective communication with the client is the key to recognising the relationship between the human and his companion animal. Case<sup>17</sup> further states that owners of companion animals are able to live their lives through the lives of their animals. This takes place when the person subconsciously sees the animal as an extension of his own ideas. Such owners harbour fantasies about their animals and if these fantasies should come into conflict with reality, such animals could experience problems. Austin<sup>6</sup> is of the opinion that veterinarians should become more involved in the interaction between clients and their companion animals, as well as in the impact of such interaction on society. In order to be able to do this, veterinarians would have to be able to identify the emotional needs of their clients. Fox<sup>23</sup> states it as a fact that cats and dogs in particular take on the emotions and temperaments of their owners. The close bond between client and companion animal can therefore influence the behaviour of both parties. The clients use and influence their animals in such a way that the behaviour of the client is reflected in that of their animals<sup>39 55</sup>. It is also important that the veterinarian regard the animal which he is required to handle in relation to its owner, as the choice of a particular companion animal often reveals personal characteristics of the clients. Antelyes<sup>3</sup> is also of the opinion that veterinarians should be sensitive to the fact that the self-image of the owner is often strengthened by his companion animal.

The role and contribution of the veterinarian to the psychological aspect of his practice is most certainly not limited to outspokenly neurotic clients as is sometimes believed. Clients often use subtle ways to justify visits to the veterinarian. Antelyes<sup>4</sup> believes that companion animal ownership has deep



psychological implications and that the veterinarian who understands the emotional implications of the relationship between man and his companion animal, will be able to handle both the emotions of the client as well as the health of the patient successfully.

Antelyes<sup>5</sup> is also of the opinion that a visit to the consulting room of the veterinarian should always be regarded as a therapeutic interaction between the patient, the client and the veterinarian. It follows that reasons for keeping animals are as important as reasons for visiting the veterinarian, which means that the animal may never be dealt with separately from its owner in the consulting room.

Veterinarians amongst themselves often refer to so-called "neurotic clients" when clients continually make use of psychological consultations. One should, however, bear in mind that all people are not able to reduce tension equally well, that some owners of companion animals necessarily make greater use of their animals for this purpose and that consequently they consult the veterinarian more often. This may burden the veterinarian with a load which may be difficult to handle. Antelyes<sup>2</sup> states unequivocally that the veterinarian should, in a sense, play a supportive role in handling the relationship between people and their companion animals, as the animal is often an emotional outlet for the owner. The veterinarian should therefore not pass moral judgment on his clients.

Case<sup>18</sup> found in a study that the client's satisfaction during veterinary consultation indicated a higher correlation with how the owner was treated than with the treatment of the animal. She also indicated the importance of communication and rapport which the veterinarian should establish with the client in order to run a successful practice. The expectations of female clients appear to be higher than those of male clients. Clients should therefore be regarded as much as "patients" as the animals themselves.

Even though the psychological aspect of veterinary practice is experienced as a shock by some veterinarians and even though this may lead to grave irritation, the veterinarian will have to take it into consideration as it forms a substantial part of his practice. A veterinarian who regards this aspect of his practice as of lesser importance, who tries to ignore it, or who is unable to identify it, is not able to deliver his complete and legitimate service to the community. Such a veterinarian may not be able to comprehend that his client is completely human, and he may therefore only

associate the client with the person who pays the account, whilst only being aware of the needs of the animal under consultation. Veterinarians who attempt to disregard the human aspects of their practices, will find that their practices will suffer as a result of it. Clients who need their animals for purposes of tension-reduction, also require the aid of the veterinarian and if the consultation does not satisfy the needs of the client, other ways or another veterinarians will be found so that these needs can be satisfied. Veterinarians should also be aware that they could possibly increase their clients' tension due to a lack of understanding of their needs. This should not happen, particularly as clients may visit veterinarians for the opposite purpose. The sensitive handling of the client's emotional needs may contribute to more meaningful communication and success in the practice. On the other hand, one should realise that the consulting room of the veterinarian is not the same as that of the psychiatrist or the clinical psychologist. The veterinarian should be able to identify his clients' problems and he should be able to display understanding, but he should also be aware of his limitations. Serious cases should definitely be referred to specialists. As in the case with zoonoses where effective interdisciplinary co-operation between veterinarian and medical doctor should exist, effective communication between the veterinarian and the mental health disciplines should be established.

The veterinarian's own supportive contribution to the mental health of society (or rather the psychological aspect of his practice) should, however, not be underestimated. In the same way that patients of medical practitioners often visit the doctor due to psychological reasons, the veterinary practitioner will also be consulted by some of his clients for psychological reasons, even though the animal is used as a pretext. Geldenhuys & Du Toit<sup>25</sup> are of the opinion that two-thirds of all cases dealt with by medical practitioners are motivated by emotional or psychological factors. They state that later investigations even place the percentage higher and that there has been a progressive increase in psychosomatic illnesses in the Western world during the last few decades. It might even occur that some people prefer to visit a veterinarian rather than a medical doctor to this end, since a veterinary consultation is less personal and direct. The veterinary practitioner does not examine the client, but the animal, who is the substitute. According to Barnard & Illman<sup>7</sup> children are also used by parents as

substitutes in human medical practice, in order for the parents to express their own problems.

Levinson<sup>40</sup> states that companion animals reflect the emotional disturbances of human beings. The animals act as therapeutic aids which are able to promote emotional health. Man has neurotic as well as healthy needs and as time goes by, companion animals will be used to both ends.

The veterinarian can no longer only limit himself to promoting the physical health of companion animals. He should also be involved in the mental health of the family who owns the animal which he is treating.

It is obvious that veterinary training will have to pay greater attention to the human aspect of veterinary practice in future. A knowledge of basic human behaviour is required to fulfil this need.

### Clinical consultations

The consultations in the second category are concerned with the natural care of the animals belonging to clients. This stems from the needs of clients to establish contact with nature. One could say that the client tends, supports and protects his or her own small part of nature. The client realises that the animal, which is a biological product, has certain needs, and that he has accepted responsibility for these needs. This need to keep animals and to take care of them was most probably strengthened by the process of urbanisation which caused man's link with nature to be broken. Clinical consultations are therefore motivated by the treatment of obvious illnesses and injuries of animals, or for the prevention thereof. The focus is on the needs of the animal and the animal is therefore on the foreground. One can also describe such a consultation as being rather more animal-clinically orientated than human-psychologically orientated. This category of consultations represents the stereotype perception of veterinary services. The companion animal veterinarian is properly qualified to look after the health of animals and it forms an important part of his daily work. The human aspects which are manifested in the consulting room of the veterinarian are often underestimated and denied because of the veterinarian's proper training and the time which is spent on animals. If it should, however, be true that the absence of animals would mean that a veterinary practice cannot exist, the same would certainly apply to an absence of clients.

The interest of the client and patient are in no way placed in opposition to one another. Rather, the stress on the

human aspect was introduced with a view to striving towards a more balanced system in the consulting room. At present the human aspects most probably do not receive enough attention in the veterinary consulting room. If all attention should be given to the animal, whereas it may be the client who is in need of attention, the productivity of the veterinarian could be seriously harmed.

### Interaction between categories and consultations

It is not always possible to distinguish clearly between the two categories of consultations. The same client's visits may fall into different categories on different occasions. The client who usually consults the veterinarian for psychological reasons will also present the animal for natural care if the animal should sustain injuries or contract an illness. The client who normally presents his animal for outspoken problems may also at times consult the veterinarian for psychological reasons.

The same visit may also exhibit elements of both categories. Even though it may be easy to identify outspoken cases, a veterinarian may be largely unaware of this continual ambivalence in his practice if he is not on the lookout for it. It is important that the veterinarian should always be aware of his client's needs and that he should be sympathetic towards both categories of consultations. The companion animal veterinarian will have to fulfil both the roles of treating animals clinically as well as identifying human needs. His aid to animals is as important as his supportive aid to clients.

Eventually it will mean that the companion animal veterinarian, with his clinical skills as well as his successful handling of the psychological aspects of his practice, will be able to employ his full potential in order to provide an optimum service to society<sup>14</sup>.

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#### Journal review/Tydskrif resensie

### PHARMACOKINETICS OF THERAPEUTIC SUBSTANCES IN RACEHORSES

Australian Equine Veterinarian, Volume 7, Supplement 1, 1989, pp 54 (ISSN 1032-6626)  
Price \$A 39.00

Perhaps the most difficult question in equine medicine is the problem of 'detection times' for drugs in race horses. Racing authorities would like horses to run 'clear' of drugs, which means that analysts should not detect any drug traces in samples taken from race horses. On the other hand, veterinarians need to treat horses with appropriate medications prior to racing, in order to optimise their health and well-being. The interface between these needs, is an area of uncertainty that has bedevilled racetrack practice for most of this century and which daily grows more complicated.

The answers to these questions require specific detailed information about the disposition and elimination of drugs in race horses. Unfortunately, for many drugs, little or no useful information exists. For others, the information is buried in the analytical records of drug testing and research laboratories. Only a proportion of this data has been published and even published data is difficult to locate and evaluate. What has been sorely needed is a comprehensive review of the available literature on drug disposition in race horses.

The first such comprehensive review has just been released by Dr. Timothy Dyke, working under the aegis of the Australian Equine Veterinary Association. In a Supplement to the 'Australian Equine Veterinarian' entitled 'The Pharmacokinetics of Therapeutic Substances in Racehorses' Dr. Dyke has exhaustively collected, collated and reviewed the available literature on the disposition of drugs in horses. This review includes formulation information, information on the dose of drugs used, the breed of horse, the number of horses in the experiment, the analytical technique used, and, most importantly the 'detection time' for that particular drug in that particular experiment. Additionally, all pertinent references are included in the bibliography, and the reader is strongly encouraged to read the source material before arriving at any conclusions.

Equally important, in the introduction to this material, Dr. Dyke points out carefully the limitations of this approach. The roles of individual variability, variations between different drug preparations, the effect of dose, the effects of urinary volume and pH, and the roles of different analytical methods are emphasised as variables that must be taken into account when estimating 'detection times'. These factors, poorly understood by laymen, introduce a large measure of uncertainty into the times for which drugs may be detected in the blood and urine of race horses, but these factors must be taken into account when estimating 'detection times' for drugs in horses.

Dr. Dyke and the Australian Equine Veterinary Association are to be congratulated on their leadership in developing the most comprehensive effort to date in this area. This review is required reading for equine veterinarians who administer drugs to race horses anywhere in the world. This reviewer would like to strongly support their efforts, and offers the hope that this booklet will be continually updated. Additionally, there is an urgent need for an available computerised base of such information to inform practitioners of the scope of analytical chemistry and thus avoid inadvertent breakage of the rules of racing. Based on his head-start in this area, Dr. Dyke is an obvious first choice to further develop this approach.

My congratulations to Dr. Dyke and the Australian Equine Veterinary Association on their timely, exhaustive and imaginative approach to this serious problem.

THOMAS TOBIN

# TREATMENT OF CEREBRAL COENURIOSIS IN SHEEP WITH PRAZIQUANTEL

ANNA VERSTER\* and R C TUSTIN\*\*

## ABSTRACT

Sheep ( $n = 14$ ) with coenuriosis were treated with praziquantel in single or divided doses at total dosage rates of 50 to 500 mg kg<sup>-1</sup> live mass. Nine of the sheep also received corticosteroid, diuretic and analgesic supportive therapy. Stress was avoided as far as practically possible. Treatment of one sheep with acute coenuriosis at a total dosage rate of 500 mg kg<sup>-1</sup> praziquantel, was unsuccessful. Eight sheep were successfully treated at total dosages ranging from 100 to 500 mg kg<sup>-1</sup> live mass, whereas dosages of 50 mg kg<sup>-1</sup> were successful in 2 sheep only. Dosages of 50 mg kg<sup>-1</sup> in 2 other sheep with multiple cerebral coenuri, were unsuccessful or only partially successful in killing parasites.

Key words: Sheep, cerebral coenuriosis, praziquantel.

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## INTRODUCTION

The treatment of sheep infested with the larval stage of *Taenia multiceps* has, until relatively recently, always posed a problem. Not only was there no specific therapeutic agent available which would kill the parasite in the central nervous system, but surgical intervention was invariably unsuccessful in those animals which warranted such expensive treatment<sup>6</sup>.

Sheep with the acute disease, usually show clinical signs within 12 to 21 d of infestation. Anorexia, depression and a disinclination to move are commonly observed initially. Sheep often stand with their heads lowered and may appear blind and deaf or may wander aimlessly with a stumbling gait. In the later stages of the disease, the animals may lie down, often with their heads tucked in at their flanks. In chronic infestations, the neurological signs include circling, blindness and anorexia (Tustin & Verster, 1961, unpublished observations).

In a pilot experiment<sup>7</sup>, the efficacy of

the drug praziquantel was determined at total dosage rates of 200, 250 and 500 mg kg<sup>-1</sup>. At these dosages, the cost of treatment would probably preclude its use as a routine therapeutic agent. The purpose of this trial was to determine whether lower dosages of praziquantel would be equally effective against the parasite.

The results of both the pilot and the subsequent trials are presented here.

## MATERIALS AND METHODS

A total of 14 sheep were used. Two Merino sheep were natural cases. Dorper sheep ( $n = 12$ ) of 3 to 14 months of age were experimentally infested with a strain of *T. multiceps* originating from a naturally infested sheep from Mossel Bay and maintained at the Veterinary Research Institute, Onderstepoort, by passage through dogs and Dorper sheep.

Three different formulations of praziquantel were obtained from the supplier (Bayer SA): a) 50 mg tablets (Droncit) which were finely ground, mixed with water and the mixture dosed via a stomach tube directly into the rumen; b) a 5% (mass/volume) suspension of the drug which was also dosed by means of a stomach tube; c) 600 mg tablets (Biltricide) which were dosed by means of a balling gun.

Sheep No. 1 was infested with 50 000

eggs of *T. multiceps* to assess the efficacy of the drug in the treatment of the acute disease. With the exception of Sheep 9 and 14 which were naturally infested, the remaining 11 sheep were dosed with 5000 eggs of the parasite and drafted into the experiment when they showed clinical signs of infestation. The details of the treatment regimen of the 14 animals with praziquantel and the duration of their infestations when treatment was commenced, are summarised in Table 1. Five sheep (No. 2, 3, 4, 5 and 10) were treated with praziquantel only; the remaining 9 animals were given simultaneous supportive treatment of betamethasone (Betsolan, Glaxo) or dexamethasone, and prednisolone (Opticortenol, Ciba-Geigy), furosemide (Lasix, Hoechst) and acetyl salicylate (Aspirin, Bayer SA) as summarised in Table 2.

The sheep that did not respond to treatment were necropsied when they died. Sheep 2, 3, 5 and 10 were euthanased and necropsied 28-30 d after praziquantel administration had ceased; the remaining sheep were euthanased after longer intervals (>3 months) to determine whether the coenuri would commence growing again or if they had been resorbed.

To determine their viability, the coenuri recovered from Sheep 2, 4 and 12, were fed to dogs known to be free of cestodes, which were euthanased, necropsied and examined for cestodes 28 d after infestation.

## RESULTS

### Acute coenuriosis

Sheep 1 showed clinical signs of acute coenuriosis 17 d after infestation and was treated with 100 mg kg<sup>-1</sup> praziquantel for 5 d, as well as receiving the supportive treatment listed in Table 2 for 5 d. Its condition deteriorated and it was euthanased and necropsied on Day 21 post infestation. There were numerous migratory tracts in the brain and other organs but no apparently live larvae could be found in the central nervous system.

### Chronic coenuriosis

The clinical signs of chronic coenuriosis exhibited by sheep that were treated with a total dose of 100 to 500 mg kg<sup>-1</sup> praziquantel (i.e. Sheep 2, 3, 5, 6, 7, 8, 9 and 10) disappeared, irrespective of

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Received: July 1989 Accepted: November 1989

**Table 1: Treatment of sheep with praziquantel and the viability of the coenuri of *Taenia multiceps***

Sheep No.	Age of infestation at treatment (months)	Dose of praziquantel		Supportive treatment	Period between treatment and necropsy (d)	Viability of coenuri	
		mg kg <sup>-1</sup>	Duration of treatment (d)				
1	0,5	100	5	500	+	21	Immature
2	6	100	5	500	-	28	Dead
3	7	100	5	500	-	28	Dead
4	7	100	2	200	-	2	Dead
5	11	100	2	200	-	28	Dead
6	2	100	1	100	+	90+	Dead
7	2	100	1	100	+	90+	Dead
8	2	100	1	100	+	90+	Dead
9	Unknown	100	1	100	+	90+	Dead
10	10	50	5	250	-	28	Dead
11	3	50	1	50	+	24	Viable
12	6	50	1	50	+	90	Some viable
13	6	50	1	50	+	90+	Dead
14	Unknown	50	1	50	+	90+	Dead

**Table 2: Supportive treatment of sheep**

Drug	Dose (mg kg <sup>-1</sup> )	Treatment
Corticosteroid	1,5	Daily for 5 d
Acetylsalicylate	100	Twice daily for 5 d
Furosemide	5	36 h after treatment with praziquantel commenced; at 12 to 24 hourly intervals as necessary for 5 d

whether the drug was administered in a single or divided doses over 1 to 5 d. Sheep 2, 3, 5 and 10 were necropsied 28 - 30 d after the last treatment, while the other 4 sheep were necropsied 3-6 months after treatment. None of these animals showed clinical signs of chronic coenuriasis after treatment.

Four sheep (No. 11, 12, 13 and 14) were treated with a single dose of praziquantel at 50 mg kg<sup>-1</sup> mass. Sheep 11 did not respond to treatment and was necropsied 24 d after treatment. Twelve viable coenuri were found in its cerebrum. Sheep 12 appeared to respond to treatment, but again showed clinical signs of infestation some 3 months later. At necropsy, multiple coenuri were found in the cerebrum and although the majority were dead, 4 of the coenuri appeared viable. These coenuri were fed to a dog and cestodes were recovered from it at necropsy, 28 d later. No viable coenuri were recovered from Sheep 13 and 14 at the time of necropsy.

The coenurus removed from the brain of Sheep 2 at necropsy 28 d after treat-

ment, was fed to a dog known to be free of cestodes; no tapeworms were recovered at necropsy of the dog 4 weeks later. Sheep 4 was found dead with its horn caught in the stable gate, 48 h after the first dose of 100 mg kg<sup>-1</sup> praziquantel had been administered. At necropsy, an apparently viable coenurus was present in the brain. The whole coenurus was fed to a dog which was necropsied 28 d later, but no cestodes were recovered, indicating that the parasite had been killed by the drug.

**DISCUSSION**

According to Eslami & Bazargani<sup>2</sup>, Bankov treated sheep with acute coenuriasis successfully with 2 doses of praziquantel at 50 mg kg<sup>-1</sup> live mass. In our trials, however, 5 treatments of 100 mg kg<sup>-1</sup> (i.e. a total dose of 500 mg kg<sup>-1</sup> live mass) did not prevent the death of a lamb (Sheep 1) which was suffering from acute coenuriasis. It has been reported that in chronic infestations in sheep, the parasite is killed by praziquantel at total dosages of 80 to 100 mg kg<sup>-1</sup> or more<sup>1 5</sup>, but Eslami & Bazargani<sup>2</sup>

found that "destruction of the parasite" did not prevent the death of the sheep. In our trials, however, sheep with chronic infestations, were successfully treated with praziquantel at doses of ≥ 100 mg kg<sup>-1</sup>.

When cysticerci of *Taenia solium* in humans die after treatment, there is an inflammatory reaction in the surrounding brain tissue, sometimes accompanied by an increase in intracranial pressure which may be controlled by the use of corticosteroids and diuretics<sup>3</sup>. Although this has not been proven experimentally in sheep, circumstantial evidence suggests that it might also be the case. Sheep 4, which did not receive supportive treatment, died 48 h after the initial praziquantel treatment. After the death of this animal, Sheep 1, 6, 7, 8, 9, 11, 12, 13 and 14 received supportive treatment (Table 2). A diuretic was administered 36 h after the first administration of praziquantel; this was repeated at 12 to 24 hourly intervals if the animals became excitable. In addition to corticosteroids, acetyl salicylate was administered for its anti-inflammatory action.

Praziquantel was administered to Sheep 1-5 as ground-up tablets, or a 5% solution by means of a stomach tube. The remaining 9 sheep were treated with the human formulation of praziquantel, i.e. "Biltricide" in the form of tablets containing 600 mg of the active ingredient, administered with a balling gun. This modification also reduced the stress of the treatment to the animals. When a naturally infested ram was treated, the stress to the animal was further reduced by administering Tomanol (Phenylbutazone + isopyrin, Byk Gulden) by injection (J Joubert, 1989; personal communication).



Treatment with praziquantel at 50 mg kg<sup>-1</sup> live mass, killed all the parasites in Sheep 13 and 14, but in Sheep 11 and 12, none or only some of the parasites were killed. Sheep 11 did not respond to treatment and at necropsy, 12 viable coenuri were present in the cerebrum. In Sheep 12, some of the coenuri were killed, but 4 remained viable. In trials on the treatment of sheep infested with cysticerci of *Taenia hydatigena*, Heath & Lawrence<sup>4</sup> found that when there were many parasites present, praziquantel at 50 mg kg<sup>-1</sup> did not kill all the metacestodes and they concluded that the efficacy of the drug at a given dosage was dependent on the biomass of the parasite. The coenuri of *T. multiceps*, like the cysticerci of *T. hydatigena*, are large, and when many are present, a

larger amount of active ingredient may be required to kill all the parasites.

These results indicate that praziquantel at a dosage of 100 mg kg<sup>-1</sup> kills metacestodes, but at a dosage of 50 mg kg<sup>-1</sup> does not kill all metacestodes when large numbers are present. Although praziquantel is expensive and treatment of flock animals is not economically viable, the cost involved is justified in the case of valuable animals.

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## PARASITES OF CATTLE IN THE SOUTH WESTERN ORANGE FREE STATE

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## ABSTRACT

A number of young Friesian oxen were slaughtered (one at a time) for parasite recovery for 12 consecutive months, from April 1987 to March 1988, on a farm in the Fauresmith district of the south western Orange Free State. A total of 5 ixodid and one argasid tick species, 2 lice species, plus one nematode species and one cestode species, were recovered.

Peak numbers of adult *Hyalomma marginatum rufipes* were present from November 1987 to February 1988, while the largest numbers of *Hyalomma truncatum* were recovered during September 1987 and from November 1987 to January 1988. Adult *Ixodes rubicundus* were recovered only during April and June 1987. Adult ticks of a *Rhipicephalus* sp. near *R. punctatus* were present during April and from September to November 1987 and in January 1988. No pattern of seasonal abundance was evident for the other tick species, the lice or the helminths.

Key words: Seasonal abundance, ixodid ticks, cattle, lice, nematodes

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## INTRODUCTION

One of the objectives of integrated pest management, is the maintenance of parasite populations below levels causing economic loss<sup>8</sup>. In order to determine economic thresholds of parasitism, production losses related to pest densities must be ascertained. The first step in such an approach should be the gathering of quantitative and qualitative data on the burdens of the economically important parasite species.

In the south western Orange Free State, cattle constitute a tertiary agricultural resource after woolled and non-woolled sheep. About 21 000 cattle are farmed in 6 districts (Fauresmith, Jagersfontein, Philippolis, Trompsburg, Bethulie and Edenburg) within this area. No data on the parasite burdens of cattle within this region have been published. This paper records the numbers of external and internal parasites recovered from free-ranging Friesian cattle run on a farm in the Fauresmith district.

## MATERIALS AND METHODS

## Study Area

Parasite collections were carried out on the farm "Preezfontein", which is situated 10 km from Fauresmith (29°46'S; 25°19'E) in the south western Orange Free State. A 72 ha camp, consisting of level as well as hilly areas, was chosen on this farm as the survey locality. The vegetation of this region is classified as False Upper Karoo<sup>1</sup>. The mean annual rainfall during the period 1980-86 was 360 mm, of which 69 percent fell during the summer. Due to the irregularity of the rainfall, mild to severe droughts occur periodically. During the study period, rainfall was measured at Fauresmith.

## Survey animals

Young Friesian oxen under 16 months of age were used as survey animals and were maintained at a constant stocking density of 1 animal/6 ha during the survey period (April 1987-March 1988). In order to pre-infest the survey camp, particularly with nematodes, the cattle were placed in it during November 1986, 5 months prior to the actual start of sampling. From the end of April 1987, one animal was slaughtered each month for 12 consecutive months. Each of these animals had been dosed with

the anthelmintic febantel (Rintal, Bayer, Animal Health) at 15 mg kg<sup>-1</sup> live mass one month prior to slaughter. This implied that the nematodes recovered at slaughter had been acquired in the survey camp only during the preceding month.

## Parasite recovery

After the collection of all easily visible ticks, one half of the skin covering the head and the neck, body and upper legs and one front and one back lower leg and foot was processed for ectoparasite recovery<sup>4</sup>. The lungs, mucosa of the abomasum and the contents of the abomasum, small and large intestines were processed for helminth recovery<sup>4</sup>. The parasite burdens of the animals were determined by examining the material collected, or in the case of the helminths representative samples, equivalent to 1/50 thereof, under a stereoscopic microscope<sup>4</sup>. The nematodes were identified, using a standard light microscope.

## RESULTS

The numbers of ectoparasites collected from each of the animals are summarised in Table 1.

Five species of ixodid ticks and one argasid tick were recovered. Four of the ixodid tick species exhibited fairly clear patterns of seasonal abundance. All the animals were infested with *Damalinia bovis* and some with *Linognathus vituli*.

Only 3 animals were infested with helminths. The bovines slaughtered during October and December 1987, harboured *Cooperia oncophora* (600, fourth stage larvae and 5700 adults; and 450 adults respectively). The scolices and strobila of 3 *Thysaniezia giardi* were recovered from the animal killed during February 1988.

## DISCUSSION

## Ticks

The adults of *Amblyomma marmoreum* prefer tortoises as hosts, but the immature stages are found on reptiles, birds and mammals<sup>7</sup>. Their presence on the cattle is thus not surprising.

The peak in seasonal abundance of *Hyalomma marginatum rufipes* on the cattle (November - February) is similar to that observed on Merino sheep (December - March) on the same farm during the previous year<sup>3</sup>. It also closely corresponds to that recorded on cattle in the northern Transvaal (December - February)<sup>6</sup>.

Although 20 sheep on "Preezfontein"

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Received: May 1989 Accepted: October 1989

**Table 1: Ectoparasites recovered from 12 Friesian cattle on the farm "Preezfontein" in the south western Orange Free State**

Month	Ixodid and argasid ticks										Lice			
	<i>Amblyomma marmoreum</i>	<i>Hyalomma marginatum rufipes</i>	<i>Hyalomma truncatum</i>	<i>Ixodes rubicundus</i>	<i>Rhipicephalus sp. (near R. punctatus)</i>	<i>Otobius megnini</i>	<i>Dama-bovis</i>	<i>Linog-nathus</i>	Rainfall (mm)					
	L	N	♂	♀	♂	♀	♂	♀	♂	♀	N	N + Ad	N + Ad	
April 1987	136		2				1	4	3	3	74	44685	2	10,6
May			4	1							12	138	12	1,0
June							2	4				771	30	11,3
July												5026	2	23,3
August												4227	65	14,9
September					3	3			1	5		606	5	88,7
October			3	3					2	3		2058	11	21,8
November			15	5	4	1			2	9		611	12	58,2
December			18	4	5	2					12	104	6	43,3
January 1988	2	4	30	2	1	4			2	2	120	12		12,1
February			29	3		1					1	209		394,2
March	8		3	1								15		89,1
<b>TOTALS</b>	<b>146</b>	<b>4</b>	<b>104</b>	<b>19</b>	<b>13</b>	<b>11</b>	<b>3</b>	<b>8</b>	<b>10</b>	<b>22</b>	<b>219</b>	<b>58462</b>	<b>145</b>	<b>768,5</b>

L = larvae N = nymphs Ad = adults

were examined for ticks each week from February 1986 to January 1987, a total of only 4 adult *Hyalomma truncatum* were recovered<sup>3</sup>. The 12 cattle examined in the present survey, harboured a total of 24 adult *H. truncatum*. The peak in seasonal abundance of these ticks (September, and November - January) was earlier than that observed on cattle in the northern Transvaal (January - March)<sup>6</sup>.

Only 2 of the survey cattle became infested with adults of *Ixodes rubicundus* during April and June. This falls within the period April - September during which *I. rubicundus* was present on the sheep on the farm in the previous year<sup>3</sup>.

The small number of ticks of this species recovered, could imply either that cattle are not suitable as hosts or that they did not utilise the hilly areas in their camp. Since *I. rubicundus* is confined mainly to rocky outcrops and hills<sup>9</sup>, its acquisition by the cattle would require that they frequent these areas.

During the previous year, adults of *Rhipicephalus* sp. (near *R. punctatus*) [then referred to as *Rhipicephalus* sp. (near *R. pravus*)] were recovered from sheep on "Preezfontein" mainly during the spring and the late summer months<sup>3</sup>. Although they were only present in small numbers on the cattle, their seasonal abundance corresponds to that seen on the sheep.

The most important host for the immature stages of this tick is the rock elephant shrew, *Elephantulus myurus*<sup>2</sup>. Hence it is reasonable to assume that the adult ticks will be confined to rocky outcrops and hills. The small number of ticks found on the cattle, means either that they are not suitable as hosts or that they did not utilise the areas in their camp where the ticks were concentrated. This tick has been incriminated as a cause of paralysis in Angora goats in the

south western Orange Free State<sup>2</sup>.

Only the larvae and the nymphs of *Otobius megnini* are parasitic and they are found in the outer ear canals of a variety of hosts<sup>5</sup>. Infestation is usually associated with kraals<sup>5</sup> and hence with hosts that are confined in these kraals for shorter or longer periods of time. The cattle in this survey were raised in kraals on the farm, from where they were introduced into the survey camp at least 3 months prior to slaughter. The immature stages of this tick spend several months in their hosts' ears<sup>5</sup> and we assume that the cattle acquired the infestation in the kraals in which they were raised.

All the cattle were infested with *D. bovis*, but no pattern of seasonal abundance was evident. Small numbers of *L. vituli* were recovered from the animals examined from April to December 1987.

The small numbers of helminths recovered and the low percentage of animals infested, is probably due to the fact that only 12 animals ran in the 72 ha camp at one time, and that the period of pre-contamination (5 months) was too short. The semi-arid nature of the region is also not conducive to the survival of the free-living stages of most helminths.

The period of exposure of one month is shorter than the developmental periods of both *Bunostomum phlebotomum* and *Oesophagostomum radiatum*. The immature stages of these worms, had they been present, may have been missed on examination of the intestinal contents.

#### ACKNOWLEDGEMENTS

We wish to thank Mr. J. van Niekerk, owner of the farm "Preezfontein", for the use of the survey camp and of the facilities on the farm. The technical assistance of Mrs. Santa Meyer and

Messrs. H. Muller and L. Barkhuizen is gratefully acknowledged.

This research was funded by the Department of Agriculture and Water Supply, the Council for Scientific and Industrial Research and Bayer (Animal Health).

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## BEE STING-INDUCED HAEMOLYSIS, SPHEROCYTOSIS AND NEURAL DYSFUNCTION IN THREE DOGS

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### ABSTRACT

Three dogs showed signs of intravascular haemolysis, spherocytosis and peripheral neuropathy following severe bee envenomation. These effects were ascribed to the constituents of bee venom, especially melittin and phospholipase A. All 3 cases recovered and were discharged subsequent to prednisolone and supportive therapy.

**Key words:** Bee sting, haemolysis, spherocytosis, neuropathy

Wysoke J.M.; Bland van-den Berg P.; Marshall C. **Bee sting-induced haemolysis, spherocytosis and neural dysfunction in three dogs.**

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### INTRODUCTION

Bee envenomation of dogs is a relatively common problem in veterinary practice. Most cases are single-sting phenomena, presenting with facial, aural and periorbital oedema<sup>5</sup>. Occasionally, laryngeal oedema and respiratory distress may be noted. Treatment with corticosteroids and antihistamines usually alleviates the clinical signs and rapid recovery ensues<sup>5</sup>. The animals in the cases described below, were subjected to numerous stings and presented with clinical signs not found in an extensive review of the veterinary literature.

### CASE 1

A 3-year-old male Cocker Spaniel was presented with a complaint of listlessness and anorexia. The dog, and 2 others, had been attacked by a swarm of bees 5 d prior to presentation, with this dog being the only survivor. Subsequent to the attack, the patient was treated with antihistamines and an unknown analgesic.

Clinical examination of the patient revealed marked depression, an elevated rectal temperature of 40,2°C and an in-

creased respiratory rate of 40 min<sup>-1</sup>. The mucous membranes were pale, with a prolonged capillary refill time and petechiation. Swelling and hyperaesthesia were evident over many parts of the body, especially the face and hindquarters. The mandibular and popliteal lymph nodes were enlarged. Ataxia of both hind limbs was evident as well as a bilateral facial paralysis. Sensory function (trigeminal nerve) appeared to be intact, but motor function (facial nerve) was depressed.

Routine urine analysis revealed no abnormality. Infestation with *Ancylostoma caninum* was detected by the identification of ova on a faecal flotation. Haematological examination indicated a poorly regenerative anaemia, neutrophilic leucocytosis with a left shift, monocytosis and a marked spherocytosis (Table 1). A direct Coombs test was negative.

A diagnosis of anaemia, spherocytosis and neurological dysfunction as a result of severe bee envenomation was made. The patient was maintained on a polyionic intravenous fluid (Balsol, Labethica), prednisolone sodium succinate (Solu Delta Cortef, Upjohn) at 4 mg kg<sup>-1</sup> was administered intravenously and amoxycillin (Clamoxyl, Beecham Pharmaceuticals) at 20 mg kg<sup>-1</sup> was administered subcutaneously. By the next day, the patient showed marked clinical improvement.

Treatment on Day 2 was continued with the oral administration of amoxycillin at 20 mg kg<sup>-1</sup> twice daily and prednisolone (Prednisolone, Centaur Laboratories) at 1 mg kg<sup>-1</sup> once daily. From Days 3-8, amoxycillin was given as before with prednisolone at 0,5 mg kg<sup>-1</sup> once daily. Hindquarter ataxia improved by Day 3, while facial twitching in response to pinpricks was noticed on Day 5. Sequential haematological examination during the treatment period, showed marked erythrocyte regeneration and an improvement in the red cell parameters at the time of discharge, continued neutrophilic leucocytosis with a left shift, persistent monocytosis and decreasing spherocytosis (Table 1).

The dog was discharged in apparent good health on Day 9, with instructions to administer prednisolone at 0,5 mg kg<sup>-1</sup> every second day for 6 d.

### CASE 2

A 2-year-old crossbred Dachshund was presented with a complaint of depression and anorexia following severe bee envenomation. Clinical examination revealed a rectal temperature of 39,8°C, a respiratory rate of 40 min<sup>-1</sup> and congested mucous membranes. Swelling and hyperaesthesia of the lumbosacral area as well as hindquarter ataxia were evident.

Cystocentesis revealed a port-wine coloured urine which tested (Multistix, Miles Laboratories) strongly positive for blood. Microscopic examination of the urine sediment revealed numerous granular casts, some renal tubular epithelial cells and a few erythrocytes. Initial haematological examination indicated haemolysis, a mild anaemia, neutrophilic leucocytosis with a marked left shift and severe spherocytosis (Table 2). A direct Coombs test yielded a negative result.

Intravascular haemolysis, spherocytosis and ataxia as a result of bee envenomation were diagnosed.

Treatment with oral prednisolone at a dosage of 1 mg kg<sup>-1</sup> twice a day for the first 3 d was administered. One day after the commencement of treatment, the patient was afebrile, eating and stronger in the hindquarters. Despite clinical im-

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Table 1: **Haematological parameters of Cocker Spaniel with anaemia, spherocytosis and neurological dysfunction secondary to bee envenomation (Case 1)**

	Normal range	Day 1	Day 2	Day 5	Day 8
Haemoglobin (g $\ell^{-1}$ )	120-180	90	71	69	107
Red cell count ( $\times 10^{12} \ell^{-1}$ )	5,5-8,5	3,07	2,53	2,59	3,69
Haematocrit ( $\ell \ell^{-1}$ )	0,37-0,55	0,24	0,22	0,23	0,32
MCV (fl)	60-77	76	86	88	86
MCHC (g $\text{dl}^{-1}$ )	32-36	37	32	30	30
White cell count ( $\times 10^9 \ell^{-1}$ )	6,0-15,0	26,29	47,71	40,4	22,93
Neutrophils (mature) ( $\times 10^9 \ell^{-1}$ )	3-11,5	3,39	17,65	9,69	4,81
Neutrophils (immature) ( $\times 10^9 \ell^{-1}$ )	0-0,3	14,35	26,71	12,00	12,13
Monocytes ( $\times 10^9 \ell^{-1}$ )	0,15-1,35	4,17	0,47	2,82	4,58
Normoblasts (%)	None	4	13	53	36
Reticulocytes (%)	0-1,5	1,5	11	25,4	ND
Spherocytes (%)	None	40	40	40	30
Coombs' test	Negative				

Table 2: **Haematological parameters of crossbred dog with haemolysis, spherocytosis and ataxia secondary to bee envenomation (Case 2)**

	Normal range	Day 1	Day 3	Day 4	Day 5
Haemoglobin (g $\ell^{-1}$ )	120-180	135	110	99	103
Red cell count ( $\times 10^{12} \ell^{-1}$ )	5,8-8,5	5,1	4,31	3,93	3,93
Haematocrit ( $\ell \ell^{-1}$ )	0,37-0,55	0,35	0,30	0,27	0,29
MCV (fl)	60-77	69	70	68	75
MCHC (g $\text{dl}^{-1}$ )	32-36	39	36	37	36
WCC ( $\times 10^9 \ell^{-1}$ )	6,0-15,0	28,89	27,60	14,32	14,46
Neutrophils (mature) ( $\times 10^9 \ell^{-1}$ )	3-11,5	18,14	18,49	8,18	7,41
Neutrophils (immature) ( $\times 10^9 \ell^{-1}$ )	0-0,3	6,65	4,69	1,98	0,87
Monocytes ( $\times 10^9 \ell^{-1}$ )	0,15-1,35	1,15	3,58	1,61	1,63
Normoblasts (%)	None	ND	2	4	3
Reticulocytes (%)	0-1,5	ND	ND	2,6	9
Spherocytes (%)	None	50	50	50	40
Coombs' test	Negative				

provement, the haematological parameters worsened over the next 3 d with a decrease in the haematocrit and continued spherocytosis. An erythrocyte fragility test indicated increased erythrocyte fragility. Treatment with prednisolone at 1 mg  $\text{kg}^{-1}$  orally, was con-

tinued until the patient was discharged on Day 5. On the day of discharge, the reticulocyte count had risen to 9,2% and the haematocrit had risen slightly (Table 2). There was no evidence of haemolysis at this stage. Following discharge, the dose of prednisolone was

decreased to 0,5 mg  $\text{kg}^{-1}$  for a further 3 d.

### CASE 3

An 8-year-old male Toy Poméranian was presented with a complaint of anorexia and depression 4 d after bee envenomation.



**Table 3: Haematological parameters of a Pomeranian with spherocytosis and neural dysfunction secondary to bee envenomation (Case 3)**

	Normal range	Day 1	Day 4	Day 6
Haemoglobin (g l <sup>-1</sup> )	120-180	69	67	69
RCC (x10 <sup>12</sup> l <sup>-1</sup> )	5,5-8,5	2,19	2,06	2,19
Haematocrit (l l <sup>-1</sup> )	0,37-0,55	0,20	0,19	0,20
MCV (fl)	60-77	91	92	91
MCHC (g dl <sup>-1</sup> )	32-36	35	35	35
WCC (x10 <sup>9</sup> l <sup>-1</sup> )	6,0-15,0	15,0	29,7	15,0
Normoblasts (%)	None	25	28	16
Reticulocytes (%)	0-1,5	23	28	18
Spherocytes (%)	None	85	20	12
Coombs'test	Negative			

**Table 4: Composition of bee venom<sup>2</sup>**

Constituent	Main effect
Apamin	Central nervous system effects (depression)
Histamine	Pain Acute circulatory effects
Melittin	Direct haemolysis
Mast cell degranulating peptide	Degranulation of mast cells
Phospholipase A	Haemolysis Affects smooth muscle and synaptic junctions Decreases blood pressure
Hyaluronidase	Increases permeability of connective tissue

Clinical examination revealed severe depression and mild swelling of the dorsum of the nose and upper lips. Rectal temperature and respiratory rate were normal. A waterhammer pulse with a rate of 120 beats per minute was present. The oral and ocular mucous membranes were pale and all superficial lymph nodes were enlarged. Marked hindquarter weakness and ataxia were evident. Faecal examination revealed orange-brown faeces with a soft consistency and a few *Ancylostoma* ova on flotation examination. The urine was dark yellow in colour. Urine dipstick showed no abnormalities, yet a few granular casts and bilirubin crystals were seen on microscopic examination of the urine sediment. Haematological examination indicated an appropriately regenerative anaemia and spherocytosis (Table 3). Spherocytosis and neural

dysfunction as a result of bee envenomation were diagnosed. The oral administration of prednisolone at 2 mg kg<sup>-1</sup> once daily and amoxycillin at 20 mg kg<sup>-1</sup> twice daily was initiated. Habitus and appetite were markedly improved after 3 d, yet little change occurred in the haematological parameters despite a significant decrease in the number of spherocytes (Table 3).

The dose of prednisolone was halved to 1 mg kg<sup>-1</sup> once daily on Days 4-6 and reduced again on Day 7 to 0,5 mg kg<sup>-1</sup> for one more day. The dog was discharged on Day 7.

## DISCUSSION

Bee envenomation in dogs is not an uncommon phenomenon in veterinary practice. Most cases present with the fairly characteristic facial swelling as

well as oedema of the eyelids and ears. These cases usually respond well to a single administration of corticosteroids as antihistamines are less effective once envenomation has occurred<sup>5</sup>. The cases presented above, evoked interest as the characteristic facial swelling was not a dominant clinical sign in these dogs, nor is one accustomed to seeing such profound depression and clinical evidence of anaemia and haemolysis following bee envenomation.

As bee venom is composed of biogenic amines, peptides and enzymes, the clinical manifestation will depend on the amount of venom injected<sup>2</sup>. The clinical and laboratory findings in these 3 cases can be adequately ascribed to the various constituents of bee venom which are listed in Table 4.

The intravascular haemolysis can be ascribed to the direct effects of melittin as well as the indirect effects of phospholipase A<sup>2</sup>. Melittin besides its basicity, is a powerful surface tension reducing agent which results in alteration of the stomata of the red blood cell (RBC) membrane, and contributes directly to the haemolytic phenomenon<sup>2</sup>. Phospholipase A, however, causes an indirect haemolysing effect, due to its conversion of extracellular lecithin to lysolecithin which in turn has a surface-acting as well as a solubilising effect on lecithin and cholesterol<sup>2</sup>. Since lipids are an important component of RBC membranes, this disaggregation will result in increased permeability. Besides potentiating the haemolytic effects of melittin, the increased permeability is also responsible for the marked spherocytosis exhibited in these cases<sup>2</sup>.

Melittin also blocks the neuromuscular junctions by exhibiting

a decamethonium-like effect which is not reversible by the administration of physostigmine. Experimental work done on rats, found this block to be irreversible<sup>2</sup>. If this effect was the cause of the posterior ataxia exhibited in all 3 cases and the facial paralysis seen in Case 1, the effects had improved at the time of discharge, presumably indicating a transient effect. Bell's palsy, optic neuritis, generalised polyneuropathy and myasthenia gravis have been reported in humans following bee stings<sup>6</sup>. A similar neuropathic syndrome appeared to have affected all 3 animals in the cases presented above.

The cardiac and respiratory effects of both apamin and melittin may explain death in some cases of severe envenomation. Intravenous injection of apamin in mice, results in extreme hypersensitivity and respiratory incoordination<sup>2</sup>. The respiratory effects can be negated by transecting peripheral nerves which indicate that these effects are initiated at a central level<sup>2</sup>. At low dosages, melittin acts as a positive inotrope<sup>2</sup>. At higher dosages administered to felines, blood pressure decreases to dangerously low levels due to a severe bradycardia with occasional ventricular extrasystolic beats<sup>2</sup>. Respiration is simultaneously depressed with periods of prolonged apnoea. These effects are vagally mediated, since transection of this nerve reverses the circulatory effects of melittin administration<sup>2</sup>.

Fatalities due to hymenoptera venom, are more often the result of anaphylaxis than of direct toxicosis<sup>6</sup>. As with most hypersensitivity reactions, previous sensitisation is a prerequisite for this to occur<sup>6</sup>. The canine equivalent of the bronchial constriction and vasculogenic shock seen in humans, has not yet been described.

Due to the multicomponent nature of bee venom, anti-histamines alone cannot be depended upon for reversal of the clinical signs of bee envenomation<sup>5</sup>. Topical and oral anti-histamines do however ameliorate the local tissue reaction caused by the venom<sup>1 5</sup>. Inhibition of the hypersensitivity reaction is best achieved with the administration of high doses of corticosteroids<sup>5 6</sup>. In the 3 cases reported here, spherocytosis and intravascular haemolysis were prominent. As a result, the dose of prednisolone administered, was that commonly used for the treatment of autoimmune haemolytic anaemia<sup>8</sup>.

The maintenance of adequate urine production is of prime importance in cases which develop intravascular haemolysis due to the potential development of haemoglobinuric nephrosis with consequent acute renal failure. Fluid support with a balanced electrolyte solution is suggested. Should urine production be less than 1-2 ml kg<sup>-1</sup> hour<sup>-1</sup>, diuresis with intravenous dextrose at 2 gm kg<sup>-1</sup> and furosemide at 2-4 mg kg<sup>-1</sup> administered intravenously is advocated<sup>4</sup>.

Cardiac function and elevation of blood pressure are best supported with the use of positive inotropic agents such as dopamine or dobutamine<sup>3 7</sup>. Pharmacological antagonism of the severe anaphylactic reaction is also reversed with intravenous adrenaline<sup>7</sup>.

Local tissue necrosis may necessitate the use of broad-spectrum antibiotic administration and topical antiseptic application.

Daily monitoring of the packed cell volume should be performed in cases that manifest a haemolytic episode. This will dictate which patients require blood transfusions to ensure survival.

It is recommended that the high doses

of prednisolone be maintained until the patient is afebrile and the packed cell volume begins to increase. At this stage, the dose of prednisolone may be gradually decreased.

Most cases of bee envenomation respond adequately to a single administration of a corticosteroid. Those patients that present with marked depression and anorexia should be subjected to a routine urinalysis and haematological examination so that a haemolytic crisis may be detected, and appropriate therapy instituted.

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## AMOEBIC MENINGOENCEPHALITIS IN A SHEEP

J J VAN DER LUGT\* and HELENA E VAN DER MERWE\*\*

### ABSTRACT

A case of amoebic meningoencephalitis in a sheep, suspected of being caused by *Acanthamoeba* sp., is described. An adult ewe was necropsied 5 days after it had developed anorexia and nervous signs, which included ataxia and walking in circles. Significant gross lesions were restricted to the central nervous system and consisted of thickening of the meninges and extensive necrosis of the cerebrum and cerebellum. The cerebral changes were characterised microscopically by a multifocal to coalescent necrotising granulomatous meningoencephalitis as well as choroid plexitis, ventriculitis and diffuse vasculitis. Numerous amoebic trophozoites and a few encysted forms morphologically compatible with *Acanthamoeba* sp. were present in the affected areas, particularly in the walls of blood vessels and perivascular spaces.

**Key words:** Meningoencephalitis, amoebic, *Acanthamoeba* sp., sheep

Van der Lugt J.J.; Van der merwe H.E. **Amoebic meningoencephalitis in a sheep.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 33-36 (En.) Section of Pathology, Veterinary Research Institute, P.O. Box 12502, 0110 Onderstepoort, Republic of South Africa.

### INTRODUCTION

Amoebae are protozoa belonging to the subphylum Sarcodina which characteristically move by the formation of pseudopodia<sup>1 4</sup>. A few genera include species known to be pathogenic to man and animals, some of which are natural parasites in the intestinal tract of their hosts. Others are free-living, highly ubiquitous microorganisms of soil and water habitats<sup>1 14</sup>.

The parasitic amoeba *Entamoeba histolytica* is the cause of amoebic dysentery in man, primates and occasionally in other species<sup>2 10</sup>. Among domestic animals, *E. histolytica* infection is most frequently observed in dogs and only rarely in cats, swine and cattle<sup>2</sup>. Amoebiasis in man and dogs is characterised by an ulcerative colitis. Extra-intestinal complications in the form of amoebic abscesses in various organs, especially in the liver and lungs, are relatively common in man but rare in dogs<sup>2 4 10</sup>.

Free-living amoebae of the genera *Naegleria* and *Acanthamoeba* cause disease in man and animals. Spontaneous infection by *N. fowleri* is the cause of primary amoebic meningoencephalitis (PAM) in man, and the lesions are essentially confined to the brain<sup>8 9</sup>. Several pathogenic species of *Acanthamoeba* are recognised and, in addition to granulomatous amoebic encephalitis (GAE)<sup>12</sup>, can give rise to keratitis, uveitis and granulomatous lesions in various organs in humans<sup>6 9</sup>.

Reports of spontaneous infection in domestic animals by free-living amoebae are rare. McConnell et al<sup>13</sup> described multiple necrohaemorrhagic foci in the lungs of a bull associated with amoebic trophozoites and encysted forms morphologically compatible with *Hartmanella* (*Acanthamoeba*) sp. Abid<sup>1</sup> reported pneumonia in a calf, caused by a suspected *Hartmanella* sp. while Culbertson<sup>7</sup> mentioned "a zoo-confined ovine (Nelson bighorn) in which typical amoebic rhinitis and encephalitis were found". *Acanthamoeba* infection in a dog, manifested by multifocal necrohaemorrhagic foci in the heart, lungs, liver and pancreas was documented by Ayers et al<sup>3</sup>. The causative organisms were identified on their morphologic features. In a case of acanthamoebiasis in a dog, the lesions in

the brain, lungs and kidneys consisted of areas of suppurative necrosis and haemorrhage which contained numerous trophozoites and cysts. The causative organism was identified as *A. castellani* by indirect immunofluorescence<sup>15</sup>.

For many years the classification of the free-living amoebae was confusing and amoebae causing disease in mammals were probably misidentified as *Hartmanella* in earlier literature. These amoebae are now classified as belonging to the genus *Acanthamoeba*<sup>5 8 9</sup>. According to Beaver et al.<sup>6</sup>, species of *Hartmanella* are generally not pathogenic to man and animals.

Sheep are susceptible to experimental infection with *N. fowleri* following intranasal installation of the organism<sup>16</sup>. In this paper the authors describe what they believe to be the first case of natural infection by free-living amoebae in a domestic sheep.

### HISTORY AND CLINICAL SIGNS

A group of adult Döfne merino ewes (n = 10) were housed at an artificial insemination centre in Potchefstroom. The animals were kraaled at night and fed a commercially-available maintenance ration. During the day they had access to natural pasture in an adjacent paddock. Municipal water was available in a trough.

One of the ewes became anorexic and developed nervous signs including ataxia, circling and a head tilt. A diagnosis of suspected cerebro-cortical necrosis was made and the animal was treated parenterally with vitamin B<sub>1</sub> for 3 consecutive days without response. The animal was sacrificed for necropsy, 5 d after the onset of clinical signs.

### PATHOLOGY

Significant macroscopical changes were limited to the central nervous system. The meninges covering the cerebrum and cerebellum were diffusely thickened and brownish-grey. On incision, almost the entire cerebrum and cerebellum appeared necrotic, manifested by a friable consistency and a brown discolouration. Other lesions included severe fatty changes in the liver and widespread necrosis of fat.

Specimens of the cerebrum were fixed in 10% buffered formalin, routinely

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Received: April 1989 Accepted: August 1989

processed and stained with haematoxylin and eosin (HE). Selected sections were also stained with the periodic acid-Schiff (PAS) reaction, Giemsa, Gomori's methenamine silver (GMS), Masson's trichrome and Gram's stain.

Microscopical examination of the cerebrum revealed widespread lesions in the meninges, brain substance, choroid plexus and ependyma as well as in the blood vessels throughout these tissues. The leptomeninges were moderately to severely thickened by deposition of loosely-arranged fibrous connective tissue and a cellular infiltrate consisting of numerous macrophages, lymphocytes and lesser numbers of neutrophils and plasma cells, as well as an accumulation of fibrin in some areas (Fig. 1). The inflammatory cells formed focal dense accumulations in the subarachnoidal space. Several macrophages contained yellowish-brown granular pigment resembling lipofuscin. The intima and

media and to a lesser extent the adventitia of medium-sized meningeal blood vessels, were expanded by diffuse infiltrations of predominantly lymphocytes and macrophages and prominent adventitial fibrosis was evident in some of these vessels. Many smaller blood vessels were necrotic and infiltrated by neutrophils, and occasionally contained thrombi. In some areas, a sub-meningeal proliferation of loose fibrovascular connective tissue extended into the underlying parenchyma (Fig. 1).

Large numbers of amoebic trophozoites were recognised in the affected meninges. The trophozoites, in association with inflammatory cells, generally infiltrated the vascular walls and perivascular spaces and were often grouped in clusters (Fig. 2). Rarely, trophozoites were seen lying free in the subarachnoidal space.

Large, focally extensive to confluent

areas of coagulative to lytic necrosis were distributed throughout the grey and white matter (Fig. 3). Lytic necrosis occurred most commonly in the outer cerebral cortex, and these areas were infiltrated by large numbers of foamy macrophages and to a lesser extent neutrophils. The necrotic tissue frequently contained moderate accumulations of mineralised debris (Fig. 3).

Several large foci of granulomatous encephalitis were evident within and at the edges of the necrotic areas (Fig. 3). The nature of the inflammatory reaction varied in different regions. In some areas the reaction consisted predominantly of macrophages, some plasma cells and lymphocytes and a few giant cells, accompanied by fibroplasia, while lymphocytes and plasma cells predominated in other foci. Large accumulations of mainly lymphocytes and plasma cells infiltrated the choroid plexus, ependyma and adjacent periven-

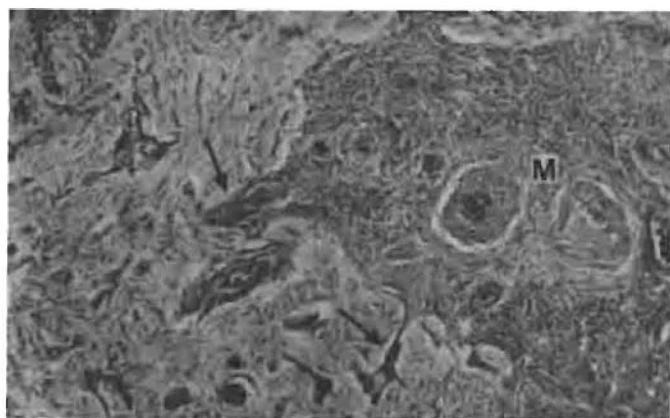


Fig. 1: Thickening of the meninges (M) at the base of a sulcus due to fibrous connective tissue and a cellular infiltrate. Proliferations of connective tissue (arrows) extend into the underlying parenchyma. HE X 80

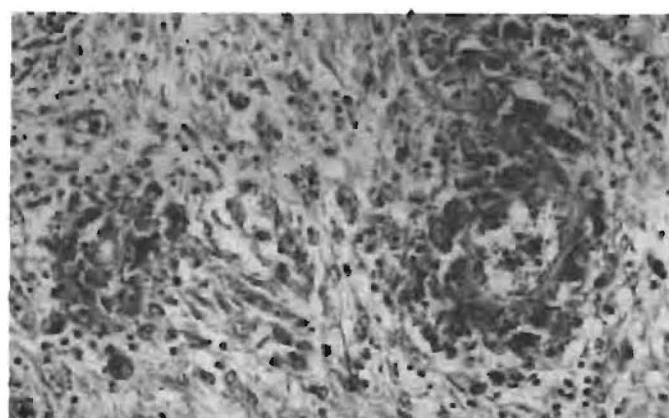


Fig. 2: Numerous trophozoites present in the walls of two blood vessels and perivascular spaces in the meninges. HE X 300



Fig. 3: Focus of granulomatous encephalitis (G) adjacent to an area of coagulative necrosis (N) containing mineralised debris (arrow). HE X 200

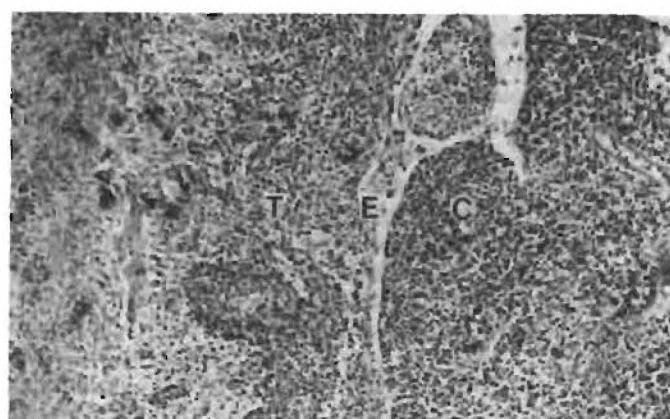


Fig. 4: The choroid plexus (C), ependyma (E) and adjacent periventricular nervous tissue (T) infiltrated by large numbers of lymphocytes and plasma cells. HE X 80

tricular tissue (Fig. 4).

A severe vasculitis which resembled the lesions described in the meninges, occurred in the majority of blood vessels throughout the cerebrum (Fig. 5). In some areas, marked angiogenesis and proliferation of perivascular connective tissue extended into the surrounding necrotic brain substance.

Numerous amoebic trophozoites and a few encysted forms were recognised in the affected tissues. The trophozoites were commonly arranged in clusters and were most numerous in and around blood vessels. Large groups of degenerative trophozoites associated with a minimal inflammatory reaction were evident in some necrotic portions of the cerebrum. A few of the cysts were phagocytosed by macrophages. Trophozoites varied in shape and measured approximately 15-35  $\mu\text{m}$  in diameter with a distinct cytoplasmic

border. The cytoplasm was abundant, finely granular, vacuolated (often with a single large vacuole) and was amphophilic to weakly basophilic with HE, bluish-purple with Giemsa and moderately positive with the PAS reaction. One, or rarely 2, centrally-located or slightly eccentric nuclei were usually present. The nuclei were round, 4-6  $\mu\text{m}$  in diameter and contained a large central, deeply-staining basophilic body or karyosome (nucleolus) often surrounded by a halo (Fig. 5-7). The encysted forms were round, approximately 13-18  $\mu\text{m}$  in diameter, with a double wall forming a wrinkled or occasionally smooth outer layer or ectocyst, and a circular or smooth inner layer or endocyst. A large centrally-located basophilic body that contained several dense granules was present in each cyst (Fig. 5, 6 & 8). The cysts stained deep purple with Giemsa, while the walls were

weakly positive with GMS and strongly positive with the PAS reaction.

## DISCUSSION

The morphological characteristics and staining of the trophozoites and cysts in this animal, conformed to the description for free-living amoebae of the genus *Acanthamoeba*<sup>8 12</sup>. Differentiation of species in the genus *Acanthamoeba* on a morphological basis is difficult, if not impossible, and for this purpose immunoperoxidase and immunofluorescent staining techniques are usually employed<sup>5 6</sup>. The organisms in this case could be differentiated from *Naegleria*, since, in *Naegleria* infections, cysts are not formed in tissues of the host<sup>8</sup>. Although the trophozoites of *Acanthamoeba* (15-50  $\mu\text{m}$ ) are larger than those of *Naegleria* (10-35  $\mu\text{m}$ ), trophozoites of the 2 genera may be morphologically indistinguishable by

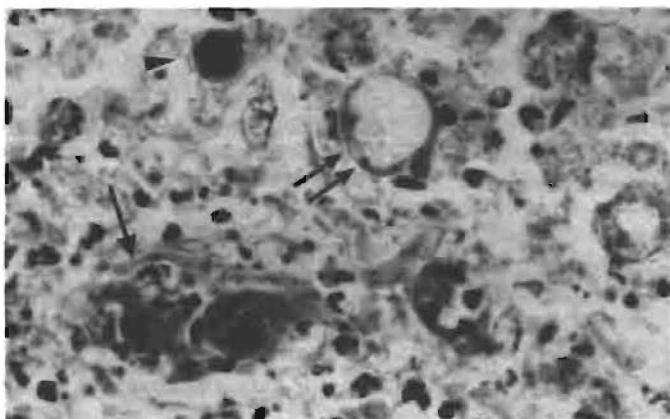


Fig. 5: Necrotising vasculitis and thrombosis (arrow) in the cerebellar grey matter. A degenerated trophozoite containing a large intracytoplasmic vacuole (double arrows) and a phagocytosed cyst (arrowhead) are evident. HE X 450

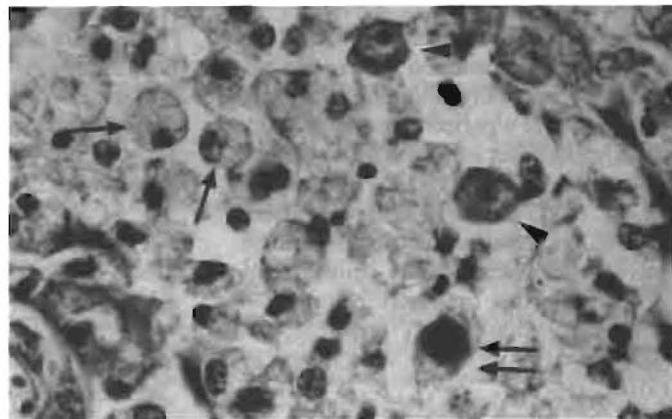


Fig. 6: Area of lytic necrosis in the cerebrum. Note the morphology of trophozoites (arrowheads) and macrophages (single arrows). A cyst is also evident (double arrows). HE X 500

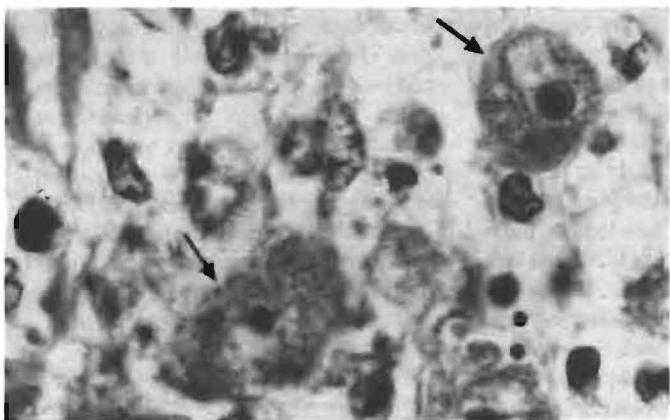


Fig. 7: The cytoplasm of the trophozoites (arrows) is abundant, finely granular and vacuolated with a single large vacuole. The single round nucleus contains a centrally-located, deeply-staining karyosome. HE X 1000

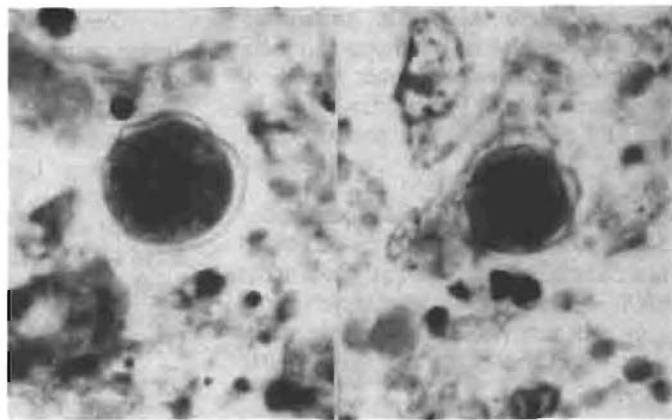


Fig. 8: The cysts are round and double-walled with a wrinkled outer layer and a smooth inner layer, and contain several dense granules. HE X 1300



light microscopy<sup>6, 8</sup>. Several authors emphasised the difficulty they experienced in differentiation of trophozoites from macrophages in tissue sections<sup>3, 5, 13</sup>. In this respect the large centrally-located karyosome is an important distinguishing feature of both *Acanthamoeba* and *Naegleria* spp<sup>8</sup>. Infection of the brain with *Entamoeba histolytica* should be considered in the differential diagnosis, although this condition is extremely rare in domestic animals<sup>4</sup>. Furthermore, *E. histolytica* trophozoites may be identified by the presence of a small karyosome with peripherally-arranged chromatin granules<sup>6</sup>.

The brain lesions encountered in this sheep resembled those of granulomatous amoebic encephalitis (GAE) caused by *Acanthamoeba* in man (necrosis and granulomatous meningoencephalitis)<sup>8, 12</sup>. This is in contrast to the more acute lesions of haemorrhagic necrosis with a minimal inflammatory reaction described in primary amoebic meningoencephalitis (PAM) caused by *Naegleria* spp.<sup>8, 12</sup>. It appears that the relatively slow invasion of tissues by *Acanthamoeba* spp. tends to evoke a granulomatous reaction. The disease, therefore, is usually of gradual onset and proceeds over a period of weeks or months. On the other hand, PAM is an acute disease with a short clinical course of 5 to 16 d<sup>6</sup>. The lesions in the sheep studied, indicated a chronic infection by an *Acanthamoeba* sp. The clinical signs observed in this case were of only 5 d duration, although it is probable that nervous signs were not detected at an earlier stage.

In man, meningoencephalitis caused

by *N. fowleri* is almost invariably associated with a history of recent swimming in fresh water or lakes. The disease tends to affect adolescents and young adults, although predisposing factors have not been identified<sup>6, 9</sup>. It is believed that the amoebae spread to the brain via the nasal mucosa and cribriform plate<sup>8</sup>. Infections of the nervous system caused by *Acanthamoeba* spp. usually occur in immunosuppressed or chronically ill patients<sup>6, 9</sup>. The multifocal distribution of lesions in these infections, points to haematogenous spread of the amoebae to the central nervous system, probably from a primary focus of infection in the skin, lungs or kidneys<sup>8</sup>. Gross lesions other than in the brain were not observed in this sheep. Unfortunately representative samples from a range of tissues were not submitted for histopathological examination. A primary site of infection or other underlying disease conditions could therefore not be excluded in this case.

#### ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Sections of Pathology and Photography, Veterinary Research Institute, Onderstepoort, for the preparation of the histopathological sections and photomicrographs, respectively.

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## ACCIDENTAL PENTOBARBITAL POISONING IN A LIONESS

ANNA VERSTER\*, H H E SCHRÖDER\*\* and J W NESBIT\*\*\*

### ABSTRACT

A lioness was accidentally poisoned by consumption of meat of a horse euthanased with pentobarbital. The post mortem examination was negative but thin-layer gas-chromatographic and mass-spectrometric methods revealed a pentobarbitone concentration of 1,14 mg kg<sup>-1</sup> in the chloroform extracts of the liver of the animal.

**Key words:** Lioness, pentobarbital poisoning

Verster, Anna; Schröder, H.H.E.; Nesbit, J.W. **Accidental pentobarbital poisoning in a lioness.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 37-38 (En.) Department of Parasitology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

Although there have been a number of case reports<sup>1 4 5 6 7 8 10 12 13 16</sup> dealing with toxicosis in domestic pets and zoo animals resulting from the consumption of meat of animals euthanased by barbiturates, this appears to be the first report of accidental barbiturate poisoning in a lioness in the Republic of South Africa.

The animal was acquired in October 1984 as a cub of approximately 3 months for investigations on the life cycles of cestodes. Up to March 1988, she was successfully maintained on a diet of horsemeat supplemented with vitamin and mineral mixtures. Occasionally the horsemeat originated from animals that had been euthanased with barbiturates. On a previous occasion (August 1986), the lioness showed moderate sedation for less than 24 h following ingestion of meat from a horse euthanased with thiopentone sodium. She recovered spontaneously.

The present report concerns accidental poisoning with pentobarbital some 18 months later. At this time she was fed 7 kg of horsemeat every second day. The first sign of poisoning was noted on the morning after she had been fed in the early afternoon of the previous day.

Initial ataxia progressed to heavy sedation within 3 h. The preceding episode and the clinical signs suggested barbiturate toxicosis. The animal was moved into her sleeping quarters and her condition monitored on an hourly basis. The following morning she was still heavily sedated and was twice treated with doxapram hydrochloride [(Dopram, Robins (Lennon-Petersen))] to resuscitate her. These attempts resulted in a brief, albeit temporary, response before she relapsed into heavy sedation.

When examined at midday on the third day, she appeared to be recovering in that she responded to tactile stimuli and was able to focus her eyes on her keeper. In spite of this encouraging improvement she was found dead an hour later, 72 h after being fed horsemeat presumed to contain barbiturate.

The post mortem examination revealed an animal in excellent condition and lesions limited in both nature and extent. The macroscopical findings included mild generalised congestion, congestive splenomegaly as well as mild enlargement and pallor of both the liver and the kidneys. Specimens of the brain and spinal cord, lungs, heart, superficial and visceral lymph nodes, liver, spleen, pancreas, kidneys and skeletal muscle were collected and preserved in 10% buffered formalin for microscopical examination. In addition, 0,2 kg of liver, was collected and frozen for chemical determination. The microscopical findings included moderate to severe congestion of the spleen, mild hydropic degeneration of the liver and kidney,

mild oedema of the central nervous system and an accumulation of mucus, heavily infiltrated with neutrophils and alveolar macrophages in the bronchioles of the lungs. No specific diagnosis was feasible, based on the morbid anatomical findings and, because of the clinical history, a diagnosis of suspected barbiturate poisoning was made.

The toxic compound was successfully identified by thin-layer chromatographic<sup>14</sup>, gas-chromatographic<sup>9 11 15</sup> and mass-spectrometric<sup>3</sup> techniques applied to chloroform extracts of the liver. The thin-layer chromatographic results indicated the presence of only one unknown compound which was found in the acidic extract, the  $R_f$  value of which was close to seco- and pentobarbitone. The gas chromatographic analysis gave a retention index (R.I.) of 1765. At a window of 50 R.I. units, several barbiturates, including amonal-, pento-, vin-, sec- and narco-barbital, were possible. However, the gas chromatographic peak with a retention time of 10 501 min gave a 9 916 match quality (10 000 = 100%) for pentobarbitone. No other drugs with quality of more than 9 500 could be found. A molecular mass of 226,25 was obtained and this is also consistent with pentobarbitone. Furthermore, its retention time and eight-peak mass spectrum (156, 141, 43, 41, 157, 98, 69 and 55 amu) were virtually identical to those of an authentic standard (156, 141, 43, 41, 157, 98, 55, 57 amu). The amount of pentobarbitone was determined by comparing the area under its gas chromatographic/mass selective detector peak with that of a known concentration of pentobarbitone. The pentobarbitone concentration was found to be 1,14 mg kg<sup>-1</sup> of liver.

Since no other compounds were identified by analytical chromatographic techniques applied to the acidic and basic chloroform extracts of the liver tissue, it was concluded that the only drug present was pentobarbitone.

The low level of pentobarbitone found in the lioness' liver, places doubt on a diagnosis of acute barbiturate poisoning. The biological half-life of pentobarbitone in felines is unknown, but in dogs it is 8,2h<sup>2</sup>. As the lioness was under heavy sedation for several days

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Received: June 1989 Accepted: August 1989

before she died, it may be assumed that the total dose she ingested was much higher but at the time of her death had been metabolised.

We cannot account for the fact that the animal died suddenly, shortly after she appeared to be recovering.

This communication is to alert veterinarians and conservationists of the dangers of feeding carnivores with meat of questionable origin.

## ACKNOWLEDGEMENTS

Prof G E Swan is thanked for helpful comment on the manuscript.

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## Book review/Boekresensie

### VETERINARY MEDICINE REPORT

The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, U.S.A. Price: (International) Single issue US\$ 28.00 Annual subscription US\$ 58.50 (ISSN 0895-7703)

*Veterinary Medicine Report* is published 3 times a year and features topics of current interest to the small animal veterinarian.

Three to 4 themes are featured in each issue of more than 130 pages. Each theme is dealt with under the section New Developments, Problem Solving and Point/Counterpoint. In the first section, New Developments, the theme is reviewed comprehensively, bringing the reader up to date with current knowledge. Relevant case studies are presented in the section Problem Solving, and in Point/Counterpoint, 2 authors discuss controversial aspects of each theme. Additional sections are Consultant's Corner, where reader's questions are answered, State of the Art, New Techniques, and Literature Review, which contains journal abstracts and comments relevant to the themes dealt with in the issue. Volume 1, Number 2, 1989 focuses on aspects of feline infectious disease, ophthalmology, degenerative lumbosacral stenosis in dogs, and external skeletal fixation. Other aspects of interest to the small animal practitioner are featured, including a section on practice management.

The text is written in an easy-to-read conversational style and key phrases are highlighted. The tables, graphics, illustrations and photographs are numerous and of exceptional quality. The report contains no advertisements.

The report has an important place in the library of all small animal practitioners dedicated to veterinary continuing education. This is a journal with a difference and is a welcome addition to the veterinary literature.

D. EVEZARD

## AN OUTBREAK OF SUSPECTED PHALARIS STAGGERS IN SHEEP IN THE WESTERN CAPE PROVINCE

A VAN HALDEREN\*, JENNIFER R GREEN\* and D J SCHNEIDER\*

### ABSTRACT

An outbreak of suspected *Phalaris* staggers in South Africa is described. Approximately 40 out of 600 sheep that had been grazing for 3 weeks on a wheat stubble land heavily infested with *Phalaris* grass, were affected. Clinical signs included nervous tremors, ataxia and convulsions. New cases occurred even after removal from the affected pasture. The most prominent microscopic lesion seen in the 2 sheep autopsied, was an accumulation of intracytoplasmic brown pigment in the neurons of the brain.

**Key words:** Sheep, *Phalaris* staggers, intracytoplasmic neuron pigment.

Van Halderen A.; Green J.R.; Schneider D.J. **An outbreak of suspected *Phalaris* staggers in sheep in the western Cape Province.** *Journal of the South African Veterinary Association* (1990) 61 No. 1, 39-40 (En.) Regional Veterinary Laboratory, Private Bag X5020, 7600 Stellenbosch, Republic of South Africa.

### INTRODUCTION

*Phalaris* toxicosis has been described in sheep and cattle grazing on specific species of *Phalaris* grasses (canary grasses) under certain environmental conditions<sup>1,3,4</sup>. In South Africa *P. minor* has been implicated in outbreaks of *Phalaris* staggers in the western Cape Province (D.J. Schneider, 1978, Regional Veterinary Laboratory, Stellenbosch, unpublished data). *P. minor* is an introduced weed and is common on disturbed lands<sup>2</sup>. Poisoning occurs where *Phalaris* is the dominant plant in pastures and new, lush growth appears to be most toxic<sup>1,5</sup>.

Three distinct syndromes have been described in sheep: a sudden death or cardiac form; an acute form with transient nervous signs and a chronic poisoning or *Phalaris* staggers which is a progressive neurological disorder that occurs when sheep have been grazing *Phalaris* pastures for 2 to 3 weeks<sup>4,5</sup>. Signs of hyperexcitability, tremors, stiffness and convulsions are precipitated or exacerbated by exercise and sheep may either recover gradually if left un-

disturbed or eventually die<sup>1,3,4,5</sup>. Three tryptamine alkaloids closely related to the neurotransmitter serotonin have been implicated as the causative toxins<sup>3,5</sup>.

### HISTORY

Six hundred Merino ewes (year-olds and culls) were pastured on a 102 ha wheat stubble land on a farm in the Moorsburg district of the western Cape Province. After 3 weeks on these pastures, the sheep were brought in for shearing on 11th August 1988. Signs of tremors, stiffness and convulsions were noticed in 8 of these sheep when chased. Affected sheep were treated with calcium borogluconate and magnesium hypophosphite (MFC, Maybaker). Response to treatment was poor and 6 d later, 5 sheep had died and approximately 32 new cases had appeared. The flock had not been returned to the suspected camp and had been in a lupin land since the first signs were noticed. Eighty cattle that had grazed with the sheep in the same camp for 2 weeks, were immediately removed although no clinical signs of disease were seen in these animals.

### CLINICAL SIGNS

A live, year-old sheep was submitted to the Regional Veterinary Laboratory, Stellenbosch. This sheep showed signs

of mild tremors, ataxia, hyperexcitability and head shaking, but when approached the tremors became so severe that the sheep fell on its side and paddled. There was a marked increase in heart and respiratory rates, but body temperature remained normal. These clinical signs gradually subsided when the sheep was left undisturbed. A second sheep collected on the farm for autopsy showed identical signs.

### LABORATORY INVESTIGATIONS:

Blood was collected from the anterior vena cava of the above-mentioned 2 sheep in 10 ml heparinised vacuum tubes. Calcium, magnesium, phosphorus, blood urea nitrogen, haemoglobin and total plasma proteins were measured by wet chemistry spectrophotometry using a Spectronic 21 digital spectrophotometer (Bausch & Lomb Inc., Rochester, New York, U.S.A.) Sodium and potassium were determined by the flame photometry method using an IL543 digital flame photometer (Instrumentation Laboratory, Viale dell' Industria, 3-20037, Paderno Dugnano, Italy).

The 2 sheep were euthanased and full post mortem investigations were conducted. Brain, lung, muscle, liver, kidney, heart and small intestinal samples were collected in 10% buffered formalin and were routinely prepared and stained with haematoxylin and eosin for light microscopy.

Liver samples were collected in analytical formalin for trace element determination by means of the atomic absorption method using a PYE UNICAM SP9 atomic absorption spectrophotometer (Philips Pye Unicam Ltd., York Str., Cambridge CB1 2PX, England).

Fresh liver and rumen content samples were collected from the first sheep for toxicological examination for lead and heavy metals, using the atomic absorption method (Philips Pye Unicam).

Brain, spleen, liver and lung samples were collected from the first sheep for bacterial culture. Medulla samples were

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Received: April 1989 Accepted: October 1989

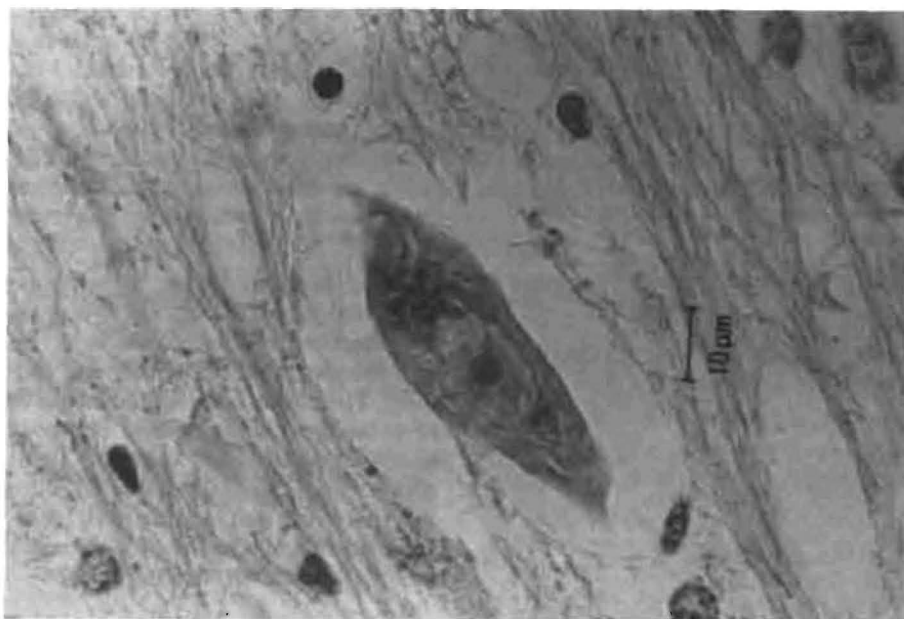


Fig. 1: Pigment in cytoplasm of neuron : HEx 1000

plated out on Columbia blood agar base (Oxoid, Basingstoke, England) for *Listeria* isolation and incubated aerobically and in CO<sub>2</sub>. The other organ samples were plated out on Columbia agar and on MacConkey Agar no. 3 (Oxoid)

## RESULTS

Plasma concentrations of phosphorus, calcium, magnesium, potassium, sodium, and total plasma proteins were within normal range for both sheep. Haemoglobin and haematocrit values as well as blood urea nitrogen were also within normal range.

Liver and rumen content collected from the first sheep were negative for lead and heavy metals.

No significant pathogenic bacteria were isolated from tissue samples collected from the first sheep.

Very low liver copper levels respectively 9 and 20 ppm (dry basis), were recorded for the 2 sheep. Molybdenum, manganese, iron and zinc values were within the normal range.

The only macroscopically visible

pathology in both cases was a mild lung oedema. Pigmentation of the brain and kidney cortex as has been described with *Phalaris* staggers was not macroscopically evident<sup>4 5</sup>.

The most significant finding in both cases was a dark brown, intracytoplasmic pigment in the neurons of the lateral geniculi, thalamus and brain stem. In the first sheep autopsied, there was also intracytoplasmic pigment present in the renal epithelium. There was also mild demyelination of the medulla oblongata in both cases, although this may have been associated with the low copper levels.

A mild perivascular lymphocyte infiltration was evident in the medulla oblongata of the second sheep.

Other changes found in both cases were moderate congestion and oedema of the lungs and mild hydropic degeneration of the liver parenchyma.

## DISCUSSION

The clinical signs and pathological changes were consistent with those described with *Phalaris* staggers<sup>1 3 4 5</sup>. The farm was visited on 25th August

1988, but unfortunately the affected camp had been ploughed over about a week before. The farmer confirmed, however, that canary grass grows abundantly on the farm every year and that heavy growth of young canary grass was noticed in the affected camp prior to ploughing. *Phalaris* staggers was diagnosed in cattle and sheep on 2 neighbouring farms in August and September 1978, when *Phalaris minor* grew abundantly after good rains following an unusually dry winter (D.J. Schneider, 1978, Regional Veterinary Laboratory, Stellenbosch, unpublished data). In this case, however, the rainfall for the previous 4 months was within the normal range. Approximately 15 animals were still visibly affected at the time of the visit to the farm. By 6th January 1989, all but 2 of the remaining affected animals appeared to have recovered completely. No signs of staggers were seen in the 80 head of cattle that had grazed in the same camp for 2 weeks.

## ACKNOWLEDGEMENTS

The Director (Animal Health) is thanked for permission to publish this article. We also wish to thank Dr. E. Dalton, Moorreesburg, for referring the case, Mr. P. van der Merwe for the photography and Mrs. R. de Kock for typing the manuscript.

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## AN OUTBREAK OF GRANULOMATOUS VULVITIS IN FEEDLOT HEIFERS

R O GILBERT\* and E E OETTLÉ\*\*

### ABSTRACT

An unusual form of vulvitis occurred in outbreak form affecting at least 18 of a group of 35 to 40 heifers soon after their introduction to a feedlot in the western Transvaal area of the Republic of South Africa. Initially, affected animals showed a knob-like swelling of the ventral commissure of the vulva. This grew in size up to approximately 6 cm in diameter and then appeared to burst open and give rise to a granulomatous lesion. Bovine herpesvirus type-1, *Moraxella bovis*, *Mycoplasma bovis* and *Mycoplasma bovis* were isolated from 2 affected heifers referred for diagnostic investigation. *Mycoplasma canadense* was isolated from one of the heifers. Attempts to reproduce the disease by transferring an excised portion of the lesion to unaffected animals, resulted only in lesions typical of uncomplicated infectious pustular vulvovaginitis. It is proposed that that multifactorial aetiology, including synergistic pathogenic action between the organisms isolated, was responsible for this outbreak. Administration of oxytetracycline to affected animals was followed by prompt resolution of symptoms. No further outbreaks have been reported.

**Key words:** Bovine herpesvirus 1, *Moraxella bovis*, *Mycoplasma* spp, vulvitis.

Gilbert R.O.; Oetlé E.E. An outbreak of granulomatous vulvitis in feedlot heifers. *Journal of the South African Veterinary Association* (1990) 61 No. 1, 41-43 (En.) Department of Theriogenology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

### HISTORY

A group of 35 to 40 Brahman heifers, ranging in age from 14 to 18 months, was brought into a small, on-farm feedlot from veld grazing. Their condition was good, and they were fed a high energy, high protein diet ad libitum. No specialised feedlot preconditioning took place, and no medication or vaccinations were administered at this time. No medicated feed additives were used. It is not known whether growth-promoting implants were administered to the animals. Almost immediately after their confinement, an unusual form of vulvitis was observed in the group. Initially, affected animals showed a knob-like swelling of the ventral commissure

of the vulva (Fig 1). This grew in size over a period of days to approximately 6 cm in diameter and then appeared to burst open and give rise to a granulomatous lesion (Fig 2). The condition appeared to be highly contagious, and within 2 weeks, at least 18 animals were affected. Horses and sheep were maintained on the same farm. Affected animals were not depressed and showed no signs of systemic illness. They continued to eat well and gain weight. Two animals were referred for investigation to the Department of Theriogenology of the Faculty of Veterinary Science of the University of Pretoria.

### PHYSICAL AND LABORATORY EXAMINATION

Abnormal clinical findings were limited to the genital tract and were similar in both animals. The primary lesion in each case was a large (8 cm or more) granulomatous lesion of the ventral vulvar commissure, covered with a thick scab. Under the scab the elevated tissue was smooth and bright and bled easily

and profusely when disturbed. The lesion straddled the mucocutaneous junction and affected perivulvar skin. Within the vestibulum, numerous smaller lesions adjacent to the granulomata were visible. They consisted of multiple vesicles, ulcers and nodules, all approximately 3 mm in diameter (Fig 3). No lesions were detected cranial to the vestibulovaginal junction. Although these heifers had reportedly not been exposed to a bull, one was found to be approximately 130 d pregnant.

Both heifers had a leucocytosis characterised by neutrophilia, mild immature neutrophilia and mild lymphoblastic and monoblastic activity. Appropriate specimens from the edges of the granulomatous lesions were submitted for aerobic and anaerobic bacterial culture and fungal, viral, mycoplasmal and chlamydial isolation. Bovine Herpesvirus type 1 was isolated from both heifers. Both also yielded *Moraxella bovis* and *Mycoplasma bovis* as well as *Mycoplasma bovis*. *Mycoplasma canadense* was isolated from one of the heifers. Samples from one heifer yielded an unidentified, gram-negative, anaerobic bacillus.

Histopathological examination of the lesion showed subacute to chronic inflammatory reaction of the subepithelial tissues characterised by infiltration of lymphocytes and neutrophils. Herpes-like intranuclear inclusions were seen.

### TREATMENT

Based on culture results, the 2 hospitalised heifers were treated systemically with oxytetracycline hydrochloride (Liquamycin 100, Pfizer) at 10 mg kg<sup>-1</sup> once daily by intramuscular injection for 3 d. In addition, topical oxytetracycline in aerosol form (Liquamycin Spray, Pfizer) was applied twice daily until the lesions regressed. Both animals responded well to treatment and were discharged. Similar treatment was applied to the rest of the affected animals in the group and they responded equally satisfactorily. The chronic nature of the lesions was reflected by the presence of considerable residual fibrosis, sometimes deforming the vulva in recovered animals.

### Transmission experiments

The 2 hospitalised cases were confined for several days before receiving any treatment with 4 unaffected cows of mixed breed. Material from the vulvar

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Received: June 1989 Accepted: November 1989





Fig 1: Early stage of the vulvar lesion showing swelling of the ventral vulvar commissure

lesions of the patients was transferred to 2 of these cows; the other 2 animals were merely left in contact with the affected cases. Only the animals to which material was transferred developed lesions, and these consisted only of vesicular and erosive lesions typical of uncomplicated herpesvirus infection. Herpesvirus type-1 was isolated from these cases, but the other pathogens isolated from the patients were not. Histopathological examination results were typical of infectious pustular vulvovaginitis with focal acute ulceration. Intranuclear inclusions were detected in surrounding epithelial cells.

## DISCUSSION

This form of vulvitis has not previously been described. None of the infectious agents isolated from these cases is individually capable of causing lesions of this nature. It is suggested that the aetiology in this case is multifactorial, involving pathogenic synergism between the isolated organisms, possibly exacerbated by the stressful conditions of intensive housing and nutrition.

Bovine Herpesvirus-1 is well known as a cause of infectious pustular vulvovaginitis in cattle. This agent also causes infectious bovine rhinotracheitis, conjunctivitis, balanoposthitis and, rarely, encephalitis. It plays a prominent role in undifferentiated bovine respiratory disease and abortion<sup>9</sup>. Infectious pustular vulvovaginitis is characterised by perineal pain (often indicated by a raised tail) and oedema, pustules, plaques of white necrotic material and mucopurulent discharge of the vestibular and vaginal mucosae. The

disease can be transmitted by natural breeding or sometimes by infected cattle sniffing the perineal area of susceptible cows<sup>9</sup>. In common with other herpesviral infections, latent infection with reactivation of the virus in times of stress, is possible.

Infectious bovine rhinotracheitis virus has been implicated in pathogenic synergism with other organisms. Depressed lymphocyte blastogenesis has been documented in calves infected with virulent type-1 bovine herpesvirus<sup>3</sup>. Detrimental effects of the virus on neutrophil functions, including diminished random migration and phagocytic ability and reduced Fc receptors on the cell membrane, have also been reported<sup>4 11</sup>. Enhancement of pneumonic pasteurellosis by infectious bovine rhinotracheitis virus has been postulated to result from impairment of cellular immune mechanisms by the virus with resultant diminished clearance of the bacterial pathogen<sup>4 11</sup>.

*Moraxella bovis* is not a common pathogen of the genital tract, being better known for its role in the aetiology of infectious bovine keratoconjunctivitis or "pink-eye". This disease is most prevalent in summer and it is thought that ultraviolet radiation enhances the pathogenicity of *M. bovis* by affecting cell-mediated immune responses as well as inducing changes in the cornea<sup>5</sup>. A pathogenic interaction between *M. bovis* and bovine herpesvirus-1 has been reported<sup>5 6</sup>. Calves vaccinated by intranasal or ocular administration of a live attenuated infectious bovine rhinotracheitis vaccine, were exposed 4 d later to experimental infection with *Moraxella bovis*. Vaccinated calves



Fig 2: Later progression of vulvitis, showing severe ulcerative, granulomatous lesion

developed more severe ocular lesions than unvaccinated control calves. Corneal perforation was observed only in vaccinated calves, and frequency of isolation of *Moraxella bovis* was significantly greater in control calves. These authors also postulate that impaired bacterial clearance due to depressed cellular immunity caused by the concomitant viral infection, was responsible for the increased severity of lesions produced. *Moraxella bovis* produces a skin necrotising toxin. Diminished bacterial clearance would allow accumulation of a greater local concentration of this toxin. The activity of the toxin in the genital tract has not



Fig 3: Vestibular signs associated with vulvitis consisting mainly of small vesicles, nodules and ulcers



been evaluated, but killed cultures of *Moraxella bovis* produce a severe local reaction and necrosis when injected intradermally into laboratory animals<sup>5,6</sup>. Prior erosion of the vulvar epithelium by bovine herpesvirus type-1 may enhance the pathogenic effect of *Moraxella bovis*.

Several species of the genus *Mycoplasma* have been found to infect the genitalia of cows. Trichard & Jacobsz<sup>14</sup> cultured cervicovaginal mucus from 1 053 cows in 151 South African herds. They found animals from 48% of herds to be positive for one (29%) or more (19%) species of *Mycoplasma*. *Mycoplasma bovis* was the most common isolate. In a single case *Mycoplasma bovis* and *M. canadense* were isolated from the same cow. *Mycoplasma bovis* was not isolated from any of the cows in this study. Apart from *Mycoplasma mycoides* ss. *mycoides*, *Mycoplasma bovis* is regarded by some to be the most pathogenic bovine mycoplasma. This organism was, however, isolated from the prepuce of six of 1 099 bulls, or semen of one of 986 samples examined<sup>14</sup>.

Although they may be present in clinically normal individuals, *Mycoplasma* spp have been implicated in various forms of bovine genital tract pathology. *Mycoplasma bovis*, and *M. bovis* appear to be the most important pathogens of the genital tract, being involved in such conditions as lowered sperm motility (*M. bovis*), vesiculitis, epididymitis (*M. bovis* and *M. bovis*) and endometritis<sup>2</sup>. *Mycoplasma canadense* has been isolated from aborted bovine fetuses<sup>1</sup> and from a cow with a history of infertility<sup>13</sup>.

A variety of *Mycoplasma* spp has been found in association with *Moraxella bovis* in naturally occurring cases of infectious bovine keratoconjunctivitis. These include *Mycoplasma bovis*, *M. bovis* and *M. bovis*<sup>10</sup>. In an experimental setting, preinfection of calves with *Mycoplasma bovis* caused prolonged ocular colonisation of *Mora-*

*xella bovis*, and more severe ocular lesions compared to control calves<sup>12</sup>.

The pathogens isolated from the subjects of this report, or closely related organisms, have therefore been shown to have synergistic pathogenic action in ocular pathology. It seems likely that the dramatic lesions seen were the result of the combined infection. Failure to reproduce the lesions experimentally, can be accounted for in several ways. The animals exposed to infectious material were mature cows, while the affected heifers were 14 - 18 months of age. The immune status of the experimental cows was not known. Prior exposure could not be determined. The experimental cows were housed in small groups and fed hay ad libitum in contrast to the intensively housed and fed heifers. Indeed, the intensive management of the infected heifers is considered a potentially important factor in the pathogenesis, as it facilitates spread of pathogens in a stressed population.

The good response of the affected animals to treatment with tetracycline antibiotics, provides further support that the isolated organisms were instrumental in the pathogenesis. Most *Mycoplasma* spp are sensitive to tetracyclines, and this antibiotic has been found to be effective in cases of *Moraxella bovis* infection as well<sup>7,8</sup>.

It is our opinion that this unusual form of vulvitis was the result of stressful management conditions and the pathogenic synergism of bovine herpesvirus type-1, *Moraxella bovis* and *Mycoplasma* spp. We suggest that this outbreak provides an outstanding example of multifactorial disease aetiology in modern animal husbandry systems.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr M.C. Williams for histopathological examination of tissues, and to Dr Dion Schaap for referral of the cases.

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## EQUINE PHARMACOLOGY AND THERAPY

### Proceedings of the 11th Bain-Fallon Memorial Lectures 1989, Australian

Equine Veterinary Association, Australia 1989 pp vi and 242, price \$A 60 (ISBN 0 7316 6093 5)

The 11th Bain-Fallon Memorial Lectures presented by the Australian Equine Veterinary Association were held at Pelican Beach Resort, Coffs harbour, New South Wales from May 22nd to 26th 1989.

An impressive array of speakers headed by Professor Desmond Baggot of the Irish Equine Centre and Professor Tom Tobin of the University of Kentucky, discussed a wide range of subjects related to the theme of the lectures. Both have vast experience in their respective fields. Professor Baggot dealt, in particular, with the selection of the most appropriate drugs for treatment of specific conditions, with specific emphasis on drug reactions, interactions and comparative absorption and disposition of drugs. Attention was also given to the treatment of selected bacterial infections in the horse, including neonatal foals, and the clinical pharmacology of drugs affecting the gastro-intestinal tract. Professor Tobin discussed the use of drugs in the racing horse, the action of certain substances, procedures applied to measure any performance-modifying activity and control measures that authorities, particularly in the United States, have instituted to prevent illegal use. Analytical methods, including recently-developed immunoassay procedures were covered. Presentations included the use of non-steroidal anti-inflammatory drugs, cortico-steroids, narcotics, stimulants, anabolics, tranquilisers, analgesics and local anaesthetics in racing horses as well as the use of medication to control exercise-induced pulmonary haemorrhage. From his submission on detection and clearance times for drugs, it was obvious that it is not possible to set hard and fast guidelines that ensure negative pre-or post-race tests below certain permitted levels for every circumstance of pre-event drug administration.

Although much of Professor Tobin's material had application in the United States, it was inappropriate for Australia where racing authorities had adopted a policy of 'no detectable residues', not only of stimulants and depressants, but also of many of the recognised therapeutic agents that are used in the treatment of equine ailments.

The routine use of 'saline drenches' was questioned and could not be justified as producing any beneficial effect, unless large volumes of fluids and electrolytes needed to be administered for dehydration. No adverse effects will be seen, however, provided that a solution reasonably close to isotonic is used. The usual amount of electrolytes in 2.3 litres of a 'pre-race drip that represents 1% of total body weight, were regarded as insignificant.

Of particular interest to equine practitioners were papers on sedatives, anaesthetics and anaesthetic emergencies in the horse. Detomidine was included among the agents considered in a most comprehensive review of a wide range of pre-anaesthetic and anaesthetic agents. Management of purchasing, storage and marketing of commonly-used drugs was central to economic practice and guidelines were proposed in a well-considered, well-reasoned submission by Dr. Reg Pascoe.

The attendance of 143 equine practitioners was an indication of the interest within the profession in the 11th Bain-Fallon lectures. The opportunity was taken to keep abreast of the ever-continuing advances in pharmacology and therapeutics and to obtain an informed appreciation of recent amendments to the Rules of Racing.

The Proceedings of the lectures in which all the prepared papers are published will form a most valuable reference source to all those engaged in equine practice.

J.M. BOURKE

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## TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

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