South African Veterinary Association
Tydskrif van die
Suid-Afrikaanse Veterinêre Vereniging

Junie/June 1991 Volume ● Jaargang 62 No. 2

SA ISSN 0038-2809
Dewey Cat No 636 089

All correspondence:
The Director,
SAVA, Jl S Afr. vet.Ass.,
P.O. Box 25033,
Monument Park,
0105 Pretoria.
Tel. (012) 346-1150

Alle briefwisseling:
Die Direkteur,
SAVV, Tydskr S.Afr.vet.Ver.,
Posbus 25033,
Monumentpark,
0105 Pretoria.
Tel. (012) 346-1150

Editor/Redakteur:
J. van Heerden

Administrative Editor/ Administratiewe Redakteur:
Vacant/Vakant

Front page/Voorblad:

Two-year old colt BOLD SPEED, winner of the Administrators Champion Juvenile Stakes (Gr. 1).

Agents in Great Britain/Agente in die Verenigde Koninkryk:
Baillière, Tindall & Cassel,
8 Henrietta St.
Covent Garden,
London

Printed by/Gedruk deur:
Berla Printers/Drukkery,
Pretoria

Contents ● Inhoud

Geldelike steun deur die Departement Nasionale Opvoeding word met dank erken.
Financial subvention by the Department of National Education is gratefully acknowledged.

Phenobarbitone and feeding in dogs

Thurman and co-workers (JSAVA 59: 86-89) reported an apparent effect of food on the systemic availability of phenobarbitone administered by mouth in dogs. Their data showed that feeding dogs immediately after dosing resulted in an AUC_{0-24} (area under the serum drug concentration - time curve from 0 to 24 h after dosing) which was 10% less than that obtained after dosage on an empty stomach. The peak mean serum drug concentration also appeared to be reduced by feeding while times to peak concentrations were unaffected.

The authors suggested that these findings indicated that ingesta reduced phenobarbitone absorption. However, comparison of AUC_{0-24} values might be inadequate for assessing changes in the extent of absorption, given the long half-time of elimination of the drug (mean 29,3 h). The flatter slope of the terminal portion of the curve shown for fed dogs suggests absorption might have been delayed, even though peak times were unchanged. In rats, food intake delayed phenobarbitone absorption by slowing gastric emptying, thus increasing the time for the drug to reach its main absorption site in the small intestine[1]. By contrast, no consistent effect of food was evident in a clinical study involving human infants[2].

As the authors noted, even a 10% reduction in phenobarbitone absorption might have no effect on control of epilepsy. However, if higher doses are given with food in compensation, and if absorption is delayed rather than reduced, phenobarbitone might accumulate to toxic concentrations. If such adjustments are made, it would be advisable to monitor serum or plasma drug concentrations to ensure they remain in the safe and effective range.

There appears to be an error on page 88, as the factor 0,232 should convert μmol to mg, not mmol to mg as shown (i.e. 1 μmol = 0,232 mg phenobarbital).

1. Kajima S, Smith R B, Doluisio J T 1971 Drub absorption V Influence of food on oral absorption of phenobarbital in rats. Journal of Pharmaceutical Sciences 60: 1639-1641
2. Jalling B 1974 Plasma and cerebrospinal fluid concentrations of phenobarbital in infants given single doses. Developmental Medicine and Child Neurology 16: 781-793

A D J Watson, Department of Veterinary Clinical Sciences, The University of Sydney, NSW 2006, Australia

Penobarbitone and feeding in dogs

At steady state AUC_{0-24} is adequate to describe the extent of absorption. AUC over a dose interval at steady state is usually equivalent to AUC after a single dose extrapolated to infinity. The only reason it is not so in this case is that enzyme induction occurs with phenobarbitone.

Although the mean curves in our paper (JSAVA 59: 86-89) appear to indicate a flatter terminal slope after feeding, elimination rate constants were not significantly different on Days 22 and 24 (paired t-test $p = 0,56$, median K_E for Day 22 was 0,025 h^{-1} and for Day 24 was 0,024 h^{-1}). It is most unlikely that absorption could be slowed to such an extent that it would affect 8-24 h samples. The mean, standard deviation, and range of gastric emptying time, small intestinal transit time, and small intestinal emptying time of normal dogs is $76 \pm 16,7$ (30-120), $73 \pm 16,4$ (30-120), and $214 \pm 25,1$ (180-300) min respectively[1].

Since phenobarbitone in the therapeutic range obeys linear elimination kinetics, a 10% dose compensation and thus 10% accumulation would be expected to increase serum concentrations by 4 to 11 μmol ℓ^{-1} (therapeutic range 40-110 μmol ℓ^{-1}). This is most unlikely to lead to toxicity of clinical significance.

We acknowledge the typographical error on page 88 and thank Dr. Watson for bringing this to our attention.

1. Miyabayashi T, Morgan J P, Atilola M A O, Muhumuza L 1986 Small intestinal emptying time in normal Beagle dogs. A contrast radiographic study. Veterinary Radiology 27: 164-168.

G D Thurman, R Miller, L MsFadyen; University of Durban-Westville, Private Bag X54001, 4000 Durban

USE OF THE CARDIOPULMONARY FLOW INDEX TO EVALUATE CARDIAC FUNCTION IN THOROUGHBRED HORSES

A J GUTHRIE*, VALERIE M KILLEEN*, MARIA S G MÜLDERS** and J F W GROSSKOPF***

ABSTRACT

The ratio of the cardiopulmonary blood volume to stroke volume is called the cardiopulmonary flow index (CPFI). The CPFI can be determined indirectly from the simultaneous recording of a radiocardiogram and an electrocardiogram. The CPFI and cardiac output were measured simultaneously in horses (n = 10) that were diagnosed as having cardiac disease. The diseased subjects were probably all exposed to feed contaminated with the ionophore, salinomycin, and all showed clinical signs indicative of chronic toxic myocarditis. The results obtained from these subjects were compared with those from control animals and significant differences ($P < 0,05$) were found between the mean CPFI of the control horses and those with macroscopically visible myocardial fibrosis on post mortem examination. No significant differences were found between the means of the cardiac output measured in either of the groups of horses. The effect of pharmacological acceleration of the heart rate on the CPFI was also studied. Significant differences ($P < 0,05$) were found between the mean CPFI and the slopes of the regression lines of CPFI on heart rate of the control and principal groups of horses. These differences were greatest at heart rates near to the resting heart rates of the individuals. The CPFI was found to be a more sensitive measure of cardiac function than cardiac output, in the horses.

Key words: Equine, cardiopulmonary flow index, cardiac function.

Guthrie A.J.; Killeen V.M.; Mülders M.S.G.; Grosskopf J.F.W. **Use of the cardiopulmonary flow index to evaluate cardiac function in Thoroughbred horses.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 43-47 (En.) Department of Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

In recent years, toxic myocarditis, particularly ionophore intoxication, has become an important clinical entity in horses. Cases of monensin[1 2 6 7 9 16], salinomycin[13] and naracin (R.H. Katzwinkel 1986 Private Practitioner, Gillitts, Natal, RSA, personal communication) poisoning have been reported in various countries. Horses with chronic monensin toxicity present with a history of poor performance and unthriftiness and on necropsy have been shown to have cardiac myopathy and replacement fibrosis[9].

Many diagnostic procedures have been adapted for use in horses and are now used as an aid to diagnose conditions that affect the function of the equine cardiovascular system. The cardiac output is used as a measure of cardiac function in many species, including the horse[3 5]. Horses with mild cardiac disease show a mild to severe drop in performance, although they show no signs which can be detected using existing non-invasive diagnostic methods[4]. A diagnostic technique that is sufficiently sensitive to detect a mild reduction in cardiac function could thus prove valuable in research and clinical practice.

The ratio of the cardiopulmonary blood volume to stroke volume can be determined indirectly from the simultaneous recording of a radiocardiogram (RCG) and an electrocardiogram (ECG). The indirect measurement of the ratio of the cardiopulmonary blood volume (CPBV) to stroke volume (V_s) is called the cardiopulmonary flow index (CPFI)[12 14 15]. This technique has been used in normal sheep, dogs, baboons, humans and horses, as well as in sheep, humans and horses with heart disease and sheep with pulmonary emboli[12 14 15]. From the data obtained in these studies, it was concluded that the CPFI is a sensitive and reliable index to describe the efficiency of the pumping function of the heart, within different models of heart disease in experimental subjects[12 14 15].

The purpose of the present study was to evaluate the CPFI as an index of cardiac function relative to the cardiac output in cardiovascularly sound Thoroughbreds and in Thoroughbreds exposed to feed probably contaminated with toxic levels of salinomycin; to evaluate the effects of changes in heart rate on the CPFI in these normal and diseased subjects; and to evaluate the sensitivity of the CPFI for the differentiation of healthy Thoroughbred horses from Thoroughbred horses with cardiovascular disease.

MATERIALS AND METHODS

Thoroughbred horses (n = 10) with a history of probable exposure to feed contaminated with toxic levels of salinomycin and that showed clinical signs indicative of cardiac disease, were donated for use as the principal subjects in this study. In general, the clinical signs were mild, but included reports of poor racing performance, electrocardiographic abnormalities, including arrhythmias (atrial fibrillation, intraventricular block, premature ventricular concentrations) and elevated serum enzyme activities following exercise. Three clinically normal Thoroughbred horses with no previous history or current signs of cardiac disease were used as control subjects in these investigations. All subjects were maintained under identical conditions of management and feeding during a 3-week adaption period prior to this study and for the entire duration of the investigations. All subjects had a portion of the left common

*Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa
**Department of Veterinary Pharmacology and Toxicology, University of Pretoria
***Department of Veterinary Physiology, University of Pretoria

Received: January 1991 Accepted: April 1991

carotid artery subcutaneously relocated prior to the commencement of the adaptation period. To reduce psychophysiological influences associated with the experimental protocol, animals were exposed to the laboratory environment on at least 3 occasions prior to data collection. Laboratory exposure included all parts of the experimental protocol and the laboratory personnel.

The cardiac output was measured using the Fick principle, with oxygen as the indicator substance. Mixed expired gas was collected by placing an airtight facemark over the horse's muzzle and allowing the subject to exhale via flow-directed valves into a meteorological balloon. The facemask was held in place by attaching it to the head-piece of a bridle. The airtight seal on the mask consisted of a thin rubber shroud over a piece of expanded foam rubber. The facemask was connected to the flow-directed valves by a 10 cm length of 5 cm-diameter flexible plastic tubing. The inspiratory side of the valves was open to the environment. The expiratory side was connected to the 200 ℓ meteorological balloon. A separate balloon was used to collect the gas during each of 3 one-minute sampling periods. The volume of gas in each balloon was measured, using a spirometer (Warren E. Collins, Braintree, MA). A 50 ml sample of the gas from each balloon was analysed for O_2 and CO_2 content using a semi-automated gas analyser (ABL3, Radiometer A/S, Copenhagen). Arterial and mixed venous blood samples were collected anaerobically into heparinised syringes during each of the one-minute sampling periods. Arterial blood was sampled via a previously-placed 18 G cannula, using aseptic technique, into the transposed portion of the carotid artery. Mixed venous blood was sampled, using a catheter, fashioned from polyethylene tubing (Clay Adams, New Jersey, NJ) introduced aseptically into the jugular vein via a 15 G hypodermic needle and then passed into the pulmonary artery. Correct positioning of the pulmonary artery catheter was confirmed both prior to and following sample collection by recording the intravascular pressure, and observation of the characteristic pulmonary artery waveform. Blood samples were sealed and placed in an iced water bath until they were analysed. Blood gas analyses were performed on these samples within an hour of collection, using a semi-automated blood gas analyser (ABL3, Radiometer A/S, Copenhagen). The haemoglobin concentration of these samples was measured, using a photometer and associated dual diluter (Coulter Electronics Inc., Hialeah, FL).

The oxygen consumption was calculated from the true oxyen fraction of the mixed expired gas and the gas volume. Oxygen content of arterial and venous blood samples was calculated from the haemoglobin concentration and saturation (calculated from oxygen tension) of the blood samples. Cardiac output was calculated by dividing the oxygen consumption by the arterio-venous oxygen content difference. These calculations were performed using a personal computer (International Business Machines, Boca Raton, FL) and dedicated software.

The radiocardiogram (RCG) was recorded by injecting a bolus of 185-370 MBq of 99m-Technitium (^{99m}Tc) into the left jugular vein via a catheter and then recording the gamma ray activity, with a gamma ray probe, as the isotope passed through the right and subsequently the left ventricles. The catheter used for the introduction of the isotope into the jugular vein, was made from polyethylene tubing and was approximately 30 cm long. It was introduced aseptically into the vein through a 15 G hypodermic needle. The gamma ray probe consisted of a collimated scintillation crystal and a discriminator, producing a signal that was recorded on a multichannel physiological recorder (Mingograf 62, Siemens-Elema AB, Solna, Sweden). The probe was supported by a stand which could be adjusted for height and which allowed variable positioning of the probe in both the horizontal and vertical planes. For recording of the RCGs, the probe was positioned on the left side of the horse with the collimator approximately one cm away from the chest wall at a point overlying the 6th intercostal space at the costochondral junction. The collimator was directed upwards at an angle of approximately 20° and forward at an angle of approximately 30°, thus pointing toward the point of the right shoulder. When the collimator is positioned correctly, the RCG is characterised by a left ventricular peak that is between 50 and 100% that of the right ventricular peak[12]. A simultaneous recording of the ECG was made on the multichannel physiological recorder using the semi-orthogonal Y lead. The CPFI was calculated by dividing the time between the two peaks of gamma activity on the RCG (CPTT) by the average duration of the R-R interval for the 10 heart beats that included the entire passage of the isotope through both ventricles. All procedures were carried out and all radioactive material was handled according to local statutes.

The cardiac output and CPFI were measured simultaneously. This was achieved by starting the mixed expired gas and blood sample collection and then injecting the bolus of ^{99m}Tc approximately 10s after the beginning of sample collection. Gas and blood samples were collected over a period of one minute, after which the meteorological balloon and heparinised blood samples for blood gas analysis were sealed and the latter placed in a bath of ice-water. This procedure was repeated 3 times on each individual during each experimental period, with approximately a one minute delay between each procedure. This entire experimental procedure was repeated on 3 separate occasions, with at least 7d between each procedure.

Response of CPFI to pharmacological acceleration of heart rate was studied in the cardiovascularly sound horses and

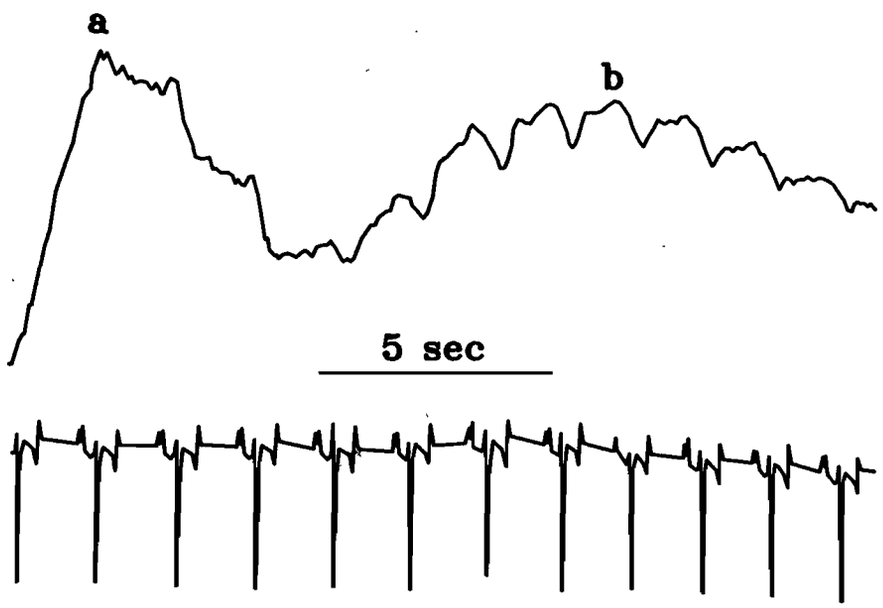


Fig. 1: **A typical tracing of the RCG (above) and ECG from a cardiovascularly sound subject. a and b - right and left ventricular peaks of gamma activity. Cardiopulmonary transit time (CPTT) was measured between a and b**

diseased subjects. This study was performed approximately one month after the initial study described above was completed. In these subjects a dose of 0,1 mg of isoproterenol (Isuprel, Sterling Drug (SA) (Pty) Ltd., Durban) was injected intravenously and the CPFI was measured one, 3 and 5 min after administration of the drug. This procedure was repeated 3 times in all subjects with at least 7 d between recordings.

Following these studies, all principal subjects were euthanased and autopsies performed. Subjects with and without macroscopically visible foci of myocardial replacement fibrosis were assigned to different groups. Principal subjects without macroscopically visible myocardial fibrosis, were assigned to Group 2 and those with such lesions were assigned to Group 3.

All statistical analyses were performed using the SAS (SAS Institue Inc., Cary, NC) package of computer programmes. The cardiovascularly sound horses were treated as a separate group (Group 1). CPFI data and cardiac output data from the 3 groups of horses were separately analysed, using an analysis of variance procedure with group, and the animals within each group, as factors and the heart rate as a covariate. Between-animal variation was used when group means were compared.

CPFI data from the horses with pharmacologically elevated heart rates (Groups 1 and 3) were analysed, using a regression analysis between the heart rate and CPFI with the group acting as a covariate. The interaction between the group and the heart rate was included in the model to test whether the slopes of the regression lines of the CPFI on the heart rate differed between the 2 groups.

Table 1: **Mean (±SD) and number of observations (n) for age, mass, cardiac output, cardiac index, cardiopulmonary flow index, heart rate, stroke volume and cardiopulmonary blood volume in control horses and horses probably exposed to feed contaminated with toxic levels of salinomycin**

Variable	Group 1	Group 2	Group 3
Age (yr)	8,3 ± 2,3 (3)	4,7 ± 0,5 (7)	5,3 ± 0,6 (3)
M_b (kg)	530 ± 4 (3)	468 ± 11 (7)	489 ± 23 (3)
$\dot{Q}$ (ℓ min^{-1})	26,01 ± 13,31 (20)	24,27 ± 5,89 (27)	24,34 ± 6,20 (12)
CI (ml min^{-1} kg^{-1})	49,18 ± 25,23 (20)	51,71 ± 12,50 (27)	49,37 ± 11,20 (12)
CPFI	5,94* ± 0,51 (28)	6,31 ± 0,55 (50)	8,09* ± 0,51 (21)
HR (beats min^{-1})	42,59 ± 8,89 (34)	37,89 ± 12,03 (53)	38,67 ± 6,34 (24)
V_s (ml)	628 ± 167 (20)	677 ± 120 (27)	650 ± 116 (12)
CPBV (ℓ)	3,97 ± 1,09 (17)	4,20 ± 0,81 (24)	4,99 ± 0,71 (24)

Group 1 - cardiovascularly sound horses, Group 2 - horses probably exposed to toxic levels of salinomycin without macroscopically-visible myocardial fibrosis and Group 3 - diseased horses with macroscopically-visible myocardial fibrosis
M_b = Body mass, $\dot{Q}$ = Cardiac output, CI = Cardac index, CPFI = Cardiopulmonary flow index, HR = Heart rate, V_s = Stroke volume, CPBV = Cardiopulmonary blood volume
*- signifies that the means are significantly different at $P < 0,05$

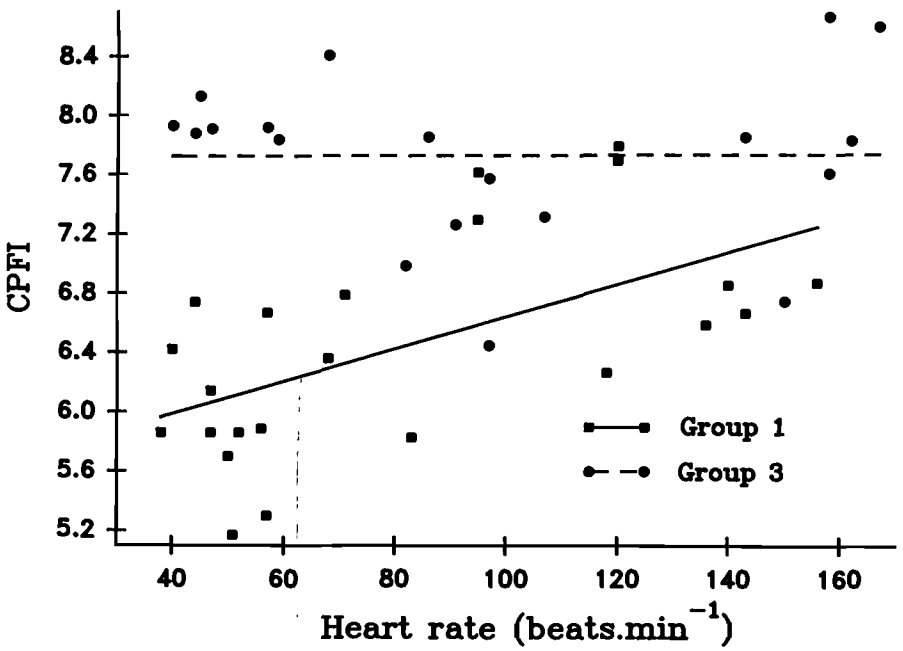


Fig. 2: **Linear regression of cardiopulmonary flow index (CPFI) on heart rate for controls (Group 1) and horses with macroscopically-visible myocardial lesions (Group 3) following intravenous injection of 0,1 mg of isoproterenol**

RESULTS

An example of the simultaneous recording of the radiocardiogram (RCG) and the electrocardiogram (ECG) is shown in Fig. 1. The first peak of activity was observed as the radioisotope passed through the right ventricle and the second peak was observed as the blood containing the isotope passed through the left ventricle. In this specific recording, the cardiopulmonary transit time was 9,80 s and the mean R-R interval of the 10 heart beats that included the entire RCG was 1,685 s. The CPFI was therefore 5,82.

The means, standard deviations and number of observations of the measured variables are tabulated in Table 1 for the sound group of horses (Group 1) and for the 2 groups of horses that were probably exposed to toxic levels of salinomycin (Groups 2 and 3). The mean CPFI of the horses in Group 1 was significantly different from that of the horses in Group 3 at the 95% confidence level. The coefficient of variation of repeated CPFI measurements on the same subject, ranged from 5,1 to 9,1% in the control subjects (Group 1), between 0,0 and 8,7% in Group 2 subjects and between 1,6 and 3,3% in Group 3 subjects.

Fig. 2 depicts the effects of pharmacological acceleration of heart rate on the CPFI in control (Group 1) and principal subjects (Group 3). The descriptive statistics for linear regressions of CPFI on heart rate for these groups of horses are shown in Table 2. The slopes of the 2 regression lines and the mean CPFI values from the 2 groups of horses were significantly different at the 95% confidence level.

Table 2: **Descriptive statistics of linear regressions of CPFI on heart rate for control horses (Group 1) and horses exposed to salinomycin (Group 3) after intravenous administration of 0,1 mg of isoproterenol**

Group	Y intercept	Gradient	n	r	P
Control	5,548	0,011*	23	0,58	<0,05
Group 3	7,717	0,00018*	20	0,02	>0,05

*-signifies that the slopes are significantly different at $P<0,05$

DISCUSSION

Recording of the data necessary for calculation of the CPFI was a simple procedure. The procedure was well-tolerated by all subjects and no adverse reactions were observed in any of the subjects. At least 3 measurements could be made in succession before the RCG became affected by background activity. Calculation of the CPFI from raw data was simple. Recording and analysis of data could be accomplished in a very short time. On the other hand, the collection of samples, analysis of samples and calculation of cardiac output was a difficult and time-consuming task.

The mean cardiac indexes in the 3 groups of horses studied, were very similar and ranged from 49,18 to 51,71 ml min^{-1} kg^{-1}. These results compare favourably with that of 63 ml min^{-1} kg^{-1} reported for resting horses using similar techniques[11]. These results are less than cardiac indices measured using thermodilution (72,61 ml min^{-1} kg^{-1})[8] and dye dilution techniques (80 ml min^{-1} kg^{-1})[10].

The mean CPFI of the cardiovascularly sound horses used in the present study was 5,94, whereas that of the control group of horses in the studies of Van Aarde and co-workers, was 6,75[12]. These differences could have been due to differences in methodology. In the present study, the ^{99m}Tc solution was introduced into the jugular vein using a 30 cm catheter, whereas the previous studies were performed using a hypodermic injection needle. The RCG can be affected by differences in collimator positioning[12]. In both studies, RCGs were only analysed if the left ventricular peak was between 50 and 100% the amplitude of the right ventricular peak. Thus differences between the results from this study and those of previous studies due to differences in collimator positioning, should have been minimised. The differences between the results could also be due to the fact that the horses used in the present study had a known history of being free from any signs of cardiac disease, whereas the horses used by Van Aarde and co-workers[12] were from a more heterogeneous population and had less well-documented histories.

Significant differences ($P<0,05$) were found to exist between the mean CPFI of cardiovascularly sound horses and principal subjects with macroscopically visible myocardial replacement fibrosis. Means of cardiac output for all 3 groups were similar and no significant differences were found to exist between any of the groups, even after standardisation of the cardiac output for body mass (cardiac index) and for heart rate. These results suggest that the CPFI is a more sensitive cardiac function test than measurements of the cardiac output or cardiac index.

In studies of the influence of exogenously-induced tachycardia on CPFI, significant differences were found to exist between the mean CPFIs and the slopes of the regression lines of CPFI on heart rate from the 2 groups of horses studied (at $P<0,05$). In the cardiovascularly sound group of horses, CPFI and heart rate increased concurrently. Van Aarde et al. showed similar findings in a group of 12 sound horses[12]. In this study, the linear regression equation of CPFI on heart rate was, $CPFI=4,16+0,0686 \times HR$, $r=0,3647$[12]. This equation is comparable with that of the control animals in the present study. In similar studies in diseased subjects, Van Aarde et al. found that the gradients of regression lines of CPFI on heart rate were steeper than those of control horses[12]. These authors ascribed these differences to increases of CPBV and concurrent decreases of V_s in the subjects with cardiovascular disease[12]. In the present study, the slope of the regression line of CPFI on heart rate of diseased subjects was less steep than that of control subjects. Furthermore, stroke volume was very similar in all groups of animals studied, while mean CPBV was increased, although not significantly, in the affected animals. This suggests that the cardiac changes associated with toxic myocarditis result in increases of CPBV without a reduction in stroke volume.

These data show that in the diseased subjects used in these studies, CPFI deviated more at a heart rate that was close to the resting heart rate of the individual. Increase of CPBV without concurrent increases in V_s suggest that toxic myocarditis associated with salinomycin intoxication may result in more severe reduction of left ventricular than right ventricular function. These data also suggest that the CPFI is a sensitive index of cardiac function in resting Thoroughbred horses. The CPFI may thus provide a reproducible and practical diagnostic technique for detecting mild cardiac pathology in Thoroughbred horses.

ACKNOWLEDGEMENTS

The authors wish to thank the University of Pretoria for financial assistance, Prof H T Groeneveld for statistical advice, Mr W Maluleka, Mr J Tjale, Mr H Kgaha and Mr J Vilikazi for technical assistance as well as Mr G Le Roux and Mr A Hattingh for engineering services.

REFERENCES

1. Amend J F, Nichelson R L, King R S, Mallon F M, Freeland L 1986 Equine monensin toxicosis: useful ante-mortem and post-mortem clinicopathologic tests. Proceedings of the Annual Convention of the American Association of Equine Practitioners 31: 361-372
2. Beck B E, Harris W H 1979 The diagnosis of monensin toxicosis: a report on outbreaks in horses, cattle and chickens. Proceedings of the American Association of Veterinary Laboratory Diagnosticians 22: 269-282
3. Eberly V E, Gillespie J P, Tyler W S 1964 Cardiovascular parameters in the Thoroughbred horse. American Journal of Veterinary Research 25: 1712-1716
4. Holmes J R 1968 The equine heart: problems and difficulties in assessing cardiac function on clinical examination. Equine Veterinary Journal 1: 10-18
5. Kubo K, Senta T, Sugimoto O 1973 Cardiac output in the Thoroughbred horse. Experimental Reports of the Equine Health Laboratory 10: 84-89
6. Langston V C, Galey F, Lovell R, Buck W B 1985 Toxicity and therapeutics of monensin: a review. Veterinary Medicine 80: 75-84
7. Matsuoka T 1976 Evaluation of monensin toxicity in the horse. Journal of the American Veterinary Medical Association 169: 1098-1100
8. Muir W W, Skarda R T, Milne D W 1976 Estimation of cardiac output in the horse by thermodilution techniques. American Journal of Veterinary Research 37: 697-700
9. Muylle E, Vendenhende C, Oyaert W, Thoonen H, Vlaeminck K 1981 Delayed monensin sodium toxicity in horses. Equine Veterinary Journal 13: 107-108
10. Thomas D P, Fregin G F, Gerber N H, Ailes N B 1980 Cardiorespiratory adjustments to tethered-swimming in the horse. Pflügers Archiv 385: 65-70
11. Thornton J, Essen-Gustavsson B, Lindholm A, McMiken D, Persson S 1983 Effects of training and detraining on oxygen uptake, cardiac output, blood gas tensions, pH and lactate concentrations during and after exercise in the horse. In: Snow D H, Persson S G B and Rose R J (ed.) Equine Exercise Physiology Granta Editions, Cambridge: 470-486
12. Van Aarde M N, Littlejohn A, Van der Walt J J 1984 The ratio of cardiopulmonary blood

volume to stroke volume as an index of cardiac function in horses. Veterinary Research Communications 8: 293-302
13. Van Amstel S R, Guthrie A J 1986 Salinomycin poisoning in horses: case report. Proceedings of the Annual Convention of the American Association of Equine Practitioners 31: 373-382
14. Van der Walt J J, Van Rooyen J M 1977 Use of technetium-99m to determine haemodynamic changes during the development of ventricular failure with gousiekte. South African Medical Journal 52: 375
15. Van der Walt J J, Van Rooyen J M, Cilliers G C, Van Ryssen J C J, Van Aarde M N 1981 Ratio of the cardiopulmonary blood volume to stroke volume as an index of cardiac function in animals and man. Cardiovascular Research 15: 580-587
16. Whitlock R H, White N A, Rowland G N, Plue R 1978 Monensin toxicosis in horses: clinical manifestations. Proceedings of the Annual Convention of the American Association of Equine Practitioners 24: 473-486

THE AMMONIA TOLERANCE TEST IN HORSES

J S VAN DEN BERG*

ABSTRACT

Clinically normal horses (n=8) with ages ranging from 5 to 8 years, were starved for 12 h and their plasma ammonia concentrations were measured. The mean fasting plasma ammonia concentration was 17,8 ± 3,8 μmol ℓ^{-1}. After dosing ammonium chloride at a dose rate of 0,02 g kg^{-1}, there was a significant increase in plasma ammonia concentration, with a maximum rise after 20 min ($P < 0,05$). To investigate the influence of temperature on plasma ammonia concentrations of stored samples, 8 plasma samples were stored at -20°C and 4°C respectively. The plasma ammonia concentrations were measured after 6, 12 and 24 h in each of the stored samples. Plasma ammonia concentrations increased significantly after 12 and 24 h when stored at 4°C ($P < 0,05$). When plasma was stored at -20°C there was no significant increase from baseline concentrations during 24h ($P > 0,05$).

Key words: Plasma ammonia, ammonia tolerance test, horses

Van den Berg J.S. **The ammonia tolerance test in horses.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 48-50 (En.)
Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa

INTRODUCTION

Detection of chronic liver damage in the horse has its limitations due to the fact that the clinical pathologist is limited to the sulfobromophthalein (BSP) clearance test, clotting factors, plasma protein, blood ammonia and bile acid concentrations[5]. However, as pharmacological grade BSP dye is no longer available, other methods of assessing liver function should be investigated.

Plasma ammonia is produced by microbial deamination of urea and exogenous dietary amines in the intestinal tract[13]. Ammonia is absorbed from the intestine and carried via the portal venous blood to the liver where it is converted to urea[13]. When hepatic function is severely impaired, or when collateral communication between portal and systemic veins develops, ammonia concentrations may increase in systemic blood[4].

Plasma ammonia concentrations in normal horses and in horses with chronic liver disease, are well-documented in the literature and summarised in Table 1.

Table 1: **Reference values for plasma ammonia concentrations in equines**

Reference	Normal (μmol ℓ^{-1})	Chronic liver disease (μmol ℓ^{-1})
1	18,3-22,4	57,1-374,0
3	7,7-63,7	64,3-454,3
8	68,7	309,0
11	68,7	76,7-164,0
12	5,2-7,3	11,2
16	15,6	--
17	5,0-9,7	227,4-308,6
18	83	431,0

*Department of Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa

Received: October 1990 Accepted: January 1991

of blood around the liver[4]. With 60% or more loss of hepatic mass in the dog, the plasma ammonia concentration may remain within normal limits. However the post-dosing plasma ammonia concentrations of these dogs will be about 5 times the baseline concentration[4].

The toxic effects of ammonia in the horse are well-documented[9] and hepatic encephalopathy is a well-recognised disease entity in equines[17 18]. By dosing urea at 450 g per pony, 7 out of 8 ponies died within 12 h[9]. The lethal dose of ammonia for farm animals is 0,5-1,5 g kg^{-1} and clinical signs of toxicity can be seen with a minimum dose of 0,3 to 0,5 g kg^{-1} in the horse[13]. Care should therefore be taken in dosing ammonia to horses with high basal concentrations of ammonia.

The collection, handling and storage of samples for ammonia determination have received considerable attention[10 15 16]. Blood samples should be collected in ammonia-free heparin and the plasma separated from the blood cells within 30 min[16]. The plasma can then be stored at 4°C for a maximum period of 2 h before the ammonia concentration is determined. Anticoagulants such as sodium citrate, potassium oxalate and sodium fluoride will give erroneously high results[16], but no studies have been done on EDTA plasma for ammonia determination in the horse. EDTA was the anticoagulant recommended by the company marketing the enzymatic UV test reagents for ammonia determination, used in this trial.

Meyer et al.[15] showed that by performing a standardised ammonia tolerance test, normal dogs could be clearly separated from dogs with portosystemic shunts. The ammonia tolerance test in dogs is performed by fasting the animals for 12 h[15]. A baseline plasma sample is collected before dosing with 20% ammonium chloride solution at a rate of 0,1 g kg^{-1} per os[4 15]. A 30 min post-dosing plasma sample is obtained for ammonia assay[4 15]. In healthy dogs, the post-dosing plasma ammonia concentration is about 2-2,5 of the baseline concentration[4]. Higher values than this, indicates reduced functional hepatic mass secondary to shunting

The objectives of this trial were to establish baseline values for the ammonia tolerance test in clinically normal horses and to investigate the influence of temperature on the plasma ammonia concentration in stored samples.

MATERIALS AND METHODS

Apparently clinically normal, Thoroughbred geldings (n=8) were used in this trial. Their ages ranged from 5 to 8 years. All horses were housed individually and fed a mixture of lucerne and teff unless

otherwise stated. To ensure that the liver function in each of the horses was normal, total serum protein, albumin and globulin concentrations were measured in each horse. Only horses with albumin concentrations exceeding 30 g ℓ^{-1} were included in the trial. One gram of sulfobromophthalein (BSP) was injected intravenously and blood samples were collected in heparin from the opposite jugular vein after 4 and 9 min. The BSP concentration was measured and plotted against a standard curve on semilog paper and the T 1/2 calculated. Only horses with a T 1/2 for BSP of less than 3,8 min were included in the trial. The normal T 1/2 for BSP excresion in horses is 3,8 min[2 3 14].

Each horse was starved for 12 h and then weighed. Venous blood was collected in EDTA and centrifuged at 4 000 r.p.m. (Roto - uni II, Optolabor) for 5 to 10 min as soon as possible after collection. The plasma was separated and analysed for ammonia within 2 h. The plasma ammonia concentration was determined, using an enzymatic UV method with Boehringer Mannheim GmbH reagents (Boehringer, Mannheim, West Germany) and an LP6 spectrophotometer (Dr Lange). Each sample was analysed in triplicate.

The remainder of each plasma sample was split into 2 groups of 3 × 1 ml aliquots and stored in capped plastic tubes at 4°C and -20°C respectively. A sample from each group was analysed in triplicate for ammonia concentrations after 6, 12 and 24 hours to determine the effect of storage on ammonia concentrations.

If the basal ammonia concentrations were within acceptable limits (below 80 μmol ℓ^{-1}) 0,02 g kg^{-1} ammonium chloride in a 20% solution was dosed via stomach tube to all the starved horses. The horses were allowed free access to feed after dosing. Post-dosing blood samples were obtained every 10 min for a period of one hour and processed as described above.

Analysis of variance was used to determine if there was a significant change in the measured ammonia concentrations after dosing and after storage. A 95% confidence interval was regarded as significant. Student's t test with a 95% confidence interval was used for testing significance of the baseline ammonia concentration and the increase after 20 min. Results were reported as mean ± standard deviation.

RESULTS

The mean fasting plasma ammonia concentration in the horses (n = 8) was 17,8 ± 3,8 μmol ℓ^{-1}. There was a significant increase in plasma ammonia concentration after dosing ammonium chloride with the maximum increase after 20 min ($P < 0,05$). Post-dosing plasma ammonia concentration are summarised in Table 2. Plasma ammonia concentrations increased significantly from baseline levels after 12 and 24 h when stored at 4°C ($P < 0,05$). When plasma was stored at -20°C there was no significant increase from baseline concentrations after 24 h ($P > 0,05$). The changes in plasma ammonia concentrations after 6, 12 and 24 h are summarised in Table 3.

Table 2: **Plasma ammonia concentrations in 8 horses after ammonium chloride administration**

Time (min)	Ammonia concentration (μmol ℓ^{-1})	Range (μmol ℓ^{-1})
0	17,8 ± 3,8	10,1 - 23,9
10	43,7 ± 43,3	13,8 - 141,8
20	92,7 ± 72,9	18,1 - 230,2
30	58,6 ± 46,0	15,8 - 160,0
40	28,7 ± 13,5	17,0 - 57,3
50	25,2 ± 6,8	17,0 - 38,6
60	24,3 ± 5,4	18,1 - 32,0

DISCUSSION

The fasting plasma ammonia concentrations measured in this trial are considerably lower than the concentrations reported by others[8 12 18]. Since diet can influence the concentration of ammonia production in the intestinal tract and subsequent absorption and transport to the liver, it is therefore important to compare fasting plasma ammonia concentrations with reference values[4].

Plasma ammonia concentrations increased significantly after dosing ammonium chloride at a dose rate of 0,02 g kg^{-1} orally to starved horses. This dose rate is considerably less than the one used by Evans et al.[6] where ammonium chloride was used to acidify urine in horses. If an ammonia tolerance test is done when the baseline plasma ammonia concentration is high, consideration should be given to the possible pathophysiological consequences of hyperammonemia[4].

The post-dosing plasma ammonia concentration of 92,7 ± 72,9 μmol ℓ^{-1} (at 20 min) in this trial is higher than the post-dosing increase reported in dogs[4 15]. This may be due to the post-dosing sample in dogs only being collected after 30 min[15]. The ammonia tolerance test is used to diagnose congenital portocaval shunts and acquired shunts due to chronic fibrosing liver disease in the dog[4]. According to the opinion of Engelking et al.[5] based on unpublished data, the ammonia tolerance test in horses showed promise as a diagnostic aid for detecting hepatic failure[5].

Plasma ammonia concentrations may decrease during storage, due to vaporous loss as equilibrium is established between aqueous and gaseous phases[10]. Hemolysed blood samples should be rejected because erythrocytes have ammonia concentrations 2,8 times higher than that of plasma[7]. Animals should be fasted at least 6 h before sample collection, because the ingestion of protein has been shown to increase plasma ammonia concentrations[16].

Results of this study showed that ammonia concentrations in plasma will increase significantly when stored at 4°C, which is in support of the results reported by Ogilvie et al.[16]. This may occur due to

Table 3: **Changes in plasma ammonia concentrations (mean ± SD) after storage for 6, 12 and 24 h**

		Plasma ammonia concentration* after: (μmol ℓ^{-1})		
Temperature	**n**	**6 h**	**12 h**	**24 h**
4°C	8	+ 4,6 ± 3,5	+ 8,9 ± 4,7	+ 11,4 ± 3,3
-20°C	8	+ 2,7 ± 3,1	+ 4,5 ± 6,3	+ 4,1 ± 2,0

*Changes in plasma ammonia concentrations expressed as measured concentration minus baseline concentration

deamination of proteins like glutamine or due to breakdown of adenyl pyrophosphate and/or adenylic acid[10]. However, this study has shown that equine plasma can be stored at -20°C for up to 24 h before the ammonia determination is carried out.

Although values from the present study can be used as a reference, it is advisable that each laboratory determine its own base-line values, due to inter-laboratory variability. The clinical usefulness of the ammonia tolerance test to diagnose congenital or acquired portocaval shunts in horses, needs to be determined.

REFERENCES

1. Byars T D 1983 Chronic liver failure in horses. The Compendium on Continuing Education for the Practising Veterinarian 5: S423-S430
2. Coffman J R 1981 Bilirubin: its intermediates and sulfobromophthalein (BSP) clearance (T 1/2). Equine Clinical Chemistry and Pathophysiology. Veterinary Medicine Publishing, Kansas: 110-115
3. Divers T J 1983 Liver disease and liver failure in horses. Proceedings of the 29th Annual Convention of the American Association of Equine Practitioners: 213-223
4. Duncan J R, Prasse K W 1986 Liver. Veterinary Laboratory Medicine. Iowa State University Press: 132-135
5. Engelking L R, Paradis M R 1987 Evaluation of hepatobilliary disorders in the horse. The Veterinary Clinics of North America: Equine Practice 3: 563-583
6. Evans J A, Lambert M B T 1974 Estimation of procaine in urine of horses. Veterinary Record 95: 316-318
7. Gau N 1984 Nonprotein nitrogenous compounds. In: Kaplan L A, Pesce A J (ed.) Clinical Chemistry Theory, Analysis and Correlation. C V Mosby Company, Toronto: 1230-1233
8. Gulick B A, Lui I K M, Qualls C W, Gribble D H, Rogers Q R 1980 Effect of pyrolizidine alkaloid induced hepatic disease on plasma amino acid patterns in the horse. American Journal of Veterinary Research 41: 1894-1898
9. Hintz H F, Lowe J E, Clifford Y J, Visek W J 1970 Ammonia intoxication resulting from urea ingestion by ponies. Journal of the American Veterinary Association 157: 963-966
10. Hitt M E, Jones B D 1986 Effects of storage temperature and time on canine plasma ammonia concentrations. American Journal of Veterinary Research 47: 363-364
11. Lessard P, Wilson W D, Olander H J, Rogers Q R, Mendel V E 1986 Clinicopathological study of horses surviving pyrrolizidine alkaloid *(Senecio vulgaris)* toxicosis. American Journal of Veterinary Research 47: 1776-1780
12. Lindsay W A, Ryder J K, Beck K A, McGuirk S M 1988 Hepatic encephalopathy caused by a portacaval shunt in a foal. Veterinary Medicine 83: 798-805
13. Lloyd W E 1986 Urea and other nonprotein nitrogen sources. In: J L Howard (ed.) Current Veterinary Therapy, Food Animal Practice, Volume 2. W B Saunders Company, London: 354-355
14. Mendel V E, Witt M R, Gitchell B S, Gribble D N, Rogers Q R, Segal H J, Knight H D 1988 Pyrrolizidine alkaloid-induced liver disease in horses: an early diagnosis. American Journal of Veterinary Research 49: 572-578
15. Meyer D J, Strombeck D R, Stone E A, Zenoble R D, Buss D D 1978 Ammonia tolerance test in clinically normal dogs and dogs with portosystemic shunts. Journal of the American Veterinary Association 173: 377-379
16. Ogilvie G K, Engelking L R, Anwer M S 1985 Effects of plasma sample storage on blood ammonia, bilirubin and urea nitrogen concentrations: cats and horses. American Journal of Veterinary Research 46: 2619-2622
17. Reed S M, Andrews F M 1986 The biochemical evaluation of liver function in the horse. Proceedings of the 32 nd Annual Convention of the American Association of Equine Practitioners: 81-93
18. Turner T A 1983 Hepatoencephalopathy in a horse. Equine Practice 5: 9-18

THE PATHOLOGY OF SUBCLINICAL INFECTION OF *ENCEPHALITOZOON CUNICULI* IN CANINE DAMS PRODUCING PUPS WITH OVERT ENCEPHALITOZOONOSIS

ELIZABETH F MC INNES* and C G STEWART**

ABSTRACT

The macroscopic, microscopic and clinical pathology and the serology of 2 clinically normal Staffordshire Bull Terrier bitches, both of whom produced pups with confirmed encephalitozoonosis, is described. Mild histopathological changes, similar to those seen in the infected pups, were observed. The spores of *Encephalitozoon cuniculi* were seen in the renal tubules of the kidney of one of the bitches. The serum urea concentrations of one of the bitches was elevated. A positive titre against *E. cuniculi* was obtained in both of the bitches. A 10-year-old girl who had had close contact with one of the infected litters of pups, seroconverted to *E. cuniculi.* Her two siblings were serologically negative.

Key words: Encephalitozoonosis, canine, dam, pathology, serology, *Encephalitozoon cuniculi,* human infection.

McInnes Elizabeth F.; Stewart C.G. **The pathology of subclinical infection of *Encephalitozoon cuniculi* in canine dams producing pups with overt encephalitozoonosis.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 51-54 (En.) Department of Pathology, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa.

INTRODUCTION

Encephalitozoonosis is a microsporidian infection that affects a wide variety of mammals[18]. In carnivores it is a sporadic, severe disease of the neonate usually culminating in the death of the animal. Although canine encephalitozoonosis in young animals has been well described, the pathology of the bitch bearing the infected litter, has not been investigated. Dams of pups infected with encephalitozoonosis usually appear to be clinically healthy[18].

Szabo & Shadduck[20] inoculated adult dogs intravenously with viable *Encephalitozoon cuniculi* spores. Histologic examination of the kidney, brain and liver revealed microfocal plasma cell and lymphocyte aggregates at the renal corticomedullary junction and in the medullary interstitium. No organisms were present in these areas or in other regions of the kidney.

Stewart et al[17] examined an adult bitch dosed with *E. cuniculi* and found plasma cell infiltrates in the meninges, lungs and spleen, together with multifocal plasmacytic interstitial nephritis and membranoproliferative glomerulonephritis. The animal did not develop clinical signs.

Experimentally infected adult vervet monkeys *(Cercopithecus pygerythrus)* were found to display macroscopic and microscopic lesions in the liver and kidney and rarely in the brain. The organisms were occasionally seen in the kidney and liver of the vervet monkeys. However, no clinical signs were recorded in these cases[22].

Several cases of clinical encephalitozoonosis have been reported in man[3 8 21], although identification of the aetiological agent of some of these cases has been disputed[6]. Antibodies to *E. cuniculi* have been found in persons living in or visiting the tropics and antibodies to *E. cuniculi* have also been noted in people suffering from malaria, tuberculosis, filariosis, schistosomosis and other diseases[1 7 14].

In the present study, 2 bitches from separate kennels, and their respective pups, which both developed typical encephalitozoonosis, were examined. Serum from 3 children, with close contact with one of the infected litters, was tested for antibodies to *E. cuniculi.*

MATERIALS AND METHODS

Pup A was one of a litter of 5 produced by an 18-month-old Staffordshire Bull Terrier bitch (Bitch A) at kennel A. Encephalitozoonosis had been diagnosed in a littermate a week previously. Two of the pups had already died and the remaining pup (still alive) was reported to have shown mild nervous signs (star gazing). Three children in the household at kennel A had had very close contact with the bitches and their litters.

A 5-year-old Staffordshire Bull Terrier bitch (Bitch B) and her pup were from a breeding kennel (B) of Staffordshire and Pit Bull Terriers where heavy losses had occurred in the litters over the previous 2 years.

Neither of the bitches showed any clinical signs of disease and they did not reveal any abnormalities upon physical examination. Serum was collected from each bitch and her respective pup, and also from all the other dogs on both properties (n=16). This was tested for antibodies to *E. cuniculi* as previously described[16].

The serum urea and creatinine concentrations of both bitches and their respective pups were determined, using the described methodologies[23].

Urine was collected from each animal. It was centrifuged at 2000G for 10 min and the sediment was stained with Gram's stain and examined.

Peripheral blood smears were made, using blood obtained from ear veins. These were stained with RapiDiff (Clinical Sciences Diagnostics, Division of C.S.M.L. Pty Ltd. P.O. Box 38939, Booysens, 2016) and examined under a light microscope.

Sterile thiopentone (Intraval Sodium, Maybaker (SA) Pty Ltd) was used to euthanase the bitches and a complete necropsy was conducted on both bitches and their pups.

*Department of Pathology, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa
**Department of Infectious Diseases and Public Health

Received: November 1990 Accepted: February 1991

All the organs were examined grossly and specimens of the kidney, liver, lung, heart, stomach, pancreas, uterus and brain were placed in 10% neutral buffered formalin. Samples were routinely embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin, with Gram's stain and Masson's Trichome stain for histological examination. Numerous serial sections were cut from the kidneys of both bitches in order to locate the *E. cuniculi* organisms. One kidney from each animal was removed aseptically and primary kidney cultures were prepared as described[15].

Following confirmation of the disease in kennel A, serum was collected from the 3 children living in that household. After testing this serum, urine was collected from the sero-positive child, centrifuged at 900 G for 10 min and half the sediment was stained with Gram's stain. Phosphate-buffered saline was added to the remaining sediment which was then centrifuged at 900 G for 10 min and the sediment added to established Madin-Darby cell line of Canine Kidney cells (MDCK, Flow Laboratories). These cells were subpassaged twice weekly for 2 weeks. They were then stained with Giemsa stain and examined by light microscopy for the presence of *E. cuniculi*.

RESULTS

The clinical behaviour of both bitches was normal. Pup A was approximately 2 months old. It was slightly pot-bellied and enlarged kidneys were palpable. No other abnormalities were evident. Pup B showed a mucopurulent, bilateral ocular discharge and crusting around the nares. No further abnormalities were detected upon physical examination of the pup.

The results of the serology from all of the dogs on each property are shown in Table 2. Bitch A had a reciprocal titre of 640 and that of her pup was 160. Bitch B had a reciprocal titre of 40 and her pup had a titre of 640.

The results of urine examination and isolation of *E. cuniculi* in tissue culture are shown in Table 3. The urine sediment of Pup A revealed many Gram positive *E. cuniculi* spores. No *E. cuniculi* spores were present in the urine sediment of Pup B. No spores were seen in the urine from the child which had developed antibodies to *E. cuniculi* and spores were not detected in the MDCK tissue cultures.

Bitch A showed a mild diffuse pulmonary congestion. The renal capsule was difficult to strip and 2 focal pinpoint white spots were present on the cortical surface. The uterus contained a brownish necrotic substance.

Bitch B showed moderate congestion of the liver and spleen. The renal capsule was focally adherent.

The postmortem examination conducted upon Pup A, revealed a distended abdomen, increased consistency of the liver and mild proteinuria. The renal capsule was firmly adherent and the cortical surface mottled. The cortex was a pale, yellowish colour and petechiae were present on the cut surface. Small wedge-shaped whitish streaks extended from the cortex into the medulla.

Table 1: **Serum creatinine and urea concentrations of Bitch A and B and their respective pups**

Blood chemistry	Bitch A	Bitch B	Pup A	Pup B	Normal
S-urea mmol ℓ^{-1}	9,1	4,6	12,1	1,4	3,6-8,9
S-creatinine μmol ℓ^{-1}	111,0	91,0	72,0	47,0	< 133

Table 2: **Serological titres of dogs from Kennels A and B and the three children that had contact with the dogs from Kennel A**

Origin	Host	Total tested	Reciprocal titres							Total positive
			0	20	40	80	160	320	640	
Kennel B	Canine	20	15	1	1	1	1	-	1	5
Kennel A	Canine	5	1	-	-	1	1	-	2	4
Kennel A	Human	3	2	-	-	-	-	1	-	1

The only finding in Pup B was that the renal capsule was moderately difficult to strip.

The serum urea and creatinine concentrations are given in Table 1. No abnormalities or parasites were detected in the peripheral blood smears of any of the 4 dogs.

A moderate, multifocal, subacute, granulomatous interstitial nephritis was present in all sections of the kidney in Bitch A. The lesions consisted of foci or linear infiltrations comprised predominantly of plasma cells. A small centre of coagulative necrosis with haemorrhage, karyorrhexis, karyolysis and neutrophils was occasionally present. Rarely, the necrotic areas contained a few individual *E. cuniculi* spores. Only one colony of *E. cuniculi* spores was observed within a renal tubule, despite the numerous serial sections that were cut and examined. The mean size of the spores was 2,38 × 1,25 μm and they stained Gram positive.

The lesions in Bitch B were mild and consisted of subacute interstitial nephritis, characterised by tiny foci of lymphocytes, macrophages and plasma cells in the cortical and medullary interstitium. Despite numerous serial sections that were cut and examined, no

Table 3: **Demonstration of *E. cuniculi* by means of histopathology, urine examination and primary kidney tissue cultures**

	Urine examination	Tissue culture isolation	Histopathology
Bitch A	-	-	+
Pup A	+	+	+
Bitch B	-	-	-
Pup B	-	-	+

\+ = positive for *E. cuniculi*
\- = negative for *E. cuniculi*

parasites were seen.

The renal lesion in both bitches was accompanied by a mild, subacute, multifocal, granulomatous hepatitis, interstitial pneumonia, endometritis and encephalitis. The inflammatory infiltrate was predominantly lymphoplasmacytic in all areas. No *E. cuniculi* organisms were discerned in the sections stained by either HE or Gram methods.

Histopathology of Pup A revealed severe, subacute, multifocal, lymphoplasmacytic interstitial nephritis. Large interstitial foci of inflammatory cells occurred predominantly at the corticomedullary junction of the kidney. Several *E. cuniculi* organisms were seen in Pup A. These varied from densely-packed colonies to a few individual organisms present in the zones of inflammation. Often the colonies were not accompanied by any cellular response.

In addition to the renal lesions noted in Pup A, subacute, plasmalymphocytic intersticial myocarditis, multifocal granulomatous meningoencephalitis, multifocal hepatitis, mild gastritis and interstitial pneumonia were also noted. A small colony of Gram's positive *E. cuniculi* organisms was observed within an acinar cell of the pancreas.

In Pup B the renal lesions were extremely mild. Most glomeruli were unaffected, but occasionally, mild periglomerular fibrosis was encountered. Many serial sections of the kidney were recut and examined, but only 2 colonies of *E. cuniculi* were seen. Mild, subacute, perivascular, multifocal, lymphoplasmacytic meningoencephalitis, interstitial pneumonia and hepatitis were also noted in Pup B.

DISCUSSION

In contrast to the paucity of information available on the pathology in a bitch bearing a litter of pups infected with *E. cuniculi*, the pathology of blue fox vixens bearing infected pups has been extensively investigated[9,10]. Vixens are reported to be healthy and, with the exception of a thickening of the lamina propria of the uterine walls by predominantly mononuclear cells, no macroscopic or histopathological lesions are seen[10]. Parasites have not been detected in the organs of vixens giving birth to infected pups[10], although they must be present, since transplacental transmission has been conclusively demonstrated[11]. In this study, an inflammatory infiltrate into the submucosa of the uterine wall was noted, similar to that described in blue foxes[9].

In addition to blue foxes, transplacental transmission has been reported in rabbits[12], mice[13] and sporadically in the squirrel monkey (*Saimiri sciureus*)[2]. Circumstantial evidence for transplacental transmission has been reported in dogs[19], but has never been conclusively proved. The presence of lesions and a positive titre in both bitches and the presence of organisms in Bitch A, would suggest that the dams were the source of infection for the pups. Although transplacental transmission is the most likely means of transmission, it is possible that the organism could infect the pups during the birth process, or that the pups were infected from an environmental source.

The mild, lymphoplasmacytic, interstitial nephritis seen in these bitches appears to be similar to the lesions reported by Stewart[17] and Szabo & Shadduck[20]. Most of the extrarenal lesions seen in the 2 bitches could be described as a mild, focal, lymphoplasmacytic infiltration into various organs. The majority of the lesions appear to be a milder form of the lesions described in young pups suffering from encephalitozoonosis[4]. This type of lesion was seen in the liver, brain, lung and uterus of both bitches. It is significant however, that the renal lesions were fairly extensive in both bitches, although neither of the bitches displayed any untoward clinical or clinicopathological abnormalities. This leads one to speculate whether the renal lesions could have remained dormant and later manifested as a chronic interstitial nephritis entity in old age[18].

E. cuniculi organisms were observed in the kidney of Bitch A. This is a significant finding, previously unreported and it shows that the bitch can act as a source of infection for the pups. Stewart[17] and Shadduck & Szabo[20] observed a non-specific plasmalymphocytic interstitial nephritis in their experimentally-infected adult dogs, but did not find any parasites present in the organs examined. In addition, no parasites were noted in the blue fox vixens studied by Mohn[10], although parasites were seen in their placentae.

It will be noted from the results that although *E. cuniculi* organisms were observed in the kidney of Pup B, it showed rather mild lesions and did not display any clinical abnormalities. Encephalitozoonosis can manifest as a mild, moderate or severe, fulminating disease[20]. In cases of mild encephalitozoonosis where the animal is not overly sick, it may be possible that the renal lesions remain and eventually lead to chronic interstitial nephritis[18].

As may be noted from the histopathological results, *E. cuniculi* organisms were detected in the pancreas of Pup A which displayed an overt case of encephalitozoonosis. This particular lesion in the pancreas has been previously described only in vervet monkeys[22], where a lymphoplasmacytic infiltrate was noted. However the presence of the *E. cuniculi* organisms was not reported in this particular study. This finding serves to emphasise the widespread dissemination of the organism.

The serum urea concentrations of Bitch A and Pup A were elevated. This finding is in accordance with those of Botha[5] and is indicative of renal damage caused by the organisms.

The child that developed *E. cuniculi* antibodies, did not display any symptoms and has remained in good health to the present date. It is likely that this child became infected from Pup A or a litter mate. Pup A had large numbers of spores in the urine which would have allowed ample opportunity for infection. The fact that the other 2 siblings did not develop antibodies in spite of a similar exposure, would suggest that humans are not readily infected with *E. cuniculi*.

Both of the bitches and their respective offspring had positive serum titres to *E. cuniculi*, indicating that this is probably the most sensitive means of diagnosis. Organisms were seen in the histopathological specimens of 3 out of 4 dogs. However, in the case of Pup B, serial sections had to be cut and examined before organisms were seen. This illustrates the difficulty that may be experienced in confirming cases of encephalitozoonosis. Urine examination and preparation of kidney tissue cultures did not prove to be a very accurate means of diagnosis. These observations support the statement of Botha[5] that careful evaluation of clinical signs, clinical pathology, gross and histopathological findings, culturing and serological results is required to obtain a definitive diagnosis of encephalitozoonosis.

ACKNOWLEDGMENTS

The authors wish to thank the staff of the Departments of Pathology and Infectious Diseases and Public Health, Faculty of Veterinary Science as well as the staff of the Chemical Pathology Laboratory, Faculty of Medicine, Medical University of Southern Africa. The authors would also like to thank Professor J. Boomker for the referral of the cases.

REFERENCES

1. Anonymous 1983 Parasitic disease surveillance. Antibody to *Encephalitozoon cuniculi* in man. World Health Organisation. WHO Weekly Epidemiological Record 5:30-32
2. Anver M R, King N W, Hunt R D 1972 Congenital encephalitozoonosis in a squirrel monkey *(Saimiri sciureus)* Veterinary Pathology 9: 475-480
3. Bergquist R, Stintzing G, Smedman L, Waller T, Andersson T 1984 Diagnosis of encephalitozoonosis in man by serological tests. British Medical Journal 288:992
4. Botha W S 1982 Die patologie van enkefalitozoönose by honde. M.Med. Vet (Path) Tesis, Universiteit van Pretoria
5. Botha W S, Van Dellen A F, Stewart C G 1979 Canine encephalitozoonosis in South Africa.

Journal of the South African Veterinary Association 50: 135-144
6. Bywater J E C 1979 Is encephalitozoonosis a zoonosis? Laboratory Animals 13: 149-151
7. Hollister W S, Canning E U 1987 An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *Encephalitozoon cuniculi* and its use in determinates of infection in man. Parasitology 94: 209
8. Matsubayashi H, Koike T, Mikata I, Takei H, Hagiwara S 1959 A case of encephalitozoon-like body infection in man. Archives of Pathology 67: 181-187
9. Mohn S J, Nordstoga K 1982 Experimental encephalitozoonosis in the blue fox. Neonatal exposure to the parasite. Acta Veterinaria Scandinavica 23: 344-360
10. Mohn S F, Nordstoga K, Dishington I W 1982 Experimental encephalitozoonosis in the blue fox. Clinical, serological and pathological examination of vixens after oral and intrauterine inoculation. Acta Veterinaria Scandinavica 23: 490-502
11. Mohn S F, Nordstoga K, Moller O M 1982 Experimental encephalitozoonosis in the blue fox. Acta Veterinaria Scandinavica 23: 211-220
12. Owen D G, Gannon J 1980 Investigation into the transplacental transmission of *Encephalitozoon cuniculi* in rabbits. Laboratory Animals 14: 35-38
13. Perrin T L 1943 Spontaneous and experimental encephalitozoon infection in laboratory animals. Archives of Pathology 36: 559-567
14. Singh M, Kane G J, Mackinlay L 1982 Detection of antibodies to *Nosema cuniculi* (protozoa: Microsporidia) in human and animal sera by the indirect fluorescent antibody technique. South-east Asian Journal of Tropical Medicine and Public Health 13: 110-113
15. Stewart C G, van Dellen A F, Botha W S 1979 Canine encephalitozoonosis in kennels and the isolation of *Encephalitozoon cuniculi* in tissue culture. Journal of the South African Veterinary Association 50: 165-168
16. Stewart C G, Botha W S, van Dellen A F 1979 The prevalence of *Encephalitozoon cuniculi* in dogs and an evaluation of the indirect fluorescent antibody test. Journal of the South African Veterinary Association 50: 169-172
17. Stewart C G, Collett M G, Snyman H 1986 The immune response in a dog to *Encephalitozoon cuniculi* infection. Onderstepoort Journal of Veterinary Research 53: 35-3
18. Stewart C G, Reyers F, Snyman H 1988 The relationship in dogs between primary renal disease and antibodies to *Encephalitozoon cuniculi*. Journal of the South African Veterinary Association 59: 19-21
19. Stewart C G, Botha W S 1989 Canine encephalitozoonosis. Zimbabwe Veterinary Journal 20: 89-93
20. Szabo J R, Shadduck J A 1988 Immunologic and clinicopathologic evaluation of adult dogs inoculated with *Encephalitozoon cuniculi*. Journal of Clinical Microbiology 26: 557-563
21. Terada S, Reddy K R, Jeffers L J, Cali A, Schiff E R 1987 Microsporidian hepatitis in the acquired immunodificiency syndrome. Annals of Internal Medicine 107: 61-62
22. Van Dellen A F, Stewart C G, Botha W S 1989 Studies of encephalitozoonosis in vervet monkeys *(Cercopithecus pyggerythrus)* orally inoculated with spores of *Encephalitozoon cuniculi* isolated from dogs (*Canis familaris*). Onderstepoort Journal of Veterinary Research 56: 1-22
23. Van Heerden J 1985 Disease and mortality of captive wild dogs *Lycaon pictus*. South African Journal of Wildlife Research 16: 7-11

THE SAFETY OF DIMETRIDAZOLE ALONE AND IN CONJUNCTION WITH OXYTETRACYCLINE IN HEREFORD CROSSBRED STEERS

G E SWAN[1], A SHAKESPEARE[2], MARIA S G MÜLDERS[1], P P MINNAAR[1], T W NAUDÉ[1] and H T GROENEVELD[3]

ABSTRACT

Dimetridazole was given intraruminally alone, and in conjunction with oxytetracycline to healthy, 10-11 month-old Hereford cross-bred steers (n = 6). Intraruminal treatment with dimetridazole was given through a fistula at 75 mg kg^{-1} daily for 5 d, while the oxytetracycline was injected intramuscularly at 10 mg kg^{-1} on Days 1 and 3 of the dimetridazole treatment. The animals were observed at various intervals throughout the trial period for adverse reactions, including effects on ruminal activity and motility, changes in live-mass, venous acid-base balance, haematology and ruminal and serum ammonia concentrations.

Dimetridazole, either when used alone or in conjunction with oxytetracycline, had a marked effect on ruminal function. Within 6 h of dosing, the ruminal pH fell to below 5, but then returned to pretreatment values over the next 24-48 h. This was followed by the eradication of the ruminal protozoal population in all animals tested and an increase in the methylene blue reduction time to more than 6 min. Ruminal motility remained unaffected throughout this period. During the week of treatment, the mean live-mass of the animals dropped by 20 ± 9,9 kg in the dimetridazole treated group and by 13,3 ± 2,8 kg in the animals treated with both dimetridazole and oxytetracycline. A mild to severe watery diarrhoea, which continued for 1 to 2 d, occurred in 4 animals after the first dimetridazole treatment. A compensated metabolic acidosis and an increase in haematocrit were observed. An initial transient rapid rise in rumen ammonia concentrations did not result in a concurrent rise in serum ammonia concentrations. Except for one animal, all the others recovered without intervention by the end of the trial period. However, in the one exception, it was necessary to administer fresh rumen content to re-establish ruminal activity. No significant differences were observed between the 2 treatment groups for any of the observations made.

Key words: Dimetridazole, oxytetracycline, safety, trichomoniasis, cattle, acid-base, rumen function.

Swan G.E.; Shakespeare A.; Mülders M.S.G.; Minnaar P.P.; Naudé T.W.; Groeneveld H.T. **The safety of dimetridazole alone and in conjunction with oxytetracycline in Hereford crossbred steers.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 55-59 (En.) Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

INTRODUCTION

The control of trichomoniasis in bulls has been achieved by therapeutic intervention[2]. Treatment is directed primarily towards infected bulls, since they represent the major source of transmission of the disease and also because the disease is regarded as self-limiting in cows[8]. Numerous topical treatments for trichomoniasis in bulls have been described[3 10]. These treatments are normally very elaborate, but are not always effective[9]. Systemic treatment, using various nitro-imidazole compounds such as metronidazole[8], ipronidazole[17] and dimetridazole[9 10], have been shown to be more effective. Metronidazole (Flagyl, Maybaker) appears to be less effective and is too costly for general use. Ipronidazole was previously the drug of choice, but has since been withdrawn from the market. Consequently the only systemically effective drug still available is dimetridazole. Dimetridazole has been administered per os to bulls at dosage rates of 50 to 100 mg kg^{-1}, daily for 5 d[1 4 11 12]. Most reports recommend the lower dosage rate. Dimetridazole dissolved in 20% sulphuric acid or dimethyl sulphoxide (DMSO) has been used for intravenous administration[16]. Numerous animals have been treated per os with no apparent adverse reactions, other than a mild indigestion, temporary reduction in appetite and a fall in milk yield[6]. The cause of these effects are not known, but has been ascribed to the effect of dimetridazole suppressing ruminal fermentation[6]. In the case of intravenous dimetridazole dissolved in 20% sulphuric acid, side-effects such as dyspnoea, ataxia, recumbency for up to 15 min and weakness for periods of up to 2 d, were noted[1]. These effects were transitory, but were regarded as unacceptable by the investigator.

Antimicrobial treatment, such as with oxytetracycline or penicillin, is given 1-2 d before dimetridazole treatment to reduce the numbers of preputial bacteria[17]. Preputial bacteria, particularly *Micrococcus spp,* are reported to cleave the imidazole ring of nitro-imidazole compounds in vivo and thereby reduce its efficacy. Without antibiotic pretreatment, efficacy of ipronidazole against trichimoniasis was reduced from 93% to 73%[17].

Recently, mortalities occurred in bulls following the use of dimetridazole (Emtryl base, Maybaker) at 75 mg kg^{-1} orally per day for 5 d (J Brandt 1989 Private Practitioner, Jan Kempdorp, personal communication). In one case, 8 out of 69 Hereford bulls died, while in another, 2 out of 110 Hereford cross bulls died. The animals exhibited nervous signs such as hyperexcitability, incoordination, chattering of the teeth and they eventually died. These effects appeared

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa
[2]Department of Medicine
[3]Department of Statistics

after the fourth treatment and progressed rapidly thereafter. Postmortem examination of 3 animals and histopathology of samples from one animal did not reveal any specific abnormality, except a rumen pH of 9 that was measured in one animal. Oxytetracyline HCl (Contromycin, Panvet) was administered at 20 mg kg^{-1} concurrently on Days 1 and 3 of the dimetridazole treatment.

Dimetridazole remains the only effective systemic drug available for the treatment of trichomoniasis in bulls. In the light of the unexplained mortalities, it was considered necessary that the safety of the product, particularly in combination with antibiotics, such as oxytetracycline be re-evaluated.

MATERIALS AND METHODS

Hereford crossbred steers (n=6), aged 10-11 months with a live-mass ranging from 202 to 288 kg, were used in the trial. The animals were purchased from a local feedlot which in turn had purchased them from a single owner in the eastern Cape Province. Forty eight days before the start of the trial, the animals were transferred to the research facilities at the Faculty of Veterinary Science, University of Pretoria. The animals were each identified by a numbered eartag.

The animals were kept together in a single open paddock. Each animal was fed 0,5 kg feed-concentrate per day in the morning and had ad libitum access to *Eragrostis* hay. Fresh borehole water was freely available in an automatic drinking bowl.

The animals were allocated to the treatment groups by restrictive randomisation, according to live-mass. Replicates of 2 animals each, one animal on each treatment, were formed after ranking by live-mass from the heaviest to the lightest animal. Allotment of the animals to replicates started from the heaviest animal and proceeded to the lightest animal. Within each replicate, the animals were randomly allocated to the treatment groups by means of a table of random numbers.

A rumen fistula, for the purpose of collecting rumen samples, was placed surgically in each animal on Day -39.

Each animal received one of the following treatments:

1. Dimetridazole at 75 mg kg^{-1} per day for 5 d.
2. Dimetridazole at 75 mg kg^{-1} per day for 5 d and oxytetracycline (Liquamycin 100, Pfizer) at 10 mg kg^{-1}, on Days 1 and 3. (Day 0 is the first day of dimetridazole treatment).

The animals were treated according to the live-mass measured on Day 0, after 12 h fasting and withholding of water. The dimetridazole was suspended in approximately 500 ml water and dosed through the rumen fistula. Oxytetracycline was administered intramuscularly in the neck. A maximum of 20 ml was administered per injection site.

Each animal was examined clinically, the measurements including heart rate, respiratory rate, rectal temperature and ruminal movements on Days -21, -7, -3, -1, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. Live-mass was measured on Days -21, 0, 7, 14, 21 and 71. The animals were observed daily for adverse reactions.

Venous blood was collected from the jugular vein in heparinised 2 ml syringes for determination of blood pH and acid-base balance on Days -21, -14, -7, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. The blood was kept on ice and the

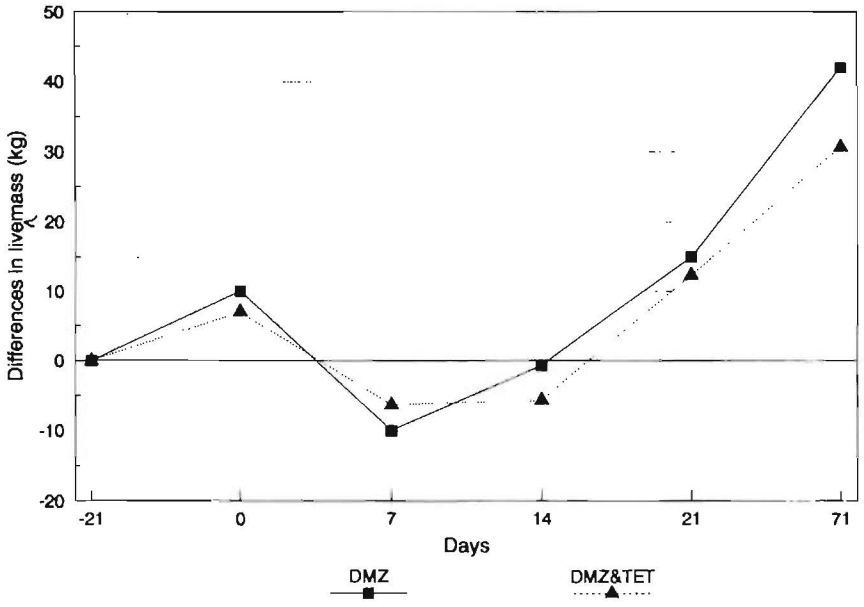


Fig. 1: **Changes in mean live-mass gain (kg) relative to individual live-mass at the start of the trial (Day-21) following treatment with either dimetridazole (DMZ) alone or in conjunction with oxy-tetracycline (DMZ&TET) in cattle**

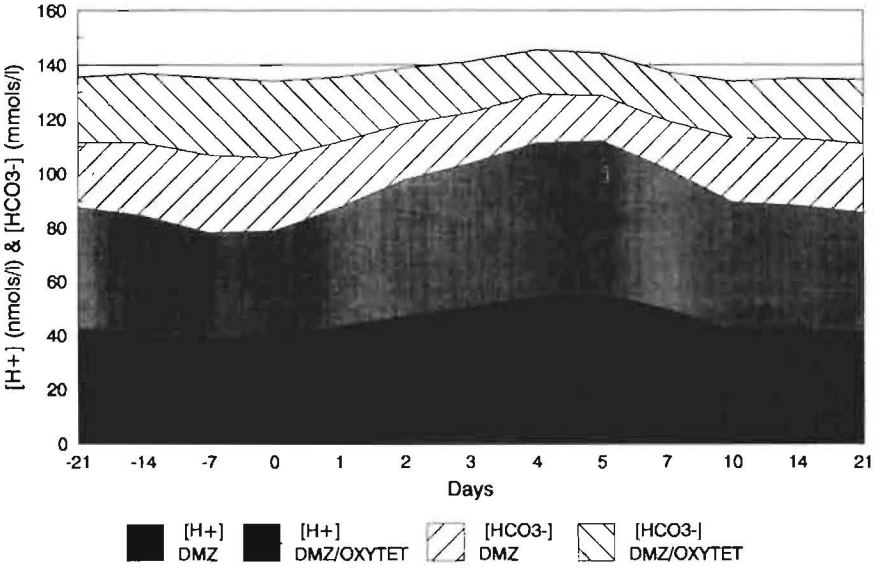


Fig. 2: **An area graph depicting changes in the mean venous hydrogen-ion (shaded areas) and bicarbonate-ion (hatched areas) concentrations before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&OXYTET) in cattle**

pH, pCO_2 and pO_2 determined within one hour of collection on a bloodgas acid-base analyser (ABL3, Radiometer). Additional venous samples were collected in EDTA and plain vacuum tubes on Days -21, -14, -7, 0, 4, 7, 10, 14 and 21 for haematology and the determination of plasma Na^+, K^+ and Cl^-. Calcium concentrations were determined on blood collected on Days 0, 1, 2, 3, 4, 5 and 7. Sodium and potassium concentrations were determined by means of an ion sensitive electrode (Baker Analyte model + 1, Baker Instrument Corp.) directly on the sample, whereas chloride and calcium concentrations were determined on a RA1000-automated chemical analyser (Technicon Instrument Corp.).

Serum samples for the determination of blood enzymes ((aspartate transaminase (AST), creatine kinase (CK)), creatinine and ammonia were collected from each animal on Days -14, 0, 4, 14 and 21 and on Days 0, 1, 2, 3, 4, 5, 7, 10 and 14, respectively. Serum ammonia concentrations were determined immediately before and 2 h after treatment on Days 0, 1, 2 and 3. AST, CK and creatinine serum concentrations were determined using a RA1000-automated chemical analyser (Technicon Instrument Corp.). Ammonia was determined by a described method[5].

Evaluation of rumen activity included rumen pH, proteolytic activity using the methylene blue reduction test[7] and microscopic examination of rumen micro-organisms. These evaluations were done on Days -21, -14, -10, -7, 0, 1, 2, 3, 4, 5, 7, 10, 14 and 21. On Days 0, 1, 2 and 3 there were 2 examinations, one before and another 2 h after treatment. Rumen ammonia concentrations were determined at the same time as the serum ammonia concentrations, using the same method.

Treatment groups were compared by means of the Mann-Whitney test.

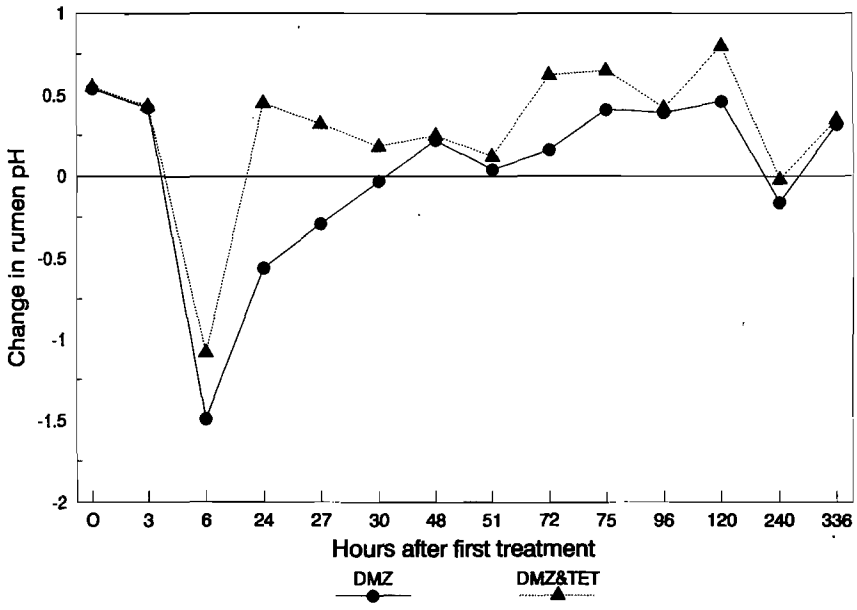


Fig. 3: **Mean rumen pH changes relative to the mean pretreatment rumen pH values following treatment with either dimetridazole (DMZ) alone or in conjunction with oxy-tetracycline (DMZ&TET) in cattle**

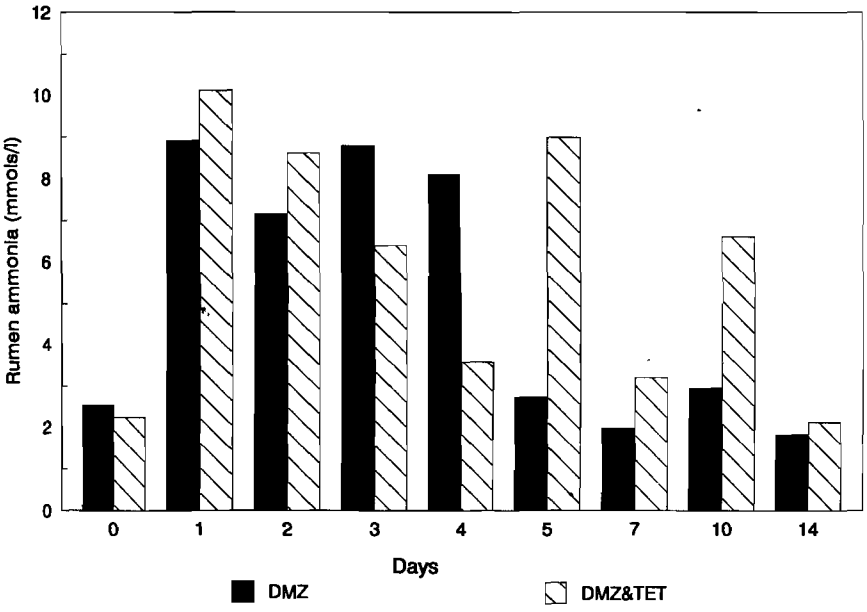


Fig. 4: **Changes in mean rumen ammonia concentration (mmols ℓ^{-1}) before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&TET) in cattle**

RESULTS

A mild to severe watery diarrhoea occurred in 2 animals of each group 1-2 d after the first dimetridazole treatment and continued for 1 to 2 d. The rumen contents became dry and impacted 3 d after dimetridazole treatment. Most of these animals had recovered by Day 10, although, in 2 cases (one from each treatment group), 1-1,5 ℓ of water was added to the rumen contents at various intervals from Days 3 to 10 for the purpose of collecting samples. In addition, one animal had to be treated with fresh ruminal contents on Day 8, whereafter it started to recover.

Decreases in live-mass gains in both treatment groups following treatment are presented in Fig. 1. The differences in live-mass loss between the treatment groups were, however, not significant.

A mild to moderate metabolic acidosis developed during Days 1 to 5 (i.e. during and following the treatment period) (Table 1 and Fig. 2). These levels returned to normal over the next 2 weeks, but with the dimetridazole/-oxytetracycline treatment group lagging behind the dimetridazole group (although not significantly different). The venous pCO_2 mimicked the changes in mean venous bicarbonate concentrations.

The changes in mean rumen pH after treatment relative to the mean pretreatment values are illustrated in

Table 1: **Individual and mean (±standard deviation) venous pH recordings before and after treatment with either dimetridazole (DMZ) alone or in conjunction with oxytetracycline (DMZ&TET) in cattle**

Treatment group	Animal No.	Venous pH recordings before and after treatment (days) -21	-14	-7	0	1	2	3	4	5	7	10	14	21
DMZ	1	7,34	7,37	7,41	7,41	7,34	7,32	7,30	7,26	7,26	7,32	7,42	7,40	7,37
	4	7,39	7,41	7,41	7,41	7,37	7,34	7,32	7,32	7,30	7,32	7,39	7,40	7,39
	6	7,39	7,39	7,41	7,40	7,37	7,32	7,26	7,22	7,21	7,27	7,32	7,33	7,39
	Mean	7,37	7,39	7,41	7,41	7,36	7,33	7,29	7,27	7,26	7,30	7,38	7,38	7,38
	SD	0,02	0,02	0,00	0,00	0,01	0,01	0,02	0,04	0,04	0,02	0,04	0,03	0,01
DMZ&TET	2	7,32	7,43	7,41	7,42	7,38	7,36	7,32	7,30	7,34	7,36	7,40	7,40	7,33
	3	7,35	7,34	7,39	7,41	7,39	7,36	7,34	7,25	7,23	7,31	7,38	7,39	7,40
	5	7,37	7,40	7,42	7,38	7,31	7,19	7,19	7,19	7,19	7,20	7,22	7,24	7,34
	Mean	7,35	7,36	7,41	7,40	7,36	7,30	7,28	7,25	7,25	7,29	7,33	7,34	7,36
	SD	0,02	0,03	0,01	0,02	0,04	0,08	0,07	0,04	0,06	0,07	0,08	0,07	0,03

DMZ-dimetridazole TET-oxytetracycline SD-standard deviation

Fig. 3. The ruminal pH of both groups of animals decreased sharply within the first 6 h of the first dimetridazole treatment and then rose back to pretreatment values over the next 24-48 h. In one animal treated with both dimetridazole and oxytetracycline, a ruminal alkalosis developed from Day 3 through to Day 5, but resolved itself thereafter.

The protozoal population was eliminated after the second dimetridazole treatment in all animals and only returned after Day 14. The time value of the methylene blue reduction test increased above the accepted 6 min interval by Day 2 and only returned to values less than 6 min by Day 14. Ruminal contractions were detectable throughout the trial period and were subjectively judged to be fairly consistent with regards to frequency, length of contraction and amplitude.

The rumen ammonia concentrations varied considerably over the experimental period. Despite this, the concentration apeared to rise sharply after the first treatment in both treatment groups (Fig. 4). However, further changes to ammonia concentrations in the rumen before and 2 h after treatment, were inconsistent. Serum ammonia concentrations remained low throughout the trial period.

Apart from an increase in haematocrit during the treatment period, no other abnormal changes in haematology, electrolyte concentrations or serum enzymes were observed throughout the trial period.

DISCUSSION

The adverse effects that occurred after the administration of dimetridazole alone or in conjunction with oxytetracycline in this trial, were in all probability caused by the suppression of rumen microbial activity and were not due to any direct systemically-toxic effect of the drugs per se. According to Owens & Goetsch[13] inadequately-controlled microbial fermentation may be reflected by the incidence of bloat, ammonia toxicity, nitrate toxicity and acidosis.

Nitro-imidazoles are bactericidal to most gram-negative and many gram-positive anaerobic bacteria[15]. They become effective after entry into a bacterial cell and reduction of the nitro-group to a number of unstable intermediates, including antibacterial active metabolites[14]. Reduction takes place under anaerobic conditions in the presence of a low redox potential such as that found in the rumen. Consequently, it may be expected that high concentrations of active, intermediate metabolites will be formed. The most susceptible rumen microbes are generally the gram-negative lactolytic anaerobic organisms. Suppression of these organisms following the administration of dimetridazole, will result in the accumulation of lactic acid as the predominant acid end-product. This could explain the initial development of a ruminal acidosis. Progression of the antibacterial action per se, or as a result of further dimetridazole administration, could result in suppression of a broader spectrum of the ruminal microbes, thereby inhibiting the production of the volatile fatty acids, which will lead to a rise in ruminal pH. The decrease in the redox potential of the rumen results from the suppression of bacteria that normally scavenge available ruminal O_2 to maintain the rumen in a reduced state. Most bacteria in the rumen are obligatory anaerobes and therefore find O_2 toxic[13].

The apparent elimination of ruminal protozoa by dimetridazole, may not in itself result in any detrimental effects on animal health. Studies conducted with defaunated animals, either by chemical or by dietary change, showed that animals were not adversely affected[18]. However, the destruction of these organisms could have resulted in the initial sharp rise in ruminal ammonia concentrations.

The systemic metabolic acidosis that developed, is believed to have resulted from the ruminal acidosis that occurred. This metabolic acidosis would have been aggravated by the diarrhoea. Adequate respiratory compensation prevented any further serious systemic effects.

Oxytetracycline administered parenterally can result in the disturbance of ruminal flora. However, since no significant differences were observed between the two treatment groups, it does not appear that oxytetracycline contributed to the adverse effects in this case. In our opinion, the population was too small to make any valid comparison. It is also possible that the oxytetracycline dose administered in this trial was too low in comparison to the dose administered to the animals that had died in the field. Further research would be needed to clarify these aspects.

Although the adverse effects observed in this trial did not result in any mortality, they were serious enough to have jeopardised the lives of these animals. Various possible contributing factors under field conditions, such as withholding water, concurrent dietary disturbance of rumen function and presence of disease, could have adversely tipped the scale and have resulted in death. Furthermore, it is possible that the adverse effects on the rumen flora would be different in animals that are kept under extensive conditions as a result of differences in the type of microbial populations.

More research is required to evaluate these different factors and to specifically examine the influence of dimetridazole on the ruminal flora.

ACKNOWLEDGEMENTS

The authors wish to thank Maybaker (Pty) Ltd for their financial support. We also acknowledge the technical advice we received from Dr A C Wellington and Prof J G van der Walt.

REFERENCES

1. Abbit B 1980 Trichomoniasis in cattle. In: Morris D A (ed) Current Therapy in Theriogenesiology. W B Saunders, Philadelphia: 482-488
2. Ball L, Mortimer R G, Cheney J M, Olson J D 1983 Trichomoniasis: diagnosis, pathogenesis, treatment and control. In: Proceedings of the American Association of Bovine Practitioners: 163-165
3. Bartlett D E 1948 Further observations on experimental treatment of *Trichomonas foetus* infection in bulls. American Journal of Veterinary Research 9: 351-359
4. Bon Durant R H 1985 Diagnosis, treatment and control of bovine trichomoniasis. The Compendium of Continuing Education for the Practising Veterinarian 7: S179-188
5. Chaney A L, Marbach E P 1962 Modified reagents for determination of urea and ammonia. Clinical Chemistry 8: 130-132
6. Clark R T J, Reid C S W, Young P W 1969. Bloat in cattle XXXII. Attempts to prevent legume bloat in dry and lactating cows by partial or complete elimination of the rumen holotrich protozoa with dimetridazole. New Zealand Journal of Agricultural Research 12: 446-466
7. Dirksen G 1979 Digestive system. In: Rosenberger G (ed) Clinical Examination of Cattle. 2nd edn. Berlin, Felgentreff and Goebel: 204-212
8. Gasparini G, Vaghi M, Tardani A 1963 Treatment of bovine trichomoniasis with metronidazole (8823 R P). Veterinary Record 75: 940-943
9. Kimsey P B, Darien B J, Kendrick J W, Franti C E 1980 Bovine trichomoniasis: diagnosis and treatment. Journal of the American Veterinary Medical Association 177: 616-619
10. Mahoney M C, Christensen J F, Steer J 1954 Trichomoniasis and its treatment in the dairy bull. In: Proceedings of the 91st Annual Meeting of the American Veterinary Medical Association: 73-76
11. McLoughlin D K 1965 Dimetridazole, a systemic treatment for bovine venereal trichomoniasis. 1. Oral administration. Journal of Parasitology 51: 835-836
12. McLoughlin D K 1967 Drug tolerance by *Tritrichomonas foetus*. Journal of Parasitology 53: 646-648
13. Owens F N, Goetsch A L 1988 Ruminal fermentation. In: Church D C (ed) The Ruminant Animal. Digestive Physiology. Prentice Hall: 145-171
14. Pratt W B, Fekety R 1986 The antimicrobial drugs. Oxford University Press, Oxford
15. Prescott J F, Baggot J D 1987 Antimicrobial therapy in veterinary medicine. Blackwell Scientific Publications, United States of America: 213-217
16. Ribot J, Uilenberg C 1965 Essias de traitement de la trichomonose du Taureau par le Dimetridazole. Revista de Medicina Veterinaria 11: 787-790
17. Skirrow S, Bon Durant R, Farley J, Correa J 1985 Efficacy of ipronidazole against trichomoniasis in beef bulls. Journal of the American Veterinary Medical Association 187: 405-407
18. Yokoyama M T, Johnson K A 1988 Microbiology of the rumen and intestine. In: Church D C (ed.) The Ruminant Animal. Digestive Physiology. Prentice Hall: 145-171

THE EFFECT OF THE LAPAROSCOPIC INSEMINATION TECHNIQUE ON THE OESTROUS CYCLE OF THE EWE

T L TALJAARD*, S J TERBLANCHE**, H J BERTSCHINGER** and L J VAN VUUREN**

ABSTRACT

This investigation was designed to determine whether or not the technique of intrauterine insemination affects the length of the subsequent oestrous cycle. Dorper ewes (n=31) were divided into treatment and control groups. All the ewes were synchronised using 40 mg fluorogestone acetate intravaginal sponges for 14 d and 300 IU pregnant mare serum gonadotrophin on the day of sponge removal. A standard semen diluent was deposited laparoscopically in each uterine horn of ewes in the treatment group. Teaser rams were used to detect oestrus. Progesterone profiles were used to confirm oestrus. The mean oestrous cycle length of 17,83 ± 0,69 d for the group in which the diluent was deposited by laparoscopy did not differ significantly ($P < 0,1$) from the 18,36 ± 2,11 d of the control group. The technique of laparoscopic insemination did not influence the length of subsequent oestrous cycles.

Key words: Laparoscopic insemination technique, ewes, oestrous cycle.

Taljaard T.L.; Terblanche S.J.; Bertschinger H.J.; Van Vuuren L.J. **The effect of the laparoscopic insemination technique on the oestrous cycle of the ewe.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 60-61 (En.) Department of Veterinary Physiology, Faculty of Veterinary Science, Medical University of Southern Africa, P.O. Box 230, 0204 Medunsa, Republic of South Africa.

INTRODUCTION

The intra-uterine laparoscopic insemination of sheep with frozen semen has become a widely-used technique[5 8 9 10]. Ewes that fail to conceive to laparoscopic insemination at synchronised oestrus, are usually bred to rams in the subsequent oestrus. In South Africa many farmers allege that ewes show prolonged inter-oestrous intervals following laparoscopic insemination (J. Steyn, Taurus, Bloemfontein - unpublished results). According to Maxwell[8], the manipulation and resulting stress associated with the intra-uterine insemination technique close to the time of ovulation, interferes with the passage of ova from the ovarian surface to the oviduct as well as with oviduct transport. Furthermore, ovarian examination may be detrimental to sperm transport and future embryonic survival[9].

It has also been shown that the additional manipulation associated with insemination into the tip of the horns rather than into the middle, reduces the lambing percentage[9]. The purpose of this study was to determine whether or not the technique of intra-uterine insemination affects the length of the first inter-oestrus period after insemination.

MATERIALS AND METHODS

Dorper ewes (n=31) of mixed ages with an average mass of 50,8 ± 7,4 kg and with a condition score[15] of 2 were used. They were kept in a small camp and fed ca 1,4 kg lucerne hay per day and a commercial sheep lick (Pascor F, Silgro) ad lib from 6 weeks before the start and throughout the duration of the experiment (July - August 1988). The ewes were randomly divided into 2 groups. Although 71% of the ewes were two-tooth, an even distribution of age was maintained. Group A (n=15) was the treatment group and Group B (n=16) served as controls.

Sponges impregnated with 40 mg fluorogestone acetate (Chronogest, Intervet, Kempton Park, SA) were inserted intravaginally into all ewes on Day -16 and subsequently removed on Day -3 at 23:30. PMSG (Folligon, Intervet, Kempton Park, SA) was administered intramuscularly at the same time at a rate of 300 IU per ewe. On Day -1 both groups were starved and exposed to 2 testosterone-treated wethers (200 mg testosterone cypionate intramuscularly every 2 weeks for the duration of the experiment; Testan, Centaur, Johannesburg, SA) fitted with harnesses containing marking crayons. The teasers were removed on Day 0, reintroduced on Day 3 and left with the ewes until the end of the experiment. The colour of the crayons was changed every 2 weeks. Marking of ewes was recorded daily.

On Day 0 (08:00), semen diluent[2] was deposited laparoscopically[3 6] in each uterine horn of sheep in Group A using a transcap and an aspic (IMV, France). All ewes were sedated with 0,25 ml xylazine (Rompun, Bayer, Isando, SA) 20 min prior to laparoscopy. The laparoscopic sites were infiltrated with local anaesthetic (Lignocaine 2%, Centaur, Johannesburg, SA) after disinfection. With the exception of blood sampling, Group B ewes were not handled during oestrus.

Blood samples were collected by jugular venipuncture into 10 ml heparinised vacuum tubes on Days 0, 7, 18, 25 and 36 of the trial. Additonal blood samples of ewes were collected on days when they were marked by the teaser rams if these did not coincide with the days mentioned above. Progesterone concentrations were determined by means of radio-immunoassay (Progesterone Coat-A-Count, DPC, Los Angeles, USA). Progesterone profiles were used to determine ovarian activity and the occurrence of silent oestrus.

Results were compared statistically using Student's *t* test.

RESULTS

Nine of 15(60%) ewes in Group A and 10 of 16 (63%) ewes in Group B were marked. One ewe in Group A and one in

*Department of Veterinary Physiology, Faculty of Veterinary Science, Medical University of Southern Africa, P.O. Box 230, 0204 Medunsa, Republic of South Africa
**Department of Theriogenology, University of Pretoria

Received: January 1991 Accepted: April 1991

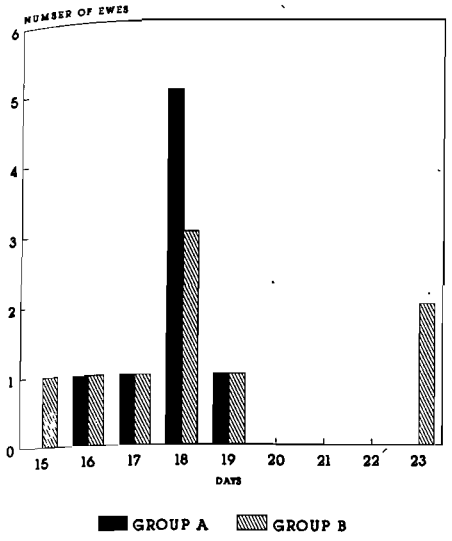


Fig. 1: **The distribution of the oestrous cycle length after synchronised heat of Group A and Group B ewes based on the number of ewes marked by the teaser rams**

Group B were excluded from the calculations because they were marked on Day 5 and Day 6 respectively. Fig. 1 shows the distribution of oestrous cycle length after synchronised heat in each of the 2 groups. The mean oestrous cycle length for Groups A and B were respectively 17,7 ± 0,83 and 18,56 ± 2,63 d. The difference was not statistically significant (P > 0,01).

Teaser rams only marked 57% (8/14) and 60% (9/15) of the ewes in each group. Serum progesterone concentrations suggested that a further 4 of 14 (29%) and 5 of 15 (33%) ewes in Groups A and B respectively, that had not been marked at the end of the first post-synchronisation cycle, had basal concentrations on Day 18, with subsequent rises to mid-cyclic concentrations. These ewes had indeed cycled with ovulation on about Day 19. Thus, using the progesterone profiles, the number of ewes cycling and the mean corrected cycle length of Groups A and B were 12 of 14 (86%) and 17,83 ± 0,69 and 14 of 15 (93%) and 18,36 ± 2,11 days respectively (Fig. 2). This difference was also not statistically significant (P > 0,1).

DISCUSSION

The mean cycle lengths in this trial were in agreement with results recorded by Boshoff[1] who found a mean oestrous cycle length of 17,7 ± 3 d post synchronisation. According to progesterone profiles, the percentage ewes cycling between 15 and 23 days after the synchronised oestrus, was comparable with results reported by various authors[2 3 11 14]. However, teaser rams only marked a low percentage of ewes. Failure of teasers to mark ewes during oestrus is well-documented[4 6 13] and progesterone profiles showed that silent oestrus occurred during this trial. Silent oestrus is a common phenomenon in sheep and occurs particularly at certain times of the year, in young animals, during lactation or with poor nutrition[12 14]. Most of the sheep used in this trial, were two-tooth ewes and the experiment was carried out during July and August. These factors could therefore explain the high incidence of silent oestrus in both treated and control ewes.

In our trial, the laparoscopic insemination technique did not result in a lengthening of the oestrous cycle. However, silent oestrus or lack of marking of some ewes in both groups, could have been interpreted as a lengthening of the cycle. Other factors that may cause lengthening of the cycle, include PMSG dosage and embryonal death. High doses of PMSG may stimulate follicular development and luteinisation without ovulation, which can lengthen the inter-oestrus period[2 16]. Laparoscopic insemination in ewes with high ovulation rates as a result of PMSG stimulation, can result in a higher than normal incidence of embryonal death[7 8 10], which, in turn, could lead to a late return to oestrus and thus prolonged inter-oestrous intervals[12]. Neither of these factors were applicable during our trial, but they are still relevant to field laparoscopic exercises.

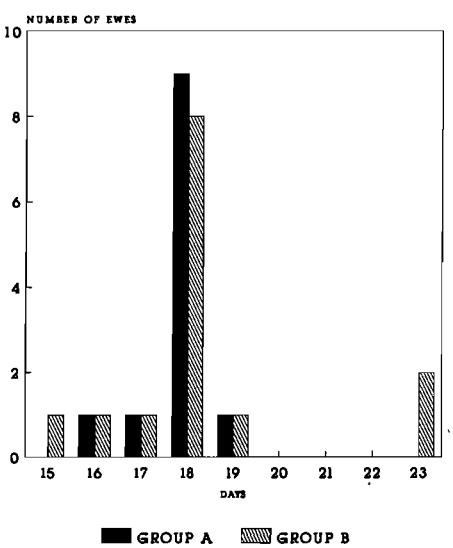


Fig. 2: **The distribution of the oestrous cycle length after synchronised heat of Group A and Group B ewes based on progesterone profiles**

REFERENCES

1. Boshoff D A, Gouws D J, Nel J A 1975 Reproductive patterns of five sheep breeds under extensive conditions. Suid-Afrikaanse Tydskrif vir Veekunde 5: 37-43
2. Evans G, Maxwell W M C 1987. Salamon's Artificial Insemination of sheep and goats. Butterworths, Durban: 126-166
3. Hackett A J, Hidiroglou M 1984 Effects of PMSG on progesterone levels in ewes treated with fluorogestone acetate or prostaglandin $F_{2\alpha}$. Animal Reproduction Science 6:191-197
4. Hunter G L 1964 The effects of season and mating on oestrus and fertility in the ewe. Proceedings of the South African Society of Animal Production: 196-206
5. Killeen I D, Caffery G J 1982 Uterine insemination of ewes with the aid of a laparoscope. Australian Veterinary Journal 59: 95
6. Knight T W, Dalton D C, Hight G K 1979 Identification of barren ewes by vasectomised rams. New Zealand Journal of Experimental Agriculture 7: 125-130
7. Le Roux P J 1975 Time-dose relationships of PMSG and MAP-intravaginal sponges and its effect on embryonic mortality in Karakul ewes. South African Journal of Animal Science 5: 33-36
8. Maxwell W M C 1986 Artificial insemination of ewes with frozen-thawed semen at a synchronised oestrus. 1. Effect of time of onset of oestrus, ovulation and insemination on fertility. Animal Reproduction Science 10: 301-308
9. Maxwell W M C 1986 Artifcicial insemination of ewes with frozen-thawed semen at a synchronised oestrus. 2. Effect of dose of spermatozoa and site of intra-uterine insemination on fertility. Animal Reproduction Science 10: 309-316
10. Maxwell W M C, Wilson H R, Butler L G 1984 Fertility of ewes after intra-uterine insemination with frozen semen. Animal Production in Australia 15: 448-451
11. Pedrana R G 1987 Oestrus synchronisation in sheep and goats. In: Refersher Course for Veterinarians Proceedings No. 96 Artificial Breeding in Sheep and Goats. Post-Graduate Committee in Veterinary Science, University of Sydney: 37-53
12. Plant J W 1981 Infertility in the ewe. In: Refresher Course for Veterinarians Proceedings No 58 Refresher Course on Sheep. Post-Graduate Committee in Veterinary Science, University of Sydney: 675-705
13. Restall B J, Wilkins J, Kilgour R, Tyrrell R N, Heamshaw H 1976 Assessment of reproductive wastage in sheep. 3. An investigation of a commercial sheep flock. Australian Journal of Experimental Agriculture and Animal Husbandry 16: 344-352
14. Robertson T J 1967 The control of the ovarian cycle in sheep. Sydney University Press, Sydney: 6-7, 43-46, 84, 115
15. Russel A J F, Doney J M, Gunn R G 1969 Subjective assessment of body fat in live sheep. Journal of Agricultural Science, Cambridge 72: 451-454
16. Zarco L, Stabenfeldt G H, Kindahl H, Quirke J F, Granström E 1984 Persistence of luteal activity in the non-pregnant ewe. Animal Reproduction Science 7: 245-267

HEREDITARY LAMINITIS IN JERSEY CALVES IN ZIMBABWE

M J HOYER[1*]

ABSTRACT

The clinical signs and radiological findings of a rare laminitis-like condition in Jersey calves (n=6) are described. Regular hoof-trimming proved very beneficial. Pedigree studies of the affected calves strongly suggest a recessive autosomal inheritance.

Key words: Jersey cattle, laminitis, hereditary

Hoyer M.J. **Hereditary laminitis in Jersey calves in Zimbabwe.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 62-64 (En.) Department of Clinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe.

INTRODUCTION

In 1949 a laminitis-like syndrome in young Jersey calves was reported from California[6]. A hereditary origin was suspected and a 'recessive monogenic autosomal gene' was thought to be the cause of the disease, as was suggested by pedigree studies and a breeding experiment[6]. Later a similar condition was recognised in 19 Jersey calves in South Africa[2] and in 3 calves in the USA[7]. A breeding experiment carried out, using an affected bull in the South African study, very strongly suggested the presence of a simple autosomal recessive gene. Edwards[3] described 5 cases of hereditary laminitis in Jersey calves in the UK. In this group a direct link could be established with the bull incriminated in the South African study. In both groups, parentage could be traced to a common male ancestor, 16 generations back. This bull, registered on the Isle of Jersey in 1912, was thought to be the original mutant (H P A De Boom 1989 173 Marija Street, Wonderboom, RSA, personal communication).

The present report describes a case of hereditary laminitis in 6 pedigree heifer calves in a Jersey herd near Harare.

CASE REPORT

Early in 1988, Jersey heifer calves (n=6) were presented with signs of acute lameness. The calves ranged from 3,5 to 6 months old and were housed and fed in groups amongst calves of the same age. They were reluctant to move, stood with arched backs and cow-hocked hindlegs. Weak muscular tremors were observed in the shoulder muscles of some of the calves. If forced to move, the calves walked stiff-legged and very gingerly on all 4 feet. Their general body condition was good and on physical examination, all vital signs appeared normal. On palpation, some hooves felt warm and sensitive at the coronary band, and in some cases a strong pulse could be felt in the common digital arteries in the front legs. No abnormalities were found in the limbs proximal to the hooves. A nutritional problem was suspected and the calves were taken off concentrates. The acute signs resolved within a few days, but within the next weeks the hooves became elongated and distinct horizontal laminitic rings started to appear on the walls. The anterior hoof walls became dished and the soles more prominent (Fig. 1 & 2). One of the affected calves was taken to the University of Zimbabwe Veterinary Hospital for a radiographic examination, together with an unaffected calf of exactly the same age for comparison. The radiographs of the affected calf, showed shortening of the third phalanx when compared to those of the unaffected calf. The animals remained slightly lame, but regular corrective foot-trimming (shortening of the toe and flattening of the sole) proved very beneficial. The natural pigmentation of the horn prevented any possible haemorrhages from being detected. Nine months after the first onset of signs, only one calf still needed regular trimming. The others appeared normal, apart from slight flattening of the hooves and the occurrence of some horizontal lines. This calf which needed trimming was brought into the clinic for a follow-up radiographic study. This revealed a broken foot axis, flattening of the hoof, a convex anterior wall, shortening and upward rotation of P3. No abnormalities could be detected in P1, P2 and the articular surfaces.

All 6 calves grew normally and at 12 to 13 months of age, were artificially inseminated by the owner, using semen of unrelated sires. By this time all of them have calved. All calves born were clinically normal and have not developed any signs of the foot disorder.

A brief study of the parentage of these calves born to different dams, revealed that all had the same sire, an American bull (FO) of which semen was imported into Zimbabwe. Other calves born to different bulls, but raised under the same conditions did not show any signs of the disease. A herditary defect was suspected and a questionnaire was sent to all Jersey breeders in Zimbabwe, who had purchased semen of the suspected carrier bull (FO). None of the addressed farmers had noted the condition on their farms in offspring of this particular bull.

An extensive study of the pedigree of the affected calves was carried out (Fig. 3) and revealed the following: five of the 6 calves had a maternal great-grandsire in common, a locally-bred Jersey bull (Sek). This bull was known to be a carrier of another hereditary defect known in the Jersey breed, namely recto-vaginal constriction. The sixth calf had, on its maternal side, a sire strongly related to the maternal grandsire of the sire of the calves.

Late in 1988, a full brother of one of the affected calves, was born with severe front-limb arthrogryposis and palatoscizes. This animal was euthanased. The postmortem examination supported the clinical findings. This condition has been proven to be of hereditary origin in Charolais, Hereford and Shorthorn breeds as well as in piglets[5], and suggests that the American bull is a carrier of more than one hereditary defect.

DISCUSSION

The clinical signs and the radiographic findings, although less severe, correlate closely with those described by other

[1*]Department of Clinical Veterinary Studies, Faculty of Veterinary Science, University of Zimbabwe, P.O. Box MP 167, Mount Pleasant, Harare, Zimbabwe

Received: November 1990 Accepted: February 1991

Fig. 1 & 2: **Hind feet of a 14-month old Jersey heifer with hereditary laminitis. Note the laminitic rings and the dished appearance of the hooves**

authors[1 2 3 4 7]. In the present cases, the animals did not develop a severe chronic laminitis necessitating slaughter on humane grounds. A possible reason for this could be that in the present outbreak, the calves were presented at an early stage and almost from the onset, the condition was treated by regular corrective hoof trimming. This therapy may be of considerable value in managing the disorder.

The fact that only 6 half-sibling calves, were affected amongst a group of calves, born to different bulls, under the same high standard management and nutritional conditions, makes a hereditary origin very likely and a nutritional or metabolic aetiology very remote, and ties in with the findings of previous studies. The pedigree of the sire of the calves (FO) showed a high degree of inbreeding and a common ancestor in the maternal line of 5 of the 6 calves (SEK). Unfortunately, insufficient information on the parentage of this Zimbabwean bull is available and it proved impossible to establish whether or not a relationship exists with the South African and British lines, as reported by Edwards[3].

A single recessive autosomal gene is suspected to be responsible for the condition. Criteria, which taken together, strongly suggest a recessive trait[3 7 8], are:

1. The parents of affected animals need not be necessarily affected, the defect may skip generations.
2. All offspring of 2 affected parents are affected.
3. Approximately equal numbers of males and females are affected.
4. The average genetic relationship bet-

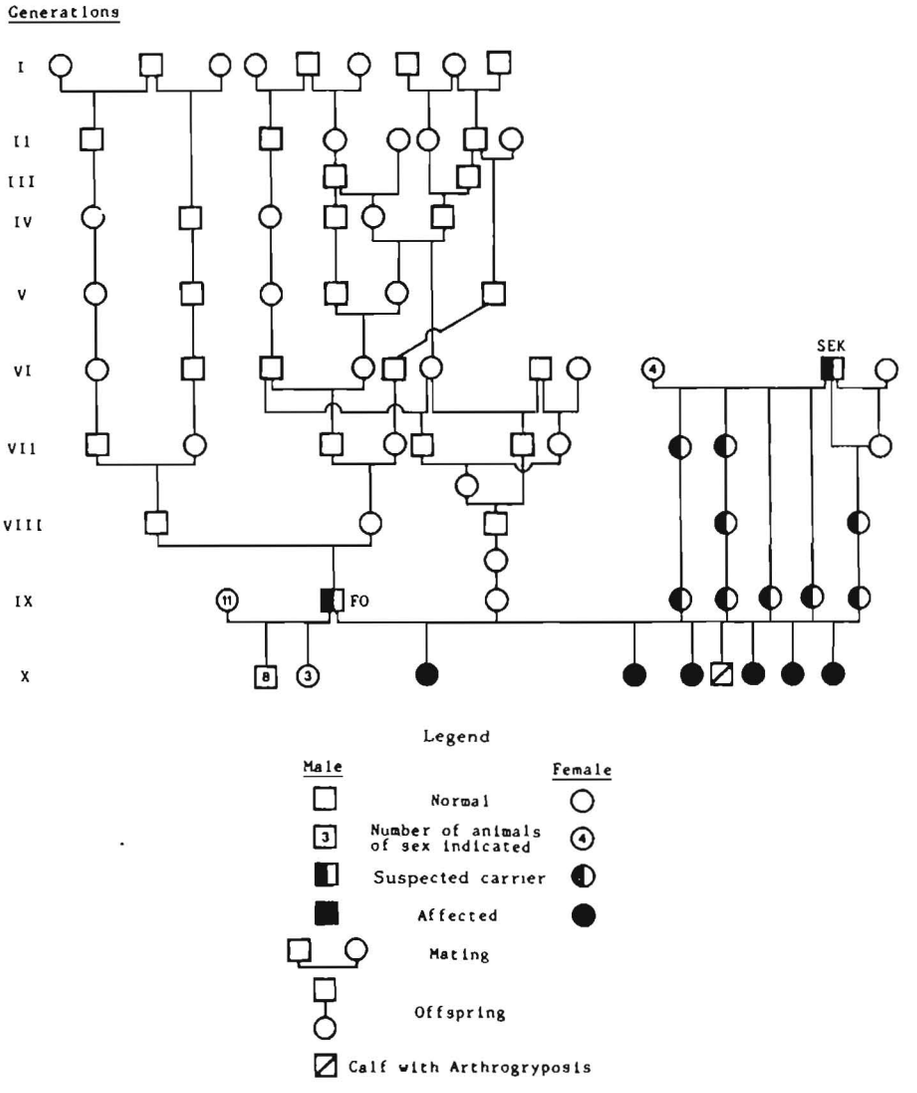


Fig. 3: **Genealogic diagram showing the close relationship between the 6 Jersey calves affected with hereditary laminitis and the inbreeding which occurred in the pedigree of the American Jersey bull FO. The bull SEK in the diagram is the locally-bred Zimbabwean Jersey bull**

ween normal parents of affected individuals is greater than between normal parents that have not produced affected offspring.

Criteria 1 and 4 were met in this case. Criterium 2 was proven by De Boom et al.[2] in 1968 where breeding experiments with an affected bull and affected cows produced 100% affected calves. Unfortunately, it is not known whether the third criterium was fulfilled, as all bull calves born on this farm are culled within a few days following birth.

ACKNOWLEDGEMENTS

The author whishes to thank all the Zimbabwean Jersey breeders and the Animal Breeders Company in Harare, who contributed to this survey. Special thanks to Mr and Mrs Meadows and the American Jersey Cattle Club who provided all the information necessary to investigate the pedigree of the affected animals. Thanks are also due to Dr J Ndikuwera, Prof E A Usenik and Prof F W G Hill.

REFERENCES

1. Blood D C, Radostits O M 1986 Veterinary Medicine 7th edn ELBS/Bailliere Tindall, London: 1363
2. De Boom H P A, Adelaar T F, Terblanche M 1967 Hereditary laminitis of Jersey calves: A preliminary report. Proceedings of the Third Conference on South African Genetics: 1-5
3. Edwards G B 1972 Hereditary laminitis in Jersey cattle. Proceedings of the World Association for Buiatrics Congress, London: 663-668
4. Edwards G B 1982 Acute and subacute laminitis in cattle. Veterinary Annual 22, Scientechnica Bristol: 99-106
5. Huffel X, De Moor A 1987 Congenital multiple arthrogryposis of the forelimbs in calves. Compendium on Continuing Education for the Practising Veterinarian 9: F333-338
6. Mead S W, Gregory P W, Regan W M 1949 A hereditary digital anomaly of cattle. Journal of Heredity 40: 151-154
7. Merritt A M, Riser W H 1968 Laminitis of possible hereditary origin in Jersey cattle. Journal of the American Veterinary Medical Association 153: 1074-1084
8. Nicholas F W 1987 Veterinary Genetics. Oxford Science Publications, Clarendon Press, Oxford: 217-226

A CRANIOPHARYNGIOMA IN A SEVEN-YEAR-OLD DOG

G N ECKERSLEY*, JUDITH K GEEL** and N P J KRIEK***

ABSTRACT

A seven-year-old male Border Collie was presented with a history of lethargy, episodic circling, incoordination and polydypsia. Physical examination revealed depression, obesity and bradycardia. A neurological examination indicated the possible presence of a space-occupying lesion in the brain. Results of the clinical investigation revealed hyposthenuria, sinus bradycardia and increased concentration of protein in the cerebrospinal fluid. A computerised axial tomography scan revealed a mass in the region of the hypophysis. The dog was euthanased and a post mortem examination confirmed the presence of a craniopharyngioma.

Key words: Dog, computerised axial tomography scan, craniopharyngioma.

Eckersley G.N.; Geel J.K.; Kriek N.P.J. **A craniopharyngioma in a seven-year-old dog.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 65-67 (En.) Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa.

INTRODUCTION

A review of the literature on brain tumours[4-6 8 11 12 17] and pituitary gland tumours[2 3 7 14] revealed that craniopharyngiomas are extremely rare[7-10 13 15].

Craniopharyngiomas are benign tumours that are derived from epithelial remnants of the oropharyngeal ectoderm of the craniopharyngeal duct (Rathke's pouch)[2 3]. Compared to all other types of pituitary neoplasms, craniopharyngiomas occur in younger dogs and they are present in either suprasellar or infrasellar locations[3]. Craniopharyngiomas in young dogs are often large and grow along the ventral aspect of the brain, where they may incorporate several cranial nerves. In addition, they extend dorsally into the hypothalamus and thalamus[3].

The clinical signs of this type of pituitary tumour are often a result of combined hormonal derangements. A lack of pituitary tropic hormone secretion results in atrophy and subnormal functioning of the adrenal cortex and thyroid[3]. Dwarfism, due to subnormal secretion of somatotropin, prior to the closure of growth plates, and gonadal atrophy may also be features of this tumour[3]. Large tumours may also result in diabetes insipidus wtih polyuria, polydypsia and hyposthenuric urine. This is due to these tumours interfering with the synthesis and release of antidiuretic hormone[3 15]. Eigenmann[7] reported a case of panhypopituitarism with growth hormone deficiency, secondary hypothyroidism and secondary hypoadrenocorticism associated with diabetes insipidus in an adult dog, that had a suprasellar tumour suspected to be a craniopharyngioma. Cranial nerve deficits and other neurological syndromes may be caused by these tumours extending into the hypothalamus and compressing surrounding brain tissue[3].

Craniopharyngiomas consist of alternating solid and cystic areas. The solid areas are composed of nests of epithelial cells (cuboidal, columnar or squamous) with focal areas of mineralisation. The cystic spaces are lined by columnar or squamous epithelial cells and contain keratin debris and colloid[2 3 8 10].

To the best of our knowledge this is the first reported case of a craniopharyngioma in an old dog.

*Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa
**Department of Experimental and Clinical Pharmacology, University of the Witwatersrand
***Department of Pathology, Medical University of Southern Africa

Received: January 1991 Accepted: March 1991

CASE REPORT

A 7-year-old male Border Collie, body mass 20 kg, was presented with a history of lethargy, episodic circling and incoordination of 2 months duration. However, the patient had shown polyphagia and polydypsia over a longer period. The abnormal findings on physical examination were moderate obesity, a dull dry haircoat, bradycardia (heart rate 64 beats min^{-1}) and moderate depression. A complete neurological examination revealed depression, a slightly incoordinated gait with a tendency to circle to either side. Cranial nerve examination revealed no abnormalities. The forelimb and hindlimb reflexes were slightly exaggerated indicating an upper motor neuron lesion, while the attitudinal and postural reactions indicated a proprioception deficit in all 4 limbs. The neurological examination indicated a lesion in the brain. Investigative procedures included a bloodsmear, haematology, chemical pathology, urinalysis (specific gravity 1,007) faecal analysis, radioimmunoassays (cortisol 118 nmol ℓ^{-1}, T4 (total) 25 nmol ℓ^{-1}) electrocardiography (sinus bradycardia; heart rate 64 beats min^{-1}) cerebrospinal fluid analysis (protein 0,85 g ℓ^{-1}, total nucleated cell count very low) and a computerised tomography scan (CAT).

Analysis of these results revealed hyposthenuria, a high concentration of protein in the cerebrospinal fluid (CSF) and sinus bradycardia. The CAT scan (Somatom 2) (Fig. 1) confirmed the presence of a mass in the region of the pituitary gland, causing pressure on the hypothalamus. Contrast studies with iopamidol (Jopomiron, Berlimed) slightly enhanced the outline of the mass. Because of the likelihood of the mass in the brain being a tumour, and the progressive nature of the condition, the owners requested euthanasia.

A complete post mortem examination was performed. Selected sections of various tissues as well as the lesion in the brain, were taken in 10% formalin for histopathological examination. The sections were routinely processed, cut and stained with haematoxylin and eosin (H & E).

A single lesion was seen in the brain. This extended as a focal cystic lesion from the hypophyseal stalk to the hypothalamic region. It was fluctuating, thin-walled, about 2 cm in diameter, and

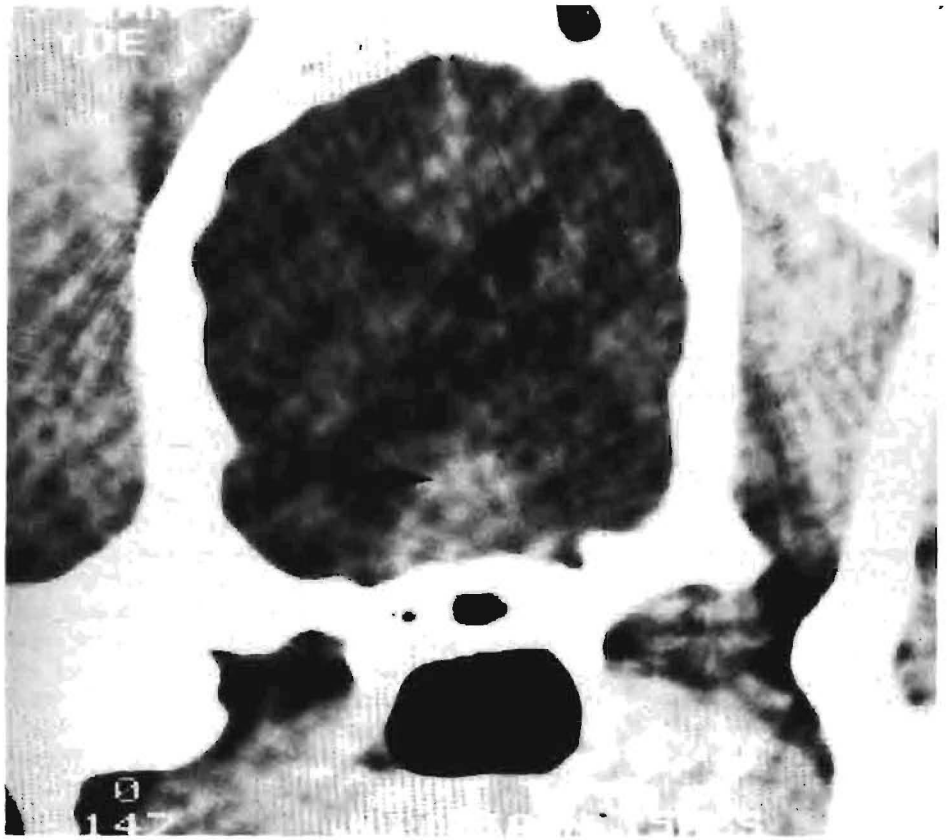


Fig. 1: **Contrast-enhanced transverse CAT scan of the dog with a craniopharyngioma. A mass (black arrow) can be seen in the region of the hypophysis and hypothalamus**

contained a pus-like, fluid content. After fixation, the content oozed out as the brain was sectioned to process specimens for histological purposes.

The histological lesions adjacent to the cyst were manifested by scant malacia and focal haemorrhage. The latter area was also infiltrated by scattered gitter cells, and focal large aggregates of haemosiderin-laden macrophages. The cyst wall was lined by an epithelium thrown into uneven folds producing cystic spaces containing amorphous cellular detritus and cholesterol clefts. A few of the spaces contained small calcified masses resembling corpora amylacia. The lining epithelium varied in appearance. It consisted mostly of undifferentiated columnar epithelium which in areas appeared to be compressed into a cuboidal to squamous epithelium. Occasionally there was an abrupt transition from undifferentiated columnar epithelium to a ciliated columnar epithelium in which the cytoplasm was eosinophilic. In the interstitial spaces, small aggregates of lymphocytes and plasma cells occurred. Large ovoid cells with a centrally-placed nucleus and ample granular eosinophilic cytoplasm occurred singly or in small groups in the supporting connective tissue framework.

Fig. 2: **Low magnification of the wall of the craniopharyngioma showing the papillary arrangement, cholesterol clefts and accompanying inflammatory reaction (HE, x40)**

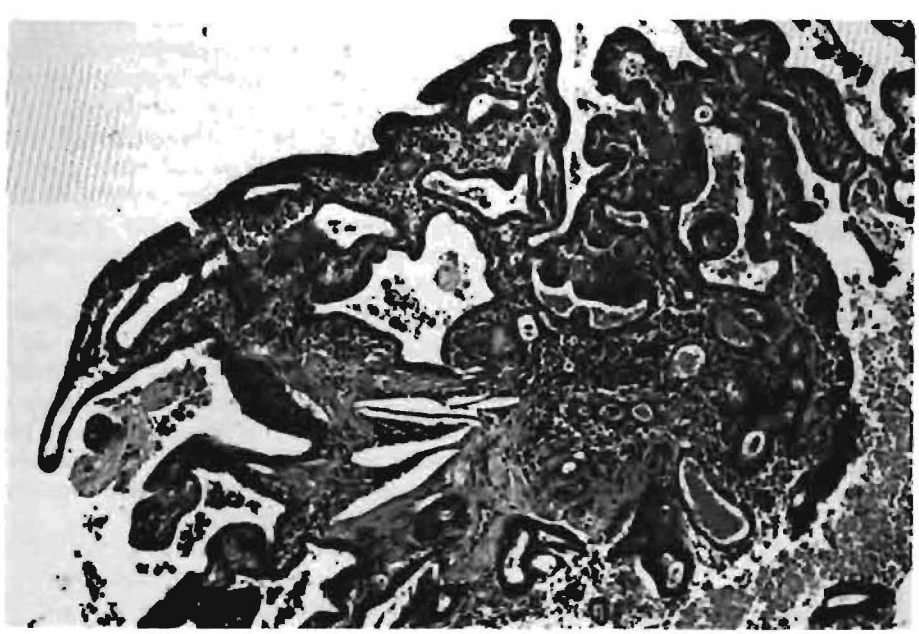
DISCUSSION

The tumour in the brain was diagnosed as a craniopharyngioma. The diagnostic features were consistent with what has previously been described in the literature[2,3,8,10]. Because these tumours are congenital tumours due to maldevelopment, this tumour must have been present since birth. This dog was asymptomatic until it reached 7 years of age.

All the abnormal clinical, neurological and laboratory findings can be related to the presence of the hypophyseal tumour. The pituitary gland had not been destroyed, as was evident at post mortem as well as on radioimmunoassays for cortisol and thyroxine, where these hormone concentrations were found to be within normal limits. There were also no growth defects or other clinical signs of growth hormone deficiency. The growth of the tumour into the hypothalamus resulted in central diabetes insipidus. Polyphagia with resultant obesity has previously been described in dogs with pituitary neoplasms[3], however, the reason for this is not known. It is also possible that the tumour affected the functioning of the osmoreceptors in the hypothalamus resulting in polydypsia with a secondary polyuria. Elevated concentrations of protein in the cerebrospinal fluid frequently occurs in association with brain tumours[6], as was the situation in this case. Sinus bradycardia is also a recognised entity in patients with space-occupying lesions in the brain[6,16].

ACKNOWLEDGEMENTS

We would like to thank the staff of the Animal Hospital and Departments of Chemical Pathology and Radiology of the Faculty of Medicine at the Medical University of Southern Africa, and the staff of the Pathology Department of the Faculty of Veterinary Science for their assistance in the case. We would also like to thank Dr C Kilian of Bedfordview Veterinary Clinic for referring this case.



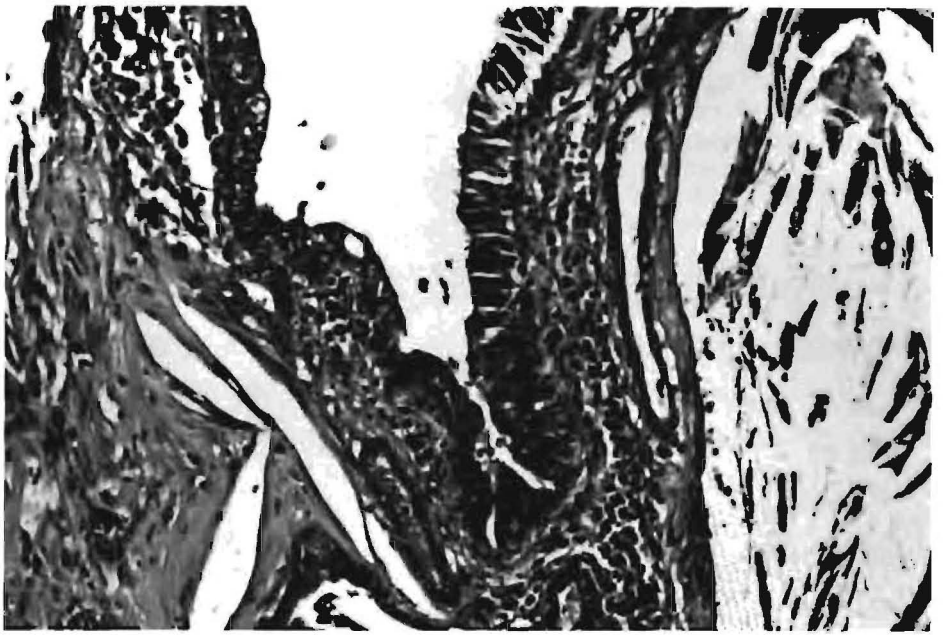


Fig. 3: **High magnification of the craniopharyngioma showing the transition from non-ciliated to ciliated epithelium. Also note the inflammatory response and the accumulated, lipid-rich debris below the neoplastic epithelium (HE x400)**

REFERENCES

1. Braund K 1984 Neoplasia of the nervous system. The Compendium on Continuing Education for the Practising Veterinarian 6: 717-722
2. Capen C 1978 Tumours of the endocrine glands. In: Moulton J (ed) Tumours in Domestic Animals 2nd edn. University of California Press, Berkeley, Los Angeles: 384-385
3. Capen C, Martin S 1983 Diseases of the pituitary gland. In: Ettinger S J (ed) Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat 2nd edn. W B Saunders Company Philadelphia: 1539
4. Cardy D 1978 Tumours of the nervous system and eye. In: Moulton J (ed) Tumours in Domestic Animals 2nd edn. University of California Press, Berkely, Los Angeles: 430-442
5. Chrisman C 1982 Behaviour and personality disorders. In: Problems in Small Animal Neurology. Lea & Febiger, Philadelphia: 115-151
6. Eckersley G N, Bastianello S S, Van Heerden J, Williams J H 1989. An expansile secondary hypophyseal mastocytoma in a dog. Journal of the South African Veterinary Association 60: 113-116
7. Eigenmann J E 1983 Pituitary-hypothalamic diseases. In: Ettinger S J (ed) Textbook of Veterinary Internal Medicine. Diseases of the Dog and Cat. 3rd edn. W B Saunders Co. Philadelphia: 1590-1593
8. Frankhauser R, Luginbuhl H, McGrath J 1974 Tumours of the Nervous System. In: Bulletin of the World Health Organization 50: 69
9. Hawkins K L, Diters R W, McGrath J T 1985 Craniopharyngioma in a dog. Journal of Comparative Pathology 95: 469
10. Holliday T, Higgins R, Turrel J 1987 Tumours of the nervous system. In: Madewell B (ed) Veterinary Cancer Medicine 2nd edn. Lea & Febiger, Philadelphia: 605
11. Le Couteur R, Turrel J 1986 Brain tumours in dogs and cats. In: Kirk R W (ed) Current Veterinary Therapy IX. Small Animal Practice. W B Saunders Company, Philadelphia: 820-825
12. Loden D, Norten F, Wolfla L 1983 Diagnosis of intracranial lesions by computerized tomography in three dogs. Journal of the American Animal Hospital Association 79: 303-308
13. Neer M T, Reavis D V 1983 Craniopharyngioma and associated central diabetes insipidus and hypothydroidism in a dog. Journal of the American Veterinary Medical Association 182: 519
14. Sarfaty D, Carillo J M, Peterson M E 1988 Neurologic, endocrinologic and pathologic findings associated with large pituitary tumours in dogs: eight cases (1976-1984). Journal of the American Veterinary Medical Association 193: 854-856
15. Saunders L Z, Rickard C G 1952 Craniopharyngioma in a dog with apparent adiposogenital syndrome and diabetes insipidus. Cornell Veterinarian 42: 490
16. Tilley L 1985 Cardiac arrhythmias. In: Essentials of Canine and Feline Electrocardiography. 2nd edn. Lea & Febiger, Philadelphia: 250-252
17. Turrell J M Fibe J R, Le Couteur R A, Higgins R J 1986 Computed tomographic characteristics of primary brain tumours in 50 dogs. Journal of the American Veterinary Medical Association 188: 851-856

MOLLUSCUM CONTAGIOSUM IN THREE HORSES

LUCIA LANGE*, SALLY MARETT**, CHARLOTTE MAREE*** and TRUUSKE GERDES//

ABSTRACT

Suspected molluscum contagiosum was diagnosed in 3 horses in the Chingola district of Zambia. The horses were found to be suffering from a slow progressive skin disease with lesions on the chest, shoulders, inner and lateral aspects of the fore- and hindlimbs, the face, fetlocks, pasterns and on the lateral surfaces of the body, The lesions varied from 4 to 20mm in diameter, were hairless but covered by soft keratin projections which, when removed, left a raw elevated base tightly adherent to the epidermis. These lesions bled profusely when the animals were groomed. Older lesions were well circumscribed, raised above the surface, devoid of hair and after removal of grey-white keratin flakes, had a depigmented waxy appearance. Microscopically cytoplasmic inclusions containing many pox virions were found. Attempts at culturing the virus were unsuccessful.

Key words: Molluscum contagiosum, equine, horse, pox virus.

Lange Lucia; Marett Sally; Maree Charlotte; Gerdes Truuske **Molluscum contagiosum in three horses.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 68-71 (En.) P.O. Box 6222, 7538 Welgemoed, Republic of South Africa.

INTRODUCTION

Pox virus infections in horses are infrequently reported in the literature. A transient, highly infectious papular eruption over almost the entire body surface was recorded by McIntyre[8]. The cause of this condition was not determined, but it was thought to be due to an unclassified pox virus[1 16 11]. Horsepox as such is a benign, contagious disease of horses that may manifest either as the "buccal form" or as the "greasy heel form"[3 13]. Buccal horsepox is characterised by the development of pocks which ulcerate, within the oral cavity, on the inner surfaces of the lips, on the gums, the tongue and anterior nares. Eruptions may also be present on the face. The greasy heel form of horsepox, also known as "Jenner's horsepox" has eruptions on the flexor surfaces of the fetlocks and other joints in the lower part of the limb[3 13].

A more generalised disease affecting the face, oral cavity, flexor surfaces of the cannon bone and coronary band was recently reported in a donkey[3]. Another manifestation of pox virus infection in horses is Uasin Gishu skin disease described in Kenya[4 5 6 7]. This disease is very similar to the recorded cases of molluscum contagiosum[2 9 12]

In this report a skin disease of horses in the Chingola district of Zambia is described.

MATERIALS AND METHODS

The histories of a 17-year-old Thoroughbred mare, a 9-year-old Miniature pony stallion and a 16-year-old Thoroughbred cross gelding were recorded as far back as possible. The mare and gelding were hospitalised and the behaviour of the lesions was observed for at least 4 months. The development of the lesions in the stallion was monitored by regular visits to the animal for at least one year. The appearance and progress of the lesions were carefully recorded.

Biopsies from recent and older lesions were fixed in 10% formalin for microscopic examination and crusts from similar lesions were collected for virus isolation. Tissues were routinely processed and sections were stained with haematoxylin and eosin (HE) for light microscopic examination. Selected blocks of formalin-fixed skin were post-fixed in 4% glutaraldehyde and routinely processed for electron microscopy.

Ultra-thin sections were stained in 1% uranyl acetate and 0,2% lead citrate and examined with the transmission electron microscope.

The scab material submitted for virus isolation was homogenised in phosphate-buffered saline (PBS) containing 200 units ml^{-1} penicillin and 200 μg ml^{-1} streptomycin. After centrifugation at 500 x 9 for 20 min, confluent monolayer cultures of bovine kidney cells (CFK and MDBK) were inoculated with 0,5 ml of supernatant fluid. After 60 min the culture flasks were washed and maintained with serum-free Eagles medium containing 100 units penicillin and 100 μg ml^{-1} streptomycin. Virus isolation was also attempted in experimental animals[6]. Briefly, embryonated eggs were inoculated by the CAM route with supernatant fluid and candled daily. The CAMs were examined on Day 6 post inoculation for lesions. Baby mice, aged 7 d, were inoculated intraperitoneally with material from the first and second CAM passages. Membranes were homogenised in PBS and prepared as outlined above and also used for attempted virus isolation on monolayers of bovine, monkey and rabbit kidney cells (CFK, MK2 and RK13).

RESULTS

In the 17-year-old Thoroughbred mare, the initial lesions were first seen 6,5 years previously. The 9-year-old Miniature pony stallion had had lesions for more than one year and the 16-year-old Thoroughbred cross gelding had lesions of at least 6 months duration.

These horses were the only ones in their stables to be affected and when 2 horses were moved to hospital stables for observation and tests, the in-contact horses did not develop lesions even after 4 months of sharing the same stables and grooming utensils. The lesions started on specific areas of the body (this differed from horse to horse) and spread slowly to involve other parts of the body surface. They healed very slowly and then recurred to affect the same areas as were previously affected.

In all 3 horses lesions were seen on the chest, shoulders and limbs. The inner and lateral aspects of both the fore- and hindlimbs were also affected. Two horses

*P.O. Box 6222, 7835 Welgemoed, Republic of South Africa
**Private practitioner, Zambia
***Department of Pathology, University of Pretoria
//Section Virology, Veterinary Research Institute

Received: October 1989 Accepted: January 1991

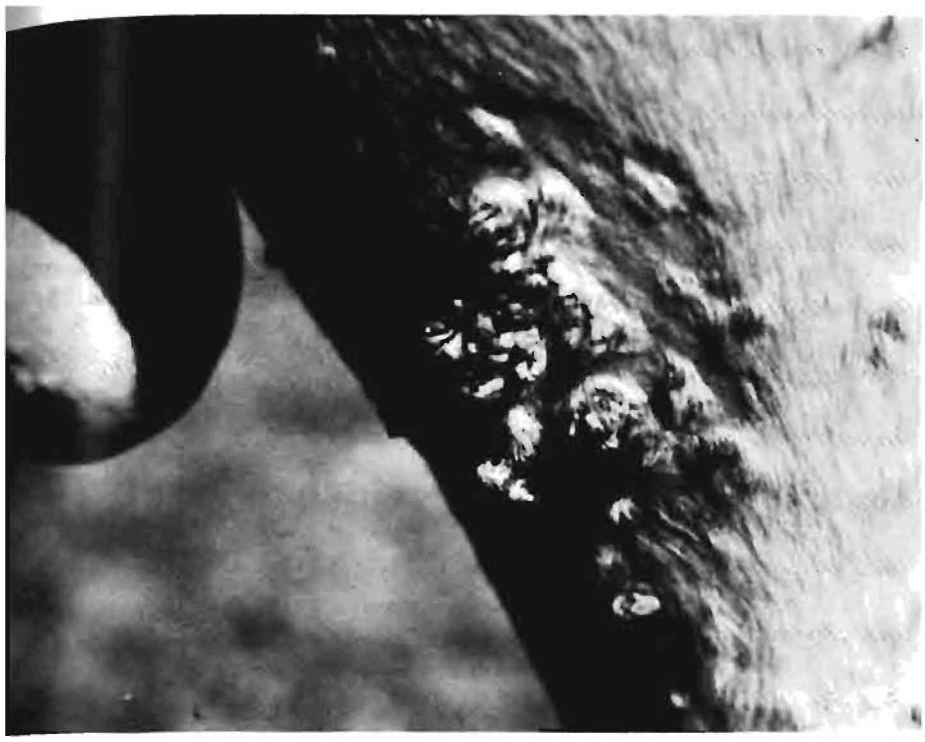


Fig. 1: **Inner caudal thigh of a Miniature pony illustrating early lesions consisting of outward projecting spicules of keratin. Note the few small lesions on the scrotum**

had lesions down to the pasterns and one had lesions involving the fetlocks. The mare initially had lesions on the muzzle and face, which spread to involve the entire body surface excluding the neck. The gelding had no lesions on the head and neck although the rest of the body was involved. In the stallion no lesions were found on the head, neck and body, but a few were seen on the caudal scrotal skin. No lesions were present, at any time during the course of the disease, on the mucous membranes of the conjunctiva, nostrils, mouth, vulva or sheath.

The very early lesions looked like insect bites on which the hair was raised over a slightly elevated firm plaque in the skin. This stage was seldom noticed.

The more acute lesions were alopecic and they consisted of soft keratinised white spicules up to 3 mm in height (Fig. 1). When the hyperkeratinised projections were scratched off, a slightly raw base which was raised above the surface of the skin and very tightly adherent to the epidermis, was left. These lesions bled profusely when the animals were groomed. Some of the lesions coalesced to form cauliflower-like growths of up to 30 mm in diameter. Lesions of several months duration were of the same size as those mentioned above. They were irregularly spherical, well-circumscribed and raised above the surface, some were elevated up to 2 mm above the surrounding normal skin. Some of these lesions were covered by hair that stood on end, while others had partial hair loss with the remaining hairs epilating easily. The larger lesions were completely devoid of hair. All of these lesions were covered by grey-white, soft keratin scales which could easily be removed by scratching or grooming. This left a depigmented, slightly-elevated area covered by powdery scales. The hair would eventually grow again to cover the lesion completely. The lesions on the pasterns were characterised by groups of hard growths that formed finger-like projections 5 mm wide and up to 20 mm long. They were tightly adherent to the skin and difficult to remove. When pulled off a raw, white, hard raised area was left.

The most striking histological feature was the abrupt transition from unaffected epidermis to prominent hyperplasia of the epidermis (Fig. 2). The hyperplasia was characterised by solid masses of stratum spinosum cells that encroached downward into the dermal connective tissue without the formation of rete pegs. The prickle cells in these hyperplastic areas were swollen, due to the presence of cytoplasmic inclusions which varied in appearance. Some were eosinophilic and clearly distinguishable from the vacuolated cytoplasm, while others had a more homogeneous faintly basophilic appearance with a few eosinophilic granules scattered on the periphery of the inclusion (Fig. 3). These 2 types of inclusions were seen in the deeper layers of the stratum spinosum. Inclusions consisting of coarse basophilic granules were present closer to the surface of the epidermis (Fig. 4). As the size of the inclusions increased, areas within the inclusions were more sparsely populated with the granular structures leading to a vacuolated appearance. The nuclei of affected cells were eccentric, pycnotic or absent. As the cells were pushed towards the surface, the inclusions attained a deeply-basophilic appearance and the cells became shrunken and keratinised. Groups of these cells were sloughed onto the surface as keratin squames. In some biopsies, follicular and surface hyperkeratosis was prominent, while in others the stratum corneum was very thin or absent. The dermal cell infiltration was limited to a few scattered neutrophils in the subepidermal connective tissue and moderate numbers of lym-

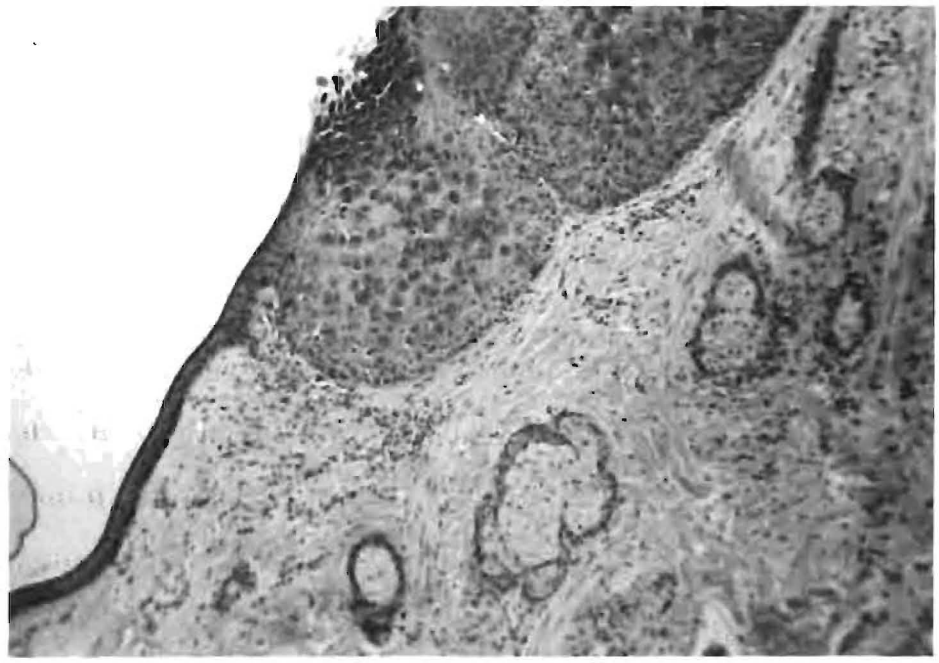


Fig. 2: **A section of the skin illustrating abrupt transition from unaffected epidermis to prominent epidermal hyperplasia. HE X 100**

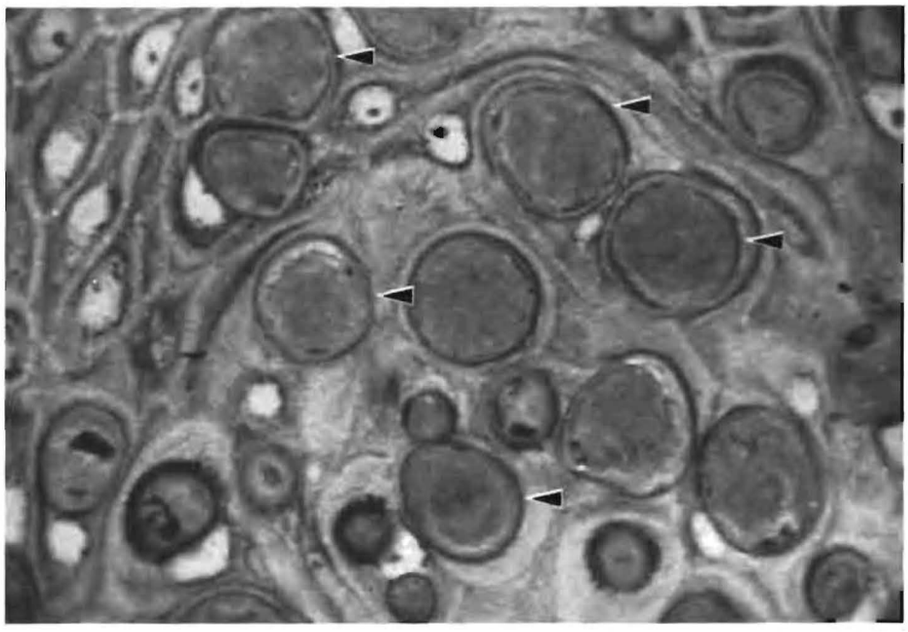


Fig. 3: **Large cytoplasmic inclusions (arrows) characterised by a pale blue homogeneous substance with peripherally-arranged fine eosinophilic granules. HE X 1000**

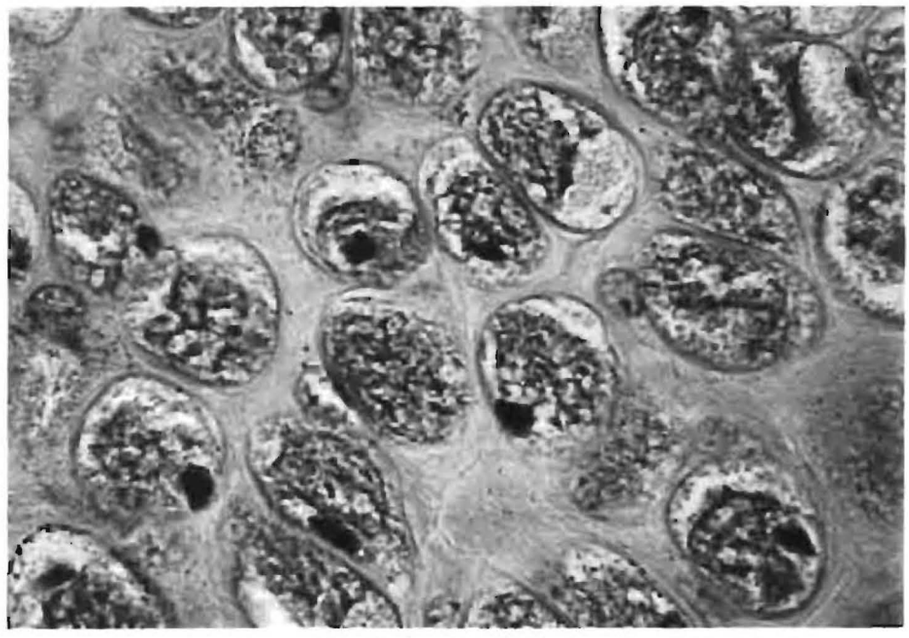


Fig. 4: **Cytoplasmic inclusions consisting of very coarse basophilic granules. HE X 1000**

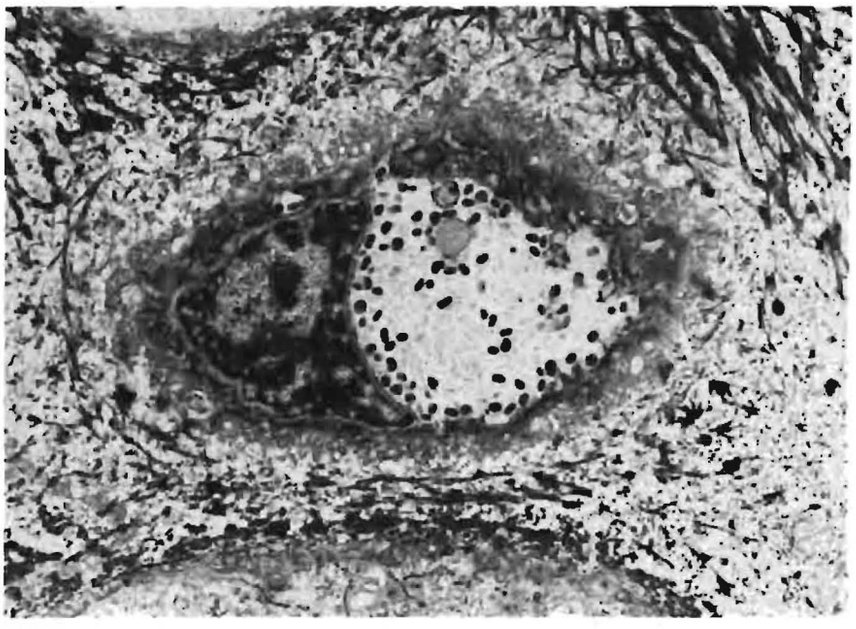


Fig. 5: **Electron micrograph of a stratum spinosum cell that appears separated from surrounding cells. The cytoplasm is filled with a finely-granular substance and viral particles arranged on the periphery. X 5900**

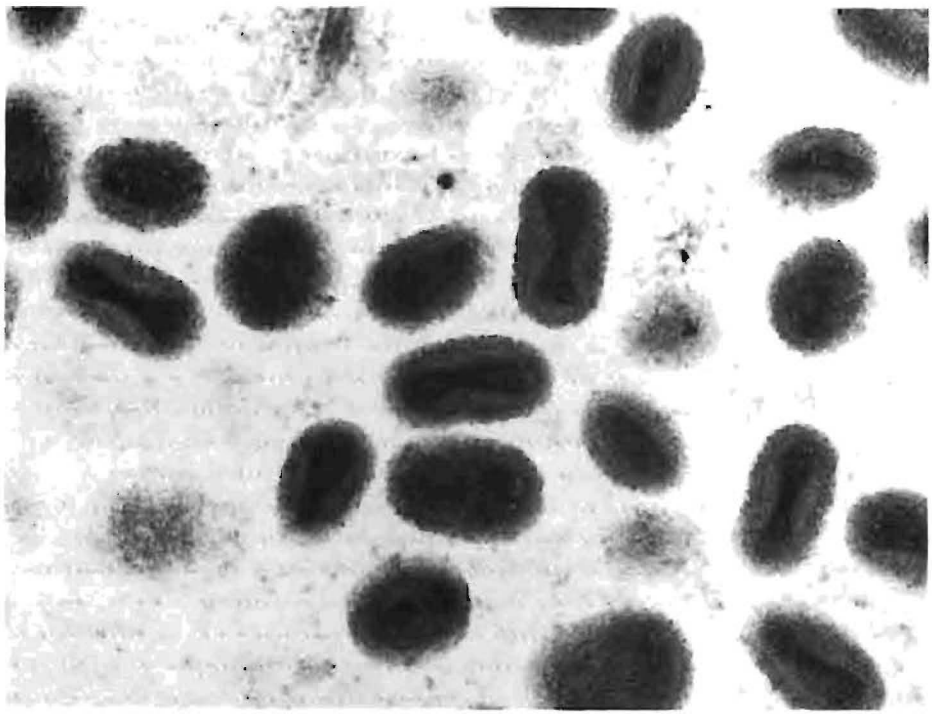


Fig. 6: Typical pox virus particles in the cytoplasm of a stratum spinosum cell. X 59000

phocytes in areas below the affected epidermal cells.

In the early lesions affected, stratum spinosum cells appeared to have withdrawn their cytoplasmic processes and to have separated from each other. Fragmented tonofilaments were scattered in the intercellular space. The cytoplasm of the cells was replaced by viral particles interspersed with a fine granular substance (Fig. 5). Affected cells in older lesions, and stratum spinosum cells situated closer to the skin surface, were rounded off and the cytoplasmic processes and tonofilaments were indistinct. The cytoplasm of these cells was filled with many viral particles and very little granular substance. The loose ribosomes and mitochondria which would normally have been present in the cell cytoplasm were not seen in the cells infected with viral particles. The viral particles had the typical appearance of pox viruses. The inner core consisted of a dumb-bell shaped electron-dense structure which was surrounded by a less electron-dense laminated capsule. The mature viral particles measured 150x250 nm (Fig. 6).

Four successive blind passages at approximately 14 d intervals were carried out on all the kidney cell monolayers, and cultures were discarded when no cytopathogenic effect was observed. Similarly, 5 CAM passages were carried out and discontinued when no obvious pock lesions were obtained. Some pock-like lesions were initially seen but could not be maintained by further egg passages. Furthermore, the baby mice did not develop any pock lesions and no deaths occurred.

DISCUSSION

The overall appearance of the lesions in these 3 horses suggests that we were dealing with the condition known as "molluscum contagiosum"[2 9 10 12]. Comparable macroscopic characteristics are poorly-contagious cutaneous eruptions of a number of months to years duration and discrete circumscribed elevated lesions with a waxy appearance[2 9 10 12 13]. Microscopic features are well demarcated foci of epidermal hyperplasia where affected prickle cells are prominently swollen and contain large intracytoplasmic inclusions, also known as "molluscum bodies"[2 10]. As the cells mature and move towards the surface, the inclusions grow in size and density and become more basophilic. The stratum corneum contains keratinocytes with deep purple molluscum bodies that exfoliate in groups[2 10 13]. With the electron microscope, many pox virions were seen within affected cells, the viral particles were similar to those previously described for molluscum contagiosum[2 9 12]. The size of the virions was similar to that reported by Cooley et al.[2], but smaller than that found by Moens & Kombe[9] and Rahaley & Meuller[12].

The skin condition under discussion is indistinguishable from Uasin Gishu skin disease of horses described in Kenya[4-7]. In Uasin Gishu skin disease, single animals were affected, the lesions were present for 12 to 30 months in some horses and lesions in various stages of development were present on the same animal at one time. The distribution of the lesions and the macroscopic and microscopic appearance were identical to those in our cases[5 7]. Kaminjolo and his co-workers succeeded in culturing the pox virus responsible for Uasin Gishu skin disease[4 6], but we were unable to grow the virus from available material.

Failure to isolate any virus from the scab material might have been due to insufficient viable virus particles being present in the field samples, the presence of a virus other than the vaccinia-related horsepox of Kaminjolo et al[4], and the pox virus particles demonstrated could have been the morphologically-similar pox virus of molluscum contagiosum[2 12] which has to date, not been isolated in tissue culture.

REFERENCES

1. Cheville N F 1975 Cytopathology in viral diseases (Monographs in Virology Vol 10) S. Karger, Basel: 1-30
2. Cooley A J, Reinhard M K, Gross T L, Fadok V A, Levy M 1987 Molluscum contagiosum in a horse with granulomatous enteritis. Journal of Comparative Pathology 97: 29-34
3. Jayo M J, Jensen L A, Leipold H W, Cook J E 1986 Poxvirus infection in a donkey. Veterinary Pathology 23: 635-637
4. Kaminjolo J S, Johnson L W, Frank H, Gicho J N 1974 Vaccinia-like pox virus identified in a horse with a skin disease. Zentralblat fur Veterinar Medizin B 21: 202-206
5. Kaminjolo J S, Johnson L W, Muhammed S I, Berger J 1975 Uasin Gishu skin disease of horses in Kenya. Bulletin of Animal Health and Production in Africa 23: 225-228
6. Kaminjolo J S, Nyaga P N, Gicho J N 1974 Isolation, cultivation and characterization of a poxvirus from some horses in Kenya. Zentralblat fur Veterinar Medizin B 21: 592-601
7. Kaminjolo J S, Winqvist G 1975 Histopathology of skin lesions in Uasin Gishu skin disease of horses. Journal of Comparative Pathology 85: 391-395
8. McIntyre R W 1949 Virus papular dermatitis of the horse. American Journal of Veterinary Research 10: 229-232
9. Moens Y, Kombe A H 1988 Molluscum contagiosum in a horse. Equine Veterinary Journal 20: 143-145
10. Montgomery H 1967 Dermatopathology Vol 1. Harper and Row, New York: 536
11. Mullowney P C, Fadok V A 1984 Dermatologic diseases of horses Part II. Bacterial and viral skin diseases. The Compendium on Continuing Education for the Practising Veterinarian 6: S16-S26
12. Rahaley R S, Meuller R E 1983 Molluscum contagiosum in a horse. Veterinary Pathology 20: 247-250
13. Yager J A, Scott D W 1985 The Skin and appendages. In: Jubb K V F, Kennedy P C, Palmer N (ed.) Pathology of Domestic Animals 3rd edn Academic Press, New York: 461-471

MOLLUSCUM CONTAGIOSUM IN A HORSE

I B J VAN RENSBURG*, M G COLLETT*, N RONEN** and TRUUSKE GERDES***

ABSTRACT

An adult stallion presented with a severe papular dermatitis of especially the neck, chest and genitalia. A marked scrotal oedema was present. Histopathological examination of skin biopsies, revealed the presence of numerous intracytoplasmic molluscum bodies in areas of focal epidermal hyperplasia. Electron microscopical examination showed the presence of typical pox virions in affected epidermal cells. Attempts at viral isolation were unsuccessful. This is believed to be the first reported case of molluscum contagiosum in a horse in the Republic of South Africa.

Key words: Pox virus, horse pox, molluscum contagiosum, Uasin Gishu disease, equine.

Van Rensburg I.B.J.; Collett M.G.; Ronen N.; Gerdes T **Molluscum contagiosum in a horse.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 72-74 (En.) Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa.

Fig. 1: **Widespread papular dermatitus particularly noticeable on the ventral neck**

INTRODUCTION

Molluscum contagiosum is a benign, mildly contagious poxviral dermatitis in man. A similar condition has been described in chimpanzees[2], kangaroos[9], South American sea lions[13] and horses[1 8 10 11]. In Africa, the disease has been reported in Zaire[10] and Zambia[8], while a form of horsepox known as Uasin Gishu disease has been reported from Kenya[4 5 6 7]. To the best of our knowledge the disease has not been reported as yet in the Republic of South Africa, although cases may have been observed but not recognised.

The disease is characterised by small raised papules that may occur anywhere on the body, but seem to occur more frequently on the skin of the face, neck, chest (trunk), inguinal region and, in males, the genitalia. The papules vary in size from 2 to 8 mm in diameter, are hypopigmented and covered by tufts of raised hair[1 10 11]. If detatched, they leave small craters which may bleed. The microscopical picture characteristically consists of a focal, abrupt proliferation of keratinocytes with numerous large intracytoplasmic inclusions known as molluscum bodies, which ultra-structurally consist of numerous pox virions.

*Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, 0110 Onderstepoort, Republic of South Africa
**Department of Medicine
***Section of Virology, Veterinary Research Institute

Received: November 1990 Accepted: March 1991

CASE HISTORY

An adult Boerperd stallion from the Hammanskraal district, Transvaal, was presented to the Department of Medicine, Faculty of Veterinary Science, University of Pretoria, showing a widespread papular dermatitis of 2-months duration. In addition, a severe scrotal oedema was evident. The horse had been kept on natural grazing, which it had shared with several other horses. It was the only affected animal on the premises.

Clinical examination of the horse revealed numerous non-pruritic grey-white papules, covered by raised tufts of hair and varying in diameter from 2 to 8 mm on the ventral neck and thorax, proximal limbs, ears, muzzle, scrotum and prepuce. The superficial layers of the papules were dry and flaky. Papules could easily be detached by manual manipulation, leaving small non-haemorrhagic craters. No vesicles or pustules were observed and there was no involvement of the mucous membranes. Although thin, the stallion was otherwise in good health.

Haematological examination showed a leukocytosis, neutrophilia with a left shift

Fig. 2: **Close-up view of the neck lesion**

and eosinophilia. Aspiration of the scrotal oedema yielded a straw-coloured exudate, containing large numbers of neutrophils and eosinophils. Dourine was excluded serologically. Skin scrapings were negative for parasites and skin cultures were negative for bacterial and fungal pathogens.

Punch biopsies of the papules were fixed in 10% buffered formalin and in 4% glutaraldehyde for light- and electron-microscopical examination respectively. These tissues were processed in a routine manner. Sections for light microscopy were stained with haematoxylin and eosin and the Feulgen reaction for DNA according to standard procedures.

Ultra-thin sections were stained with 1% uranyl acetate and 0,2% lead citrate for electronmicroscopical examination. In addition, a number of papules were manually removed from the skin for further investigation. Some were homogenised and adsorbed onto 400 mesh grids before being negatively stained with 3% phosphotungstic acid and examined electronmicroscopically.

A 10% suspension of papule material was prepared in a neutral buffer and clarified by centrifugation at 2 000 rpm for 20 min. The resulting supernatant was used to inoculate primary and low passage calf-kidney cell cultures. Infected and control cultures were maintained at 37°C and observed daily for a cytopathic effect.

Fig. 3: **Focal hyperplasia of stratum spinosum with molluscum bodies in the majority of the keratinocytes. Note release of cells containing molluscum bodies from the epidermal surface. HE X 100**

Histopathological examination of skin biopsies revealed sharply demarcated areas of papillomatous hyperplasia of the stratum spinosum of the epidermis, which projected above the surrounding normal epidermis and extended somewhat below the basement membrane into the dermis (Fig. 3). Many of the keratinocytes contained large molluscum bodies, which, in many instances, compressed the somewhat pyknotic epidermal nuclei to one side of the cell (Fig. 4). These bodies stained mildly basophilic in the suprabasilar layers and became larger, more basophilic and granular in the outermost layers. They stained positively for DNA with the Feulgen reaction. In some biopsies the cells containing molluscum bodies were being discharged from the surface (Fig. 3). The dermal reaction was mild and consisted of focal infiltrations of a few lymphocytes and plasma cells.

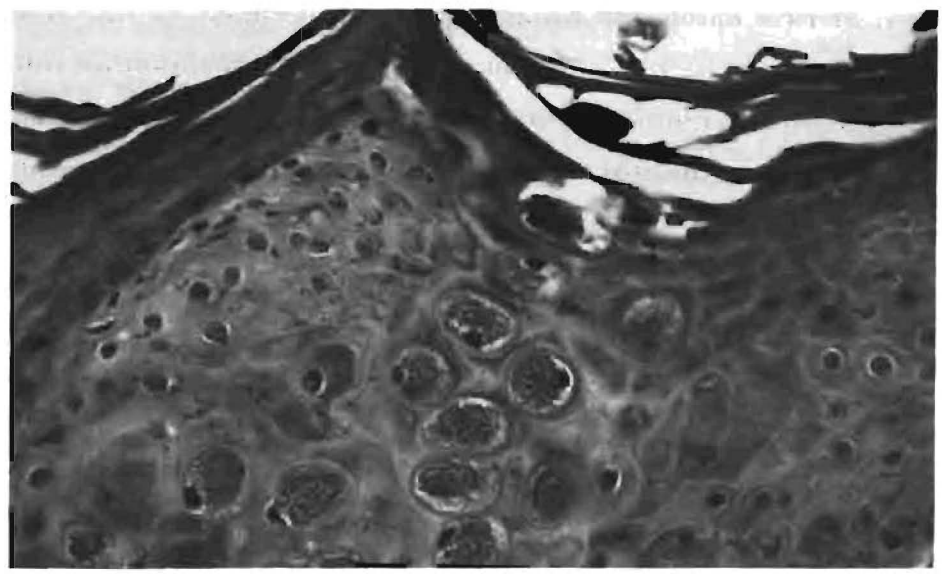


Fig. 4: **Large molluscum bodies in epidermal cells compressing the nuclei. HE X 400**

Isolation attempts on tissue culture were unsuccessful.

Ultrastructural examination of sections, as well as the negatively-stained material, revealed the presence of numerous viral particles, which conformed to the characteristics of pox viruses. In sections, the virions were mostly oval to rectangular and consisted of a biconcave, dumbbell-shaped electron-dense nucleoid and two less electron-dense lateral bodies (Fig. 5). Negatively-stained particles showed C (capsule) and M (mulberry) forms with the irregular arrangement of surface fibrils and the serrated outline clearly visible in the M forms. The particles measured 239,66 x 178,53 nm (mean of 20 particles measured).

DISCUSSION

Poxvirus infection is rare in horses. Classical horsepox allegedly occurs only in Europe, is highly contagious and is characterised by the development of papules progressing to vesicles and pustules on the skin in the "greasy heel type" (contagious pustular dermatitis) or on the face, nares, oral and nasal mucous membranes in the "buccal type" (contagious pustular stomatitis)[3 12]. The latter was recently reported in a donkey from a zoo in Kansas, USA[3].

Viral papular dermatitis, which may be a variant of horsepox, is described as a highly contagious disease of horses in the United States and New Zealand[4 12]. The disease is characterised by a papular reaction only. The causative virus has not yet been characterised and the histopathology not described. The cause of Kenyan horsepox, also known as Uasin Gishu skin disease, has been isolated and characterised[4 5 6] and is related to the vaccinia group of pox viruses.

Equine molluscum contagiosum on the other hand is only mildly contagious, if at all, and although lesions may appear anywhere on the skin, they are apparantly more common on the face, neck, chest and trunk, innerlegs and on the genitalia. The similarities in the clinical appearance, protracted course of the disease and identical morphological characteristics of the causative virus, have prompted some authors to regard Uasin Gishu skin disease and molluscum contagiosum as the same disease[8 10]. However, the relative ease with which the virus causing Uasin Gishu skin disease can be grown on primary calf-kidney cultures[5] and the inabili-

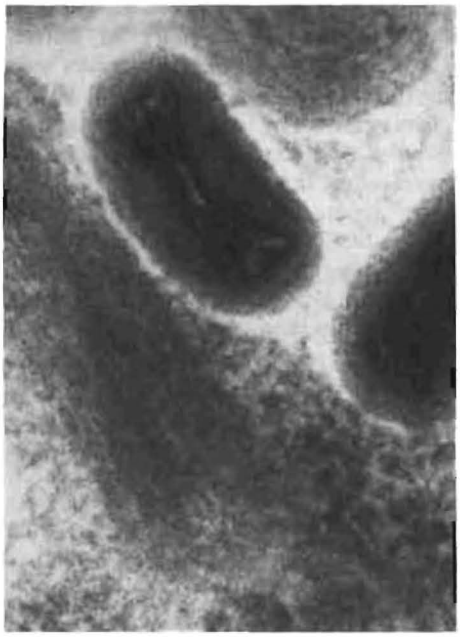


Fig. 5: **Pox virion showing the rectangular shape and biconcave electron-dense nucleoid. Electron micrograph Bar 50 nm**

ty to isolate the virus of molluscum contagiosum on such cultures, suggest that the 2 conditions are caused by related, but different viruses.

In humans molluscum contagiosum is a contagious skin disease with a worldwide distribution. It is characterised by single or multiple cutaneous nodules, 2-5 mm in diameter anywhere on the body. The clinical course of the condition, extends over weeks to several months. The histopathology is characterised by focal epidermal hyperplasia and the presence of large eosinophilic intracytoplasmic inclusions located in a cytoplasmic cavity[10 11]. (Routine haemotoxylin and eosin staining techniques used in our laboratory, stained the inclusions mildly basophilic.) The virus has not yet been isolated and therefore remains unclassified.

Similar conditions have been described in several animal species including chimpanzees[2], kangaroos[9], sea lions[13] and equines[1 8 10 11], and probably represent accidental infections in debilitated or immune-compromised hosts. The disease under these circumstances is not regarded as being contagious.

The Kenyan horsepox may have originated as a spill-over into the horse population from the small pox eradication vaccination programme, with conceivable adaptation of the virus to its new host. Since small-pox vaccination ceased in 1980 when the disease was eradicated, any pox in equines in the Republic of South Africa would have to have been imported into the country as there is no naturally-circulating vaccinia virus. As molluscum contagiosum occurs naturally in the human population, it seems possible that the horse described in this paper may have picked up the virus from a human carrier.

Although this is the first description of equine molluscum contagiosum in the Republic of South Africa, an apparently identical clinical syndrome was observed previously (B J Erasmus 1990 Veterinary Research Institute, Onderstepoort and S R van Amstel 1990 Faculty of Veterinary Science, University of Pretoria, personal communication). According to Erasmus, the scrotal oedema, which was very pronounced in the case reported in this paper, is a common finding in affected stallions. He also states that the assumed identical condition seen by him, could be transmitted to other horses by the intravenous injection of blood or saline suspensions of skin material from infected cases (which were not confirmed to be molluscum contagiosum). No transmission studies were attempted with material from the case reported in this paper. Normally, the disease has a prolonged course and spontagenous recovery may take place after several months. According to Erasmus, the administration of corticosteroids results in a flare-up of the condition in such animals. This strongly supports the view of other authors that the disease is predisposed to by immune-incompetence and debilitation[1]. The stallion reported in this paper arrived in an emaciated condition and improved during hospitalisation on a proper diet without any other specific treatment. Unfortunately, contact with the owner has been lost since the discharge of the patient and the present condition of the horse is unknown.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the information supplied by Dr B J Erasmus and Prof S R van Amstel, the assistance rendered by Miss C Maree and Mr J Putteril in the electron microscopical examination, as well as the help of Prof J W Nesbit, Mrs V Käber & Mrs H Smit.

REFERENCES

1. Cooley A J, Reinhard M K, Gross T L, Fadok V A, Levy M 1987 Molluscum contagiosum in a horse with granulomatous enteritis. Journal of Comparative Pathology 97: 29-34
2. Douglas J D, Tanner K N, Prine J R, Van Riper D C, Derwelis S K 1967 Molluscum contagiosum in chimpanzees. Journal of the American Veterinary Medical Association 151: 901-904
3. Jayo M J, Jensen L A, Leipold H W, Cook J E 1986 Pox virus infection in a donkey. Veterinary Pathology 23: 635-637
4. Kaminjolo J S, Johnson L W, Frank H, Gicho J N 1974 Vaccinia-like pox virus identified in a horse with a skin disease. Zentralblatt für Veterinär Medizin (Reihe B) 21: 202-206
5. Kaminjolo J S, Johnson L W, Muhammed S J, Berger J 1975 Uasin Gishu skin disease of horses in Kenya. Bulletin of Animal Health and Production in Africa 23: 225-233
6. Kaminjolo J S, Nyaga P N, Gicho J N 1974 Isolation, Cultivation and characterization of a poxvirus from some horses in Kenya. Zentralblatt für Veterinär Medizin (Reihe B) 21: 592-601
7. Kaminjolo J S, Winquist G 1975 Histopathology of skin lesions in Uasin Gishu skin disease of horses. Journal of Comparative Pathology 85: 391-394
8. Lange A L, Marett S, Maree C, Gerdes T 1989 An outbreak of poxvirus infection in horses. Proceedings of the Electron Microscopical Society of Southern Africa 19: 79-80
9. McKenzie R A, Fay F R, Prior H C 1979 Poxvirus infection of the skin of an Eastern Grey Kangaroo. Australian Veterinary Journal 55: 188-190
10. Moens Y, Kombe A H 1988 Molluscum contagiosum in a horse. Equine Veterinary Journal 20: 143-145
11. Rahaley R S, Mueller R E 1983 Molluscum contagiosum in a horse. Veterinary Pathology 20: 247-250
12. Timoney J F, Gillespie J H, Scott F W, Barlough J E (ed) 1988. In: Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals. 8th edn. Comstock Publishing Associates, Ithaca. p 571
13. Wilson T M, Poglayen-Neuwall I 1971 Pox in South American sea lions *(Otaria byronia)*. Canadian Journal of Comparative Medicine 35: 174-177

CHYLOTHORAX IN A KITTEN

N M DUNCAN*

ABSTRACT

Chylothorax with collapse of the lungs was found on postmortem examination of a 2-week-old Siamese kitten. The chylothorax was probably due to a lack of continuity of the thoracic duct.

Key words: Chylothorax, cat.

Duncan N.M. **Chylothorax in a kitten.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 75 (En.) Department of Pathology, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa.

The characteristics of a chylothorax effusion are classically a milky white to pink, opaque fluid that does not produce a cleared supernatant upon centrifugation, has the mature lymphocyte as the main cell-type present, clears with ether at an alkaline pH and reacts positively to Sudan III and Oil Red O stains[2 4 7 10-14]. These characteristics are due mainly to the presence of chylomicrons from the intestinal lymph.

Reported causes of chylothorax in cats, include trauma (often associated with diaphragmatic hernias[2 9]), cardiomyopathy[4 11], thymoma[3], mediastinal lymphoma[9] and dirofilariasis[5]. When a ruptured thoracic duct can be demonstrated, as with direct or indirect trauma or by tumour erosion, the pathophysiology is easy to determine. In many cases of cardiomyopathies, some neoplasms and dirofilariasis, the duct is intact. In these cases the chylothorax is thought to occur due to an increase in thoracic duct pressure or due to an increase in venous pressure in the cranial vena cava, which results in a secondary increase in thoracic duct pressure[2].

Congenital defects of the thoracic duct seen in man, include complete absence of the duct, fistulas between the duct and pleura, failure of the lymphatics to communicate and incomplete communication of the segmental components of the embryonic duct[7]. Chylothorax in very young animals is either rare or just never reported[1]. Congenital chylothorax i.e. chylothorax due to developmental defects of the thoracic duct itself is more rare. There has been one reported case of a 2-year-old Afghan hound presenting with chylothorax and abnormal terminations of the thoracic duct[6]. The only reported case of a thoracic duct defect in the cat, was in a one-year-old Siamese which presented with chylothorax due to a massive diverticulum of the thoracic duct[8].

A 2-week-old pedigreed Siamese kitten was presented for necropsy with a history of sudden death. A necropsy was performed and 5 ml of pink-tinged milky fluid was found in the thoracic cavity, the collapsed lungs were red in colour and had a rubbery consistency. On gross examination, no inflammatory changes of the pleura, dilatation of the thoracic duct or abnormalities of the thymus and heart were seen. Neither was there any indication of trauma to the thorax or to the rest of the body, and the diaphragm was intact. Attempts at introducing dye into the thoracic duct to check the patency, were unsuccessful. After staining with Sudan III, orange fat droplets were visible in the thoracic fluid and centrifugation of the fluid did not produce a cleared supernatant. Cytological examination of the fluid revealed that 95% of the cells were small mature lymphocytes, with solitary erythrocytes, neutrophils and activated macrophages making up the rest of the cell population. Smudged and lysed nuclear material was also observed.

Selected tissues were fixed in 10% buffered formalin and routinely processed for light microscopy. The only histopathological changes observed, were a marked pulmonary atelectasis and a mild periacinar lipid accumulation in the liver.

A diagnosis of chylothorax was made, based on the gross and microscopic findings, as well as on the results of the laboratory tests performed on the thoracic fluid.

The case of chylothorax presented was probably due to a lack of continuity of the thoracic duct. This is based on the exclusion of the causes of chylothorax mentioned previously[2-5 9 11].

Lack of continuity of the thoracic duct may either be a developmental defect or an acquired condition. The very young age of this kitten supports the possibility of this case being due to a developmental defect. Unfortunately chylothorax can result from minor chest trauma such as that which occurs with coughing or vomiting[8], which makes it difficult to rule out trauma as a possible cause.

*Department of Pathology, Faculty of Veterinary Science, Medical University of Southern Africa, 0204 Medunsa, Republic of South Africa

Received: January 1991 Accepted: March 1991

REFERENCES

1. Berg J 1982 Chylothorax in the dog and cat. Compendium on Continuing Education for the Practising Veterinarian 4: 986-991
2. Birchard S J, Fossum T W 1987 Chylothorax in the dog and cat. Veterinary Clinics of North America: Small Animal Practice 17: 271-283
3. Carpenter J L, Holzworth J 1982 Thymoma in 11 cats. Journal of the American Veterinary Medical Association 181: 248-251
4. Creighton S R, Wilkins R J 1975 Thoracic effusions in the cat: etiology and diagnostic features. Journal of the American Animal Hospital Association 11: 66-76
5. Donahue J M, Kneller K, Thompson P E 1974 Chylothorax subsequent to infection of cats with *Dirofilaria immitis*. Journal of the American Veterinary Medical Association 164: 1107-1110
6. Fossum T W, Birchard S J, Jacobs R M 1986 Chylothorax in 34 dogs. Journal of the American Veterinary Medical Association 188: 1315-1318
7. Harper N K 1989 Chylothorax. In: Kirk R W (ed.) Current Veterinary Therapy: Small Animal Practice 10th edn. W B Saunders Co, Philadelphia, USA: 295-301
8. Herbert W B 1964 Dyspnea due to a cystic thoracic duct in a cat. Journal of the American Veterinary Medical Association 144: 46
9. Holzworth J, Cotter S 1987 Disorders of the hematopoietic system. In: Holzworth J (ed.) Diseases of the Cat. Medicine and Surgery. Vol. I. W B Saunders Co, Philadelphia, USA: 772-773
10. Lindsay F E F 1974 Chylothorax in the domestic cat - a review. Journal of Small Animal Practice 15: 241-258
11. McConnell M F, Huxtable C R 1982 Pseudochylous effusion in a cat with cardiomyopathy. Australian Veterinary Journal 58: 72-74
12. Meinke J E, Hobbie W V Jr, Barto L R 1969 Traumatic chylothorax with associated diaphragmatic hernias in the cat. Journal of the American Veterinary Medical Association 155: 15-20
13. Quick C B 1980 Chylothorax - a review. Journal of the American Animal Hospital Association 16: 28-29
14. Schalm O W 1976 Pleural effusions in the cat. Feline Practice 6: 41-43

COBALT DEFICIENCY IN PASTURED SHEEP IN THE SOUTH-WESTERN CAPE PROVINCE

D J SCHNEIDER*, E W P HEINE* and JENNIFER R GREEN*

ABSTRACT

Annually recurrent illthrift and mortalities in a small flock of pastured sheep at the Regional Veterinary Laboratory, Stellenbosch were cured by the supplementation of cobalt. The similarities between acute cobalt deficiency and ovine white liver disease are discussed.

Key words: Cobalt deficiency, sheep, illthrift, white liver disease

Schneider D.J.; Heine E.W.P.; Green J.R. **Cobalt deficiency in pastured sheep in the south-western Cape Province.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 76-77 (En.)
Regional Veterinary Laboratory, Private Bag X5020, 7600 Stellenbosch, Republic of South Africa.

Routine daily inspection of a small flock of South African Mutton Merino/Dorper crossbred sheep kept on mixed dryland pasture consisting mainly of kikuyu (*Pennisetum clandestinum*) grass at the Stellenbosch Regional Veterinary Laboratory revealed unexpected loss in body condition in ewes, as well as illthrift and mortality in lambs. A commercial protein/mineral sheep block (Rumevite Sheep Block, Rumevite/Agricura Animal Production) containing inter alia 8 mg cobalt per kg, was available ad lib. The consumption of these blocks was lowest during the spring months when the quality and quantity of the grazing was best, and highest in autumn when poor grazing prevailed.

Since 1976 it had annually been noticed, paradoxically, that the sheep were in best condition during autumn when the grazing was dry and of poor quality, and in poorest condition when they were on the lush spring grazing. Ewes were usually in excellent physical condition when lambing, between April and July, and the lambs usually grew well during the first 6-8 weeks after birth. Lambs born in the latter part of the period were affected at a younger age than those born earlier in the lambing season. By October/November most lambs were unthrifty and pot-bellied. From 1977 to 1979, 22 out of 54 lambs born, died between the ages of 1 to 7 months.

*Regional Veterinary Laboratory, Private Bag X5020, 7600 Stellenbosch, Republic of South Africa

Received: April 1990 Accepted: March 1991

Most lambs wasted away gradually. Some, however, died within 2 d of first showing signs of stiffness and lethargy, and a small number died acutely while in good condition and without having shown any clinical signs of disease. In addition to the poor condition, drooping upper eyelids, a serious ocular discharge as well as a mild diarrhoea and anaemia were seen clinically in most subacute and chronic cases. Recumbency, nystagmus and icterus were seen occasionally, but photosensitivity was not observed. From about February each year, all the sheep began to improve. This improvement was more marked in adult than in young sheep.

Post mortem investigations on 22 lambs that died or were euthanased in extremis, revealed that 14 were emaciated while the condition of the remainder varied from reasonable to good. Other changes were non-specific; the most important of which was a slightly enlarged, pale, sometimes yellowish, friable liver (found in 14 cases). The changes in the liver were more prominent in the acute cases. Pulmonary oedema and congestion were also more often seen in acute cases.

Chronic cases manifested ascites and a mild hydrothorax which was usually associated with localised atelectasis and pneumonia. Most chronic cases were anaemic while a slight icterus was seen in 2 of the acute cases. No blood parasites were observed on examination of peripheral blood smears.

Organs from 21 of the 22 lambs were examined histopathologically. One of the lambs was in an advanced stage of decomposition. Moderate to very severe fatty changes (n=18), mild to moderate bile duct proliferation (n=20), mild peribilary fibrosis (n=5), a brownish-grey pigment in the cytoplasm of the Von Kupffer cells (n=11) and mild to moderate splenic haemosiderosis (n=9) were observed in liver specimens. A moderate nephrosis was found in 2 cases. Copper, iron, zinc and manganese concentrations in the liver specimens assessed by atomic absorption, and serum concentrations of phosphorus and magnesium were found to be within the normal range.

As cobalt deficiency was considered to be a possible cause of the illthrift and since laboratory assays for cobalt or cyanocobalamin were unavailable to us at that stage, it was decided to determine the effect of cobalt supplementation administered to a group of the ewes and their lambs.

A flock of sheep (n=30) was divided into control (5 ewes, 5 young ewes, 6 lambs) and treatment (5 ewes, 9 lambs) groups. The mean body mass per lamb (23,65 kg) was approximately the same in each group. All animals grazed together. For a period of approximately 3 months, the treatment group was dosed with cobalt chloride at a dosage of 7 mg of cobalt per ewe and 5 mg per lamb per week. A protein/mineral sheep block (Rumevite Sheep Block) was available ad lib. After the initial period of 3 months, all animals were supplemented with cobalt. For the first 6 months, 7 mg of cobalt was given per os weekly. Thereafter all lambs were given 2 ml of hydroxycobalamin (Neocytamin injection, 1000 mcg per ml. Milvet Ethicals) subcutaenously at the ages of 2 weeks and 2 months. All sheep older than 4-5 months, received one "Cobalt Heavy Pill for Sheep" (Top Brand, Adelaide+Wallaroo Fertilizers Ltd. Australia) per os by means of a special dosing gun.

At the end of the first month, the average mass per lamb in the treatment group was 3,3 kg more than that of the lambs in the control group. After the second month, the average mass of the lambs in the treatment group was 6,7 kg more and after another month, it increased to 14,8 kg per lamb.

Two of the 6 lambs in the control group became lethargic and emaciated and one died. The second one was euthanased in extremis. The clinical signs and

pathological changes seen at necropsy, were similar to those of the natural cases described earlier.

After supplementation of cobalt, the average mass of the surviving lambs in the control group increased from 28,8 to 38,7 kg over a period of 40 d. Since the cobalt/vitamin B_{12} supplementation was implemented, the illthrift and related mortality in the flock have disappeared completely. The mortality figure for lambs aged between 1 and 7 months was 22 out of 54 (40,7%) for the years 1977 to 1979. For 1980, the year of the experiment described in this report, it was 2 out of 15 (13,3%) and for the years 1981 to 1989, the mortality figure was 2 out of 308 (0,45%).

Although this small diagnostic trial has many scientific shortcomings, e.g. the small number of lambs per group, the method of dividing the lambs into the 2 groups, the fact that sex was not taken into account and the fact that twin lambs had to be in the same group, the dramatic response obtained by cobalt supplementation suggests strongly that cobalt deficiency was the primary cause of this illthrift problem and the related mortalities. The diagnosis is further supported by the improved condition of the lambs and the dramatically-reduced mortality figures after continuous cobalt supplementation was implemented.

The use of cobalt pellets is preferred, because the weekly dosage of cobalt to pastured sheep is unpractical, the intake of licks by sheep unreliable (especially along the coast), and the top-dressing of cobalt on pastures only economically justifiable in high production, intensive grazing systems[8]. Problems encountered with this method of cobalt supplementation are the loss of these pellets by the animal, and the development of an impervious coat, mostly calcium phosphate, encapsulating the pellet[8]. A steel screw administered with the pellet or 2 pellets together, has been used successfully to reduce the formation of this coating[8].

In our experience, the cobalt pills are not readily lost, as we have recovered all at slaughter, in some cases up to 5 years after administration. In about 30% of cases, however, the cobalt pellet had become coated with a hard, greyish-brown layer which can be expected to reduce or prevent the release of cobalt in the rumen. Contrary to reports in the literature, our attempts to prevent formation of this coating by administering a 10x12,5 mm grub screw with the pellet, was unsuccessful in many cases.

The following factors are possbily responsible for the seasonal occurrence of clinical signs of cobalt deficiency under our conditions in spring and early summer:

1. Soil contamination of the grazing is greater during late summer and autumn months, when the grazing is short and dry, than during winter and spring months, when the grazing is lush and fast-growing. This is important since most soils contain more cobalt than the plants growing on them and soil may constitute 10-25% of total dry matter intake in sheep when grazing is short and dry[6].
2. The cobalt content of fast-growing, lush grazing plants in spring is lower than that of plants during the dry summer and autumn months[2].
3. We have found that the intake of the protein/mineral supplement is always perceptibly higher during the dry months than during spring when the grazing is lush.

Ovine white liver disease (WLD) which has been described in New Zealand[1 2 3 6], Australia[6] and the United Kingdom[4 5], occurs mainly in late spring and in most cases affects young sheep of 3 to 6 months of age manifesting clinical signs of illthrift and mortality. Low concentrations of serum vitamin B_{12} and liver cobalt as well as pale fatty friable livers, are consistent findings in this condition. Histopathologically, parenchymal fatty change, bile duct proliferation and ceroid pigmentation are characteristic[6].

McLoughlin et al.[5] found lesions in the liver resembling ovine white liver disease in cobalt-deficient lambs and Mitchell et al.[6] agreed that the difference between WLD and cobalt deficiency might not be absolute. As our findings are similar to those of these authors, we are at this stage in agreement that it is not possible to distinguish between acute cobalt deficiency and white liver disease on the basis of these lesions.

ACKNOWLEDGEMENTS

We wish to thank Dr M G Collett, Mrs R de Kock, Mr T T de Villiers and Mr P van der Merwe.

The Directorate of Animal Health is thanked for permission to publish this paper.

REFERENCES

1. Black H, Hutton J B, Sutherland R J, James M P 1988 White liver disease in goats. New Zealand Veterinary Journal 36: 15-17
2. Clark R G 1981 Cobalt Deficiency in Sheep and Cattle. Proceedings Waikato University Trace Element Seminar. Ruakura Animal Health Laboratory, New Zealand
3. Clark R G, McKnight L J, Cornforlth I S, Oliver J, Jones B A H 1978 A condition resembling ovine white liver disease in lambs on irrigated pastures in South Canterbury. New Zealand Veterinary Journal 26: 316
4. Ferguson E G W, Mitchell G B B, MacPherson A 1989 Cobalt deficiency and *Ostertagia circumcinta* infections in lambs. Veterinary Record 124: 20
5. McLoughlin M F, Rice D A, Taylor S M 1984 Liver lesions resembling ovine white liver disease in cobalt deficient lambs. Veterinary Record 115: 325
6. Mitchell P J, McOrist S, Thomas K W, McCausland I P 1982 White liver disease of sheep. Australian Veterinary Journal 58: 181-184
7. Sutherland R J, Cordes D O, Carthew G C 1979 Ovine white liver disease - an hepatic dysfunction associated with vitamin B_{12} deficiency. New Zealand Veterinary Journal 27: 227-232
8. Underwood E J 1981 The Mineral Nutrition of Livestock, Commonwealth Agricultural Bureaux England. Page Bros (Norwich) Ltd.

PERINATAL LAMB MORTALITY — ITS INVESTIGATION, CAUSES AND CONTROL

K G HAUGHEY*

ABSTRACT

Methods of investigating perinatal loss in grazing sheep flocks are reviewed and evaluated. The "wet-dry" method is the simplest method for assessing minimal prevalence, whereas the differences between the numbers of single and twin foetuses present at ultrasonic determination of litter size during pregnancy, and the numbers of single and twin lambs present at lamb-marking, is the most precise. The veterinary investigation of field mortality involves full autopsy of a representative sample of dead lambs, a history of prenatal nutrition, disease and husbandry, as well as a qualitative estimate of weather conditions over the period of lamb collection. Pathological processes may be identified in over 95% of deaths and the specific cause determined in about 75% of deaths. The identification of the specific causes in the remainder of deaths, all classified as the starvation-mismothering-exposure (SME) complex, requires intensive, costly, on-site observation, and physiological and biochemical assessment. The probable causes of these deaths include prenatal physiological handicaps resulting from placental insufficiency, aberrant parent-offspring behaviour, management-induced mismothering, misadventure, inadequate milk supply or teat and udder abnormalities, and cold-induced starvation. The gross pathology and pathophysiology of birth stress and the SME complex, which are associated with at least 80% of mortality, are summarised. Birth injury to the foetal central nervous system, characterised by cranial and spinal meningeal haemorrhage is exclusive to parturient deaths and the SME complex. Observed flock prevalences range from 81% to 100% in parturient deaths, and 20% to 57% in the SME complex. The high total prevalence and experimental evidence, indicate the major causal role of birth stress in the pathogenesis of these entities. Lethal congenital malformations, infections (both congenital and acquired after birth), trace element deficiencies and predation are reviewed as minor causes. The new understanding of the pathogenesis of perinatal lamb mortality, recognises the heritable nature of birth mass, maternal pelvic dimensions, parent-offspring behaviour, and the resistance of neonates to cold. Control measures need to incorporate selection for maternal rearing ability, further refinement of prenatal nutritional management of twin-bearing ewes, disease control, provision of shelter for lambing flocks, and avoidance of husbandry practices which frustrate innate parent-offspring behaviour. A selection programme is summarised.

Key words: Perinatal, lambs, mortality

Haughey K.G. **Perinatal lamb mortality - its investigation, causes and control.** *Journal of the South African Veterinary Association* (1991) 62 No. 2, 78-91 (En.) 22 Stromlo Place, Campbelltown, New South Wales 2560, Australia.

*22 Stromlo Place, Campbelltown, New South Wales, 2560 Australia

INTRODUCTION

Perinatal lamb mortality, defined as deaths occurring shortly before, during or within 7 d of birth, is widely recognised as a major source of reproductive wastage among sheep[9]. It may account for 80% to 90% of preweaning mortality[33 74]. Numerous reports on loss in lambs reflect a serious and intractable, world-wide problem. Average perinatal loss in Australia, New Zealand and Great Britain ranges from 10% to 20% of lambs born[33 43 81]. Reliable data on the national level of loss under the wide range of environmental and management conditions in South Africa are scarce. Two recent studies reported that 12,6% of lambed ewes lost their lambs within one month of lambing[50], and that perinatal loss among intensively-managed flocks in the Western Cape, averaged 15,1% of lambs born[67]. Mortality ranging between 16% and 23% of lambs born, was recorded in apparently well-managed individual flocks of several breeds[25 73 127 128]. Despite a marked improvement of nutrition of ewes during pregnancy and early lactation, husbandry, and disease control in many flocks, perinatal losses remain unacceptably high. Recent evidence suggests that intrinsic maternal and/or foetal defects, including birth asphyxia and trauma, aberrant parent-offspring behaviour and neonatal susceptibility to cold, play a major role in perinatal lamb mortality. This highlights the fundamental importance of the ewe-lamb partnership to lamb survival.

Perinatal lamb mortality varies greatly within and between breeds, flocks, districts, seasons and management systems, and may reach more than 50% of lambs born in exceptional circumstances. Its complex and variable aetiology, necessitates that both professional advisers and flock-owners have a clear knowledge of the magnitude and the causes of the problem before cost-effective control programmes can be implemented successfully.

This paper reviews the investigation, causes and control of perinatal mortality.

METHODS OF ESTIMATING THE PREVALENCE OF PERINATAL MORTALITY

A. Wet-dry technique[40]

The method classifies ewes at lamb-marking (preferably within a month of lambing), by visual appraisal and udder palpation, as (1) barren (2) lambed and rearing a lamb(s) (3) lambed and lost lamb(s). Barren (not lambed) ewes show neither udder development nor "lambing stain" (the staining of the posterior udder surface and hocks with dried lambing discharges) and usually show better body condition and fleece quality than lambed ewes. Lambed ewes have enlarged udders containing secretion, usually show "lambing stain", have poorer body condition and fleece quality than dry ewes. The class can be further subdivided into: (a) Ewes rearing a lamb(s) have full, resilient udders containing milk. Teats and adjacent areas of the udder are soft, pliable and clean due to the lamb's sucking. (b) Ewes which have lost their lambs (lambed and lost ewes) have variably developed udders, often with pronounced cleavage between the 2 glands and stiff, dirty teats, with secretion ranging from milky to thin watery, or thick, viscous, honey-coloured matter, depending on the period elapsed since the death of progeny.

Heavy contamination of the teats and udder with mud or dust may make classification difficult, and hence unreliable. Skilled palpation to detect sucked teats will overcome this difficulty. The ingestion of oestrogenic pastures may cause udder development unrelated to pregnancy and parturition.

Perinatal mortality is expressed as:

$$\frac{\text{(No of ewes losing lambs)}}{\text{(No of ewes lambing)}} \times 0.9\ \%$$

as about 90% of losses to lamb-marking occur during the perinatal period. The result is a minimal estimate of perinatal mortality because it takes no account of ewes losing part or all of a set of multiple births.

An indication of the prevalence of twinning is estimated as:

$$\frac{\text{(Number of lambed marked)}}{\text{(Number of ewes lambing)}} \times 100\ \%$$

B. Pregnancy diagnosis by real-time ultrasound[51]

Pregnancy diagnosis by this technique, as early as day 45 of pregnancy, enables appropriate nutrition of non-pregnant, single- and multiple-bearing ewes respectively, in late pregnancy and lactation, and improved management of multiple pregnancies during lambing. Provided the flocks of single- and multiple bearers are maintained separately until lamb-marking, the discrepancy between foetal numbers present at scanning and the number of single and twin lambs present at marking, offers the most precise estimate of perinatal mortality relative to litter size. This is calculated as 90% of the discrepancy between the values. It is still necessary for culling purposes to "wet-dry" the lambed ewes in order to identify ewes which "lamb and lose" lambs.

C. Carcase collection

This method usually underestimates losses under extensive grazing conditions. Carcases may be removed by predators or scavengers and/or easily missed during pick-up, despite the most diligent searching.

"Wet-drying" is probably the most widely applied method on commercial farms, despite its tendency to underestimate mortality in highly fecund flocks. This method is cheap, simple to apply and is suitable for both extensively- and intensively-managed flocks. It allows the detection of teat and udder abnormalities and, given the repeatable[68] and heritable[66] nature of rearing ability, it identifies barren and "lambed and lost" ewes for culling. Age-specific classification of the failure classes may suggest causes of failure. For example, high prevalences of failing to lamb among maidens is often associated with low body mass at mating, due to inadequate weaner nutrition or disease.

Table 1 illustrates the importance of perinatal mortality measured by the "wet-dry" technique, relative to other sources of reproductive wastage in selected Australian Merino flocks. Row G highlights the apalling net reproductive efficiency of better-than-average flocks, expressed as the proportions of ewes joined, that actually were rearing a lamb(s). The superior performance of Flock 5 was associated with the implementation of policies incorporating pasture improvement, disease control, culling barren and "lambed and lost" ewes, and the use of twin-born rams. Lambs marked/ewes joined, rose from 95% to 135% over a 15-year period (Haughey, unpublished data).

METHODS OF INVESTIGATING PERINATAL MORTALITY

1. Direct observation of the lambing flock by a team of skilled observers recording relevant data, including maternal behaviour[14]. It is a labour-intensive method, suitable mainly for research of parent-offspring behaviour in individual flocks. The pathological basis of mortality is often poorly-defined as complete autopsies may not be performed.
2. Autopsy, including appropriate microbiological, serological and histopathological examination, in association with a history of flock management, nutrition and disease control, and qualitative estimates of weather conditions during lambing. Competent investigators may identify the pathological processes involved in over 95% of deaths and the precise cause in about 75% of deaths[67]. The balance invariably comprise neonatal deaths typical of the starvation-mismothe-

Table 1: **Perinatal lamb mortality relative to the reproductive performance of selected, mixed-age, spring-lambing Merino flocks with lamb-marking percentages above the Australian average (70%) [Haughey, unpublished data]**

Flock	1	2	3	4	5
Performance		**% of ewes joined**			
A. Lambs marked	106	86	76	112	135
B. Ewe deaths - joining to marking	5	9	3	3	3
C. Dry ewes	15	23	11	10	7
D. Ewes lambing	80	68	86	87	90
Lambed ewes		**% ewes lambing**			
E. Ewes rearing lamb(s)	88	84	86	77	93
F. Ewes losing all lambs born	12	16	14	23	7
Minimum fecundity of lambed ewes (lambs marked/ewe lambing)	1,33	1,27	0,88	1,29	1,50
G. Ewes rearing/ewes joined	70	57	74	67	84

ring-exposure syndrome[59], the probable causes of which include prenatal foetal physiological impairment, aberrent parent-offspring behaviour, management-induced mismothering, misadventure, inadequate milk supply, teat and udder abnormalities, and cold-induced starvation. The lack of direct observation precludes specifying the precise role of these factors in pathogenesis.

3. Physiological biochemical and pathological methods including the recording of birth mass and rectal temperature, haematocrit, plasma concentrations of lactate and fructose about 15 min after birth and age at death, were claimed to differentiate causes of neonatal death associated with placental insufficiency, acute intrapartum hypoxaemia, inadequate thermogenesis and starvation[21 89].

Although a combination of the 3 methods provides the most efficient diagnosis, the high cost precludes their widespread use. Veterinarians experienced in the ecology of sheep production, are uniquely situated to use the autopsy method supplemented by a farm history in large scale surveys and the investigation of mortality in individual flocks. The autopsy method involves the postmortem examination of a sample of about 50 carcases, collected during the first 3 weeks of lambing. Carcases should be stored in the farm coolroom until submission, usually 3 times per week. Where practicable, a tag, noting relevant data including litter size, date of birth and death, should be attached to each lamb, accompanied by a subjective assessment of prevailing weather conditions over the period of collection, for example, hot, warm, or cold, wet or dry, calm or windy. At the laboratory, all unmutilated lambs are weighed as the dead mass of parturient deaths is identical to birth mass, and the dead mass of neonatal deaths is a reflection of birth mass. As the birth mass of lambs is rarely available in commercial flocks, it may be reliably estimated from the crown-rump measurement[67].

A systematic autopsy, including examination of the central nervous system (CNS), is performed[58 84]. Each carcase is classified according to its time of death relative to birth. This is of diagnostic use because specific entities tend to occur in specific time-of-death classes (Table 3): Ante-parturient death: deaths occurring before birth commenced; Parturient death: deaths occurring during or within 3 h after birth; Post-parturient death: deaths occurring more than 3 h and less than 8 d after birth.

In the absence of specific data, the age of post-parturient deaths is estimated as follows (Haughey, unpublished data):

Less than 2 d: fat catabolism is nil, slight, moderate or marked (see section on fat catabolism); if the lamb has not fed, there is no food in the abomasum or small intestine; if the lamb has fed, food has not passed beyond the small intestine; the large intestine contains meconium.

Two to 7 d: fat catabolism is nil, slight, moderate or marked; if the lamb has not fed there is no evidence of meconium in the large intestine; when the lamb has fed, there are variable amounts of milk ingesta throughout the almimentary tract, remnants of chyle are seen in the mesenteric lymphatics and the contents of the large intestine are gritty in nature, compared to the homogeneous consistency of meconium (Haughey, unpublished data).

Table 2 summarises the main features of the time-of-death classes[58 84]. Table 3 summarises the occurrence of common causes of perinatal loss relative to time-of-death classification[34 58 67].

Examination of the CNS is mandatory because of the major role of birth injury in neonatal as well as parturient death. The brain is exposed by removing the cranial calvarium with sharp-pointed foot-paring shears. The spinal cord is exposed by cutting and removing the vertebral arches.

MAJOR CAUSES OF PERINATAL MORTALITY AND THEIR DIAGNOSIS

The aetiology of perinatal lamb mortality is complex and involves the action and interaction of many factors, including weather conditions, genetic factors, deficiency of gross and specific nutrients during pregnancy, predation, infections, maternal and neonatal behaviour, adequacy of milk supply, management, and the effects of birth asphyxia and/or trauma on the foetal CNS. Many carcases show lesions of more than one origin. The multitudinous constraints to lamb survival have been reviewed by Alexander[9].

At least 80% of deaths usually fall into 2 categories at autopsy[58 67]:

1. Deaths occurring during, or within a few hours of birth due to uncomplicated birth stress. During severely cold weather (<5°C) this class may contain very small lambs dying of peracute hypothermia.
2. Neonatal deaths, classified as the starvation-mismothering-exposure (SME) complex, characterised at autopsy by evidence of starvation and cold exposure.

Maternal prenatal nutrition

For comprehensive reviews of this topic see the appropriate literature[88 89 111]. Excess or deficiency of gross nutrients during pregnancy exerts profound effects on the proportions classified as parturient deaths or the SME complex. Perinatal survival is related to birth mass by an inverted U-shaped curve, with the highest survival of lambs between 3 and 5kg[85 93]. Birth mass is influenced by maternal prenatal nutrition, litter size, placental size and foetal genotype. The level of maternal nutrition during the third trimester affects birth mass, due to the accelerated foetal growth that occurs in that period. Excessive feeding, while substantially increasing birth mass of mainly single foetuses, predisposes them to dystocia[130].

0038-2809 Jl.S.Afr.vet.Ass. (1991) 62(2):78-91

Table 2: **Major pathological, and other relevant features characterising ante-parturient, parturient and post-parturient time-of-death classes**[58 84]

Criteria at autopsy	Time of death relative to birth: Before	During or within 3h	After
Ante-parturient death			
Generalised, subcutaneous oedema, autolysis, blood-stained serosal fluid, haemoglobin staining) mummification.	+	-	-
Parturient death			
Subcutaneous oedema of presenting portion of the foetus	-	70-80%*	-
Abdominal haemorrhage	-	10-30%	-
Meningeal haemorrhage	rare	80-100%	35-55%
Post-parturient death			
Thrombi, umbilical arteries	-	variable	+
Breathing	-	variable	+
Walking	-	rarely	usually
Feeding	-	rarely	sometimes
Colour of fat depots			
Perirenal, Pericardial, Epicardial	pinkish cream, often haemoglobin-stained	pinkish cream	red-brown to pinkish cream depending on level of thermogenic activity

+ : present; - : absent; * : prevalence.

Underfeeding restricts the growth of litters, reduces their lipid reserves and neonatal vigour, and impairs colostral production, so necessary for thermogenesis during the first few hours of adaptation to neonatal existence[90 91]. The latter studies indicated that the current feeding recommendations[1] underestimate the nutritive requirements of twin-bearing ewes in late pregnancy by 100%. These handicaps create the potential for high mortality among litters because of low lamb vigour and predisposition to hypothermia. Ongoing penalties also accrue from undernutrition earlier in pregnancy, notably the death of fertilised ova, embryonic death during implantation and reduced placental size.

The importance of placental size to foetal growth has been underestimated, since a recent study[21] incriminated placental insufficiency in 24% of deaths. Placental weight accounted for almost two-thirds of the variation in birth mass[88] in a wide range of nutritional treatments. Although most of the within-group variation remained unexplained, moderate underfeeding in the second trimester retarded placental growth. Underfeeding during late pregnancy appears to further retard placental growth of twin foetuses but not that of singles. To an extent, improved nutrition during late pregnancy compensated for small placental size.

Increased litter size reduces birth mass because litters must share both the discrete number of maternal caruncles available for implantation, and the substrates available for foetal growth. Although birth mass is reputed to have a low to medium heritability[105], some sires are prepotent at siring very large lambs[86], suggesting that heritability may have been underestimated. Large birth mass associated with a heritable prolongation of gestation has been recorded in Merinos (Haughey, unpublished data).

Pathophysiological criteria[21 89] implicated prenatal physiological handicaps imposed by placental insufficiency, acute intrapartum hypoxaemia and inadequate thermogenesis, in 71% of perinatal deaths from a highly fecund flock, compared to 26% by conventional clinicopathological methods. The latter apparently did not include adequate pathological assessment of birth stress. This result emphasises the importance of interactive maternal and foetal factors in perinatal mortality.

Efficient sheep farmers recognise the detrimental effects of prenatal undernutrition by raising the level of nutrition of breeding flocks in late pregnancy. Indiscriminate supplementary feeding of mixed flocks of non-pregnant, single- and twin-bearing ewes, is wasteful because of their differing nutritive requirements. While it may improve the survival of twins, there is a danger that increased

Table 3: **Occurrence of major causes of death relative to time-of-death classification**[34 58 67*]

	Time of death: Before	During	After
Prevalence, %	<2	74-24	24-74
Cause of death			
Congential malformations	+	+	+
Congenital mineral deficiencies	+	+	+
Congenital infections	+	+	+
Birth injury	-	+	+
Infections acquired after birth	-	-	+
Predation	-	-	+
Starvation-mismothering-exposure complex	-	-	+

* - virutally all perinatal deaths fall into the parturient or the post-parturient time-of-death classes, except when abortion substantially raises the proportion of ante-parturient deaths.

birth mass of singles, will predispose to dystocia.

The advent of real-time ultrasound pregnancy diagnosis[51] for the detection of non-pregnant, single- and twin-bearing ewes early in pregnancy, allows selective feeding of these groups. The detrimental effects on placental growth of underfeeding twin-bearing ewes in early and mid-pregnancy is a powerful incentive to feed them preferentially throughout most of pregnancy. Blood glucose measurement[97], using a portable diabetic glucometer, shows promise for determining the nutritional status of twin-bearing ewes at 90 to 100 d of pregnancy.

Birth stress

Birth stress results from the effects of asphyxia and/or trauma on the foetal CNS during vaginal birth[113]. At autopsy its manifestations vary in the 2 time-of-death classes to which it is virtually exclusive, namely carcases classified as parturient deaths and the SME complex[58]. Birth stress is considered to be the primary cause of death only in those carcases in which there is no other complicating pathology e.g., infections, lethal malformations, or primary predation. In parturient deaths, gross evidence of birth stress includes: injury to the foetal CNS characterised by a variety of cranial subdural, cranial subarachnoid, and extradural, subdural and subarachnoid haemorrhages in and around the spinal meninges and spinal nerve roots (referred to hereafter as birth injury); subcutaneous oedema of the presenting portion of the foetus; abdominal haemorrhage due to rupture of the liver or tearing of the liver capsule; subpleural, subepicardial, subendocardial and thymic petechiae or ecchymoses. Contusions of the right myocardium are frequent. The skin and birth coat are frequently meconium-stained.

During the author's investigations, birth injury was the most sensitive index of birth stress in the parturient time-of-death class, with flock frequencies ranging between 80 and 100%, compared to 70 to 80% for oedema of the presenting portion of the foetus, and 5 to 40% for abdominal haemorrhage. Not all manifestations are necessarily present in the same carcase. In the SME complex, birth injury is the main manifestation of birth stress, invariably accompanied by varying degrees of catabolism of brown fat. Observed frequencies of birth injury among the SME complex, ranged from 20 to 57%. The overall mean prevalences of birth injury in 2 studies were 71% and 61%[59 67], highlighting the dominant role of birth stress in the pathogenesis of perinatal lamb mortality. The main sites of cranial subdural haemorrhage at decreasing frequency are: caudal fossa; middle fossa; adjacent to the fourth choroid plexus; over the dorsal cerebral and cerebellar surfaces. Cranial subarachnoid haemorrhage occurs most frequently along the course of the middle cerebral vessels. Most spinal birth injuries occur in the cervical segment of the spinal canal and spinal cord with lower frequencies in the thoracic and lumbar-sacral segments[67]. Some investigators have erred by confining their examination to the cervical segment only, thereby detecting only about 60% of occurrences of spinal birth injury[67].

Increasing duration of parturition is associated with falling foetal PO_2, rising PCO_2 and acidaemia (foetal asphyxia), damage to vital centres in the CNS and trauma to the spinal cord and spinal nerve roots[113 135]. Parturitional asphyxia may compound a pre-existing chronic foetal anoxia due to placental insufficiency. The prevalence of birth injury, and its severity as measured by the number of sites involved, were significantly correlated with duration and vigour of birth[62] and birth mass[67]. Parturient deaths were assumed to result from the effects of acute asphyxia on the vital centres of the foetal CNS. Less severe damage caused impaired sucking and locomotory activity[63], and increased susceptibility to hypothermia[11], due to temporary impairment of thermoregulation[42] in neonatal lambs. Birth-injured lambs succumbed to high ambient temperatures because impaired sucking activity precluded the maintenance of adequate hydration[63 122]. Neonatal mortality to 7 d of age among lambs surviving artificially prolonged vaginal birth, was double that of caesarean-born lambs[64].

The role of perinatal asphyxia and birth injury to the foetal CNS has been underestimated in perinatal lamb mortality. Perinatal mortalities and morbidities have been associated with these entities in human infants[113], primates[132], calves[61], foals[71], piglets[107] and guinea pigs[20]. Perinatal asphyxia is the main cause of depressed sucking activity in neonatal infants[38 133]. Foetal blood pH was lower and neonatal mortality higher among late birth order compared to early birth order piglets[107].

Foeto-pelvic disproportion, malpresentation of single lambs and less frequently of litters, and uterine load of polytocous ewes are the main causes of birth stress. Foeto-pelvic disproportion due to foetal oversize[67], small maternal pelvic size[49 68 87 103], or both, predispose to prolonged parturition. Although there is little published evidence to support the contention of an increased uterine load in polytocous ewes, the greater total foetal mass of litters compared to single births, must theoretically impose greater uterine work load during parturition, thereby increasing its duration, and the risk of pathological asphyxia and trauma to the CNS of the foetus during its extrusion through the birth canal. Support for this contention is set out in Table 4 which compares the mean birth masses, durations of Stage 2 labour[62], and maternal pelvic dimensions of uncomplicated single and twin births among fourth and fifth parity Merino ewes (Haughey, unpublished data).

The mean duration of Stage 2 labour for twin births was 2,01 times that for single births to deliver a 1,49 times greater foetal mass. Time to deliver the first twin was 1,75 times that for single births, despite the latter being 1,34 times heavier, with a mean interval of 19,3 min between first and second twin. Foeto-pelvic disproportion was an unlikely determinant of the duration of parturition of twins as their birth masses were significantly lower than those of single births and there was no difference between the pelvic dimensions of single and twin mothers.

Little information exists on the relationship between exercise during pregnancy and ease of birth. Penned, fat, pregnant Dorset Horn ewes, exercised on a tread-mill for 20 min daily for 3 weeks prior to parturition, had less dystocia than the unexercised controls (17% v. 50%; $p<0,05$) [J George 1983 CSIRO Pastoral Research Laboratory, Armidale, personal communication]. Lack of exercise and fat condition may contribute to perinatal mortality, particularly in winter rainfall environments where autumn-lambed flocks are often fed totally on supplements.

Starvation-mismothering-exposure (SME) complex

Post-parturient deaths are classified as the SME complex when there is evidence of hypothermia, manifested by varying degrees of brown fat catabolism, subcutaneous ("peripheral") oedema of the extremities, and changes in the adrenal cortex[60], accompanied by absence of, or inadequate amounts of milk ingesta in the alimentary tract. Carcases showing pathological features other than birth injury are excluded from the classification.

Foetal fat reserves in newborn lambs are composed of mitochondria-rich brown adipose tissue[7]. Below thermoneutral temperature, brown fat depots are important sites of non-shivering thermogenesis. Catabolism of brown fat occurs during cold exposure and independently of starvation[60]. At birth and at ambient tempera-

Table 4: **Mean (±SD), and range of birth masses, durations of Stage 2 of labour, maternal pelvic conjugate and transverse diameters of Merino single and twin births (Haughey, unpublished data)**

Parameter		Birth type				Significance of difference		
		Single	1st Twin	2nd Twin	Both Twins	t	df	P
Number		92	55	55	55			
Birth mass (kg)								
Single	vs 1st twin	7,4 ± 0,07 (1,9-6,6)	3,5 ± 0,07 (1,8-4,6)			11,07	145	0,001
	vs 2nd twin			3,5 ± 0,08 (1,9-4,7)		10,60	145	0,001
	vs both twins				7,0 ± 0,13 (3,7-9,2)	16,14	145	0,001
1st	vs 2nd twin		3,5 ± 0,07 (1,8-4,6)	3,5 ± 0,08 (1,9-4,7)		0,00	108	n.s.
Duration of Stage 2 labour (min)								
Single	vs 1st twin	73,9 ± 7,09 (5-400)	129,2 ± 13,29 (7-371)			2,70	145	0,01
	vs both twins				148,8 ± 14,33. (7-392)	4,02	145	0,001
Interval between 1st and 2nd twin					19,3 ± 2,31 (1-80)			
Maternal pelvic diameters (cm)								
Conjugate		11,6 ± 0,08 (10,3-14,4)			11,8 ± 0,12 (9,8-14,2)	1,45	145	n.s.
Transverse		8,2 ± 0,05 (7,0-9,3)			8,3 ± 0,05 (7,4-8,9)	0,20	145	n.s.

tures above thermoneutrality, the perirenal, pericardial and epicardial sites are cream-pink in colour. As the fat is depleted by thermogenesis, the fat depots change to a red-brown colour (hence the term "brown fat"), the extent and duration of the rise in metabolism modifying the degree of colour change. When the depots are red-brown, the lamb has exhausted its reserves of brown fat. Thus the colour of the perirenal, pericardial and epicardial fat depots is a sensitive qualitative measure of cold exposure and the level of energy reserves.

The degrees of fat catabolism are scored arbitrarily as: a) Nil (stable or uncatabolised fat) - the texture of the fat is firm, and the colour at all sites is cream-white or slightly pink; b)Slight - the prescapular, perirenal, and pericardial fat depots are distinctly pink, and the epicardial site (along the coronary grooves) is cream-white or slightly pink; c) Moderate - the prescapular, perirenal and pericardial fat depots are distinctly red-brown, similar in appearance to liver tissue. Epicardial fat is cream-white or slightly pink. Texture is less firm than the previous 2 classes; d) Marked - fat at all sites is gelatinous in texture and red-brown in colour.

Varying degrees of yellowish, subcutaneous oedema (up to 5mm thick) often occur in the distal limbs, and less frequently at the base of the tail, face, muzzle and ears ("peripheral" oedema). Duration and severity of exposure to cold weather affect the prevalence and degree of oedema. Under controlled conditions, oedema was first detected in newborn lambs with damp birth coats 4,5 h after exposure at 1°C in "still" air. Oedema extended from the coronets to above the carpus or tarsus after exposure for 8 to 10 h. After mild exposure (19°C), it was confined to the plantar and volar aspects of the pasterns. Its detection requires adequate reflection of the skin from the medial or lateral aspects of the distal limbs, tail, face and muzzle.

Changes in the adrenal cortex include cortical hypertrophy and focal petechiation, which are typical manifestations of severe systemic stress[116].

Thermo-neutral temperature in "still-air" for the newborn lamb is about 28°C. Below 28°C, thermoregulatory mechanisms are invoked to maintain homeothermy by shivering, catabolism of brown fat, and peripheral vasoconstriction to reduce heat loss[3 4 5 6 41]. The average lamb has sufficient energy reserves, mainly brown fat, to sustain maximum metabolic rate ("summit metabolism")[5 41] for about 20 min. When heat loss exceeds heat production, body temperature falls below that required for

normal metabolism and function, energy reserves are exhausted unless replenished from colostrum, and death results from primary or secondary hypothermia[65]. The extent and time of onset of thermogenesis is modified by ambient temperature[3], wind velocity[4], the thickness and wetness of the birth coat, skin thickness and birth mass[5 41]. Small lambs are vulnerable to hypothermia because of a wider surface area to mass ratio and lower energy reserves compared to those of large lambs[5 41].

During sporadic outbreaks of severe, cold, wet, windy weather (<5°C), catastrophic mortality may result from primary hypothermia. During blizzards the rate of body cooling may be so rapid in small lambs that death intervenes before brown fat can be catabolised, or before food can be digested even if the lamb has fed. In addition, severe weather depresses sucking ability[15 120]. "Sheep weather alerts", derived from physiological data[3 4 5], forecast the meteorological conditions conducive to primary hypothermia[94]. Fortunately in South Africa the severe weather conducive to primary hypothermia in newborn lambs, rarely occurs more than 2-3 times per 6-week-lambing period. Most deaths classified as the SME complex, result from secondary hypothermia. Secondary hypothermia is the result of exhaustion of substrates necessary for thermogenesis because of starvation during ambient temperatures higher than 5°C. Common causes of failure to feed, include birth injury to the foetal CNS[63], aberrant maternal[13], or neonatal behaviour or misadventure[123], udder or teat abnormalities, agalactia[77], and management-induced mismothering[102]. Invariably fat depots are moderately or markedly depleted and "peripheral" oedema is prevalent[60]. The mean percentages of the SME category, failing or ceasing to feed, approximate 50 ± 10%[67]. Pathological evidence in birth-injured lambs, suggests that cessation of feeding may be due to traumatic injury to the spinal cord and nerve roots resulting in loss of mobility and sucking dexterity, whereas failure to feed may be due to asphyxic damage to the feeding centres of the brain, resulting in suppression of sucking drive[67].

The initiation of a successful partnership between mother and offspring, involves exclusive bonding within a few hours of birth[8 10]. The visual, auditory and olfactory cues learned during this period, allow mutual recognition and acceptance so that close or proximate contact is maintained to allow frequent suckling. Maternal behavioural traits that should facilitate strong bonding, include[10]: the seeking of isolation for birth; the selection of a safe, sheltered birth site; birth of short or average duration; absence of interference with, or by, other parturient ewes; intense persistent grooming of all members of a litter; absence of aggression towards her own progeny; co-operation with the lamb's first attempts to suck; remaining on the birth site for at least 5 h; agitation at the absence of a lamb; the ability to keep the litter together after leaving the birth site; active defence of the lamb in the presence of a predator or a dog. Desirable behavioural traits of newborn lambs include[10]: standing soon after birth; sucking soon after standing; a well-defined "prone" response to handling; ability to follow the mother closely, and re-unite with her when separated; absence of separation from the mother. Breed comparisons indicate genetic diversity in some of these traits, including ease of birth[52 53], time spent on the birth site and ability to care for twins[13].

Many intrinsic and extrinsic factors adversely affect parent-offspring bonding. Maternal factors include: genotype[13]; maternal inexperience[12]; dystocia[2]; the birth of multiples[8 67]. Lamb factors include: genotype[120 123]; multiple birth type[8]; asphyxic or traumatic birth injury to the foetal CNS[63]; hypothermia[15 120]. Husbandry factors include: high stocking density of lambing ewes[14], including that induced by oestrus synchrony[78]; disturbance of lambing and recently-lambed ewes by human interference and supplementary feeding[102]; the prevalence of damaged teats and udders[77]. Nutritional factors include: prenatal undernutrition[101], low pasture availability near the birth site[14] and a poor milk supply[90]. Severe prenatal undernourishment of ewes, for example, during drought, may prevent or delay the onset of lactation. Affected ewes show poorly developed udders containing scanty, viscous, honey-coloured secretion in addition to severely-depressed maternal behaviour.

Genetic aids

The main thrust for more effective control of perinatal mortality must come from addressing the causes of birth stress and the SME complex as these entities are the largest components of mortality. These entities are currently minimised by prenatal nutrition, supervision and obstetrical assistance at lambing, and the provision of shelter, warmth and food to affected neonates. Despite their widespread and even intensive application, perinatal mortality has not been reduced below a seemingly intractable level of about 15% of lambs born[9], suggesting the involvement of unrecognised aetiological factors. For example, the mean perinatal mortality in 15 intensively-managed, including pen-lambed, South African flocks was 15,1%, ranging from 8,9% to 41,0%[67]. Intrinsic defects of the ewe-lamb partnership, and the compounding effects of some husbandry practices are now recognised as major causes of birth stress and the SME complex. Some of these defects can be manipulated genetically.

A. Selection for maternal rearing ability, including rearing of twins

The heritability of lifetime maternal rearing ability has been estimated variously between 0,1 and 0,2[32 99]. The reliability of these heritability estimates has been challenged because of possible pedigree errors[14]. More than 60% of rearing failures among ewes lambing on 4 occasions, occurred in slightly more than 25% of the flock[29 68]. In one study[29], ewes which always reared a lamb, or failed only once, weaned lambs on a mean of 90% of occasions, whereas ewes failing to rear on 2, 3 or all occasions, weaned a lamb on a mean of 59% of occasions, illustrating the wide variation in rearing ability within flocks. Ewes which reared a lamb at maiden lambing, weaned on average 8% more lambs over the next 3 years compared to ewes which lost their lamb at maiden lambing[68], suggesting that performance at maiden lambing is a useful indicator of subsequent rearing performance. The mean survival to weaning of line-bred single and twin descendants of ewes with high lifetime rearing ability, was 13% and 15% higher respectively than those descended from maternal ancestors of low rearing ability[66]. Selection for ability to rear at least one lamb also selects indirectly for fertility and fecundity. Twin-bearing ewes have a greater probability of rearing at least one lamb compared to single-bearing ewes and therefore escape culling for rearing failure.

The recommendation to select for twinning without reference to the ewe's ability to rear the additional progeny, is to be deprecated because it results in a cosmetic improvement in weaning percentage at the expense of a higher mortality among twins, compared to single lambs. Selection for ability to rear twins, improved both weaning percentage and lamb survival[18]. The reasons for the improvement were not specified, but it clearly reflects increased fitness of the ewe-lamb partnership.

Selection for rearing ability has been used by co-operative breeding schemes, commercial sheep farmers and a few progressive studs for the last 20 years[104].

A typical selection programme for improving lamb survival is summarised below:

1. Identify and eliminate obvious causes of perinatal mortality e.g. prenatal undernutrition, disease.
2. Identify and cull ewes which lose all lambs born, require obstetrical assistance, or any other intervention to

ensure the survival of their progeny. Identify the surviving progeny of these ewes at the time of the intervention and cull them at weaning. Identification of "lambed-and-lost" and "not-lambed" ewes is most accurately carried out at lamb-marking by the "wet-dry" technique. Selection for ability to rear a lamb to lamb-marking, is probably as effective as selecting for ability to wean a lamb as most lamb deaths between marking and weaning are due to extrinsic causes.

3. Cull barren ewes, after eliminating other causes of infertility, e.g. ram infertility, anoestrus. Ability to conceive and maintain pregnancy has a low heritability.
4. If possible, select for ability to rear twins. Selecting for ability to rear one lamb indirectly selects for twinning.
5. Avoid lambing husbandry practices which disrupt ewe-lamb bonding, e.g. high stocking densities, supplementary feeding during daytime, disturbance of recently-lambed or about-to-lamb ewes. Lambing flocks should be conditioned to the presence of trained shepherds moving quietly among them.
6. Ensure good nutrition of lambs, ewe hoggets, and maiden replacements to 2,5 years of age, when the pelvic centres of ossification fuse, to maximise pelvic size.
7. For selection to be successful, the efficient ewes must be joined to rams born of ewes with high rearing ability, otherwise the strategy is futile. An adequate supply of suitable rams demands that co-operative breeding schemes and studs include rearing ability in their selection programmes.
8. The programme must be backed by good nutrition, disease control and judicious husbandry including the provision of shelter and shade.

Culled ewes and their salvaged progeny should be sold preferably for slaughter. Alternatively they may be run as dry sheep for wool production or joined to black-faced mutton breed rams for easy identification of progeny. Unlike Australia and New Zealand where cull mutton is practically worthless, selection for rearing ability can be implemented in South Africa at minimal cost because of the high price of mutton. The programme may also be phased in over 4 to 5 years by implementing it in successive intakes of maiden replacements so that at the end of that period all lambed ewes, except maiden replacements, have always reared a lamb. Provided we are brave enough to implement the ruthless culling required, substantial improvement in lamb survival seems certain in the medium term. The programme is doomed unless rams selected for rearing ability are available. The stud industry, as custodians of the heritable production characteristics of the national flock, has a responsibility to include selection for rearing ability in their breeding programmes. Given their conservative attitudes, they are unlikely to do so without pressure from commercial breeders. Failure of the New Zealand studs to take up the challenge 20 years ago, resulted in the newly-established group-breeding schemes capturing 10% of the ram market in recent years - a result which has now forced the New Zealand stud industry to adopt similar selection procedures (B J McGuirk 1985 CSIRO Division of Animal Production, Prospect, personal communication). Reported results include: 10 years aggressive selection in a Romney co-operative ram breeding flock, halved lamb mortality to weaning (7% of lambs born) in a flock dropping over 80% of twins compared to the district mortality average (15%) in flocks with twinning rates up to 35%[104]; 95% survival to weaning after 15 years selection in an "easy-care" Romney flock, due in part to superior mothering[79], compared to 86% survival in a control flock with a comparable twinning rate[75]; an average of 95% survival to weaning in the Marshall Romney after 7 years natural selection on harsh hill country[17]; a 7% improvement in the survival of both singles and twin Merino lambs after 9 years selection compared to a control flock[18]; a 9% improvement in Merino lamb survival over controls in 6 years[37]. The relative contributions of culling in current generations and true genetic improvement to these results was not specified. All results were obtained in wholly pasture-fed flocks managed with minimum labour - so-called "easy-care" sheep.

B. Selection for specific components of lamb survival

Because the heritability of some specific components of lamb survival is higher than that for rearing ability, it has been suggested that selection for these traits would improve lamb survival more rapidly[32].

1. The size of the maternal pelvic conjugate diameter

At least 60% of perinatal mortality appears to be associated with birth stress[59 67]. Both elements of birth stress due to foeto-pelvic disproportion, namely birth mass and pelvic size, have a genetic basis. Effective selection for optimal birth mass would be difficult because of the wide variation in prenatal nutrition, the occurrence of twins, and the disadvantage and practical difficulties of measuring birth mass at lambing. The dimensions of the mature maternal pelvic conjugate diameter were highly correlated with lifetime rearing ability (lambs weaned/lambs born)[68], and the mean conjugate diameter of ewes with high rearing ability was larger than that of ewes with low rearing ability[29]. The genetic correlation between the size of the conjugate diameter of Merino ewes and lamb survival was 0,73, with the heritability of the dimension estimated at 0,30[19]. Thus, direct selection for the size of the mature maternal conjugate diameter, using radiography[69], appears to offer considerable scope for reducing not only parturient deaths, but also the birth-injured component of the SME complex. Little data are available on the pelvic dimensions of rams. The technique is expensive and there is a need for a cheaper technology. Attempts to exploit the high correlation between some external anatomical measurements and pelvic dimensions[49 103] and the development of a pelvimeter (Haughey, unpublished data) have been unsuccessful.

2. Selection for cold resistance in newborn

Neonatal resistance to hypothermia has a useful heritability, estimated between 0,27 and 0,44[119 121 134], with a value of 0,76 being reported in Australian Merinos (J Slee 1989 CSIRO Division of Animal Production, Prospect, New South Wales, personal communication). Although heritabilities of this magnitude offer considerable scope for improving lamb survival, the selection technique is onerous, involving measurement of the physiological response of individual lambs to cold in a progressively-cooled water-bath[124 134]. The use of rams born during, and surviving severe weather conditions may also be an option. As birth stress has a powerful depressant effect on neonatal thermogenesis[42], it is not yet clear whether the trait may be partly a reflection of ease of birth.

3. Selection for parent-offspring behaviour

This technique requires labour-intensive observations during lambing[14]. The rate of genetic progress cannot be predicted yet, as the heritability has not been estimated, but this may soon be known (G Alexander 1989 CSIRO Division of Animal Production, Prospect, New South Wales, personal communication).

Direct selection for cold resistance and parent-offspring behaviour are unlikely to find widespread application in the industry because of the costly labour-intensive selection techniques. Although it offers considerable scope for rapid genetic gain, selection for pelvic size is probably disqualified in most circumstances by its high cost. The availability of rams selected for these traits presents additional difficulties. Selection for rearing ability is the most practical technique for wide-

spread use for the reasons outlined earlier.

Nutritional aids

Provided it is cost-effective, competent ultrasonic pregnancy diagnosis of litter size facilitates more effective and less wasteful prenatal feeding of single and twin pregnancies. The risk of dystocia and birth stress is minimised in single pregnancies if the ewes are fed separately. Low-level prenatal protein supplementation of pasture-fed ewes, according to litter size, increased the birth mass and survival of single, twin and triplet lambs[82]. Lambing ewes are more likely to remain longer on the birth site in the presence of plentiful pasture. The high effective stocking densities and the inevitable stampede which accompany daytime supplementary feeding of lambing ewes increase mismothering. Lambing when plentiful pasture is available, is preferable. Where supplementary feeding cannot be avoided, Australian experience suggests that the associated problems can be minimised by feeding out at night. In the southern Cape, substantial cost-benefits resulted from winter or early spring lambing on plentiful pasture compared to autumn-lambed, supplementary-fed flocks due to increased conception, twinning, survival and growth rate of lambs, and lower feed costs[72] (I A Herbst 1989 Veterinarian, Caledon, personal communication).

Husbandry aids

Twin lambs, particularly, are prone to mismothering due to the difficulty of ewes keeping the sets of multiples together, maternal desertion, or lamb-stealing by ewes on the point of lambing. Perinatal mortality was correlated significantly with fecundity and stocking density at lambing[67], and mismothering increased disproportionately at stocking densities exceeding 18 lambing ewes/ha[14]. The prevalence of lamb-stealing was related to the number of ewes lambing at any one time[78]. Twin-bearing ewes, particularly, require low stocking densities at lambing (not more than 15 ewes/ha) to prevent mismothering. Nutritional management and husbandry which disrupt parent-offspring bonding are to be avoided. Conditioning lambing flocks to the presence of shepherds is accomplished conveniently during routine prenatal husbandry or feeding. If this is impossible, the lambing flock should experience minimal disturbance. "Drifting" unlambed ewes off the lambing camp, is successful provided the lambing and lambed ewes are not unduly disturbed. The choice of sheltered lambing camps reduces evaporative and convective heat loss from newborn lambs and therefore minimises losses from exposure. There is a lack of researched designs of lambing camps, including their orientation to adverse weather, the type and positioning of shelter, and the siting of fence lines and watering points to prevent frustration of parent-offspring behaviour. Despite the practical problems, shearing ewes within 3 to 4 weeks of lambing, improved twin survival, because shorn ewes sought the shelter provided[11]. Ewes with teat and udder abnormalities, easily identified at "wet-drying", should be culled. Obstetrical assistance, and the treatment of mismothered and hypothermic lambs with warmth, stomach-tubing, intraperitoneal dextrose and foster-mothering are traditional methods of improving lamb survival. Given a genetic basis for the major causes of perinatal lamb mortality, namely birth stress, aberrant parent-offspring behaviour and neonatal cold resistance, the retention of the affected ewes and their surviving progeny in the breeding flock is contraindicated. That practice can only ensure the continued accumulation of genetically-determined defects, leading inevitably to decreasing fitness of ewe-lamb partnerships in the evolutionary sense. Indeed, it is probable that the present unsatisfactory state of lamb survival has been compounded in part by centuries of lambing husbandry.

MINOR CAUSES OF PERINATAL MORTALITY

In general, less than 20% of perinatal mortality is due to lethal congenital malformations, infections (both congenital and acquired after birth), mineral deficiencies, predation and unknown causes[34 59 67]. Individual entities may cause sporadic heavy mortality in some flocks, seasons and districts. Because of their relative unimportance, a comprehensive review of specific entities will not be undertaken.

Lethal congenital malformations

Lethal congenital malformations occur usually at low prevalence in the ante-parturient, parturient and post-parturient time-of-death classes. They affect all body systems[36], with the highest frequency in the CNS. Multiple malformations are common. Hyperkeratinised plaques on the hooves, accessory digits and the horn buds are frequent. Pathogenesis is due mainly to environmental factors[36], including foetal viral infections[22 95 98], maternal ingestion of phytoteratogens[24], maternal hyperthermia during organogenesis[57], and less frequently, chromosomal anomalies[27]. The sporadic nature of outbreaks, low level of loss, and ignorance of causes often preclude the adoption of control measures. Avoiding vaccination for bluetongue, Rift Valley fever and Wesselsbron disease, and the grazing of teratogenic plants during pregnancy, will prevent losses due to those causes.

Congenital infections

Generally, infections occur widely at low prevalence. Nationally, they probably form a small component of total perinatal mortality[67]. Infections may be congenital or acquired after birth.

A variety of bacterial and viral agents cause ante-parturient, parturient or post-parturient death (Table 5). They are endemic to many flocks[70], but with the exception of sometimes spectacular abortion "storms", they rarely cause serious economic loss. Vertical transmission from ewe to foetus occurs during pregnancy, resulting in foetal death, abortion, or foetal growth retardation, because of placentitis, the direct effects on foetal well-being, or both. Pregnant ewes infected with *Coxiella burnetii,* the cause of the zoonosis, Q fever, pose a threat to the health of farm, laboratory and abattoir staff[80]. The ovine condition is usually inapparent and self-limiting, with localisation of the organisms mainly in the placenta and birth fluids[80]. Less frequently *Coxiella* congenital infection is characterised by placentitis, abortion and the birth of weak lambs[26 96 106]. Septicaemic infections of pregnant ewes, e.g. *Salmonella* spp, may cause secondary abortion showing non-specific foetal pathology.

Many congenital infections result in characteristic gross lesions of the placenta and/or foetus. Table 5 summarises the gross placenta and/or foetal pathology associated with specific infections. Submission of the correct specimens, as well as autopsy, are essential to efficient diagnosis. Diagnostic laboratories should be consulted as to the appropriate diagnostic material. The following are usually appropriate:

1. Placenta, including cotyledons - fresh and fixed in formo-saline.
2. Fresh foetuses, parturient time-of-death class, delivered rapidly to the laboratory in chilled insulated containers - otherwise
   (a) foetal lung and liver - fresh and fixed
   (b) foetal abomasum and contents -fresh
   (c) foetal heart blood, CSF, or effusions from serous cavities
   (d) foetal brain - fixed
   (e) serums from affected ewes

When levels of loss are low or sporadic abortion "storms" occur, no recommendations can be made, apart from observing routine hygiene. Abortion in sheep

seems to have a low repeatability, presumably because an effective immunity is acquired. Aborted ewes can be retained in the flock with impunity, comforting advice to an unfortunate sheep breeder (Haughey, unpublished data). Persistent economic loss due to infections with *Campylobacter fetus, Chlamydia* spp (Enzootic abortion), *Brucella ovis,* bluetongue, Wesselsbron disease and Rift Valley fever, can be controlled by vaccination. While the vaccination of ewes against *Coxiella burnetii* prevented placentitis and the birth of weak lambs, it did not prevent the shedding of organisms[26]. A vaccine against Akabane infection is being tested in Australia.

Infections acquired after birth

A wide variety of bacterial infections have been incriminated. Prevalence rises with intensive management systems e.g. penned lambing. Most are acquired at, or soon after birth although their pathological manifestations may extend beyond the perinatal period[67].

Common pathogens include[34 67 76]:

(a) *Clostridium septicum, Clostridium chauvoei* and *Clostridium novyi,* cause gangrene around the umbilicus and localised or generalised sero-fibrinous peritonitis.
(b) *Pasteurella haemolytica* and *Pasteurella multocida* cause pneumonia and localised or generalised sero-fibrinous peritonitis.
(c) Infection by *Staphylococcus aureus, Streptococcus* spp, *Corynebacterium* spp, *Fusobacterium necrophorum,* and other bacteria cause pyaemia with multiple purulent foci in the liver, kidneys, heart, muscles and joints.
(d) *Eschericia coli* causes syndromes characterised by enteritis, septicaemia or leptomeningitis.
(e) *Erysipelothrix insidiosa* and *Chlamydia* spp cause polysynovitis.

Diagnosis is confirmed by microbiological and histopathological examination of appropriate specimens. Vaccination of ewes in late pregnancy provides effective colostral immunity against infections with *Clostridial* spp. The similar use of *Pasturella haemolytica* vaccine is of equivocal efficacy[54]. Often losses do not warrant the cost of vaccination programmes, but they may be mandatory when penlambing is practised. Routine hygiene and the changing of bedding daily may help to minimise losses in the latter system.

Deficiencies of trace elements

Congenital swayback, congenital goitre and congenital white muscle disease associated with deficiency of copper, iodine and selenium, respectively, are usually endemic to certain soil types with sporadic outbreaks of heavy mortality[23 55 92]. Modern pasture production techniques have led to the emergence of trace element deficiencies in districts where they have not been recorded previously. As deficient and normal tissue levels of the various trace elements and the amount of supplement required for prevention of the syndromes vary between districts and countries, advice should be sought from the local authorities when deficiencies are newly-diagnosed.

Copper deficiency[48 55 125], either primary, or secondary to excess molybdenum and/or sulphate in the diet, is characterised by paralysis and other nervous signs, bone fragility, progressive emaciation any time from birth to 4 months of age and "steely wool" in adult sheep. The congenital form is associated with acute deficiency and affected lambs show nervous signs, including chorea, inco-ordination or paralysis due to extensive demyelination leading to cavitation of cerebal white matter. Histopathological examination of brain and spinal cord, and liver copper concentrations of $< 10 mg\ kg^{-1}$ dry matter, confirm the diagnosis. Levels between 10 to 90 mg kg^{-1} are definitely on the low side of normal (150-700 mg kg^{-1}). With acute deficiency, other syndromes of copper deficiency are likely to occur in all sheep and cattle grazing the same pastures. Sheep breeds vary in their susceptibility to copper deficiency[125].

Supplementation of the diet[55 125] with copper by pasture top-dressing, oral or parenteral administration, prevents copper deficiency. As the pathogenesis of copper deficiency is incompletely understood, the recommendations are guides only:

(1) Pasture topdressing, often in the form of copperised superphosphate, applied at recommended levels. Extravagant use may lead to copper toxicity.
(2) Oral administration in the form of slow-release proprietary preparations e.g. copper "needles", glass boluses, once annually to ewes at joining or early pregnancy.
(3) Parenteral administration of pro-

Table 5: **Congenital infections associated with perinatal mortality and abortion**

Agent	Gross Lesions
Bacteria	
Listeria monocytogenes[35]	placentitis, multiple focal abscesses, 1-2 mm, in
Yersinia pseudotuberculosis[70]	liver, occasionally in lungs and kidneys
Histophilus ovis[131]	placentitis
Brucella ovis[115]	placentitis, hyperkeratinised placques on horny hooves
Chlamydia spp[16]	placentitis
Campylobacter fetus var. fetus[114]	occasionally oedematous chorio-alantois, hepatomegaly, large circumscribed yellowish, necrotic liver lesions, 10-30mm, in 30 to 40% of cases
Coxiella burnetii[80 96]	usually no visible lesions, placentitis less frequently
Protozoa	
Toxoplasma gondii[39]	Yellow-white flecks, 1-2mm, in foetal cotyledons, leucoencephalomalacia
Viruses	
Akabane[98] Bluetongue, including vaccinal virus[95]	hydranencephaly, hydrocephalus, micrencephaly, scoliosis, kyphosis, arthrogryposis
Border disease[22] (Hairy Shaker disease)	hydranencephaly, hairy birth coats, some affected lambs are choreic
Rift Valley fever[30]	enlarged, yellow-brown to dark red, friable liver, with grey-white necrotic foci, 1-2mm, icterus occassionally
Wesselsbron disease[31]	infection during organogenesis - similar lesions to Akabane. Infection later in pregnancy - icterus, enlarged yellow to orange-brown liver

prietary preparations of copper, including copper edetate or glycinate, once annually at recommended dose rates. Copper glycinate sometimes causes severe local reactions (Haughey, unpublished data).

Congenital goitre[23 126] is characterised by grossly enlarged thyroids (>2g) in lambs[84]. The entity is usally endemic to soils which are variably deficient in iodine. Other factors must also be involved in the pathogenesis to explain the variation in severity and frequency of outbreaks. Feeding *Brassica* spp, heavily fertilised star grass and clovers containing goitrogens during late pregnancy, and the reduced amount of soil ingested in good seasons during pregnancy have so far been incriminated. A biochemical defect, inherited as a simple recessive, preventing the biosynthesis of thyroid hormone has been identified in Merinos[46 47]. Thyroid enlargement may be so great as to cause dystocia (>200g)[118].

Goitre due to simple iodine deficiency or the ingestion of goitrogens can be prevented by[23 44]:

(1) drenching ewes at monthly intervals during the third and fourth months of pregnancy with a solution containing 280 mg potassium iodide per dose.
(2) Providing salt licks containing 120 g potassium iodate per tonne throughout pregnancy.
(3) Oral administration to maiden ewes of a proprietary intra-ruminal device containing slow-release iodine with a claimed 3-year effective life[45].
(4) Intramuscular injection of preparations containing iodine in poppy seed oil, 2 months before lambing.

The extremely sporadic, even rare, occurrence of goitre often does not warrant the cost of preventive measures. The latter can be justified only in flocks experiencing regular outbreaks.

Congenital white muscle disease (WMD)[28 92 117] is manifested by subendocardial circumscribed, dirty-white plaques in the ventricles due to necrosis and calcification of the myocardium. Its detection requires routine opening of the ventricles during autopsy. Lambs die suddenly during or shortly after birth. The selenium status of flocks may also be ascertained by determining blood glutathione peroxidase levels in 15 to 20 young sheep. Other manifestations of selenium deficiency, including ewe infertility, delayed WMD affecting voluntary muscle, and unthriftiness of young sheep, often occur in affected flocks. The prevention of congenital WMD[92] usually has to be integrated with control of other selenium-responsive syndromes occurring in affected flocks. Selenium compounds have a relatively low therapeutic index, necessitating their prudent use. Congenital WMD may be prevented by: oral administration of 5 mg of Se as a solution of sodium selenite or sodium selenate to ewes one month before due date of lambing; oral administration of selenium "bullets" during pregnancy; parenteral administration of selenium. In some countries selenium salts have been incorporated in vaccines.

Experimentally, the application of selenium-fortified superphosphate or selenium prills to pasture has proved a safer but more expensive method of controlling selenium deficiency than sodium selenate or selenite administered orally[92].

Predation

The role of predation in perinatal mortality by carnivorous, omnivorous, and occasionally, avian species, is often overestimated because investigators fail to distinghuish between primary predation (the killing of an otherwise viable lamb), secondary predation (the killing of a lamb of low viability), and scavenging[83]. Australian and South African studies using these classifications, showed that primary predation caused low losses despite popular opinion[67 83 108 109], although sporadic catastrophes occurred in some seasons, districts, and even camps. Difficulties arise when prey are wholly consumed on site or removed from camps, as occurs with large predators, necessitating the use of indirect methods to estimate losses[100]. A substantial portion of the carcase should be skinned, as not only are external appearances misleading regarding the degree of multilation, but the site and nature of wounds may also indicate the species or genus of the predator or scavenger involved. The killing and feeding methods, and the inter-canine tooth-skin puncture distances of some Australian, South African and American sheep predators have been characterised[56 108 110 112 129]. The distinguishing pathological features of predation and scavenging are[83]:

Primary predation - carcases are characterised by lethal ante-mortem wounds showing severe haemorrhage and contusions, with no evidence of other pathological processes. When the prey has fed, there is evidence of active absorption of digested milk in mesenteric lacteals. Body fat is not catabolised.

Secondary predation - is characterised by severe ante-mortem mutilation in a carcase in which other pathological processes are evident e.g. marked fat catabolism, lethal malformation or infection.

Scavenging - is characterised by varying degrees of multilation and consumption of a carcase carried out after death, indicated by the absence of contusions and haemorrhage around the site of mutilation, and evidence of other pathological processes indicative of the cause of death.

PROSPECTS FOR IMPROVING LAMB SURVIVAL

The identification of heritable components conducive to improving fitness of the ewe-lamb partnership for survival, a more precise understanding of the prenatal handicaps imposed by undernutrition of twin-bearing ewes, and the ability to diagnose litter size early in pregnancy thereby allowing refinement of parental nutritional management, offer excellent prospects for improving lamb survival. Those prospects can be approached confidently as ruthless selection for rearing ability, along with selection for other production traits including improved fecundity, have achieved survival to weaning of 95% of lambs born in pasture-fed "easy-care" flocks in New Zealand. The conservatism of studs, in not adopting similar selection procedures to increase the availability of suitable sires, is likely to blight progress. The market forces imposed by the establishment of group-breeding schemes, eventually overcame that difficulty in New Zealand - albeit over a period of 20 years. The hard fact is, that we will not improve lamb survival beyond its present unsatisfactory level until we implement programmes aimed at minimising all the constraints imposed by genetics, nutrition, husbandry, disease and the weather.

REFERENCES

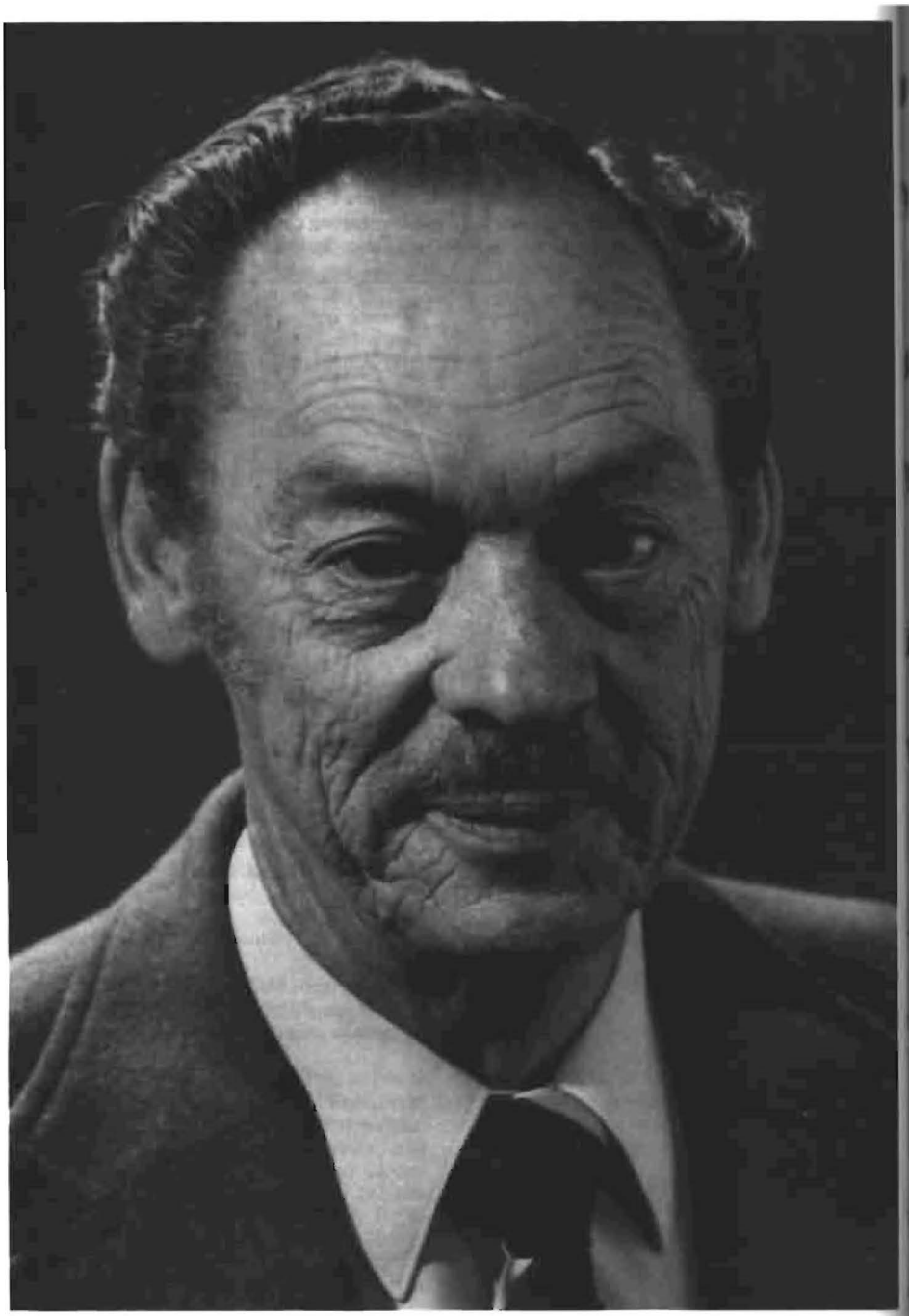
1. Agricultural Research Council 1980 The nutrient requirements of ruminant livestock. Commonweatlh Agricultural Bureau, Slough: 115-117
2. Alexander G 1960 Maternal behaviour in the Merino ewe. Proceedings of the Australian Society of Animal Production 3: 105-114
3. Alexander G 1961 Temperature regulation in the new-born lamb. III. Effect of environmental temperature on metabolic rate, body temperature and respiratory quotient. Australian Journal of Agricultural Research 12: 1152-1174
4. Alexander G 1962 Temperature regulation in the new-born lamb. IV. The effect of wind and evaporation of water from the coat on metabolic rate and body temperature. Australian Journal of Agricultural Research 13: 82-89
5. Alexander G 1962 Temperature regulation in the new-born lamb. V. Summit metabolism. Australian Journal of Agricultural Research 13: 100-121
6. Alexander G 1962 Energy metabolism in the new-born lamb. Australian Journal of Agricultural Research 13: 144-164
7. Alexander G 1979 Cold thermogenesis. In: Robertson D (ed.) Environmental Physiology 3rd International Review of Science 20: 43-156
8. Alexander G 1980 Husbandry practices in

relation to maternal-off-spring behaviour. In: Wodzicka-Tomasczewska M, Edey T N, Lynch J J (ed.) Reviews in Rural Science no 4. University of New England, Armidale: 99-107
9. Alexander G 1984 Constraints to lamb survival. In: Lindsay D R, Pearce D M (ed.) Reproduction in Sheep. Australian Academy of Science in conjunction with the Australian Wool Corporation, Canberra: 199-209
10. Alexander G 1988 What makes a good mother?: components and comparative aspects of maternal behaviour in ungulates. Proceedings of the Australian Society of Animal Production 17: 25-41
11. Alexander G, Lynch J J, Donelly J B, Mottershead B 1980 Reduction in lamb mortality due to grass wind-breaks: results of a five year study. Proceedings of the Australian Society of Animal Production 13: 329-332
12. Alexander G, Stevens D, Bradley L R 1988 Maternal behaviour in ewes following caesarian section. Applied Animal Behaviour Science 19: 273-277
13. Alexander G, Stevens D, Kilgour R, De Langen H, Mottershead B E, Lynch J J 1983 Separation of ewes from twin lambs: incidence in several sheep breeds. Applied Animal Ethology 10: 301-317
14. Alexander G, Stevens D, Mottershead B 1983 Problems in the accurate recording of lambing data. Australian Journal of Experimental Agriculture and Animal Husbandry 23: 361-368
15. Alexander G, Williams D 1966 Teat-seeking activity in new-born lambs: the effects of cold. Journal of Agricultural Science Cambridge 67: 181-189
16. Aitken I D 1983 Enzootic (Chlamydial) abortion. In: Martin W B (ed.) Diseases of Sheep. Blackwell Scientific Publications, Oxford: 119-123
17. Anderson A R, Marshall R R 1967 Developing hard hill country and the use of a special strain of labour-saving Romneys. Sheepfarming Annual 30: 35-46
18. Atkins K D 1980 Selection for skin folds and fertility. Proceedings of the Australian Society of Animal Production 13: 174-176
19. Atkins K D, Haughey K G 1989 Pelvic dimensions and their association with lamb survival. In: Genetic Improvement in Reproductive Rate in Merino Sheep. Final report project K/1/1065 New South Wales Department of Agriculture and Fisheries: 57-58
20. Bailey C J, Windle W F 1959 Brain damage in guinea-pigs due to *asphyxia neonatorum*. Experimental Neurology 1: 467-482
21. Barlow R M, Gardiner A C, Angus K W, Gilmour J S, Mellor D J, Cuthbertson J C, Newlands G, Thompson R 1987 Clinical, biochemical and pathological study of perinatal lambs in a commercial flock. Veterinary Record 120: 357-362
22. Barlow R M, Patterson D S P 1982 Border disease of sheep: a virus-induced teratogenic disorder. Advances in Veterinary Medicine Supplement to the Journal of Veterinary Medicine No. 36 Paul Parey, Berlin: 70-71
23. Barry T N 1983 Iodine. In: Grace N D (ed.) The Mineral Requirements of Grazing Ruminants. Occasional publication No 9 New Zealand Society of Animal Production: 48-55
24. Binns W, James L F, Shupe J L, Everett G 1963 A congenital cyclopiantype malformation induced by maternal ingestion of a range plant *Veratrum californicum*. American Journal of Veterinary Research 24: 1164-1175
25. Brand A A, Cloete S W P, De Villiers T T 1985 Faktore wat lamvrektes by die Elsenburg Dormer - en SA Vleismerinokuddes beïnvloed. South African Journal of Animal Science 15: 155-161
26. Brooks D L, Ermel R W, Franti C E, Rupanner R, Behymer D E, Williams J C, Stephenson J C 1986 Q Fever vaccination of sheep: challenge of immunity of ewes. American Journal of Veterinary Research 47: 1235-1238
27. Bruere A N 1974 The discovery and biological consequences of some important chromosome anomalies in populations of domestic animals. First World Congress on Genetics Applied to Livestock Production 1: 151-175
28. Campbell E A 1983 Selenium. In: Animal Health in Australia Nutritional Deficiencies and Diseases of Livestock. Australian Bureau of Animal Health. Canberra 3: 186-192
29. Cloete S W P, Haughey K G 1988 Preliminary note on mature pelvic dimensions and rearing efficiency of Merino ewes. South African Journal of Animal Science 18: 171-174
30. Coetzer J A W 1977 The pathology of Rift Valley fever. 1. Lesions of natural cases in new-born lambs. Onderstepoort Journal of Veterinary Research 44: 205-212
31. Coetzer J A W, Theodoridis A, Van Heerden A 1978 Wesselsbron disease. Pathological, haematological and clinical studies in natural cases and experimentally infected new-born lambs. Onderstepoort Journal of Veterinary Research 45: 93-106
32. Cundiff L V, Gregory K E, Koch R M 1982 Selection for increased survival from birth to weaning. Second World Congress on Genetics Applied to Livestock Production 5: 310-337
33. Dalton D C, Knight T W, Johnson D L 1980 Lamb survival in sheep breeds on new Zealand hill country. New Zealand Journal of Agricultural Research 23: 167-173
34. Dennis S M 1974 Perinatal lamb mortality in Western Australia. 1. General procedures and results. Australian Veterinary Journal 50: 443-449
35. Dennis S M 1975 Perinatal lamb mortality in Western Australia. 6. Listeric abortion. Australian Veterinary Journal 51: 75-79
36. Dennis S M, Leipold H W 1979 Ovine congenital defects. The Veterinary Bulletin 49: 233-239
37. Donnelly F B 1982 A practical attempt to breed for better lamb survival. Proceedings of the Australian Society of Animal Production 14: 30-34
38. Dreir T, Wolff P H 1972 Sucking, state and perinatal distress in newborns. Biology of the Neonate 21: 16-24
39. Dubey J P, Beattie C P 1988 Toxoplasmosis in sheep. In: Toxoplasmosis in Animals and Man CRC Press, Florida: 61-80
40. Dun R B 1963 Recording the lambing performance of ewes under field conditions. Australian Journal of Experimental Agriculture and Animal Husbandry 3: 228-231
41. Eales F A, Small J 1980 Summit metabolism in newborn lambs. Research in Veterinary Science 29: 211-218
42. Eales F A, Gilmour J S, Barlow R M, Small J 1982 Causes of hypothermia in 89 lambs. Veterinary Record 110: 118-120
43. Eales F A, Small J, Gilmour J S 1983 Neonatal mortality in lambs and its causes. In: Haresign W (ed.) Sheep Production. Butterworths, London: 289-298
44. Ellis K J, Overdale O R 1982 The effects on newborn lambs of administering iodine to pregnant ewes. Proceedings of the Australian Society of Animal Production 14: 660
45. Ellis K J, George J M, Raby R H 1983 Evaluation of an intra-ruminal device to provide an iodine supplement for sheep. Australian Journal of Experimental Agriculture and Animal Husbandry 23: 369-373
46. Falconer I R 1965 Biochemical defect causing congenital goitre. Nature 205: 978-980
47. Falconer I R, Roitt I M, Seamark R F, Torrigiani G 1970 Studies of the congenitally goitrous sheep. Iodoproteins of the goitre. Biochemical Journal 117: 417-424
48. Fell B F 1987 The pathology of copper deficiency in animals. In: Howell J McC, Gawthorne J M (ed.) Copper in Animals and Man. CRC Press, Florida 2: 2-28
49. Fogarty N M, Thompson J M 1974 Relationship between pelvic dimensions, other body measurements and dystocia in Dorset Horn ewes. Australian Veterinary Journal 50: 502-506
50. Fourie A J 1988 Die reproduksie doeltreffendheid van die teelooi. In: Paring, Reproduksie en Lamvrektes. Lesings aangebied deur die Wes-Kaaplandse Tak, Suid-Afrikaanse Vereniging vir Diereproduksie. Stellenbosch 20 Oktober: 2.1-2.3
51. Fowler D G, Wilkins J F 1984 Diagnosis of pregnancy and number of foetuses in sheep by real time ultra-sonic imaging. 1. Effect of number of foetuses, stage of gestation, operator and breed of ewe on accuracy of diagnosis. Livestock Production Science 11: 437-450
52. George J M 1975 The incidence of dystocia in fine-wool Merino ewes. Australian Veterinary Journal 51: 262-265
53. George J M 1976 The incidence of dystocia in Dorset Horn ewes. Australian Veterinary Journal 52: 519-523
54. Gilmour N J L, Martin W B, Sharp J M, Thompson D A, Wells P W 1979 The development of vaccines against pneumonic pasteurellosis in sheep. Veterinary Record 104: 15
55. Grace N D 1983 Copper. In: Grace N D (ed.) The Mineral Requirements of Grazing Ruminants. Occasional publication No. 9, New Zealand Society of Animal Production: 56-66
56. Grobler J H 1986 The caracal (African lynx) or "rooikat". Wildlife Technical Guides for Farmers No. 15: 1-2
57. Hartley W J, Alexander G, Edwards M J 1974 Brain cavitation and micro-encephaly in lambs exposed to prenatal hyperthermia. Teratology 9: 299-303
58. Haughey K G 1973 Vascular abnormalities in the central nervous system associated with perinatal lamb mortality. 1. Pathology. Australian Veterinary Journal 49: 1-8
59. Haughey K G 1973 Vascular abnormalities in the central nervous system associated with perinatal lamb mortality. 2. Association of the abnormalities with recognised lesions. Australian Veterinary Journal 49: 9-15
60. Haughey K G 1973 Cold injury in newborn lambs. Australian Veterinary Journal 49: 554-563
61. Haughey K G 1975 Meningeal haemorrhage and congestion associated with the perinatal mortality of beef calves. Australian Veterinary Journal 51: 22-27
62. Haughey K G 1980 The role of birth in the pathogenesis of meningeal haemorrhage and congestion in newborn lambs. Australian Veterinary Journal 56: 49-56
63. Haughey K G 1980 The effect of birth injury to the foetal nervous system on the survival and feeding behaviour of lambs. In: Wodzicka-Tomasczewska M, Edey T N, Lynch J J (ed.) Reviews in Rural Science No 4. University of New England, Armidale: 109-111
64. Haughey K G 1982 Postnatal mortality, bodyweight gain and wool production to two years of age of lambs surviving caesarean or stressful vaginal birth. Australian Veterinary Journal 58: 173-180
65. Haughey K G 1984 Improved lamb survival - a challenge to the industry, both stud and commercial. Wool Technology and Sheep

Breeding 31: 139-145
66. Haughey K G 1984 Can rearing ability be improved by selection? In: Lindsay D R, Pearce D M (ed.) Reproduction in Sheep. Australian Academy of Science in conjunction with the Australian Wool Corporation, Canberra: 210-212
67. Haughey K G 1989 Studies on causes of perinatal lamb mortality in the Western Cape and the pelvic dimensions of ewes relative to their lifetime rearing performance. Report on activities during a visiting research fellowship 1987-88, Department of Agriculture and Water Supply, Elsenburg, Republic of South Africa: 37
68. Haughey K G, George J M, McGuirk B J 1985 The repeatability of rearing performance of Merino and Dorset Horn ewes and its relationship with mature pelvic size. Australian Journal of Experimental Agriculture and Animal Husbandry 25: 541-549
69. Haughey K G, Gray C M 1982 A radiographic technique for pelvimetry in unanaesthetised ewes and a comparison of three methods of estimating the area of the pelvic inlet. Australian Veterinary Journal 58: 51-59
70. Haughey K G, Hughes K L, Hartley W J 1967 The occurrence of congenital infections associated with perinatal lamb mortality. Australian Veterinary Journal 43: 413-420
71. Haughey K G, Jones R T 1976 Meningeal haemorrhage and congestion associated with the perinatal mortality of foals. Veterinary Record 98: 518-522
72. Herbst I A 1989 Flock health programmes (South Africa). Proceedings 19th Seminar Sheep and Beef Cattle Society. New Zealand Veterinary Association incorporating the Second International Congress for Sheep Veterinarians: 97-108
73. Heydenrych H J 1975 'n Studie van kuddestatistieke, nie-genetiese faktore, genetiese parameters en seleksievordering met betrekking tot die Tygerhoek Merinokudde. Ph.D. (Agric.) treatise. University of Stellenbosch. English summary. Agricultural Research 1976, Department of Agricultural Technical Service, Pretoria 1977: 125-127
74. Hight G K, Jury K E 1970 Hill country sheep production. II. Lamb mortality and birthweights in Romney and Border Leicester X Romney flocks. New Zealand Journal of Agricultural Research 13: 735-752
75. Hight G K 1978 Sheep selection experiment. Annual Report 1976-77 Whatawhata Hill Country Research Station, New Zealand Ministry of Agriculture and Fisheries: 1-3
76. Hughes K L, Haughey K G, Hartley W J 1971 Perinatal lamb mortality: infections occurring among lambs dying after parturition. Australian Veterinary Journal 47: 472-476
77. Jordan D J, O'Dempsey N, Stephenson R G A, Wilson K 1984 The effect of udder damage on milk yield, lamb growth and survival. In: Lindsay D R, Pearce D M (ed.) Reproduction in Sheep. Academy of Science in conjunction with the Australian Wool Corporation, Canberra: 220-222
78. Kilgour R 1982 Better lambing procedures. Proceedings of the Ruakura Farmers Conference 34: 9-14
79. Kilgour R, De Langen H 1980 Neonatal behaviour of "easy-care" sheep. In: Wodzicka-Tomasczewska M, Edey T N, Lynch J J (ed.) Reviews in Rural Science No 4. University of New England, Armidale: 117-118
80. Lang G H 1990 Coxiellosis (Q fever) in animals. In: Marrie T J (ed.) Q Fever Volume 1. The Disease. CRC Press, Boca Raton Florida: 24-48
81. Luff A 1980 A service for all seasons. Final Report Sheep Fertility Service. New South Wales Department of Agriculture, Sydney: 14-15
82. Lynch J J, Leng R A, Hinch G N, Nolan J, Bindon B M, Piper L R 1990 Effects of cottonseed supplementation on birthweights and survival of lambs from a range of litter sizes. Proceedings of the Australian Society of Animal Production 18: 516
83. McFarlane D 1964 The effects of predation on perinatal lamb losses in the Monero, Oberon and Canberra districts. Wool Technology and Sheep Breeding 11: 11-14
84. McFarlane D 1965 Perinatal lamb losses. 1. An autopsy method for investigation of perinatal losses. New Zealand Veterinary Journal 13: 116-135
85. McMillan W H 1983 Hogget lamb mortality. Proceedings of the New Zealand Society of Animal Production 43: 33-36
86. McSporran K D, Buchanan R, Fielden E D 1977 Observations on dystocia in a Romney flock. New Zealand Veterinary Journal 25: 247-251
87. McSporran K D, Fielden E D 1979 Studies on dystocia in sheep. II. Pelvic measurements of ewes with histories of dystocia and eutocia. New Zealand Veterinary Journal 27: 75-78
88. Mellor D J 1983 Nutritional and placental determinants of foetal growth and consequences for the newborn lamb. British Veterinary Journal 139: 307-324
89. Mellor D J 1988 Integration of perinatal events, pathophysiological changes and consequences for the newborn lamb. British Veterinary Journal 144: 552-569
90. Mellor D J, Murray L 1985 Effects of maternal nutrition on udder development during late pregnancy and colostrum production in Scottish Blackface ewes with twin lambs. Research in Veterinary Science 39: 230-234
91. Mellor D J, Murray L 1985 Effects of maternal nutrition on the availability of energy in the body reserves of fetuses at term and in the colostrum from Scottish Blackface ewes with twin lambs. Research in Veterinary Science 39: 235-240
92. Millar K R 1983 Selenium. In: Grace N D (ed.) The Mineral Requirements of Grazing Ruminants. Occasional publication No. 9, New Zealand Society of Animal Production: 38-47
93. Mullaney P D 1969 Birth weight and survival of Merino, Corriedale Polwarth lambs. Australian Journal of Experimental Agriculture and Animal Husbandry 9: 157-163
94. Nixon-Smith W F 1968 Cold stress and sheep survival. Part 2 Working Paper 40/4167 Bureau of Meteorology Commonwealth of Australia, Melbourne
95. Osburn B I, Silverstein A M, Prendergast R A, Johnson R I, Parshall C J 1971 Experimental viral-induced congenital encephalopathies. 1. Pathology of hydranencephaly and porencephaly caused by bluetongue vaccine virus. Laboratory Investigations 25: 197-205
96. Palmer N C, Kierstead M, Key D W, Williams J C, Peacock M G, Vellend H 1983 Placentitis and abortion in sheep and goats in Ontario caused by *Coxiella burnetii*. Canadian Veterinary Journal 24: 60-61
97. Parr C A, Campbell I P 1984 The glucose test. In: Lindsay D R, Pearce D T (ed.) Reproduction in Sheep. Australian Academy of Science in conjunction with the Australian Wool Corporation, Canberra: 185-186
98. Parsonson I M, Della-Porta A J, Snowden W A 1981 Akabane virus infection in the pregnant ewe. 2. Pathology of the foetus. Veterinary Microbiology 6: 209-224
99. Piper L R, Hanrahan J P, Evans R, Bindon B M 1982 Genetic variation in individual and maternal components of lamb survival of Merinos. Proceedings of the Australian Society of Animal Production 14: 29-32
100. Plant J W, Marchant R, Mitchell T D, Giles J R 1978 Neonatal lamb losses due to feral pig predation. Australian Veterinary Journal 54: 426-429
101. Putu I G, Poindron P, Lindsay D R 1988 A high level of nutrition during late pregnancy improves subsequent maternal behaviour of Merino ewes. Proceedings of the Australian Society of Animal Production 17: 294-297
102. Putu I G, Poindron P, Lindsay D R 1988 Early disturbance of Merino ewes from the birth site increases lamb separations and mortality. Proceedings of the Australian Society of Animal Production 17: 298-301
103. Quinlivan T D 1971 Dystocia in sheep: preliminary observations on within- and between-breed differences in various skeletal measurements. New Zealand Veterinary Journal 19: 73-77
104. Quinlivan T D 1981 Co-operative ram breeding. Proceedings No. 58 Refresher course in Sheep. Post-Graduate Committee in Veterinary Science. University of Sydney: 280
105. Rae A L 1982 Information on selection programmes. In: Coop I E (ed.) Sheep and Goat Production. Elsevier Scientific Publishing Company, Amsterdam: 27
106. Raju N R, Collings D F, Saville P H 1988 Abortion in black bellied Barbados sheep in Fiji caused by *Coxiella burnetii*. Australian Veterinary Journal 65: 225-226
107. Randall G C B 1978 Perinatal mortality: some problems of adaptation at birth. In: Brandly C A, Cornelius C E (ed.) Advances in Veterinary Science and Comparative Medicine 22: 53-81
108. Roberts D H 1986 Determination of predators responsible for killing small livestock. South African Journal of Wildlife Research 16: 150-152
109. Rowe-Rowe D T 1975 Predation by black-backed jackals in a sheep-farming region of Natal. Journal of the South African Wildlife Management Association 5: 79-81
110. Rowe-Rowe D T 1983 Killing and feeding methods of some carnivores. Wildlife Management Technical Guides for Farmers No. 4: 1-2
111. Robinson J J 1983 Nutrition of the pregnant ewe. In: Haresign W (ed.) Sheep Production. Butterworths, London: 111-131
112. Rowley I 1970 Lamb predation in Australia: incidence, predisposing conditions, and the identification of wounds. CSIRO Wildlife Research 15: 79-123
113. Schwartz P, Anderson G W 1972 Birth lesions of the nervous system. In: Minkler J (ed.) Pathology of the Nervous System. McGraw Hill, New York 3: 2926-2952
114. Seddon H R, Albiston H E 1965 Vibriosis in sheep. In: Diseases of Domestic Animals in Australia Part 5. Bacterial Diseases. Commonwealth Department of Health, Canberra 2: 217-219
115. Seddon H R, Albiston H E 1965 Ovine brucellosis. In: Diseases of Domestic Animals in Australia Part 5. Bacterial Diseases. Commonwealth Department of Health, Canberra 2: 41-47
116. Selye H, Stone H 1950 In: On Experimental Morphology of the Adrenal Cortex 1st edn. Charles C Thomas, Springfield 105 pp
117. Shamburger R J 1983 Selenium deficiency diseases of animals. In: Biochemistry of Selenium. Plenum Press, New York: 31-58
118. Sinclair D P, Andrews E D 1954 Goitre in newborn lambs. New Zealand Veterinary Journal 2: 72-79
119. Slee J 1981 A review of genetic aspects of survival and resistance to cold in newborn lambs. Livestock Production Science 8: 419-429
120. Slee, J, Springbett A 1986 Early post-natal behaviour in lambs of ten breeds. Applied

Animal Behaviour Science 15: 229-240
21. Slee J, Stott A W 1986 Genetic selection for cold resistance in Scottish Blackface lambs. Animal Production 43: 397-404
22. Stephenson R G A, Suter G R, Le Feuvre A S 1984 Reduction of the effects of heat stress on lamb birth weight and survival by provision of shade. In: Lindsay D R, Pearce D T (ed.) Reproduction in Sheep. Australian Academy of Science in conjunction with the Australian Wool Corporation, Canberra: 223-225
23. Stevens D, Alexander G, Mottershead B, Lynch J J 1984 Role of the lamb in postpartum separation of ewes from twin lambs. Proceedings of the Australian Society of Animal Production 15: 751
24. Stott A W, Slee J 1987 The effects of litter size, sex, age, body weight, dam age and genetic selection for cold resistance on physiological responses to cold exposure of Scottish Blackface lambs in a progressively cooled waterbath. Animal Production 45: 477-491
25. Suttle N F 1981 The treatment and prevention of copper deficiency in ruminants. Proceedings 11th Seminar Sheep and Beef Cattle Society. New Zealand Veterinary Association: 24-33
126. Suttle N F, Linklater K A 1983 Iodine deficiency. In: Martin W B (ed.) Diseases of Sheep. Blackwell Sicentific Publications, Oxford: 175-176
127. Van der Merwe C A 1976 Genetiese en niegenetiese faktore wat die produksie en reproduksie-eienskappe van die Elsenburg Dormerskaapkudde beïnvloed. Ph.D.(Agric.) treatise. University of Stellenbosch. English summary. Agricultural Research 1978. Department of Agricultural Technical Services, Pretoria: 122-125
128. Vosloo L P 1967 Faktore wat die produksie en reproduksie van die Elsenburg Duitse Merino-vleisskaapkudde beïnvloed. D.Sc.(Agric.) treatise. English summary. Agricultural Research 1967-68. Department of Agricultural Technical Services Part 1 1968, Pretoria: 280-282
129. Wade D A, Bowns J E 1980 Procedures for evaluating predation of livestock and wildlife. Texas Agricultural Extension Service. Texas A & M University in conjunction with United States Fish and Wildlife Service 42 pp
130. Wallace L R 1948 The growth of lambs before and after birth in relation to the level of nutrition. Journal of Agricultural Sience 38: 367-401
131. Webb R F 1983 Clinical and pathological findings in *Histophilus ovis* infection in sheep. Research in Veterinary Science 35: 30-34
132. Windle W F 1969 Brain damage by asphyxia at birth. Scientific American 221: 77-84
133. Wolff P H 1968 The serial organisation of sucking in the young infant. Paediatrics 42: 943-956
134. Wolff J E, Baker R L, Dobbie P M, Ford A J, Jordan R B 1987 Genetic aspects of cold resistance in newborn lambs. New Zealand Society of Animal Production 47: 93-98
135. Wood C, Ng K H, Hounslow D, Benning H 1973 The influence of birth times upon fetal condition in normal deliveries. Journal of Obstetrics and Gynaecology of the British Commonwealth 80: 289-294

PROFESSOR J M W LE ROUX 1926-1991



Jan le Roux is op Maandag 11 Maart, digby sy geboorteplaas by Riviersonderend op Montagu, waar hy en Sjaan sedert 1988 woon, oorlede. Dit was 'n jaar nadat longkanker gediagnoseer is. Tot kort voor sy dood was hy besig met die Engelse vertaling van 'n Anatomiehandboek waarvan hy die hoofouteur was. Op 2 bladsye na, het hy dit voltooi.

Jan le Roux is op 5 April 1926 op Swellendam gebore. Hy matrikuleer in 1943 aan die Hoërskool Jan van Riebeeck, Kaapstad, behaal in 1948 die BVSc-graad aan die Universiteit van Pretoria en verower ook die kliniese medalje. As navorsingsbeampte (1949), senior lektor (1956), professor en hoof van die Departement Anatomie (1974) en dekaan (1982-1986) van die Fakulteit Veeartsenykunde aan sy alma mater, het hierdie stil man met sy besondere styl en humorsin die professie op 'n unieke manier gedien en uitgebou.

Sy kollegas in die Departement Anatomie kan getuig van sy onbaatsugtige ondersteuning en lojaliteit. Hy het 'n besielende visie gehad, nie alleen ten opsigte van sy eie vakgebied nie, maar ook vir die professie. Daarom was dit so gepas dat hy sy loopbaan as dekaan kon afsluit.

Sy eerste professionele aanstelling (1949-1956) was as navorsingsbeampte in die Afdeling Patologie aan die Navorsingsinstituut vir Veeartsenykunde onder leiding van prof K Schultz. Sy deeglikheid en wetenskaplike instelling is hier vasgelê, asook sy waardering vir, en lojalitleit teenoor die Navorsingsinstituut. In 1956 het hy 'n Senior Lektoraat in die Departement Anatomie van die Fakulteit Veeartsenykunde aan die Universiteit van Pretoria aanvaar. Met sy deeglike agtergrond in Patologie, was Anatomie vir hom die ideale volgende arbeids- en studieveld. Hy was gereeld elke week in die biblioteek om op hoogte te bly van publikasies in sy vakgebied. In 1959 verwerf hy die Dr Med Vet-graad aan die Tierärtzliche Hochschule in Hannover, met 'n verhandeling oor die venas van die kop van die bees. Foto's van sy disseksies is later in die beroemde handboek oor die vergelykende anatomie van die huisdiere deur Nickel, Schummer en Seiferle (Paul Parey, Berlyn en Hamburg) opgeneem.

Jan le Roux het lewenslange vriendskappe gesluit tydens hierdie jaar in Hannover. Met sy kenmerkende hoflikheid en humorsin, gerugsteun deur matriek-Duits, het hy almal se harte verower. Sy deeglikheid en akademiese uitnemendheid het die hoogste agting afgedwing en het gelei tot latere formele uitnodigings as gasdosent in 1970/71 en gasprofessor tydens Desember/Januarie 1976/77. By geleentheid van die 200-jarige herdenkingsfees van hierdie skool in 1978, is hy genooi om deel te neem aan 'n internasionale simposium oor Veterinêre Anatomie.

Na sy terugkeer uit Duitsland aan die einde van 1959, het hy die Anatomiekursus aan Onderstepoort ingrypend verander. Die benadering word sistematies-vergelykend en die lewende

0038-2809 Jl S.Afr.vet.Ass. (1991) 62(2):92-93

dier word by praktika betrek. Oppervlakanatomie en Toegepaste Anatomie word beklemtoon. Anatomie het 'n kliniès-gerigte baadjie gekry.

Die voorgraadse kursus is in die vroeë tagtigerjare sinryk gerasionaliseer en in medewerking met Malie Smuts en Christine Seegers het die eerste Afrikaanse studie- en disseksiegids oor die anatomie van huisdiere verskyn. Dit is na ons wete die enigste vergelykende studie- en disseksiegids van sy soort in Veterinêre Anatomie. Met 3 van die 5 dele reeds in Engels vertaal, het Jan met byna bomenslike ywer gepoog om voor sy dood die oorblywende dele te vertaal.

Gedurende die sestigerjare het hy moderne anatomiese tegnieke in die Departement ingevoer en toegepas. Man-alleen het hy 'n stel demonstrasiedisseksies voorberei wat sedertdien die grondslag vorm van voor- en nagraadse onderrig. Hierdie onvervangbare en unieke versameling word nou in die Le Roux-studielokaal in die nuwe Anatomiegebou gehuisves.

Prof le Roux het 'n aktiewe navorsingsprogram in die Departement van stapel gestuur wat steeds as rigsnoer dien. Gedurende sy hoofskap is twee doktorale proefskrifte onder sy leiding voorberei. Uit sy pen het 15 artikels in internasionale en plaaslike wetenskaplike joernale verskyn.

Sy volgehoue persoonlike kontak met buitelandse vakdeskundiges het as natuurlike uitvloeisel gehad dat bekende wetenskaplikes uit Duitsland, Engeland, die Verenigde State van Amerika en Nederland deur hom genooi is om die Fakulteit Veeartsenykunde, Universiteit van Pretoria te besoek. Nie alleen is hulle aan ons studente voorgestel nie, maar as huisgaste van die Le Roux's is hulle dikwels op begeleide toere van Suid-Afrika geneem.

In die middel van die sewentigerjare is 'n gesamentlike poging van dosente en studente van stapel gestuur om die studentekorps by musiek en kultuur te betrek. Prof le Roux is as voorsitter van hierdie komitee verkies. Weeklikse vergaderings en baie ure se beplanning, briewe en reëlings het gelei tot hoogs suksesvolle konserte in die Musaion, Universiteit van Pretoria, waar verrassende talent na vore gekom het en waar die res van die universiteit bekend gestel is aan die "ander" kant van veeartsenystudente.

Nog 'n bewys van prof le Roux se gewildheid onder die studente, was sy verkiesing as die nuwe "President" tydens die studente se jaarlikse pretdag in 1980. Min van die kollegas sou die takhaar-figuur as prof le Roux kon eien, nadat die studente hom "aangetrek" het. Hy het met groot sportmanskap die voorbereide "toespraak" gelees en hom ingeleef in die pret. Daar was altyd by hom 'n warm belangstelling in die wel en weë van studente. Uit eie sak het hy jaarliks die nuwe klaskomitees, tesame met die dosente, vir 'n ete na 'n restourant geneem "om mekaar te leer ken". Tydens sy hoofskap en later ook as Dekaan, het prof en mev le Roux aan die begin van elke akademiese jaar die nuwe buitelandse studente by hulle aan huis ontvang. Sy meelewing met die behoeftes van buitelanders kan teruggevoer word na sy eie gewaarwordinge as student in die buiteland.

Prof le Roux was nooit in akademiese of professionele politiek betrokke nie. Sy eerlikheid, onpartydigheid, deeglikheid en hoflikheid het in 1981 tot sy verkiesing as dekaan gelei. 'n Trotse oomblik in sy dekaansperiode was toe sy enigste kind, Marina, in Junie 1985 haar BVSc-graad ontvang het as een van die 5 topstudente in die klas.

Sy heel eerste aankondiging as dekaan was dat papierwerk tot 'n minimum beperk gaan word; gesprekvoering gaan die metode van kommunikasie en besluitneming wees. 'n Program is opgestel en elke lid van die personeel is sinvol betrek in 'n gespreksgroep op Sondagaande by hulle aan huis. Hierdie besondere program was natuurlik ondenkbaar sonder die aktiewe en heelhartige ondersteuning van sy vrou, Sjaan. Dit was vir hom belangrik om elke lid van die personeel persoonlik te leer ken. Met die buitengewone ondersteuning van mev le Roux, is al die personeel (dosente en tegnici) met hulle gades gedurende sy 5 jaar as dekaan op feestelike noenmale by hulle huis in Silverton onthaal.

Prof le Roux was bekend as 'n man van min woorde. Hy het ook bekend gestaan as iemand wat sy huiswerk gedoen het. Hy het dieselfde van sy kollegas verwag, en dit was 'n kenmerk van vergaderings wat deur hom gelei is, dat hulle nooit langer as een uur geduur het nie.

Gedurende sy termyn as dekaan het hy hom aktief vir die akademiese ontwikkeling van sy Fakulteit beywer. Drie selfstandige departemente is gestig (Veterinêre Volksgesondheid, Farmakologie en Toksikologie, en Pluimveesiektes) en 2 geborgde professorate is ingestel (in die Departemente Chirurgie en Veterinêre Etologie). Prof le Roux se dekaanskap het saamgeval met die beplanning van nuwe, moderne fasiliteite op die kampus van die Universiteit van Pretoria. Gedurende 1984 besoek hy etlike veterinêre fakulteite in Amerika, Engeland, Nederland, België en Duitsland om 'n oorsig te kry van die beste wat wêreldwyd aangebied word.

Om navorsing te prikkel en kollegas in staat te stel om hulle werk in die fakulteit bekend te stel, het prof le Roux 'n jaarlikse Fakulteitsdag ingestel.

As die Universiteit van Pretoria se verteenwoordiger op die SA Veeartsraad het hy hierdie statutêre liggaam se eksamenstelsel ingrypend laat hersien tot voordeel van die professie. Hy was tydens sy dekaanskap 'n benoemde lid van die Federale Raad van die Suid-Afrikaanse Veterinêre Vereniging.

As dekaan was hy 'n benoemde lid van die Raad en Senaat van die Mediese Universiteit van Suider-Afrika en hy het 'n waardevolle bydrae gelewer deur sy eerlike en deurdagte kommentaar.

Vir Jan le Roux was sý verhouding, en dié van die Fakulteit, met die Suid-Afrikaanse Veterinêre Vereniging altyd baie belangrik. Nuwe eerstejaarstudente is altyd onmiddellik ingelig en voorgestel aan die President of sy verteenwoordiger. Hy het oral waar hy gegaan het, die aktiwiteite van die SAVV bekendgestel en ondersteun.

Goeie buurskap tussen die Navorsingsinstituut vir Veeartsenykunde, Onderstepoort, en die Fakulteit het hom besonder na aan die hart gelê en hy het hom aktief daarvoor beywer. Jare nadat hy by die Fakulteit aangestel is, het hy steeds elke oggend by die Instituut tee gaan drink om die kollegiale kontak te behou.

Prof le Roux was lid van die Suid-Afrikaanse Veterinêre Vereniging, Openbare Sektorgroep van die Suid-Afrikaanse Veterinêre Vereniging, Wêreldvereniging van Veterinêre Anatome en die Anatomiese Vereniging van Suider-Afrika.

Ons, wat jare lank die voorreg gehad het om hom as leermeester en vriend te kon ken, bring dankbaar hulde aan hierdie onselfsugtige man en prins van veeartsenykundige opleiding.

Malie M S Smuts

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

This is a refereed journal. All submissions will be refereed by the Editorial Committee and two independent referees.

The JOURNAL is owned and published by the South African Veterinary Association, of which it is the official organ. It appears quarterly and is devoted to matters of veterinary importance generally. The statements made and opinions expressed by contributors are their responsibility only; such statements are not necessarily endorsed by the Editorial Committee, neither do the opinions reflect those of the Committee. The whole of the literary contents of this Journal is copyright.

SUBSCRIPTION — A free copy of each issue is sent to all members of the Association in good standing. The subscription rate for local non-members is R85,00 per annum, post free; overseas subscription is $86 per annum, post free, surface mail. BACK NUMBERS are obtainable at R20,00 per number.

CONTRIBUTIONS — The Editor will consider contributions of veterinary interest. Double-spaced, carefully revised, typewritten manuscrips, tables and figures should be submitted in triplicate (original plus two good copies). Layout and references should be in the style of this number. REFERENCES should not exceed 20 in number unless approved by the Editor. The number of figures and tables may be limited at the Editor's discretion unless the author contributes to the cost of reproduction. This applies particularly to reproductions in colour.

TABLES and FIGURES should be in widths of 85 mm, or 176 mm, or in sizes of 263 x 176 mm, or reducible thereto. Only the International Metric System (SI) is used in this Journal and contributors must ensure that fluid volume, length, mass, time, amount of substance, etc. are indicated in the correct SI unit. Time is expressed as: year, month, week, d (days), h (hours), min (minutes) and s (seconds). For further information refer to the "Guide for Authors" in Vol. 52, No. 2, pp 83-97.

REPRINTS should be ordered upon confirmation of publication (25 copies are sent free of charge).

TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

Alle bydraes in hierdie tydskrif is onderworpe aan redaksionele beoordeling deur die Redaksionele Komitee en twee onafhanklike beoordelaars.

Die TYDSKRIF is die offisiële mondstuk en eiendom van en word gepubliseer deur die Suid-Afrikaanse Veterinêre Vereniging. Dit verskyn kwartaalliks en word aan sake van algemene veeartsenykundige belang gewy. Bydraers tot hierdie Tydskrif maak hul stellings en lug hul menings uitsluitlik op eie verantwoordelikheid; sodanige stellings word nie noodwendig deur die Redaksiekomitee onderskryf nie en die menings gee nie noodwendig die Komitee se menings weer nie. Kopiereg word op al die letterkundige inhoud van die Tydskrif voorbehou.

INTEKENING — 'n Eksemplaar van elke uitgawe word gratis aan alle volwaardige lede van die Vereniging gestuur. Die intekengeld vir plaaslike persone wat nie lede is nie, beloop R85,00 jaarliks, posvry; oorsese intekengeld is $86 jaarliks posvry per land of seepos. VORIGE UITGAWES is beskikbaar teen R20,00 per eksemplaar.

BYDRAES — Die redaksie sal alle bydraes van veeartsenykundige belang vir publikasie oorweeg. Dubbelgespasieerde, noukeurig hersiende, getikte manuskripte en meegaande figure en tabelle moet in triplikaat (oorspronklike en twee goeie afskrifte) ingedien word. Opset en verwysing môet die styl van hierdie uitgawe volg. MEER AS 20 VERWYSINGS word slegs met die goedkeuring van die Redakteur toegelaat. TABELLE en FIGURE moet in breedtes van 85 mm, of 176 mm, of in groottes van 263 x 176 mm weergegee word, of daartoe gereduseer kan word. Die getal figure en tabelle kan na oordeel van die redaksie beperk word tensy die outeur tot die koste van reproduksie bydrae, veral kleurreproduksie. Slegs die internasionale Metrieke Stelsel (SI) word in hierdie Tydskrif gebruik, en outeurs moet sorg dat die korrekte SI eenhede vir vloeistofvolume, lengte, massa, tyd en stofhoeveelheid gebruik word. Tyd word uitgedruk as: jaar, maand, week, d (dae), h (ure), min (minute) en s (sekondes). Verwys verder na die "Riglyne vir Outeurs" in Jaargang 52, Nr 2, pp 83-97.

HERDRUKKE moet ten tye van bevestiging van plasing bestel word (25 herdrukke word gratis verskaf).