SA ISSN 0038-2809
Dewey Cat. No. 636.089

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

MARCH 1981/MAART 1981

VOLUME 52 No. 1
JAARGANG 52 Nr. 1

CONTENTS/INHOUD

Contents continued on page 1 — *Inhoud vervolg op bladsy 1*

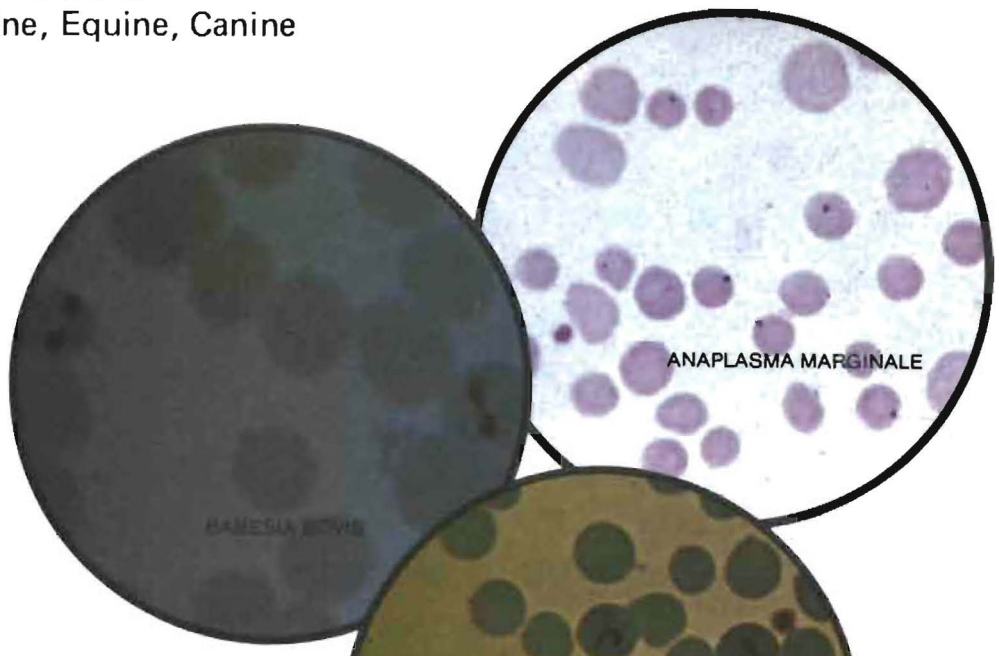
NOW FREELY AVAILABLE FROM YOUR WHOLESALER

COOPERS

Forray 65

Imidocarb Dipropionate 12% M/V

The single answer to *Anaplasmosis* and *Babesiosis*
Bovine, Equine, Canine



- **Prophylaxis** – *Babesia bovis*, 4 weeks *Babesia bigemina*, 2 - 3 months.
- **Therapy:** Cures *Anaplasmosis* and *Babesiosis*.
- **One injection** – usually sufficient for *Anaplasmosis*.
- **Small dose:** Ready-to-use solution.
- **Not** an antibiotic.
- **Safe** for pregnant animals.

COOPERS
(SOUTH AFRICA) (PTY) LIMITED
68 Rigger Road Spartan Transvaal
P.O. Box 677 Kempton Park 1620 Tel. 975-1146

A WELLCOME COMPANY

International J16266

Index to Advertisers **Advertensie-Opgaaf**

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

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 R25,00/a, post free; overseas subscriptions is R30,00/a, post free surface mail. BACK NUMBERS are obtainable at R8,00/number.

CONTRIBUTIONS – The Editor will consider contributions of veterinary interest. Double-spaced, carefully revised, typewritten manuscripts should be submitted in triplicate (original plus first two 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 × 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: a (year), week, d (days), h (hours), min (minutes) and s (seconds). For further information refer to M33a (SABS, P/Bag X191, Pretoria). REPRINTS should be ordered upon confirmation of publication. The senior author receives 25 reprints of each article free.

TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING

Die TYDSKRIF is die offisiële mondstuk en eiendom 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 slegs 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 R25,00/a, posvry; oorsese intekengeld is R30,00/a, posvry per land of seepos. VORIGE UITGAWES R8,00/eksemplaar.

BYDRAES – Die redaksie sal alle bydraes van veeartsenykundige belang vir publikasie oorweeg. Dubbelgespasieerde, noukeurig hersiende, getikte manuskripte moet in triplikaat (oorspronklike en twee afskrifte) ingedien word. Opset en verwysing moet 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 × 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 bydra, 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: a (jare), week, d (dae), h (ure), min (minute) en s (sekondes). Verwys verder na M33a (SABS, P/sak X191, Pretoria).

HERDRUKKE moet ten tye van bevestiging van plasing bestel word. Senior outeurs kry 25 herdrukke gratis.

ALL CORRESPONDENCE: Director, SAVA, Jl. S. Afr. Vet. Ass., Box 26498, Arcadia 0007 (Tel. 26-6233)

ALLE BRIEFWISSELING: Direkteur, SAVV, Tydskr. S. Afr. Vet. Ver., Bus 26498, Arcadia 0007 (Tel. 26-6233)

REDAKTEUR/EDITOR: Prof. R.C. TUSTIN

CONSULTANT EDITOR/RAADGEWENDE REDAKTEUR: H.P.A. DE BOOM

ADMINISTRATIVE EDITOR/ADMINISTRATIEWE REDAKTEUR: Mrs. C. GROENEWALD

REDAKSIE/EDITORIAL COMMITTEE: H.J. BERTSCHINGER, C. BUTTON, R.I. COUBROUGH, A.J. DE VOS, A. IMMELMAN, R.K. REINECKE, C.G. STEWART, H.M. TERBLANCHE, L.W. VAN DEN HEEVER, R.D. SYKES (Financial/Geldsake), Mrs. M.M.E. SMIT (Secretary).

AGENTS IN GREAT BRITAIN: **AGENTE IN DIE VERENIGDE KONINKRYK**

Baillière, Tindall & Cassel, 8 Henrietta St.
Covent Garden, London.

ADVERTISING RATES on application **ADVERTENSIETARIEWE op aansoek**

Financial subvention by the Department of National Education is gratefully acknowledged.

Geldelike steun deur die Departement Nasionale Opvoeding word met dank erken.

Typesetting by/Tipografie deur Dieter Zimmerman (Pty) Ltd./(Edms) Bpk.,
Printed and bound by/Gedruk en gebind deur
Sigma Press (Pty) Ltd/(Edms) Bpk., Pretoria.

REGISTRATION OF VETERÍNARIANS IN THE REPUBLIC OF SOUTH AFRICA

Because of the uncertainty which seems to exist about the requirements for registration as a veterinarian in the Republic of South Africa, a useful purpose might be served if the attention of all concerned is drawn to the present position. Until such time as the new draft Veterinary Act comes into effect, the requirements for registration under the Veterinary Act, No. 16 of 1933 (as amended by Veterinary Amendment Acts No. 49 of 1963 and No. 19 of 1972), are still applicable.

Graduates from the Faculty of Veterinary Science of the Pretoria University are accepted by the Veterinary Board as soon as they apply for registration, which is usually immediately after qualifying. The reason for this uncomplicated procedure is that the Board is fully acquainted with the standard of training maintained at this Faculty.

An arrangement dating back to the inception of the Veterinary Act makes veterinary graduates from all universities in England, Scotland and Ireland and from the Pretoria University reciprocally eligible for registration with the S A Veterinary Board and the Royal College of Veterinary Surgeons. A graduate from a university in the British Isles has only to submit a statement by the Royal College of Veterinary Surgeons that he is a registered member in good standing. This concession does not apply to graduates from universities outside the British Isles who have obtained membership of the Royal College for reasons determined by the Royal College. They have to apply to the S A Veterinary Board as graduates from the universities at which they qualified, although their registration by the Royal College will be taken into account when their applications are considered.

A reciprocal arrangement similar to that for graduates from the British Isles exists between our South African graduates and those from the Massey University of Manawatu, New Zealand.

Any person who, at the time he commences his veterinary studies, is a South African citizen and subsequently obtains his veterinary degree, is entitled to be registered as a veterinarian by the Veterinary Board on condition that the degree is obtained at one of the following institutions:

University of Utrecht,
Veterinary College of Berlin,
Veterinary College of Hanover,
Veterinary College of Munich,
Veterinary College of Leipzig,
Veterinary College of Giessen,
University of Berne,
University of Zurich,
University of Vienna,
University of Queensland,
New York State Veterinary College,
Cornell University.

Any other veterinary graduate who seeks registration must submit to the Veterinary Board documentary evidence of his academic career and professional experience as well as two testimonials of his good character from university teachers or previous employers. A statement from a professional registering body to the effect that he is a member in good standing is also required. His application will then be considered in terms of section 12 bis of the Veterinary Act. The Veterinary Board may require such an applicant to undergo an examination before it is prepared finally to consider his application. By agreement with the Faculty of Veterinary Science of the Pretoria University, which assists the Veterinary Board in setting the examination, the times determined for examination are the last week in May and October. If, however, the Veterinary Board decides that the applicant has a sufficient knowledge of and training in veterinary science, it may recommend to the Minister of Agriculture and Fisheries that the applicant be registered as a veterinarian without his having to pass an examination.

If an applicant accepted for registration under section 12 bis of the Veterinary Act is not a South African citizen and does not become one within a period not exceeding seven years (this period is determined by the Minister at the time of registration), his registration lapses. A person cannot be registered a second time under the same section of the Act.

STUDIES ON FELINE BABESIOSIS. 4 CHEMICAL PATHOLOGY; MACROSCOPIC AND MICROSCOPIC *POST MORTEM* FINDINGS

G.J. FUTTER*, P.C. BELONJE†, A. VAN DEN BERG†, AND A.W. VAN RIJSWIJK†

ABSTRACT: Futter G.J., Belonje P.C., Van den Berg A., Van Rijswijk A.W. **Studies on feline babesiosis. 4 Chemical pathology; macroscopic and microscopic *post mortem* findings.** *Journal of the South African Veterinary Association.* (1981) **52** No. 1, 5–14 (En) 22 Blue Route, Tokai Road, 7945 Retreat, Republic of South Africa.

Chemopathological changes were monitored in 20 experimentally infected and 70 clinical cases of feline babesiosis. Total serum proteins remained unchanged but there was a definite increase in γ globulin and decrease in α and β globulins. In most cases liver function was essentially normal although function tests occasionally indicated hepatic dysfunction. Renal function was unaffected. Venous blood pH remained normal throughout. *Post mortem* findings on the experimental cats included bile stasis and hepatic necrosis in some; marked internal icterus was only seen in 2 cases.

INTRODUCTION

No work has been published on either the chemical pathology or the macro- and microscopic pathology of feline babesiosis[2]. For this reason we conducted an in-depth study of these aspects of the disease in 20 experimental artificially infected cats and 70 clinical naturally infected cases.

MATERIALS AND METHODS

Animals

The 20 experimental artificially infected (Table 1) and 70 clinical naturally infected cats were the same as detailed in a previous article[3].

Data collected from experimental animals

Blood samples: Free-flowing jugular blood was taken anaerobically in lightly heparinized syringes for pH determinations. Non-heparinized samples were taken at the same time and centrifuged for serum.

Total serum proteins (TSP) were determined once weekly according to the method described by Weichselbaum[14]. Albumin, globulin and globulin fractions were determined by cellulose acetate electrophoresis using the Beckman microzone cell and a Gelman ACD 15 densitometer.

Serum alanine amino transferase (SGPT) levels were determined once weekly using the Monotest GPT Optimal u/v Test (Boehringer Mannheim).

Total and direct bilirubin were determined when the icterus index was above 7,5 using a Bilirubin kit (Merck).

Blood pH was determined twice weekly on a Radiometer BMS 3 Mk 2 Blood micro-system.

Serum urea and creatinine were determined on selected samples by the Technicon SMAC System.

Serum cholesterol was determined on selected samples by the Technicon SMAC System.

Urine taken at *post mortem* examination was analysed for urobilinogen, blood, ketones, glucose, protein and pH by the use of reagent strips (Multistix, Ames), for bilirubin by the standard Fouchet method and for osmolality by means of an automatic osmometer (Osmette A, Precision System Inc.).

At *post mortem* examination, various tissues were taken in formalin for histopathology.

*22 Blue Route, Tokai Road, Retreat, 7945
†Dept Human and Animal Physiology, University of Stellenbosch, Stellenbosch, 7600

Data collected from clinical cases

When circumstances permitted, various serum analyses were performed by the Technicon SMAC System.

Statistical methods

These were performed as outlined by Snedecor & Cochran (1967)[11].

RESULTS

Experimental animals

Serum proteins

1. Total serum protein (TSP)

(a) Splenectomised cats (Table 2)
As can be seen in Table 2, no significant variations were observed in the TSP in the splenectomised cats. A moderate increase in the mean TSP was observed from Day 20 onwards. Four of the cats showed a rise in TSP. In Cat K from Day 0 to Day 21 the TSP rose 18,5 g/ℓ. In 3 of the cats the TSP was relatively unchanged, whereas in the other 3 a reduction occurred. From Day 0 to terminal Day 20 a reduction of 17,7 g/ℓ was recorded in the TSP of Cat M.

(b) Non-splenectomised cats (Table 3)
Three of the cats showed no significant changes in the TSP. Cats Q and R, however, showed an increase in the TSP on Day 17 and then a reduction on terminal Days 39 and 42, respectively. In Cat S a rise was recorded on Day 25. No meaningful trend could be detected in the mean TSP.

2. Percentage albumin and globulin (% Alb & Glob)

(a) Splenectomised cats (Tables 4 and 5)
No significant changes were recorded in the albumin and globulin percentages in the splenectomised cats.

(b) Non-splenectomised cats (Table 6 and 7)
A reduction in the mean albumin percentage and an increase in the mean globulin percentage were recorded in the non-splenectomised cats, although there were individual fluctuations.

3. Percentage α globulin

(a) Splenectomised cats (Table 8)
There was a significant decrease in the percentage α globulin. The decrease measured for each of the cats

0038-2809/81/01/0005–0014

Table 1: DESCRIPTION OF CATS, SOURCE OF INFECTION AND SUBSEQUENT LIFESPAN

Cat	Sex	Age	Origin of infection	Number of days from inoculation until death	Remarks
1	Female	Adult	Clinical case from	114	Splenectomised – Main source
2	Female	Adult	Cape Point	118	of infection for experiment
A	Male	Adult	Cat 1	12	Splenectomised trial run
B	Female	6 weeks	Cat 2	32	Splenectomised trial run
C	Male	Adult	Cat 1	73	Splenectomised experimental
D	Female	Adult	Cat 2	35	Splenectomised experimental
E	Female	Adult	Cat 1	18	Splenectomised experimental
F	Male	Adult	Cat 2	14	Splenectomised experimental
G	Female	Adult	Cat 1	27	Splenectomised experimental
H	Female	Adult	Cat 2	21	Splenectomised experimental
I	Female	Adult	Cat 1	21	Splenectomised experimental
J	Female	Adult	Cat 2	14	Splenectomised experimental
K	Female	Adult	Cat C	45	Splenectomised experimental
M	Pregnant Female	6 months	Cat D	20	Splenectomised experimental
N	Female	4 months	Cat D	53	Non-splenectomised experimental
O	Female	4 months	Cat D	53	Non-splenectomised experimental
P	Female	8 months	Cat D	49	Non-splenectomised experimental
Q	Male	8 months	Cat D	39	Non-splenectomised experimental
R	Female	10 months	Cat D	42	Non-splenectomised experimental
S	Pregnant Female	Adult	Cat C	38	Non-splenectomised experimental

Table 2: CHANGES IN TOTAL SERUM PROTEIN (g/ℓ) IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	71,2	76,5	75,0	72,0	84,0	81,4	82,0
D	72,0	79,5	79,5	85,5	85,5	78,7	—
E	76,0	71,4	67,3	—	—	—	—
F	79,4	67,6	80,9	—	—	—	—
G	66,5	63,0	63,6	64,0	56,0†	—	—
H	68,6	75,5	81,2	—	—	—	—
I	76,6	75,2	74,9	78,0	—	—	—
J	65,8	66,4	—	—	—	—	—
K	71,1	74,2	83,7	89,6	82,6	84,9	78,9
M	77,3	66,6	67,6	59,6*	—	—	—
Mean	72,5	71,6	74,9	74,8	77,0	81,7	80,5
SD	4,7	5,4	7,2	11,8	14,0	3,1	2,3

*Day 20 †Day 27

following infection ranged from 3,3 to 9,3 percentage points. The mean percentage α globulin decreased from 19,2% on Day 0 to 10,1% on Day 42. Cat F was the only animal that did not show a decrease.

(b) Non-splenectomised cats (Table 9)
As in the splenectomised cats, the percentage α globulin also decreased in the non-splenectomised cats. The decrease measured for each cat following infection ranged from 4,6 to 11,7 percentage points. The mean percentage, however, did not fall as much (16,1% on Day 0 to 9,3% on Day 39) as in the splenectomised cats.

4. Percentage β globulin

(a) Splenectomised cats (Table 10)
A decrease in the mean percentage β globulin of 2,5 percentage points was recorded. There was, however, variation in the values for the individual cats. Two of

Table 3: CHANGES IN TOTAL SERUM PROTEIN (g/ℓ) IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection															
	0	7	11	14	17	18	20	24	25	32	35	39	42	45	46	49
N	74,8	71,3	—	—	—	76,3	—	74,0	—	75,5	—	76,2	—	—	—	70,4
O	59,6	—	65,5	—	—	65,6	—	72,9	—	70,6	—	—	—	—	69,5	69,9
P	79,9	63,6	—	76,3	—	—	74,9	—	—	—	73,4	—	—	76,2	—	74,9
Q	73,6	—	69,4	—	87,9	—	—	—	73,2	72,3	—	55,2	—	—	—	—
R	71,8	—	73,6	—	80,2	—	—	—	75,6	76,5	—	—	62,3	—	—	—
S	73,2	—	76,8	—	76,8	—	—	—	85,5	77,8	—	—	—	—	—	—
Mean	72,1	67,5	71,3	—	77,4		—	76,2		74,5	—	65,7	—	—	—	71,7
SD	6,7	5,4	4,9	—	8,0		—	5,3		3,0	—	14,8	—	—	—	2,8

Table 4: CHANGES IN PERCENTAGE ALBUMIN IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	54,4	36,9	47,9	52,5	48,2	49,0	53,5
D	46,5	43,8	—	39,5	40,2	45,0	—
E	45,8	48,2	50,1	—	—	—	—
F	38,5	38,6	29,8	—	—	—	—
G	50,1	53,5	53,3	51,3	46,8†	—	—
H	41,8	39,2	40,4	—	—	—	—
I	40,1	41,2	39,9	41,9	—	—	—
J	41,6	45,0	—	—	—	—	—
K	37,1	35,7	36,2	34,7	36,9	38,9	—
M	32,5	40,4	42,6	38,8*	—	—	—
Mean	42,8	42,3	42,5	43,1	43,0	44,3	—
SD	6,5	5,5	7,9	7,2	5,4	5,1	—

*Day 20 †Day 27

Table 5: CHANGES IN PERCENTAGE GLOBULIN IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	45,6	63,1	52,1	47,5	51,8	51,0	46,5
D	53,5	56,2	—	60,5	59,8	55,0	—
E	54,2	51,8	49,9	—	—	—	—
F	61,5	61,4	70,2	—	—	—	—
G	49,9	46,5	46,7	48,7	53,2†	—	—
H	58,2	60,8	59,6	—	—	—	—
I	59,9	58,8	60,1	58,1	—	—	—
J	58,4	55,0	—	—	—	—	—
K	62,9	64,3	63,8	65,3	63,1	61,1	—
M	67,5	59,6	57,4	61,2*	—	—	—
Mean	57,2	57,7	57,5	56,9	57,0	55,7	—
SD	6,5	5,5	7,7	7,2	5,4	5,1	—

*Day 20 †Day 27

Table 6: CHANGES IN PERCENTAGE ALBUMIN IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection																
	0	7	11	14	17	18	20	24	25	28	32	35	39	42	45	46	49
N	45,9	44,3	—	—	—	44,4	—	40,8	—	—	39,3	—	41,9	—	—	—	44,7
O	40,8	—	39,2	—	—	40,7	—	41,3	—	—	31,0	—	—	—	—	39,0	36,9
P	47,8	57,4	—	61,7	—	—	46,6	—	—	47,3	—	40,3	—	—	34,4	—	34,6
Q	52,9	—	46,4	—	41,8	—	—	—	47,1	—	42,5	—	48,2	—	—	—	—
R	53,5	—	43,2	—	44,1	—	—	—	44,3	—	40,7	—	—	46,2	—	—	—
S	41,3	—	38,1	—	42,6	—	—	—	34,0	—	37,9	—	—	—	—	—	—
Mean	47,0	50,8	41,8	—	42,8		—	41,5		—	38,3	—	45,1	—	36,7		38,7
SD	5,5	9,3	3,8	—	1,6		—	4,9		—	4,4	—	4,5	—	3,3		5,3

Table 7: CHANGES IN PERCENTAGE GLOBULIN IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection																
	0	7	11	14	17	18	20	24	25	28	32	35	39	42	45	46	49
N	54,1	55,7	—	—	—	55,6	—	59,2	—	—	60,7	—	58,1	—	—	—	55,3
O	59,2	—	60,8	—	—	59,3	—	58,7	—	—	69,0	—	—	—	—	61,0	63,1
P	52,2	42,6	—	38,3	—	—	53,4	—	—	52,7	—	59,7	—	—	65,7	—	65,4
Q	47,1	—	53,6	—	58,2	—	—	—	52,9	—	57,5	—	51,8	—	—	—	—
R	46,5	—	56,8	—	55,9	—	—	—	55,7	—	59,3	—	—	53,8	—	—	—
S	58,7	—	61,9	—	57,4	—	—	—	66,0	—	62,1	—	—	—	—	—	—
Mean	53,0	49,2	58,2	—	57,2		—	58,5		—	61,7	—	54,9	—	63,3		61,3
SD	5,5	9,3	3,8	—	1,6		—	4,9		—	4,4	—	4,5	—	3,3		5,3

Table 8: CHANGES IN PERCENTAGE α GLOBULIN IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	16,7	26,3	12,8	10,8	12,6	11,4	10,4
D	16,7	18,6	—	8,8	9,6	8,1	—
E	22,0	14,6	13,4	—	—	—	—
F	15,5	10,1	15,8	—	—	—	—
G	21,5	19,7	16,3	17,6	17,1†	—	—
H	15,7	14,7	12,4	—	—	—	—
I	20,6	14,4	14,3	11,7	—	—	—
J	20,7	15,2	—	—	—	—	—
K	19,7	19,1	12,9	11,4	9,4	10,8	—
M	22,5	13,2	13,5	13,2*	—	—	—
Mean	19,2	16,6	13,9	12,3	12,2	10,1	—
SD	2,7	4,5	1,4	3,0	3,6	1,8	—

*Day 20 †Day 27

Table 10: CHANGES IN PERCENTAGE β GLOBULIN IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	15,3	14,4	17,2	14,9	16,3	16,5	13,5
D	14,3	13,5	—	13,9	12,8	10,4	—
E	15,6	18,7	17,2	—	—	—	—
F	14,6	14,8	15,2	—	—	—	—
G	16,5	13,7	17,8	17,5	21,3†	—	—
H	17,3	15,8	14,0	—	—	—	—
I	13,1	15,9	17,4	17,3	—	—	—
J	15,7	15,1	—	—	—	—	—
K	16,9	16,4	14,2	14,4	14,0	12,8	—
M	17,7	19,1	17,4	14,6*	—	—	—
Mean	15,7	15,7	16,3	15,4	16,1	13,2	—
DS	1,4	1,9	1,6	1,6	3,8	3,1	—

*Day 20 †Day 27

Table 9: CHANGES IN PERCENTAGE α GLOBULIN IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection															
	0	7	11	17	18	20	24	25	28	32	35	39	42	45	46	49
N	16,8	12,1	—	—	13,0	—	13,2	—	—	11,7	—	11,3	—	—	—	12,5
O	12,1	—	14,2	—	11,9	—	12,9	—	—	9,6	—	—	—	—	10,6	12,9
P	16,4	6,1	—	—	—	10,1	—	—	7,9	—	7,2	—	—	14,2	—	8,3
Q	18,9	—	13,0	11,3	—	—	—	9,3	—	8,7	—	7,2	—	—	—	—
R	—	—	12,8	13,5	—	—	—	12,8	—	12,3	—	—	12,4	—	—	—
S	16,1	—	12,1	10,9	—	—	—	12,4	—	10,0	—	—	—	—	—	—
Mean	16,1	9,1	13,0	12,1		—	12,1		—	10,5	—	9,3	—	12,4		11,2
SD	2,5	4,2	0,9	1,1		—	1,6		—	1,5	—	2,9	—	2,5		2,5

the cats showed little change while the percentage rose in 3 of the cats.

(b) Non-splenectomised cats (Table 11)
A decrease in the percentage β globulin was recorded and individual variation was again found. The decrease in individual cats varied from 0,8 to 7,5 percentage points.

5. Percentage γ globulin

(a) Splenectomised cats (Table 12)
In all the cats an increase in percentage γ globulin was recorded. The individual increases varied from 2,7 to 14 percentage points, while there was a mean increase of 10,1 percentage points (22,3% on Day 0 to 32,4 on Day 35).

(b) Non-splenectomised cats (Table 13)
An increase in γ globulin ranging from 11,9 to 27,6 percentage points was recorded in the individual cats, with an increase of 15,6 percentage points in the mean γ globulin (21,0% on Day 0 to 36,6% on Day 45 and Day 46).

Serum alanine amino transferase (SGPT)

(a) Splenectomised cats (Table 14)
In all the cats SGPT increased. This usually occurred during the terminal stages of the disease. The highest individual increase ranged from 26 to 845 U/ℓ while the highest mean increase was 96 U/ℓ. The final level for Cat C on Day 73 was 328 U/ℓ.

(b) Non-splenectomised cats (Table 15)
Apart from fluctuations, the SGPT in 4 of the cats remained unchanged. On the other hand, in Cat N a level of 231 U/ℓ was recorded on Day 24 and a level of only 21 U/ℓ on Day 49. Cat R showed a significant increase in the SGPT from 32 U/ℓ on Day 39 to 664 U/ℓ on terminal Day 42.

Total, direct and indirect serum bilirubin

(a) Splenectomised cats (Table 16)
There was a moderate rise in the total bilirubin levels from Day 14 onwards. The indirect bilirubin predominated at this stage. The highest level recorded (157 μmol1/ℓ) was in Cat M on the terminal Day 20, at which the indirect and direct were approximately equal. When Cat C died on Day 73 a level of 70 μmol1/ℓ total bilirubin was recorded with an indirect bilirubin level of 51 μmol1/ℓ.

(b) Non-splenectomised cats (Table 17)
In 3 of the cats no significant change was recorded in

Table 11: CHANGES IN PERCENTAGE β GLOBULIN IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection															
	0	7	11	17	18	20	24	25	28	32	35	39	42	45	46	49
N	17,8	21,3	—	—	18,0	—	20,9	—	—	17,6	—	17,2	—	—	—	17,0
O	21,1	—	18,5	—	17,7	—	17,6	—	—	18,7	—	—	—	—	17,8	17,9
P	18,4	15,4	—	—	—	12,1	—	—	10,9	—	14,4	—	—	10,9	—	14,8
Q	12,2	—	15,1	14,8	—	—	—	10,8	—	12,9	—	13,9	—	—	—	—
R	—	—	20,0	18,2	—	—	—	15,9	—	17,5	—	—	16,9	—	—	—
S	16,5	—	16,9	16,9	—	—	—	17,3	—	13,8	—	—	—	—	—	—
Mean	17,2	18,4	17,6	17,1	—		16,5		—	16,1	—	15,6	—	14,4		16,6
SD	3,3	4,2	2,1	1,4	—		3,7		—	2,6	—	2,3	—	4,9		1,6

Table 12: CHANGES IN PERCENTAGE γ GLOBULIN IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	13,6	22,4	22,1	21,8	22,9	23,1	22,6
D	22,5	24,1	—	37,8	37,4	36,5	—
E	16,6	18,5	19,3	—	—	—	—
F	31,4	36,5	39,2	—	—	—	—
G	11,9	13,2	12,6	13,6	14,9†	—	—
H	25,1	30,3	33,1	—	—	—	—
I	26,2	28,5	28,4	29,0	—	—	—
J	22,0	24,7	—	—	—	—	—
K	26,3	28,7	36,7	39,5	39,6	37,5	—
M	27,3	27,3	26,5	33,4*	—	—	—
Mean	22,3	25,4	27,2	29,2	28,7	32,4	—
SD	6,4	6,5	9,0	10,0	11,8	8,0	—

*Day 20 †Day 27

Table 14: CHANGES IN SERUM ALANINE AMINO TRANSFERASE (U/ℓ) IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection						
	0	7	14	21	28	35	42
C	16	14	48	58	117	64	44
D	12	—	45	82	94	89	—
E	21	28	47	—	—	—	—
F	12	30	216	—	—	—	—
G	20	25	48	151	179†	—	—
H	13		69	—	—	—	—
I	28	37	64	188	—	—	—
J	32	158	—	—	—	—	—
K	89	82	37	128	158	62	55
M	25	41	174	870*	—	—	—
Mean	27	52	83	246	137	72	50
SD	23	47	65	309	39	15	8

*Day 20 †Day 27

Table 13: CHANGES IN PERCENTAGE γ GLOBULIN IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY O) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection															
	0	7	11	17	18	20	24	25	28	32	35	39	42	45	46	49
N	19,5	22,3	—	—	24,7	—	25,0	—	—	31,4	—	29,7	—	—	—	25,7
O	26,0	—	28,1	—	29,7	—	28,3	—	—	40,7	—	—	—	—	32,5	32,3
P	17,4	21,1	—	—	—	31,3	—	—	33,9	—	38,0	—	—	40,6	—	42,3
Q	16,0	—	25,5	32,2	—	—	—	32,8	—	36,0	—	30,6	—	—	—	—
R	—	—	24,0	24,2	—	—	—	27,0	—	29,5	—	—	24,6	—	—	—
S	26,1	—	32,9	29,6	—	—	—	36,3	—	38,3	—	—	—	—	—	—
Mean	21,0	21,7	27,6	28,1		—	29,9		—	35,2	—	30,1	—	36,6		33,4
SD	4,8	0,8	3,9	3,5		—	4,6		—	4,7	—	0,6	—	5,7		8,3

Table 15: CHANGES IN CERUM ALANINE AMINO TRANSFERASE (U/ℓ) IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection																	
	0	4	7	11	14	17	18	20	24	25	28	32	35	39	42	45	46	49
N	65	—	23	37	—	—	134	—	231	—	—	48	—	23	—	—	21	21
O	27	—	28	57	—	—	50	—	—	57	—	53	—	38	—	—	46	37
P	23	—	30	—	32	—	—	57	—	—	66	—	28	—	28	23	—	—
Q	50	25	—	—	—	28	—	—	—	32	—	18	—	21	—	—	—	—
R	25	21	—	—	—	21	—	—	—	25	—	23	—	32	664	—	—	—
S	21	25	—	39	—	46	—	—	—	38	—	28	—	—	—	—	—	—
Mean	35	24	27	44	—		56	—		77	—	34	—	29	346		30	29
SD	18	2	4	11	—		45	—		87	—	16	—	8	450		14	11

Table 16: TOTAL, DIRECT AND INDIRECT SERUM BILIRUBIN (μmol/ℓ) IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) *B. FELIS* INFECTED BLOOD

Cats	Day 14			Day 21			Day 28			Day 35		
	Tot	Dir	Ind	Tot	Dir	Ind	Tot	Dir	Ind	Tot	Dir	Ind
C	—	–	—	10	2	8	21	—	—	20	–	—
D	13	3	10	14	4	10	11	2	9	15	3	12
E	2	1	1	—	—	—	—	—	—	—	–	—
F	8	3	5	—	—	—	—	—	—	—	–	—
G	3	1	2	16	6	10	25†	18	7	—	–	—
H	10	2	8	—	—	—	—	—	—	—	–	—
I	—	—	—	12	4	8	—	—	—	—	–	—
K	15	6	9	31	10	21	18	5	13	21	6	15
M	17	5	12	157*	81	76	—	—	—	—	–	—
Mean	10	3	7	40	18	22	19	8	10	19	5	14
SD	6	2	4	58	31	27	6	9	3	3	2	2

*Day 20 †Day 27 Tot – Totaal Dir – Direct Ind – Indirect

Table 17: CHANGES IN TOTAL, DIRECT AND INDIRECT SERUM BILIRUBIN (μmol/ℓ) IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days 14-18			Days 20-25			Days 28-32			Days 35-49		
	Tot	Dir	Ind	Tot	Dir	Ind	Tot	Dir	Ind	Tot	Dir	Ind
N	47	7	40	50	28	22	8	—	—	6	—	—
O	7	–	—	5	—	—	8	—	—	4	—	—
P	21	4	17	20	3	17	13	—	—	33	14	19
Q	4	–	—	10	—	—	—	—	—	—	—	—
R	3	–	—	—	—	—	—	—	—	14	—	—
S	6	3	3	—	—	—	3	—	—	—	—	—

Tot – Total Dir – Direct Ind – Indirect

Table 18: CHANGES IN SERUM UREA (mmol/ℓ) IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection					
	0	7	14	21	27	35
C	—	—	6,4	—	—	—
D	—	—	—	10,8	—	14,9
E	—	6,6	7,3	—	—	—
F	28,7	—	16,4	—	—	—
G	—	5,3	—	—	12,3	—
H	7,8	—	4,4	—	—	—
I	5,7	—	—	19,7	—	—
J	—	5,5	—	—	—	—
K	—	4,2	—	—	—	—
M	14,5	—	—	18,2*	—	—

*Day 20

Table 19: CHANGES IN SERUM UREA (mmol/ℓ) IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection								
	0	3	17	24	32	39	42	45	49
N	7,0	—	—	7,5	6,8	—	—	—	9,6
O	—	—	—	7,5	—	—	—	—	9,6
P	—	8,6	8,6	—	—	—	—	8,0	30,2
Q	8,0	—	10,9	—	—	13,9	—	—	—
R	8,4	—	7,3	—	—	—	16,2	—	—
S	7,0	—	11,1	—	8,6	—	—	—	—

the bilirubin levels (mean 5 μmol/ℓ). In 3 of these cats (N, P and R), the levels of total bilirubin rose moderately, the highest levels being 50 μmol/ℓ (Cat N), 33 μmol/ℓ (Cat P) and 14 μmol/ℓ (Cat R).

Serum cholesterol

The serum cholesterol levels remained essentially unchanged throughout the experiment in both the splenectomised and non-splenectomised cats. The levels ranged from 2,03 to 5,54 mmol/ℓ (mean 3,60 ± 0,70 mmol/ℓ).

Serum urea (Tables 18 and 19)

The serum urea remained essentially unchanged in both the splenectomised and non-splenectomised cats. A moderate rise was noted in 3 splenectomised cats and one non-splenectomised cat at the terminal stage. The highest value recorded (30 mmol/ℓ) was observed in Cat P on the terminal Day 49. In Cat F 16,4 mmol/ℓ was recorded on Day 14, while on Day 0 the level was 28,7 mmol/ℓ. In Cat I a level of 19,7 mmol/ℓ was recorded on Day 21.

Serum creatinine (Table 20 and 21)

The serum creatinine remained essentially unchanged in both the splenectomised and non-splenectomised cats. The levels ranged from 58 to 187 μmol/ℓ (mean 109 ± 32 μmol/ℓ. In Cat F the initial creatinine was 171 μmol/ℓ with a level of 187 μmol/ℓ on Day 14. Cat I also showed a raised level of 186 μmol/ℓ on Day 21.

Table 20: CHANGES IN SERUM CREATININE (μmol/ℓ) IN SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. Felis* INFECTED BLOOD

Cats	Days after infection					
	0	7	14	21	27	35
C	—	—	121	—	—	—
D	—	—	—	123	—	120
E	—	113	106	—	—	—
F	171	—	187	—	—	—
G	—	83	—	—	58	—
H	85	—	125	—	—	—
I	135	—	—	186	—	—
J	—	137	—	—	—	—
K	—	127	—	—	—	—
M	77	—	—	63*	—	—

*Day 20

Table 21: CHANGES IN SERUM CREATININE (μmol/ℓ) IN NON-SPLENECTOMISED CATS FROM DAY OF INOCULATION (DAY 0) WITH *B. FELIS* INFECTED BLOOD

Cats	Days after infection								
	0	3	17	24	32	39	42	45	49
N	83	—	—	94	89	—	—	—	79
O	—	—	—	75	—	—	—	—	102
P	—	146	135	—	—	—	—	90	91
Q	116	—	103	—	—	58	—	—	—
R	92	—	108	—	—	—	89	—	—
S	104	—	96	—	94	—	—	—	—

Urine analysis (Table 22)

(a) Splenectomised cats
Slight proteinuria was detected in all cases. Bilirubinuria was detected in 3 cats and haematuria in 5 cats, with 4 of these showing raised urobilinogen.

(b) Non-splenectomised cats
Slight proteinuria was detected in all cases. Bilirubin was not detected and only traces of urobilinogen were found.

Blood pH

The blood pH remained within normal limits in both splenectomised and non-splenectomised cats throughout the experiment (mean 7,35 ± 0,06). Minor reductions (as low as 7,14) occurred in cats at the terminal stages or in those that struggled during bleeding.

Macroscopic pathology

The *post mortem* findings were characterised by extreme pallor of the viscera and thin watery blood. Marked icterus was only seen in 2 cats which were all from the splenectomised group (Cats C and M), while slight icterus was observed in six cats (D, H, I, J, K, and P). The liver was often enlarged and frequently yellow or dark brown. In a number of cases the liver surface had a mottled appearance. Cat R, with no evidence of icterus, had a yellow mottled liver which, when handled, stained one's hands. Occasionally distension of the gall bladder was present.

The intestinal tract usually contained thick yellow or brownish bile and the rectal contents were invariably yellow to orange. Urine, when present, was golden yellow with haematuria present in some of the splenectomised cats. Ample body-fat was usually present. Enlarged mesenteric lymph nodes were occasionally seen. Inspissated brown foetuses were found in Cat M which had marked icterus and liver necrosis. There was no essential difference in gross pathology between the 2 groups.

Histopathology

There was no specific uniform pattern in the histopathology. Except for Cat Q which had a normal liver, the most consistent finding in the livers of the other cats was varying degrees of centrilobular necrosis, bile stasis and extramedullary haematopoiesis. Pigment which was considered most likely to be haemosiderin was found in the liver, while an unidentified pigment was also found in the kidneys which were otherwise considered normal.

Effects of splenectomy

The effects of splenectomy on serum proteins and SGPT are presented in Table 23.

Clinical cases

For the purpose of this study the clinical cases were grouped into three categories according to the haematocrits recorded in a previous paper:[3]
Group 1: Cats with Ht greater than 0,16
Group 2: Cats with Ht 0,13 to 0,16
Group 3: Cats with Ht less than 0,13

Table 22: ANALYSES OF URINE TAKEN AT POST MORTEM EXAMINATION FROM SPLENECTOMISED (1) AND NON-SPLENECTOMISED (2) CATS INFECTED WITH *B. FELIS*

	Cats	Osmolality mOsm/kg	pH	Proteins	Glucose	Ketones	Blood	Urobilinogen	Bilirubin
1	C	1157	6	+	Neg	Neg	Neg	4	++
	D	1764	6	++	Neg	Neg	+	1	Neg
	F	810	6	++	Neg	Neg	Neg	0,1	Neg
	G	1737	6	++	+++	Neg	Neg	8	++++
	I	754	6,5	++	Neg	Neg	+++	0,1	Neg
	J	1136	6	++	Neg	Neg	++	0,1	+++
	K	—	5	++	Neg	Neg	+++	4	Neg
2	Q	2527	6	++	Neg	Neg	Neg	Neg	Neg
	R	1013	5	+	Neg	Neg	Neg	0,1	Neg
	S	1331	6	+	Neg	Neg	+	1	—

Table 23: SERUM PROTEINS AND SGPT PRE-SPLENECTOMY AND 14 DAYS POST-SPLENECTOMY

	Pre-splenectomy	Post-splenectomy	Significant differences
TSP (g/ℓ)	65,2 ± 11,6	69,9 ± 4,7	NS
% Alb	51,4 ± 17,2	40,6 ± 6,5	NS
% Glob	48,6 ± 17,2	59,4 ± 6,5	NS
% α Glob	16,5 ± 5,5	20,1 ± 2,6	$p < 0,05$
% β Glob	12,7 ± 3,8	16,8 ± 0,8	$p < 0,05$
% γ Glob	19,4 ± 12,9	22,5 ± 6,3	NS
SGPT (U/ℓ)	47,0 ± 28,0	36,0 ± 30,0	NS

NS – Not significant

Chemical pathology at initial diagnosis (Table 24)

1. SGPT

In Group 1, 14 of the 18 cats had values within the normal range (0–112 U/ℓ). An individual value of 377 U/ℓ affected the mean value of the group (105 U/ℓ ± 82). In Group 2, 4 of the 7 cats had values within the normal range. One animal had a level of 1 086 U/ℓ, the highest recorded in all the initial field cases. In Group 3, 5 out of 12 values were within the upper limits of the normal range, while a mean value of 186 U/ℓ (± 100) was recorded.

2. Total, direct and indirect serum bilirubin

The mean total bilirubin was raised in all 3 groups. In Group 1, 12 of the 20 total bilirubin values were within the normal range and 2 only marginally raised. However, one value of 143 μmol/ℓ raised the mean value to 15 μmol/ℓ (± 31), whereas this value would have been 8 μmol/ℓ without this aberrant figure. At the levels of about 20 μmol/ℓ the indirect values were higher than direct, whereas at higher levels the direct and indirect values were about equal. In Group 2, 7 of the 12 total bilirubin values were in the normal to near-normal range, while the other 5 were increased. The highest value (261 μmol/ℓ) of the 3 groups was recorded in this group. At total bilirubin levels of 27 μmol/ℓ and 43 μmol/ℓ the indirect was predominant, at 105 μmol/ℓ the indirect and direct were approximately equal, and at 261 μmol/ℓ the direct was predominant. In Group 3, 4 of the 14 total bilirubin values were within the normal range. Indirect bilirubin values were higher than direct in all cases.

3. Serum creatinine

The mean serum creatinine levels in all 3 groups were within the normal range. Only 5 raised individual values (159–207 μmol/ℓ) were recorded.

4. Serum urea

Serum urea remained essentially unchanged in Groups 1 and 2. The mean value of 13,7 mmol/ℓ (± 7,6) recorded in Group 3 was influenced by an individual high value of 37,6 mmol/ℓ.

5. TSP

TSP values were within the normal range in all cases.

6. Serum cholesterol

Serum cholesterol values were within the normal range in all cases.

Chemical pathology during recovery

1. SGPT (Table 25)

In the majority of cases, SGPT was lower when blood was taken in recovery stages of the disease. In 6 cases, however, the levels were raised.

2. Bilirubin (Table 26)

A rapid recovery to normal levels was recorded. As can be seen in one cat, a drop of 135 μmol/ℓ was recorded 11 days after admittance. However, in 3 cases the levels rose slightly.

Relapses

The haematology and clinical pathology of these cases fell within the limits found for the initial infection, ie Hb 3 to 11 g/dℓ; Ht 0,09 to 0,25; TSP 65 to 94 g/ℓ; Tot

Table 24: AGE, HAEMATOLOGY AND CHEMICAL PATHOLOGY OF 70 CLINICAL CASES OF *B. FELIS* INFECTION AT INITIAL DIAGNOSIS

	Group 1 Ht > 0,16			Group 2 Ht 0,13 – 0,16			Group 3 Ht < 0,13		
Age (months)	28	± 27	(26)	19	± 24	(19)	27	± 37	(25)
Ht	0,22	± 0,06	(26)	0,14	± 0,11	(19)	0,11	± 0,11	(25)
Hb (g/dℓ)	6,4	± 2,0	(26)	4,3	± 0,5	(19)	3,2	± 0,5	(25)
MCHC (g/dℓ)	29	± 3,0	(26)	30	± 4,0	(19)	30	± 3,0	(25)
SGPT (U/ℓ)	105	± 82	(18)	287	± 366	(7)	186	± 100	(12)
Tot Bil (μmol/ℓ)	15	± 31	(20)	42	± 75	(12)	25	± 18	(14)
Dir Bil (μmol/ℓ)	7	± 16	(20)	20	± 40	(12)	5	± 3	(14)
Ind Bil (μmol/ℓ)	8	± 15	(20)	22	± 35	(12)	20	± 15	(14)
TSP (g/ℓ)	77	± 10	(20)	79	± 11	(12)	73	± 13	(14)
S Chol (mmol/ℓ)	3,36	± 0,52	(20)	3,74	± 0,10	(12)	3,46	± 0,93	(14)
S Creat (umol/ℓ)	102	± 34	(20)	127	± 41	(12)	106	± 32	(14)
S Urea (mmol/ℓ)	8,9	± 3,9	(20)	10,40	± 3,0	(12)	13,7	± 7,6	(14)

Figures in brackets denote the number of animals.

Table 25: INITIAL (I) AND RECOVERY (R) SGPT (U/ℓ) LEVELS OF FIELD CASES OF *B. FELIS*

Group 1			Group 2			Group 3		
I	R	Days	I	R	Days	I	R	Days
131	86	17	55	67	12	213	141	10
96	72	7	103	31	10	97	215	8
99	45	7	119	1650	9	299	144	12
117	369	19	366	123	7	312	108	8
197	20	8	111	63	7	117	69	6
52	36	7				138	43	9
72	82	4				404	271	4
377	456	7						
29	29	4						
82	109	11						
56	54	4						

Table 26: INITIAL (I) AND RECOVERY (R) TOTAL SERUM BILIRUBIN (μmol/ℓ) LEVELS OF FIELD CASES OF *B. FELIS*

Group 1			Group 2			Group 3		
I	R	Days	I	R	Days	I	R	Days
11	4	17	6	4	12	32	5	10
10	3	7	17	5	10	42	9	8
7	6	7	43	8	9	25	10	12
3	1	19	11	4	7	5	3	8
17	1	8	10	2	7	8	3	6
8	2	7	2	2	11	24	2	9
3	24	4	6	11	8	39	22	4
24	1	7				71	7	7
3	10	4						
143	8	11						
6	2	4						

bil 6 to 19 μmol/ℓ; Dir bil 3 to 20 μmol/ℓ; Ind bil 3 to 25 μmol/ℓ; SGPT 114 to 522 U/ℓ; S chol 244 to 422 mmol/ℓ; S urea 12,2 to 14,5 mmol/ℓ; S creat 102 to 141 μmol/ℓ.

DISCUSSION

Serum proteins

Although the liver plays a major role in the anabolism and catabolism of serum proteins, changes in the protein levels occur only in the later stages of liver damage[10].

No real changes were recorded in the TSP of all the experimental cats and field cases. Amongst the experimental non-splenectomised cats there was a moderate increase in globulin with a concomitant decrease in albumin. This did not occur in the splenectomised cats and therefore the albumin:globulin ratio remained essentially unchanged.

Among the globulins, definite changes were found and in both the splenectomised and non-splenectomised cats, γ globulin rose while α and β globulins decreased. A similar position has been reported for canine babesiosis[7]. The increase in γ globulins is probably the antibody response of the reticulo-endothelial system to the babesia antigen[19] and it is interesting to note that the reaction in the non-splenectomised animals was, not unexpectedly, greater than in the splenectomised animals.

SGPT

In the cat and dog an increased SGPT level is a specific indication of hepatocellular necrosis or inflammation[5]. Only moderate elevations in SGPT were recorded in the splenectomised cats. Two of the non-splenectomised cats showed raised levels; Cat N 134 U/ℓ on Day 18 and 231 U/ℓ on Day 24; Cat R 664 U/ℓ on Day 42. The highest level (870 U/ℓ) was found in Cat M which had inspissated foetuses.

Of 37 clinical cases, only 14 had raised SGPT levels. It is interesting to note that in the severely anaemic Group 3 animals, 5 of the cats had normal values and the others were only moderately raised. During the recovery stage most of the SGPT levels returned rapidly to normal, although in 5 cats the levels actually rose (in 1 cat from 119 to 165 U/ℓ). In limited experiments on the toxicity of Primaquine (Primaquine phosphate, ICI), SGPT levels have all risen. As these clinical cases initially received fairly high doses of Primaquine, this could be an explanation for the increased SGPT levels.

Serum bilirubin

Moderate increases were recorded in many of the experimental cats. The indirect value was usually higher than the direct, which is to be expected in a haemolytic anaemia. At times this increase in bilirubin followed a rapid decrease in Ht. Cat M showed gross changes.

Among the non-splenectomised animals Cat N showed the highest bilirubin values. A level of 47 μmol/ℓ was recorded on Day 18 (Ht 0,07) and at this stage the indirect value predominated. On Day 24 (Ht 0,11) a value of 50 μmol/ℓ was recorded and at this stage the direct value was slightly higher than the indirect. However, on Days 32 and 49 the total values had fallen to 8 and 6 μmol/ℓ, respectively. It is interesting to note that in this case SGPT (231 U/ℓ) and bilirubin (50 μmol/ℓ) were both at their peak values on the same day and both decreased thereafter. These changes are classical for a haemolytic anaemia with subsequent hepatocellular damage due to anoxia.

An increase in serum bilirubin was recorded in 23 of 47 field cases which were analysed. Sixteen of the 23 cats showed only slight increases, 4 cats showed moderate increases and 2 animals had markedly raised values. It is interesting to note that in Group 3, 4 values were within normal limits and in others the indirect levels predominated. This would explain the lack of clinical icterus as this is not seen unless hepatocellular damage is present. During the recovery stage the serum bilirubin levels returned to normal, confirming the exceptional regenerative ability of the liver.[4]

Serum Cholesterol

No significant changes were noticed in the serum cholesterol in experimental and clinical cases.

Serum urea

No significant changes were noted in the serum urea in experimental and clinical cases except in the terminal stages in a few animals. These findings are contrary to those found in canine babesiosis, where renal damage is considerable and frequently encountered[8].

Serum creatinine

Serum creatinine was within the normal limits in both experimental and clinical cases, once again indicating that renal damage is not a feature of feline babesiosis.

Urine analysis

It is important to point out that the urine was only taken for analysis at *post mortem* examination.

In the non-splenectomised cats, the urine analysis showed only slight proteinuria and traces of urobilinogen. This can be considered a normal finding.

In the splenectomised cats bilirubinuria was detected in 3 cats and haemoglobinuria in 4 cats. Bilirubinuria is not a common finding in the cat, as the renal threshold for bilirubin is high. Thus bilirubinuria is considered to be an indication of gross intravascular haemolysis and hepatocellular disease[6]. Haemoglobinuria is also indicative of acute intravascular haemolysis.

Blood pH

The cats were generally able to maintain the blood pH within the normal range of 7,25 to 7,40.[12] This means that if there are any acid-base changes in feline babesiosis, the body is quite capable of compensating. Further investigations into this matter are required.

Post mortem findings

At *post mortem* examination, the urine of the non-splenectomised animals was normal while in some of the splenectomised cats the presence of haemoglobinuria and bilirubinuria indicated gross intravascular haemolysis and hepatocellular damage.

Other *post mortem* findings included extreme pallor of the viscera, thin watery blood and yellow to orange rectal faeces. Marked icterus was only seen in 2 cats, while slight icterus was observed in 6 cats. The liver was often enlarged and frequently yellow or dark brown in colour. Ample body fat was usually present. The most consistent histopathological finding was hepatic centrilobular necrosis, bile stasis and extramedullary haematopoiesis.

The effects of splenectomy

Initially cats were splenectomised to facilitate transmission of the parasite[12]. However, it was soon found that this was unnecessary as transmission was as successful in the whole non-splenectomised cat.

Splenectomy *per se* caused a moderate ($p < 0,05$) increase in α and β globulins. Nevertheless, the general course and effects of the disease were similar in the splenectomised and non-splenectomised animals although the latter were not as severely affected. In addition, the rise in γ globulins was, not unexpectedly, higher in the non-splenectomised animals. It is of interest to note once again that, contrary to expectation, splenectomised Cats C, 1 and 2 survived the longest amongst all the experimental cats.

ACKNOWLEDGEMENTS

We wish to thank the Clinical Pathology Laboratory of Tygerberg Hospital for analysing some of the field samples and Dr R McCully of the Medical School of the University of Cape Town for his generous assistance with the histopathology.

REFERENCES

1. Dimopoullos G T 1961 A Serum Protein and Antibody study in viral disease; a Review. Annals of the New York Academy of Science 94: 149–161
2. Futter G J, Belonje P.C. 1980 Studies on feline babesiosis 1: Historical review. Journal of the South African Veterinary Association 51: 105–106
3. Futter G J, Belonje P.C. 1980 Studies on feline babesiosis 2. Clinical observations. Journal of the South African Veterinary Association 51: 143–146
4. Futter G J, Belonje P C, Van den Berg A. 1980 Studies on feline babesiosis 3. Haematological findings. Journal of the South African Veterinary Association 51: 271–280
5. Hardy R M 1975 Diseases of the liver. In: Ettinger (ed) Textbook of Veterinary Internal Medicine, Diseases of the Dog and Cat Vol. 2. Saunders W B Company, Philadelphia
6. Jones B D 1977 Diseases of the liver. In: Proceedings of the American Animal Hospital Association 44: 201–208
7. Malherbe W D 1950 The management of drugfast biliary fever in dogs. Journal of the South African Veterinary Medical Association 21: 158–159
8. Malherbe W D 1968 A Clinical-pathological study of Babesia Canis infection in dogs. DVSc Thesis, University of Pretoria.
9. Peterman M L 1961 Plasmoprotein in bacterial infections. Annals of the New York Academy of Sciences 94: 144–148
10. Schalm O W, Jain N C, Carroll E.S. 1975 Veterinary Haematology 3rd ed. Les & Febiger, Philadelphia
11. Snedocor G W, Cochran W G 1967 Statistical methods 6th ed. Iowa State University Press, Ames
12. Spector W S 1956 Handbook of Biological Data. Saunders, Philadelphia
13. Swenson M J 1977 Physiological properties and cellular and chemical constituents of blood. In: Swenson M J (ed) Physiology of Domestic Animals 9th ed. Cornell University Press, Ithaca
14. Weichselbaum T E 1946 An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American Journal of Clinical Pathology 16: 40–49

PRE- AND POST-WEANING PERFORMANCE OF ARTIFICIALLY REARED CALVES. II THE EFFECTS OF MILK SUBSTITUTE AND MILK SUBSTITUTE-WHEY DIETS ON THE PERFORMANCE OF CALVES WITH PARTICULAR REFERENCE TO THE SELECTION OF CALVES WITH IMPROVED GROWTH POTENTIAL FROM THEIR BLOOD COMPOSITION A WEEK AFTER BIRTH

S. COUVARAS

ABSTRACT: Couvaras S. **Pre- and post-weaning performance of artificially reared calves. II. The effects of milk substitute and milk substitute-whey diets on the performance of calves with particular reference to the selection of calves with improved growth potential from their blood composition a week after birth.** *Journal of the South African Veterinary Association* **52** No. 1, 15–19 (En) Dept. of Zootechnology, Faculty Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110, Rep. of South Africa.

Sixteen Friesland bull calves were divided into two groups and raised artificially in an early weaning system. The liquid feeding of one group consisted of a commercial milk substitute and that of the other group of a 50:50 milk substitute-whey mixture. Blood samples were taken at regular intervals, starting one week after birth, and analysed for packed cell volume (PCV), blood glucose and haemoglobin (Hb); serum albumin, total protein, urea, inorganic phosphate (Pi), Ca, Mg, Na and K.

The liquid portion of the diet had no significant effect on either the blood composition of the calves or on their performance (mass gain, dry matter intake and feed conversion) at the end of the experimental period (week 13). The only significant difference ($P<0,05$) between the two groups was the body mass of the calves at weaning.

There were significant changes in the concentrations of most blood constituents with age ($P<0,001$ for Hb, PCV, albumin, globulin, Pi and K; $P<0,01$ for urea and Ca). A significant correlation ($P<0,05$) was also found between the calves' growth rates from 1 to 13 weeks and the concentration of globulin at one week of age. The regression of growth rate from 1 to 13 weeks with the blood constituents, globulin, Hb, PCV, Pi, Mg and Ca at one week of age accounted for 67,4% of the variance among calves.

The evidence suggests that the above types of liquid feeding in an early weaning system would not significantly affect the composition of the blood of calves when sampled approximately six weeks or more after weaning, provided that such calves are offered the same dry ration, and that metabolic profiles during the first week of life may provide a predictive assessment of subsequent growth performance.

INTRODUCTION

In a previous paper[2] it was pointed out that the artificial rearing of calves on dairy farms either for heifer replacements or for veal production could, depending on the cost of the milk replacer used and the calf mortality rate, be a profitable supplementary enterprise, but that systems for hand-raising calves economically in South Africa still have plenty of room for improvement.

Interest in this subject has resulted in extensive research on the one hand on nutritional aspects such as the utilization and effect of either whole milk or milk replacer (with or without varying amounts of fat) on the pre- and post-weaning performance of calves[4 8 13 17] and on the other hand on veterinary aspects such as the causes, effects, prevention and treatment of diarrhoea in calves[3 6 7 11 15].

Recently, Rowlands *et al.* (1974)[16] and Little *et al.* (1977)[9] have demonstrated significant correlations between the mass gain of calves and the concentrations of various constituents of their blood, which suggests that blood composition might be a useful aid in the selection of stock with improved growth potential. However, the calves in the first-mentioned work were fed *ad libitum* in groups, and consequently the feed intake of individuals could not be measured. In the latter work, which was designed to investigate the influences of appetite and efficiency of feed conversion on the blood composition of calves, all animals investigated were reared on the same plane of nutrition. Although several workers[5 10] have investigated the effect of a high and low plane of nutrition on the blood composition of dairy calves, these investigations were performed on animals three months and older.

The experiment described in this paper was designed to investigate the effect of the plane of nutrition (as influenced by the level of energy, protein and other nutrients supplied by the liquid portion of the diet) on live-mass gain, feed intake and efficiency of feed conversion of calves reared artificially in an early-weaning system. In view of the renewed interest in recent years in feeding liquid whey not only to pigs but also to cattle, a first aim was to compare the performance of calves fed a milk substitute-whey mixture to that of calves receiving only the milk substitute. For many small cheese factories, the cost of drying whey is high, and selling or even giving their liquid whey back to farmers to use as a feed is generally a favourable option for such plants.

A second aim was to determine to what extent metabolic profiles of calves made during the first week of age may provide a predictive assessment of subsequent growth performance. Such a tool would be useful since it would then be possible to screen calves during the first week of age for superior profile characteristics.

PROCEDURE

Animals and management

Sixteen Friesland bull calves (obtained from a local dairy) with an average age of one week and with a mean body weight of 38,7 ± 5,3 kg were divided into two groups with the same body weight (38,7 ± 4,8 kg and 38,7 ± 6,1 kg, respectively) by means of stratified sampling. The two groups were randomly allotted to a diet of which the liquid feeding consisted of either a commercial milk substitute (Nukamel R^{II}) (diet A) or a 50:50 mixture (on a volume basis) of the same milk substitute (Nukamel R^{II}) and whey (diet B). The system of management and feeding of the calves in this study was similar to that reported previously[2]. The

0038-2809/81/01/0015–0019

Table 1: THE CHEMICAL COMPOSITION OF THE MILK SUBSTITUTE AND MILK SUBSTITUTE-WHEY DIETS (VALUES EXPRESSED ON AN AS-FED BASIS)

Item	Milk substitute (Diet A)	Milk substitute + whey (Diet B)
	%	%
Dry matter	14,33	10,39
Crude protein	3,69	2,30
Lactose	7,22	6,11
Fat	1,89	0,97
Ash	1,16	0,93
Calcium	0,14	0,09
Phosphorus	0,12	0,08

chemical composition of the two liquid diets (A and B) is presented in Table 1.

Water and a calf starter-meal was supplied *ad libitum* and was made available after the first week. The calf starter-meal presented was formulated, using NRC (1971)[12] requirements for growing calves up to the age of 12–13 weeks. The ingredients and amounts used were based on the recommendations of Van der Merwe (1977)[20]. A chemical analysis of this starter meal was obtained from the Department of Animal Husbandry of the University of Pretoria (Table 2).

Calves were weighed at weekly intervals and the meal consumed by individual calves was recorded daily. Weaning, which was abrupt, took place when calves consumed 0,7 kg per day or more of the meal on two consecutive days and had reached a mass of at least 50 kg.

Table 2: COMPOSITION AND ANALYSIS OF THE STARTER MEAL AND ITS CALCULATED DIGESTIBLE ENERGY CONTENT

Item	%
Ingredient:	
Yellow maize meal	66,0
Soyabean oilcake meal	16,0
Fish meal	6,0
Lucerne meal	11,0
Mineral premix*	1,0
Chemical analysis:	
Dry matter	89,6
Crude protein	14,94
Crude fibre	5,22
Crude fat	4,73
Ash	7,26
Calcium	0,898
Phosphorus	0,671
Digestible energy (MJ/kg)	13,998

*The mineral premix consisted of 50% finely ground stock salt and 50% Fermafos-12P (a commercial phosphate-trace element mixture).

Blood sampling and analysis

Jugular blood samples were taken from each calf between 08h30 and 10h30 on each Tuesday morning beginning during the first week of life. The blood samples were analysed by the Department of Medicine, Faculty of Veterinary Science of the University of Pretoria. The constituents analysed and methods used were the following:

Haemoglobin (Hb) was estimated by the cyanhaemoglobin method, using a haemoglobinometer (Coulter Electronics).
Packed cell volume (PCV) was carried out, using a Roto Uni II (PHG) microhaemoglobin centrifuge.
Blood glucose was estimated with o-toluidin, using the Lange cuvette test.
Urea was determined enzymatically (urease).
Albumin was estimated by microzone electrophoresis (Beckman; Model R-101).
Globulin was estimated as the arithmetical difference between serum total protein and serum albumin values. Total protein was estimated by refractometer (TS meter; American Optical).
Serum inorganic phosphate (Pi) was estimated with the Lange Kit, using molybdic acid and p-semidine.
Calcium (Ca) was estimated photometrically, using glyoxal-bis (2-hydroxyanil) in methanol.
Magnesium (Mg) was estimated with a spectrophotometer, using xylidyl blue in ethanol.
Sodium (Na) and potassium (K) were estimated with a Zeiss (PMQ II) spectrophotometer (Flame photometer).

Statistical analysis

The effect of age on concentrations of each blood constituent from the first to the 13th week of life, together with the effect of type of liquid diet on body mass at weaning, body weight at 7 weeks, body mass at 13 weeks, age at weaning, mass gain (0–7 weeks and 0–13 weeks), dry matter intake (0–13 weeks), feed conversion ratio (0–13 weeks) and each blood constituent (0–13 weeks) was estimated by analysis of variance[19]. Multiple regression analysis[19] was carried out, relating growth rate to blood chemistry at one week of age.

RESULTS AND DISCUSSION

The means and standard deviations of body masses at birth, weaning, 7 and 13 weeks of age, age at weaning, mass gains, feed intake and feed conversion ratios (kg dry matter intake per kg mass gain) for the two planes of nutrition are given in Table 3. The only significant difference ($P<0,05$) between the two groups was the body mass of the calves at weaning. This was due to the fact that calves receiving the milk substitute-whey mixture (low plane of nutrition) consumed more starter meal at an earlier age than the group receiving no whey with the milk substitute (high plane of nutrition). However, by week 13 they had compensated by taking in more dry matter (118,65 ± 42,17 kg DM versus 112,47 ± 30,94 kg DM, which was not significantly different ($P<0,05$), so that at the end of the experimental period (week 13), both groups had approximately the same end mass (92,4 ± 23,2 kg and 93,6 ± 19,6 kg for the milk substitute-whey and milk substitute groups, respectively).

The means, ranges, standard deviations and coefficients of variation of blood constituents sampled during the first week of life and at the end of the experimental period (week 13) are given in Table 4. There were significant changes in the concentrations of most blood

Table 3: MEANS AND STANDARD DEVIATIONS OF BODY MASSES, AGE AT WEANING, LIVE MASS GAIN, DRY-MATTER INTAKE AND FEED CONVERSION RATIO FOR THE TWO GROUPS OF CALVES

	Group raised on milk substitute only Mean ± S.D.	Group raised on milk substitute + whey Mean ± S.D.
Body mass (kg)		
Birth	38,7 ± 4,8	38,7 ± 6,1
Weaning	61,9 ± 4,8*	56,1 ± 3,9†
7 weeks	61,6 ± 13,9	58,8 ± 13,7
13 weeks	93,6 ± 19,6	92,4 ± 23,2
Age at weaning (days)	52 ± 16	48 ± 22
Mass gain (kg per day)		
0–7 weeks	0,50 ± 0,19	0,42 ± 0,19
0–13 weeks	0,63 ± 0,16	0,60 ± 0,20
Dry-matter intake (kg)		
0–13 weeks	112,47 ± 30,94	118,65 ± 42,17
Feed conversion ratio		
0–13 weeks (kg dry matter intake/kg mass gain)	2,06 ± 0,14	2,24 ± 0,21

*,†Means with different superscripts are different ($p<0,05$)

constituents with age ($P<0,001$ for Hb, PCV, albumin, globulin, Pi and K; $P<0,01$ for urea and Ca). These are illustrated in Figure 1.

The effect of plane of nutrition (diet A vs. diet B) on the blood composition of calves at week 13 is summarized in Table 5. No significant differences in any of the constituents analyzed were found. It seems therefore that the type of liquid feeding in an early weaning system would not significantly affect the composition of the blood of calves when sampled approximately six weeks or more after weaning, provided of course that such calves are offered the same starter-meal (or dry ration)[5].

Simple correlation coefficients between blood constituents sampled during the first week after birth are shown in Table 6. Significant correlations ($P<0,05$) are 0,41 or greater.

There were several significant correlations between blood constituents, involving particularly Hb, albumin and Ca. The high negative correlation of Ca and albumin and the positive correlation between albumin and globulin is in contrast to that found by Rowlands *et al.* (1974)[16] in calves and by Payne *et al.* (1973)[14] in dairy herds. The above-mentioned authors reported a high positive correlation between Ca and albumin and a negative correlation between albumin and globulin.

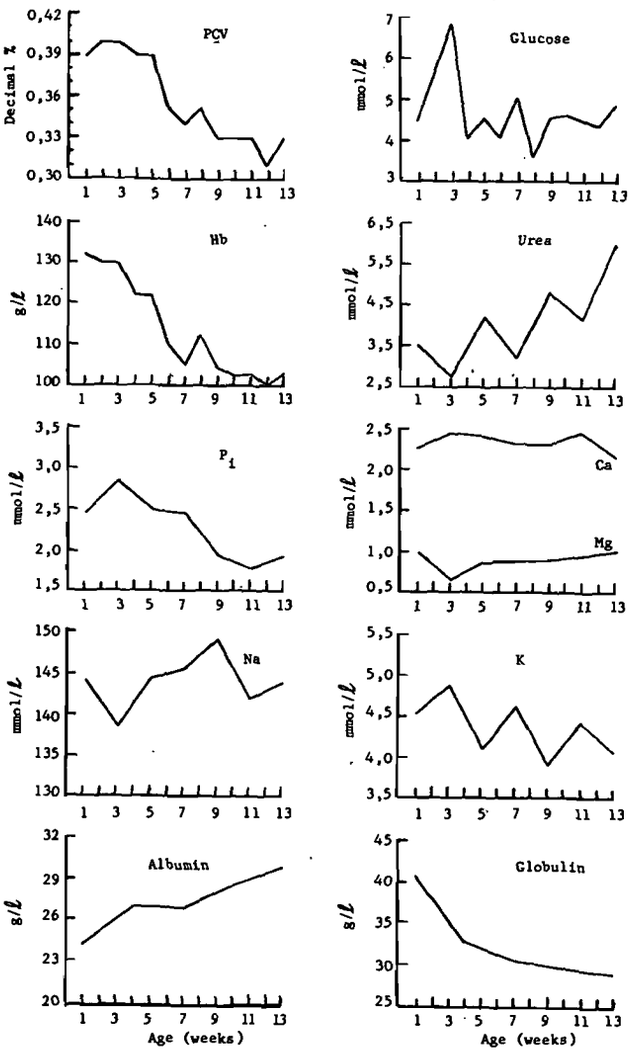


Fig. 1 Change in the mean PCV and concentrations of blood Hb and glucose and serum urea, Pi, Mg, Ca, Na, K, albumin and globulin with age for 16 calves.

Table 4: MEANS, RANGES, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF BLOOD CONSTITUENTS SAMPLED DURING WEEK 1 AND WEEK 13 ($n=16$)

Age at sampling:	1 week				13 weeks			
	Means	Range	S.D.	C.V.	Means	Range	S.D.	C.V.
Hb (g/ℓ)	131,94*	100 –195	19,65	14,89	102,63†	90–113	5,29	5,15
PCV (decimal %)	0,39*	0,20– 0,49	0,06	14,91	0,33†	0,29– 0,37	0,02	6,14
Glucose (mmol/ℓ)	4,47	2,66– 7,22	1,08	24,18	4,90	3,94– 5,77	0,59	12,01
Urea (mmol/ℓ)	3,50‡	2,14– 11,80	2,47	70,43	5,98§	4,28– 11,06	1,85	31,01
Albumin (g/ℓ)	24,24*	18,60– 30,90	3,26	13,45	29,99†	24,00– 36,00	3,24	10,81
Globulin (g/ℓ)	40,31*	23,50– 61,30	11,34	28,13	28,73†	23,10– 40,50	4,23	14,72
Pi (mmol/ℓ)	2,45*	2,07– 2,97	0,28	11,23	1,93†	1,10– 2,26	0,32	16,35
Ca (mmol/ℓ)	2,26‡	2,00– 2,50	0,13	5,85	2,14§	1,93– 2,40	0,11	5,31
Mg (mmol/ℓ)	1,00	0,66– 2,67	0,48	48,17	1,04	0,90– 1,19	0,08	7,33
Na (mmol/ℓ)	143,96	133,8 –160,0	6,91	4,80	138,84	128,6 –149,4	7,44	5,36
K (mmol/ℓ)	4,52*	4,10– 4,90	0,23	5,12	4,07†	3,80– 4,30	0,17	4,28

*,†Means with different superscripts are different ($p<0,001$)
‡,§Means with different superscripts are different ($p<0,01$)

Table 5: THE EFFECT OF PLANE OF NUTRITION ON THE BLOOD COMPOSITION OF CALVES SAMPLED AT 13 WEEKS OF AGE

Constituent	Diet A Means ± S.D.		Diet B Means ± S.D.		F value (1,14 df)
Hb (g/ℓ)	101,38	5,97	103,88	4,55	0,89
PCV (decimal %)	0,32	0,02	0,33	0,02	1,29
Glucose (mmol/ℓ)	4,89	0,51	4,92	0,69	0,01
Albumin (g/ℓ)	29,74	3,19	30,25	3,49	0,09
Globulin (g/ℓ)	27,08	2,63	30,39	5,02	2,74
Urea (mmol/ℓ)	6,07	2,12	5,89	1,69	0,03
Pi (mmol/ℓ)	1,86	0,39	2,00	0,22	0,85
Ca (mmol/ℓ)	2,15	0,09	2,13	0,14	0,13
Mg (mmol/ℓ)	1,03	0,05	1,05	0,10	0,37
Na (mmol/ℓ)	140,70	7,73	136,98	7,12	1,00
K (mmol/ℓ)	4,10	0,18	4,04	0,18	0,50

Table 7: SUMMARY OF THE MULTIPLE REGRESSION ANALYSIS RELATING GROWTH RATE (1 TO 13 WEEKS) WITH BLOOD ANALYSIS AT ONE WEEK OF AGE

Blood constituent	Regression coefficients (with standard errors)	Differences in growth performance across the 95% confidence ranges of the blood constituent concerned
Globulin (g/ℓ)	−0,0179 ± 0,0063	−0,21 kg per day
Hb (g/ℓ)	0,0224 ± 0,0104	+0,47 kg per day
PCV (decimal %)	−6,551 ± 3,500	−0,39 kg per day
Pi (mmol/ℓ)	−0,306 ± 0,157	−0,09 kg per day
Mg (mmol/ℓ)	0,188 ± 0,132	0,10 kg per day
Ca (mmol/ℓ)	0,550 ± 0,410	0,08 kg per day

Simple correlation coefficients. describing the relationships between growth rate from 1 to 13 weeks and the concentrations of individual blood constituents at one week of age were also determined. Although correlations existed with most blood constituents and rate of growth, a significant ($P<0,05$) correlation was found only with globulin.

Regression analysis was also carried out to relate growth rate from 1 to 13 weeks with the blood profile at one week of age to assess whether the blood chemistry at this time could be used to predict growth rate later in life. The regression of growth rate up to 13 weeks with globulin concentration alone accounted for 42% of the variance among calves, while the regression of growth rate up to 13 weeks with Hb concentration alone accounted for only 16,6% of the variance among calves. The regression involving all eleven constituents accounted for 70,6% of the variance among individuals. A summary of the multiple regression analysis relating growth rate (1 to 13 weeks) with blood analysis at one week of age is presented in Table 7.

Regression equations in Table 7 include only those blood constituents which met the 5% significance level for entry into the model and which therefore provided a significant contribution ($P<0,05$) to the equation, viz. globulin, Hb, PCV, Pi, Mg and Ca. The regression involving all six constituents (Table 7) accounted for 67,4% of the variance among individuals.

The practical importance of the present results is illustrated by the final column in Table 7, which has been calculated by multiplying the regression coefficients in Table 7 by the corresponding 95% confidence ranges of the respective blood constituents. These 95% confidence ranges, calculated from variances among calves, were 12 g/ℓ for globulin, 21 g/ℓ for Hb, 6% for PCV, 0,3 mmol/ℓ for Pi, 0,51 mmol/ℓ for Mg and 0,14 mmol/ℓ for Ca. In this way an assessment can be made for a particular blood constituent of the relative growth performance of calves at the top of the range compared with calves at the bottom. Thus, for example, calves with the *highest* Hb concentrations grew on average 470 g per day faster from 1 to 13 weeks than calves with the *lowest* concentrations; calves with the *lowest* globulin concentrations grew on average 210 g per day faster than calves with the *highest* concentrations, and so forth.

Not all authors have obtained similar results. Rowlands *et al.* (1974)[16] found significant correlations between calves' growth rates from 1 to 12 weeks and the concentrations of blood glucose, Hb, K, Na, albumin and Pi. Arthaud *et al.* (1959)[1] looked for an association between growth rate and concentrations of Hb and glucose in the blood, but found none. Schultze (1955)[18] failed to obtain any significant association betweeen growth rate and glucose or Hb concentrations in 29 Holstein calves. This disagreement in the literature suggests the likelihood of interactions of genetics with environment and nutrition.

The results of this work suggest that the type of liquid feeding used here in an early weaning system would not significantly affect the composition of the blood of calves when sampled approximately six weeks or more after weaning, provided that such calves are offered the same dry ration, and that metabolic profiles during the first week of life may provide a predictive assessment of subsequent growth performance.

Table 6: SIMPLE CORRELATION COEFFICIENTS BETWEEN BLOOD CONSTITUENTS*

	Hb	PCV	Glucose	Urea	Albumin	Globumin	Pi	Ca	Mg	Na	K
Hb	1,00										
PCV	0,96	1,00									
Glucose	−0,07	0,11	1,00								
Urea	−0,09	0,08	0,74	1,00							
Albumin	0,52	−0,08	0,23	0,00	1,00						
Globulin	−0,38	−0,46	−0,08	−0,07	0,53	1,00					
Pi	0,46	0,44	0,23	0,05	0,46	−0,08	1,00				
Ca	−0,62	0,06	0,45	0,54	−0,54	0,28	−0,29	1,00			
Mg	−0,03	0,07	0,29	0,00	−0,13	0,56	0,35	−0,02	1,00		
Na	0,41	0,31	−0,05	−0,36	0,54	−0,25	−0,08	−0,56	−0,12	1,00	
K	0,21	0,25	0,39	0,51	0,08	0,26	0,43	0,09	0,38	−0,32	1,00

*All correlation coefficients with absolute values ≥ 0,48 are statistically significant ($p<0,05$)

ACKNOWLEDGEMENT

The author thanks Mrs L Wynja for her technical assistance.

REFERENCES

1. Arthaud R L, Schultze A B & Arthaud V H 1959 The relationship of certain blood constituents to rate and economy of gain in beef cattle. Journal of Animal Science 18: 314–322
2. Couvaras S 1979 Pre- and post-weaning performance of artificially reared calves. I. High and low fat levels in milk replacers and cement kiln dust as an additional source of minerals in starter meals. South African Journal of Animal Science 9: 41–46
3. Frank F W 1976 New concepts in calf scours. Agricultural Science Review 4: 36–40
4. Khouri R H & Pickering F S 1968 Nutrition of the milk-fed calf. 1. Performance of calves fed different levels of whole milk relative to body weight. New Zealand Journal of Agricultural Research 11: 227–236
5. Kitchenham B A, Rowlands G J, Manston R & Dew S M 1975 The blood composition of dairy calves reared under conventional and rapid-growth systems. British Veterinary Journal 131: 436–445
6. Lewis L D & Phillips R W 1972 Water and electrolyte losses in neonatal calves with acute diarrhoea. A complete balance study. The Cornell Veterinarian 52: 596–607
7. Lewis L D, Phillips R W & Elliot C D 1975 Changes in plasma glucose and lactate concentrations and enzyme activities in the neonatal calf with diarrhoea. American Journal of Veterinary Research 36: 413–416
8. Liebenberg L H P & van der Merwe F J 1974 The utilization of milk diets with different fat content by the preruminant calf. 1. Digestibility and metabolizability of the diets and live mass gain of the calves. South African Journal of Animal Science 4: 21–26
9. Little W, Kay R M, Manston R, Rowlands G J & Stark A J 1977 The effects of age, live-weight gain and feed intake on the blood composition of young calves. Journal of Agricultural Science 89: 291–296
10. Manston R, Kitchenham B A & Baldry A J 1977 The influence of system of husbandry upon the blood composition of bulls and steers reared for beef production. British Veterinary Journal 133: 37–45
11. Mebus M A, Stair E L, Rhodes M B, Underdahl N R & Twiehaus M J 1973 Calf diarrhoea of viral etiology. Annales de Recherches Veterinaires 4: 71–78
12. National Research Council 1971 Nutrient requirements of domestic animals. Nutrient requirements of dairy cattle. Washington: National Academy of Sciences
13. Neville W E, McCullough M E, Sell O E & Baird D M 1952 The effect of three levels of milk feeding to young calves on their growth rate and feed consumption. Journal of Animal Science 11: 772–772
14. Payne J M, Rowlands G J, Manston R & Dew S M 1973 A statistical appraisal of the results of the metabolic profile tests on 75 dairy herds. British Veterinary Journal 129: 370–381
15. Radostits O M, Rhodes C S, Mitchell M E, Spotswood T P & Wenkoff M S 1975 A clinical evaluation of antimicrobial agents and temporary starvation in the treatment of acute undifferentiated diarrhoea in newborn calves. Canadian Veterinary Journal 16: 219–227
16. Rowlands G J, Payne J M, Dew S M & Manston R 1974 Individuality and hereditability of the blood composition of calves with particular reference to the selection of stock with improved growth potential. Journal of Agricultural Science 82: 473–481
17. Roy J H B 1970 The calf. Management and feeding. 3rd edition, volume 2. London: Iliffe Books Ltd
18. Schultze A B 1955 Changes of certain characters of the blood of calves during one year of life and their possible relation to rate of gain in body weight. Growth 19: 141–149
19. Steel R G D & Torrie J H 1960 Principles and procedures of statistics. London: McGraw-Hill Book Company
20. Van der Merwe F J 1977 In "Dierevoeding". Revised edition. Stellenbosch: Kosmo-uitgewery (Edms.) Bpk

FIELD OUTBREAKS OF LEUKOENCEPHALOMALACIA IN HORSES CONSUMING MAIZE INFECTED BY *FUSARIUM VERTICILLIOIDES* (= *F. MONILIFORME*) IN SOUTH AFRICA

J.G. PIENAAR*, T.S. KELLERMAN* and W.F.O. MARASAS†

ABSTRACT: Pienaar J.G.; Kellerman T.S.; Marasas W.F.O. **Field outbreaks of leukoencephalomalacia in horses consuming maize infected by *Fusarium verticillioides* in South Africa.** *Journal of the South African Veterinary Association* (1981) **52** No. 1, 21–24 (En) Veterinary Research Institute, Onderstepoort 0110, Republic of South Africa.

Four outbreaks of leukoencephalomalacia in horses in widely separated areas in South Africa are reported. The clinical signs and pathological lesions observed in each outbreak are briefly described. Mouldy home-grown maize from which *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheldon) was isolated in every instance, was involved in the outbreaks. Clinical signs and pathological lesions were identical to those seen in experimentally produced cases of *F. verticillioides* poisoning in horses.

INTRODUCTION

Mortalities in horses, preceded by marked nervous symptoms prior to death, have been known since 1850 to occur periodically in the maize-growing regions of the United States of America[2–8 18]. Thousands of horses died in Maryland around the turn of the century[4] and in some of the Midwestern states during the 1930's[2 3 7 8 18] and field cases have been reported in Illinois as recently as 1974[14]. The disease is characterized by liquefactive necrotic lesions in the white matter of the cerebral hemispheres, from which the name equine leukoencephalomalacia (LEM) was derived. Only one report is available to the authors on a similar disease affecting animals other than of the equine species, i.e. cattle; this occurred during an epizootic of LEM in Kentucky[6].

The disease was reported in South America by Rodriguez (1945)[17] who stated that outbreaks in horses had already been known to occur in Argentina for about 30 years. In the Hebej province of China, hundreds of equine animals (mostly donkeys and to a lesser extent horses and mules) died of LEM during the autumn of 1955[10]. Extensive mortalities in donkeys due to LEM have occurred in Egypt in recent times[1] and have also been noticed in Greece and probably Germany[4 25]. Clinical signs very similar to those of LEM have been described in "beanhulls poisoning", a disease of horses which is very prevalent in Hokkaido, Japan[12 21], and which has been reproduced experimentally by feeding mouldy soybean hulls[13]. The main pathological lesions of this disease have been described as "degenerative change of the nerve cells of the cerebral cortex and acute circulatory disturbance"[19], but no specific mention was made of leukoencephalomalacic lesions. Although liquefactive necrotic lesions in the white matter are not found in all cases of LEM[1 25], it is not clear at this stage whether or not "bean-hulls poisoning" is identical to LEM.

The popular names of the disease, "moldy corn disease" and "cornstalk disease", indicate the association of the disease with the ingestion of mouldy maize (*Zea mays* L.). In the U.S.A., outbreaks of the disease have usually occurred in wet seasons preceded by a drought[6] or when maize was late in maturing and early frosts occurred[2]. In Egypt it has been linked with the annual flooding of the Nile[1].

The disease was reproduced experimentally in horses by the feeding of naturally contaminated mouldy maize as early as 1902 by Butler[5]. His findings were subsequently confirmed by several workers in the U.S.A.[3 18], China[10] and Egypt[1].

Despite these early indications that the disease is a mycotoxicosis, the responsible fungus was positively identified as *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheld.) only in 1971[23–25]. Wilson & Maronpot (1971)[24] experimentally reproduced LEM in one donkey with a pure culture of an Egyptian isolate of this fungus. This was confirmed in South Africa by the induction of typical lesions in 2 horses with pure cultures of 2 local isolates of *F. verticillioides* from mouldy maize[15 16]. The South African isolates were also shown to be capable of causing a fatal hepatic syndrome in horses[11]. Recently typical LEM was again experimentally reproduced in one of 2 donkeys with an Egyptian isolate of *F. verticillioides*[9]. Toxigenic strains of this fungus that caused circulatory disturbances in mice have also been isolated from toxic mouldy bean hulls in Japan, but these strains soon lost their toxin-producing ability and have not yet been tested in horses[20].

Although maize has been produced on a large scale in southern Africa since the beginning of this century, the occurrence of equine LEM has until recently never been reported in this country. Van der Walt & Steyn (1943)[22] described outbreaks of a toxic syndrome in horses and mules in the southern Transvaal and north western Orange Free State which were characterized by liver damage, icterus and nervous symptoms. The mortality rate was almost one hundred per cent. Sugar bean (*Phaseolus vulgaris* L.) hay heavily contaminated with *F. verticillioides* and some other fungi was suspected by them as the cause of the mortality. Extensive toxicity trials with *F. verticillioides* in rabbits, however, failed to prove this fungus as toxic. In addition they did not succeed in reproducing the disease in horses by feeding the contaminated hay. Despite these negative results, these authors still maintained that the sugar bean hay was the cause of the mortalities as the circumstantial evidence incriminating it was very strong.

Since 1970, 4 confirmed outbreaks of *F. verticillioides* poisoning in horses have been encountered in South Africa. A short description of these cases is presented.

CASE REPORTS

Outbreak 1

During May 1970 specimens of fixed brain tissue from

*Veterinary Research Institute, Onderstepoort.
†National Research Institute for Nutritional Disease, P.O. Box 70, S.A. Medical Research Council. Tygerberg, Republic of South Africa.

0038-2809/81/01/0021–0024

two horses were received from Potchefstroom in the western Transvaal with the following history. The owner had lost 3 horses within one week. All had shown identical symptoms which manifested at first as marked hyperexcitability lasting several hours. The animals appeared to be blind and ran into fences and walls, totally losing comprehension of their environment. These signs of hypersensitivity passed over to outspoken depression and stupor and death followed within 12–24 hours after onset of symptoms. The horses had been fed maize chaff.

Outbreak 2

Two draught-horses died in the Benoni district in July 1973. The onset of symptoms was abrupt. The animals were noticed to be hypersensitive, appeared to be blind and walked into objects. Both seemed to have paralysis of the upper and lower lips and the tongue, which protruded from the mouth. They died within 12 hours after the onset of symptoms. One of the horses also showed a posterior ataxia manifested as a swaying and unsteady gait in the hindquarters.

Large quantities of cracked maize formed part of the ration of these horses. The maize consisted of spoiled kernels which were rejected during threshing and were heavily fungus-infected.

Outbreak 3

Within a period of a few days, 7 out of 34 Thoroughbred horses died in the Schweizer-Reneke district in 1974. These horses had been grazing on harvested maize fields for two months before the mortality started. They had been moved to a different maize field two weeks before the first death occurred. Hypersensitivity, ataxia and eventually posterior paralysis were the main symptoms reported. The maize fields were used for growing seed maize and were harvested by hand. Heavily fungus-infected ears, or parts of ears, were discarded in the field.

Outbreak 4

On a farm near Odendaalsrus in the Orange Free State, 5 horses died during October 1974. One mare appeared clinically normal when examined on a Saturday but apparently became blind and ran wildly into fences on the following Sunday and was dead on the Monday morning. The other horses that died exhibited similar nervous signs, lateral recumbency and paddling motions prior to death. The mucous membranes were hyperaemic but no icterus was present.

The horses were fed cracked mouldy home-grown maize.

LABORATORY EXAMINATIONS

Mycology

Samples of maize kernels or maize chaff involved in the field outbreaks were cultured for fungi according to the method described by Kellerman et al. (1972)[11]. *F. verticillioides* was found to be the predominant fungus present in all the samples examined (Fig. 1). In some instances this fungus was isolated from all the platings.

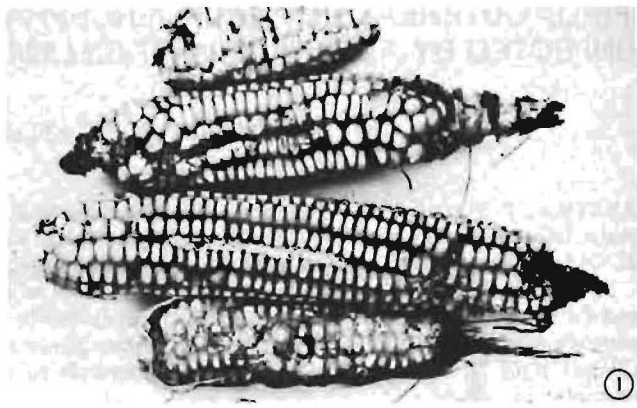


Fig. 1. Mouldy maize ears associated with a field outbreak of equine leukoencephalomalacia. The ears are insect-damaged and heavily infected by *Fusarium verticillioides.*

Gross pathology

A total of 6 brains, fixed in 10% formalin, was received, representing at least one from each of the outbreaks.

Focal areas of encephalomalacia were located in the white matter of the cerebral hemispheres in all the brains (Fig. 2). These areas were most frequently situated in the frontal poles of the cerebral hemispheres. They varied in size from cavities a few cm in diameter, with irregular edges, to small pea-sized areas of softening. Numerous haemorrhages surrounded the encephalomalacic lesions. In some cases haemorrhages also occurred unassociated with encephalomalacia in various parts of the brain. Large haemorrhages in the thalamus, subcortical white matter and midbrain regions are illustrated in Fig. 2. A striking gross feature of these lesions after fixation in formalin was a bright pea-green discolouration of the intact white matter around the areas of softening.

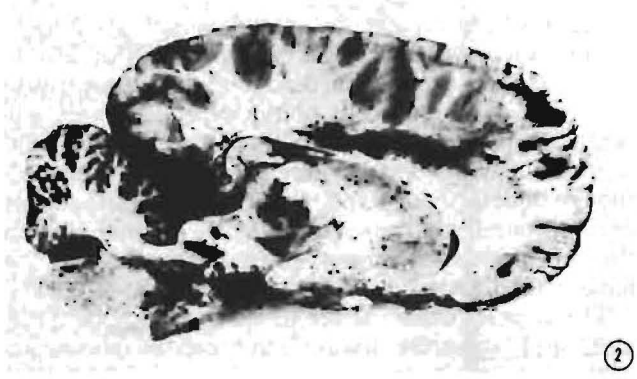


Fig. 2. Focal areas of encephalomalacia and haemorrhages in the white matter of the brain of a horse that died during a field outbreak of leukoencephalomalacia.

Microscopic pathology

Paraffin sections of the fixed brains were prepared according to standard procedures for light microscopic examination.

Marked disintegration of the white matter, seen as a disappearance of all tissue elements, leaving large empty cavities, was the salient microscopic lesion.

These cavities were surrounded by white matter showing oedema, changes of early encephalomalacia and haemorrhages. A few blood vessels in the vicinity of the encephalomalacic lesions showed a slight perivascular cell infiltration consisting of eosinophils and plasma cells. The pathological changes of experimentally produced cases in horses have been reported in detail elsewhere[15].

DISCUSSION

The lesions observed in the brains of the natural cases described here are identical to those reported in experimental cases which were produced by using culture material of 2 different South African isolates of *F. verticillioides*[15].

The clinical signs of the disease observed in the cases reported here are similar to those that have been seen in other countries. Characteristically, these consist of inappetence and unthriftiness followed by nervous signs which may include drooping of the lower lip, drowsiness and apparent blindness, causing animals to run into fences and other obstacles. Twitching of the muscles of the shoulders and thighs and paralysis of the pharynx may be present. Unsteadiness of gait with a tendency to walk to one side or to circle aimlessly are frequently seen. Eventually the animals go down in lateral recumbency. Death may follow within a few hours or the animals may live for a few days after the appearance of clinical signs. Periods of wild delirium may occur before animals go down.

Kellerman et al. (1972)[11] were initially not successful in producing encephalomalacia experimentally in horses and donkeys with pure culture material of *F. verticillioides*. In contrast, a hepatotoxic syndrome which was characterized clinically by icterus and subcutaneous oedema and pathologically by wide-spread haemorrhages accompanied by lesions in the liver varying from fatty changes to cirrhosis were seen. However, subsequent research[15] showed that both the encephalomalacic and hepatotoxic syndromes could be produced by dosing the same batch of culture material to horses. High dosages of culture material induced the liver syndrome while smaller doses given over a longer period of time (90–144 days) resulted in the encephalomalacic syndrome. Chronic liver lesions were also found concurrent with encephalomalacia in some horses, while others only showed the encephalomalacic syndrome[15].

Thus far the liver syndrome produced experimentally by dosing *F. verticillioides* culture material[11] has not been reported under natural conditions in South Africa. It is possible that the mortalities in horses described by Van der Walt & Steyn (1943)[22] could have been this condition. Icterus was a prominent feature of these outbreaks associated with the consumption of *F. verticillioides* contaminated bean hay.

The low incidence of LEM in South Africa is probably related to the dry local weather conditions. In overseas countries the occurrence of this condition is usually attended by rainy and humid conditions during late summer and autumn. Heavy rains towards the end of the growing season had been experienced during the years when field outbreaks occurred, and the resultant higher relative humidity may have created favourable conditions for the growth of *F. verticillioides* on maize. Another factor which may contribute to infection of maize by this fungus is the presence of the maize stalk borer. Damage of kernels by this insect probably enhances the growth of the fungus on maize ears. Badiali et al. (1968) have made a similar observation in Egypt. They recorded that the growth of molds on maize ears was frequently associated with the tracts left by insect larvae.

It should be noted that mouldy, home-grown maize, and not commercial maize graded according to the standard South African maize grading regulations, was involved in all the field outbreaks of LEM reported in this paper. In one outbreak, maize chaff was involved, in another spoiled kernels which were rejected during threshing, and in another the affected horses had access to heavily fungus-infected ears discarded in the field during harvesting. The danger inherent in feeding poor-quality, mouldy maize to horses is evidenced by the occurrence of field cases of LEM in all these instances. LEM should be clearly distinguished from viral encephalomyelitis, the clinical signs of which are very similar, but the disease usually occurs during the summer months and is differentiated by the occurrence of fever prior to death and the absence of leukoencephalomalacic lesions[2, 7, 8, 17, 18, 25].

REFERENCES

1. Badiali L, Abou-Youssef M H, Radwin A L, Hamdy F M, Hildebrandt P K 1968 Moldy corn poisoning as the major cause of an encephalomalacia syndrome in Egyptian Equidae. American Journal of Veterinary Research 29:2029–2035
2. Biester M E, Schwarte L H 1939 Moldy corn poisoning (Leucoencephalomalacia) in horses with history of previous attack as well as recovery from virus encephalomyelitis. North American Veterinarian 20: 17–19
3. Biester M E, Schwarte L H, Reddy C H 1940 Further studies on moldy corn poisoning (Leucoencephalomalacia) in horses. Veterinary Medicine 35:636–639
4. Buckley S S, MacCallum W G 1901 Acute haemorrhagic encephalitis prevalent among horses in Maryland. American Veterinary Review 25:99–101
5. Butler T 1902 Notes on a feeding experiment to produce leucoencephalitis in a horse with positive results. American Veterinary Review 26:748–751
6. Graham R 1912 Forage poisoning or so-called cerebro-spinal meningitis in horses, cattle and mules. Kentucky Agricultural Experiment Station Bulletin No 167
7. Graham R 1935 Results of inoculating laboratory animals with equine brain-tissue suspensions and equine brain-tissue filtrates from spontaneous cases of so-called cornstalk disease. Journal of the American Veterinary Medical Association 86:778–780
8. Graham R 1936 Toxic encephalitis or non-virus encephalomyelitis of horses. Veterinary Medicine 31:46–50
9. Haliburton J C, Vesonder R F, Lock T F, Buck W B 1979 Equine leucoencephalomalacia (ELEM): a study of *Fusarium moniliforme* as an etiologic agent. Veterinary and Human Toxicology 21:348–351
10. Iwanoff X, Chang-kuo Y, Shih-Chieh F 1957 Über die toxische Encephalomalazie (moldy corn poisoning) der Einhufer in China. Archiv für experimentelle Veterinärmedizin 11:1033–1056
11. Kellerman T S, Marasas W F O, Pienaar J G, Naudé T W 1972 A mycotoxicosis of Equidae cause by *Fusarium moniliforme* Sheldon. A prelimary communication. Onderstepoort Journal of Veterinary Research 39:205–208
12. Konishi T, Ichijo S 1970a Clinical studies on bean-hulls poisoning of horse. I. Clinical and biochemical observations in spontaneous cases (in Japanese). Research Bulletin of Obihiro University 6:242–257
13. Konishi T, Ichijo S 1970b Clinical studies on bean-hulls poisoning of horse. II Clinical and biochemical observations in experimental cases (in Japanese). Research Bulletin of Obihiro University 6:258–273
14. Lock T F 1974 Leukoencephalomalacia in two quarter horses. Modern Veterinary Practice 55:464
15. Marasas W F O, Kellerman T S, Pienaar J G, Naudé T W 1976 Leukoencephalomalacia: A mycotoxicosis of Equidae caused by

Fusarium moniliforme Sheldon. Onderstepoort Journal of Veterinary Research 43: 113–122
16. Pienaar J G 1977 Nuwere veterinêre neuropatologiese toestande in Suid-Afrika. Journal of the South African Veterinary Medical Association 48:13–18
17. Rodriguez J A 1945 Diferenciacion entre la enfermedad de los rastrojos y la meningo encefalomielitis infecciosa de los equinos. Anales de la Sociedad Rural Argentina 69:305–307
18. Schwarte L H, Biester H E, Murray C 1937 A disease of horses caused by feeding moldy corn. Journal of the American Veterinary Medical Association 90:76–85
19. Ueda A, Ono T, Yamigiwa S 1967 Neurohistopathological studies on bean-hulls poisoning of horses (in Japanese). Research Bulletin of Obihiro University 5:149–154
20. Ueno Y 1973 Akakabi toxins. Part 2 (in Japanese). Journal of the Food Hygienic Society of Japan 14:501–510
21. Ueno Y, Ishii K, Sakai K, Kanaeda S, Tsunoda H, Tanaka T, Enomoto M 1972 Toxicological approaches to the metabolites of Fusaria. IV Microbial survey on "Bean-hulls poisoning of horses" with the isolation of toxic trichothecenes, neosolaniol and T-2 toxin of *Fusarium solani* M-1-1. Japanese Journal of Experimental Medicine 42:187–203
22. Van der Walt S J, Steyn D G 1943 Recent investigations into the toxicity of plants, etc. XIII Onderstepoort Journal of Veterinary Science and Animal Industry 21:45–55
23. Wilson B J 1971 Recently discovered metabolites with unusual toxic manifestations In IFH Purchase (Ed.) Mycotoxins in Human Health, Macmillan, London
24. Wilson B J, Maronpot R R 1971 Causative fungal agent of leucoencephalomalacia in equine animals. Veterinary Record 88:484–486
25. Wilson B J, Maronpot R R, Hildebrandt P K 1973 Equine leukoencephalomalacia. Journal of the American Veterinary Medical Association 163:1293–1295

DIE GENETIESE WAARDE VAN EMBRIO-OORPLASING IN SUID-AFRIKA

D.R. OSTERHOFF

ABSTRACT: Osterhoff D.R. **The genetic value of embryo transfer in South Africa.** *Journal South African Veterinary Association* (1981) **52** No. 1, 25–28 (Afr). Department of Zootechnology, Faculty of Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110, Republic of South Africa.

The different applications of embryo transfer from the animal improvement, scientific and commercial point of view are outlined. The most important application in South Africa is the production of the next generation's dairy A.I. bulls out of elite cows after superovulation, inseminations with the best available semen and embryo transfer to many foster mothers. The specific difficulties of running breeding programmes with small active breeding populations are discussed, and it is suggested that elite cows should be brought to special farms or government stations during the period of superovulation and transfer. In this way the disadvantage of small breeding populations could to a certain extent be overcome by embryo transfer.

INLEIDING

Drie verskillende rigtings van die toepassing van embrio-oorplasings kan onderskei word, die veekundige, die wetenskaplike en die kommersiële. Wat die *veekundige* toepassing betref, lê die grootste voordeel in die skerp seleksie van bulmoeders in die kunsmatige-inseminasie-skemas van melkbeeste. Die benutting van minder waardevolle vroulike diere wat vryelik beskikbaar is, moedig hierdie toepassing aan. By dubbeldoelbeeste kan aan tweelingdragtigheid gedink word wat deur die inplasing van twee embrios verkry word. Die produksie van vleiskalwers van beste moeders kan miskien in uitsonderlike gevalle van belang wees en ook die spoedige vermeerdering van diere met spesifieke eienskappe en van rasse wat in klein getalle voorkom.

Vir die *wetenskaplike* word hier 'n groot veld van aktiwiteite aangebied, soos bevriesing van embrios, geslagsbepaling van embrios, deling van embrios in veelvoudige identiese tweelinge, vermenging van embrioselle vir die produksie van chimeras en suiwer endokrinologiese studies.

Die benutting van minder waardevolle diere vir die verbetering van sinchronisasie-, superovulasie- en embrio-oorplasingstegnieke kan vir Suid-Afrika van groot betekenis wees.

Uit die *kommersiële* oogpunt is die uitvoer en invoer van bevrore embrios van belang omdat aanpassingsprobleme van diere in die nuwe omgewing verminder of uitgeskakel kan word. Hierdie uitvoer en invoer van embrios vir die verbetering van die eie genetiese materiaal is ook van belang vir die behou van kontakte met die buitelandse veeteler en wetenskaplike.

MOONTLIKHEDE VAN EMBRIO-OORPLASING BY MELKBEESTE

Die eerste storm het oorgewaai in verband met embrio-oorplasings. Hier word gedink aan die regte benaming van die proses, die nuwe Veeteeltwet, die entoesiastiese aktiwiteite van jong veeartse en die reeds bepaalde veeartsgelde vir embrio-oorplasings. En nou is dit tyd om te besin oor die genetiese waarde van hierdie tegnieke en prakties uitvoerbare moontlikhede van embrio-oorplasings in Suid-Afrika.

Die belangrikste aspek wat embrio-oorplasing ten opsigte van die verbetering van die nasionale melkbeeskudde kan meebring, is die produksie van voldoende nakomelinge van besonder skerp geselekteerde bulmoeders. Die benutting van die nuwe tegnieke in die verbetering van vleisbeeste is van minder belang, aangesien die koste te hoog is en die moontlike winste moeilik kan balanseer.

Cunningham[1] het sommige van die resultate wat in verskillende berekeninge verkry is, opgesom en duidelik die effek van verhoogde reproduksievermoë deur embrio-oorplasings aangetoon (Tabel 1)

Uit die tabel is dit duidelik dat by vier nakomelinge per koei 59% van alle koeie vereis word vir die produksie van vervangingsverse. Word in die seleksie net die melkproduksie in aanmerking geneem (geen bykomende vleisproduksie nie), kan 'n mens genetiese voortreflikheid van die nakomelinge van 2,2% verwag. Hoe groter die getal nakomelinge per koei, hoe groter sal die genetiese voortreflikheid wees. Let daarop dat dit 'n suiwer teoretiese benadering is en nie die resultate in aanmerking neem wat werklik behaal is nie.

In Tabel 2 word die meer realistiese benadering gevolg, d.i. die effek van embrio-oorplasing waarby die moeders van toekomstige K.I.-bulle as skenkers gebruik word.

By 'n normale seleksiedruk van 3%, d.i. 3 koeie word per jaar as beste koeie van 100 aangewys om die moeders van die bulle van die volgende generasie te

Tabel 1: DIE EFFEK VAN VERHOOGDE REPRODUKSIEVERMOË VAN KOEIE OP GENETIESE VOORTREFLIKHEID VAN NAKOMELINGE[1]

Totale getal nakomelinge per koei	Persentasie koeie geselekteer as koeimoeders	Genetiese voortreflikheid van nakomelinge (as pers. van gemiddelde)
4	59,0	2,2
8	29,5	3,8
20	14,8	5,1
40	7,4	6,1
80	3,7	7,2

Tabel 2: DIE EFFEK VAN EMBRIO-OORPLASINGS IN DIE SELEKSIE VAN BULMOEDERS[1]

Huidige seleksiedruk by die uitsoek van bulmoeders		Relatiewe fenotipiese waarde by ... kalwers per bulmoeder en jaar 1	5	10	15
matig	5 %	100	131	141	147
normaal	3 %	100	127	136	141
uitsonderlik	1 %	100	117	125	125

0038-2809/81/01/0025–0028

wees, kan die gemiddelde relatiewe fenotipiese waarde by 10 kalwers deur embrio-oorplasing geproduseer met 36% verhoog word. Aangesien die genetiese vooruitgang langs die weg moeder-seun met 25% bereken word, verkry 'n mens dus 'n bykomende verbetering van 9%.

Die skatting van die genetiese verbetering deur embrio-oorplasings is ook deur ander navorsers gedoen. Hansen[2] kom op bykomende verbetering van 13% terwyl Kräusslich[3] 11% bereken het (Tabel 3).

Kosteberekening is tot dusver baie skaars en nog geen betroubare syfers is beskikbaar nie. In 'n reeks oorplasings wat op Onderstepoort uitgevoer is op 15 ontvangers is die koste met inspuitings van skenkers en ontvangers, uitspoel en inplasing van embrios op R1 200 geskat. As die kostelimiet volgens Kräusslich by R3 300 lê om een bul te produseer wat as toetsbul aanvaar kan word, kan die skema uitvoerbaar wees.

Tabel 3: SKATTING VAN GENETIESE VERBETERING DEUR SUPEROVULASIE EN EMBRIO-OORPLASING

By die verhoging van die kalwergetal tot 10 per koei word die volgende bykomende genetiese verbetering verwag:	
Cunningham (Ierland)	9 % per generasie
Hansen (Denemarke)	13 % per generasie
Kräusslich (Duitsland)	11 % per generasie
Berekening van koste wat aanvaarbaar is:	
20 % van die netto seleksieresultaat kan gespandeer word aan die embrio-oorplasingsprogram:	
In 'n populasie van 100 000 koeie kan R1 per koei opsygesit word – 30 toetsbulle is nodig – die kostelimiet is bereik by R3 300 per toetsbul geproduseer.	

MOONTLIKE EMBRIO-OORPLASINGSKEMAS BY MELKBEESTE IN SUID-AFRIKA

Die Nasionale Nageslagstoetsskema vir Suiwelrasbulle is in 1976 in die lewe geroep, 25 jaar na die begin van kommersiële kunsmatige inseminasie in Suid-Afrika. Voor 1976 was daar ook pogings om nageslagstoetsing van jong bulle uit te voer, maar in genoemde jaar is die "privaat"-skema op 'n nasionale grondslag geplaas. Die proses van evaluasie, doeltreffende identifikasie en die gebruik van meerderwaardige bulle is egter 'n groot probleem: slegs 'n klein getal bulle kan in die stoeterye en kommersiële kuddes van Suid-Afrika getoets word omrede min boere aantekeninge hou van die melkproduksie van hul koeie en die keuse dus taamlik beperk is. Volgens skatting word 17% van alle koeie kunsmatig geïnsemineer (die syfer behoort opgestoot te word tot 40%), en slegs 5% van alle koeie staan in melkaantekening (die syfer moet opgestoot word tot minstens 10%).

Verder het ons in Suid-Afrika met twee ander beperkings te doen wat die genetiese verbetering van melkrasbulle bemoeilik, naamlik-

(a) die vertraging by die vroegtydige verkryging van genoeg inligting oor die nageslagsprestasie van 'n bul; en
(b) die moeilike opsporing van potensieel goeie bulle en koeie weens die geringe belangstelling in die K.I. en melkaantekening.

Die vraag wat nou ontstaan, is: "Behaal Suid-Afrikaanse melkprodusente enige voordeel uit die nuwe embrio-oorplasingstegniek?" 'n Mens is geneig om die tegnieke as vervroeg en te duur af te skryf en te sê: dit is nie vir Suid-Afrika nie, maar skrywer is van mening dat hierdie vraagstuk spesifiek onder Suid-Afrikaanse toestande ondersoek behoort te word.

Volgens die huidige Nasionale Nageslagstoetsskema word 'n beroep gedoen op die verskillende telersverenigings en lede word uitgenooi om hulle verenigings te voorsien van gegewens van jong bulle tussen ses en agt maande oud vir oorweging as moontlike toetsbulle vir die Nasionale Toetsskema. Die eienaar van so 'n toetsbul tref self reëlings vir die verkoop aan die K.I.-Koöperasie. Die prys van die bul word dan deur onderhandeling met die K.I.- Koöperasie bepaal.

Uit die staanspoor moet beweer word dat die stelsel hoogs ondoeltreffend is- òf die prys is te hoog vir die K.I.-Koöperasie òf die diere is nie waardevol genoeg om die hele toetsprogram te deurloop nie. Met ander woorde: die uitnodiging aan telers om jong bulle aan te bied, is onaanvaarbaar en behoort onmiddellik vervang te word deur 'n stelsel van doelgerigte parings, d.i. die beste koeie (elitekoeie), waar hulle ook al mag staan, word geïnsemineer met die beste sperma beskikbaar in die land om bulkalwers vir die volgende generasie te produseer.

Uit die samestelling van melkraskoeie wat op leeftydsprestasie kwalifiseer[4], blyk dit duidelik dat net 'n totaal van 587 geregistreerde en 148 graadkoeie in die land aanwesig is wat 'n minimum van 10 kg vetgekorrigeerde melk (melk bereken op 4% bottervet) per dag van ouderdom geproduseer het. Die syfers tussen hakies in Tabel 4 dui hierdie koeie in persentasie aan uit die totale getal koeie in melkaantekening vir die toetsjaar 1975/76 – d.w.s. die effektiewe teelbevolking (the effective or active breeding population).

Tabel 4: MELKRASKOEIE WAT OP LEEFTYDS-PRESTASIE KWALIFISEER VIR LYSTING

	Getal koeie			Getal laktasies (1975/76)		
	Reg. (%)	Graad (%)	Totaal (%)	Reg.	Graad	Totaal
Fries	274 (3,3)	117 (0,9)	391 (0,9)	8 180	33 828	42 008
Jersey	253 (4,1)	29 (0,5)	280 (2,3)	6 062	6 159	12 221
Ayrshire	24 (1,7)	0 (0)	24 (0,7)	1 441	1 946	3 387
Guernsey	38 (4,6)	2 (0,1)	40 (1,6)	833	1 578	2 411
	587 (3,6)	148 (0,3)	735 (1,4)	16 516	43 811	60 017

In Tabel 5 word die volledige onderverdeling van die koeie gegee volgens die getal laktasies wat hulle voltooi het tot op 31 Augustus 1976.

'n Mens kan duidelik sien dat die grootste getal koeie verteenwoordig is in die groepe wat vyf, ses en sewe laktasies voltooi het. Dit is ook die koeie wat as bulmoeders vir die K.I.-bulle vir die volgende generasie in aanmerking behoort te kom, aangesien die jonger koeie nog nie volledig getoets is nie en die ouer koeie sekerlik nie geskik is vir superovulasie en embrio-oorplasing nie. Skrywer is van mening dat juis hierdie groep koeie vir die genetiese verbetering van die totale melkveestapel in die land gebruik behoort te word. In Tabel 6 word hierdie groepe vir die geregistreerde en

Tabel 5: SUID-AFRIKAANSE ELITEKOEIE IN DIE JAAR 1975/76

Getal Laktasies	FRIESE		JERSEYS		GUERNSEYS		AYRSHIRES	
	Geregistreer	Graad	Geregistreer	Graad	Geregistreer	Graad	Geregistreer	Graad
1	1	1	—	—	1	1	—	
2	3	1	1	—	—	—	1	
3	24	10	2	—	2	—	1	
4	56	22	11	2	9	1	4	
5	54	25	35	5	13	—	1	Geen
6	49	23	37	9	5	—	3	
7	47	18	54	5	5	—	4	koeie
8	20	11	46	3	2	—	6	
9	10	2	23	1	—	—	3	kwalifi-
10	7	3	19	2	1	—	—	
11	3	1	15	2	—	—	—	seer
12	—	—	4	—	—	—	—	
13	—	—	1	—	—	—	1	
14	—	—	1	—	—	—	—	
15	—	—	1	—	—	—	—	
Totaal	274	117	253	29	38	2	24	

Tabel 6: BESTE KOEIE IN SUID-AFRIKA (1975/76)

Getal laktasies	Getal koeie	Gem. totale hoeveelheid melk (kg)	Gem. totale hoeveelheid bottervet (kg)	Gem. V.G.M. per dag ouderdom (kg)	Gemiddelde kalfinterval (dae)
Friese					
Geregistreer:					
5	54	33 363	1 238	11,2	414
6	49	45 253	1 454	11,5	417
7	47	44 013	1 640	11,6	409
Graad:					
5	25	31 437	1 150	10,9	390
6	23	36 771	1 320	10,9	396
7	18	40 204	1 415	11,0	378
Jersey					
Geregistreer:					
5	35	25 571	1 266	10,9	395
6	37	29 863	1 454	10,9	396
7	54	33 234	1 658	11,1	399
Graad:					
5	5	23 870	1 143	10,3	364
6	9	29 037	1 389	10,5	397
7	5	31 295	1 557	10,5	379

Tabel 7: VERGELYKING VAN TWEE AYRSHIRE-ELITEKOEIE

No. van koei	Getal laktasies	Tot. hoeveelheid melk (kg)	Tot. hoeveelheid bottervet (kg)	Gemiddelde VGM per dag ouderdom (kg)	Gemiddelde kalfinterval (dae)
61558	8	45 135	1756	11,2	377
63240	7	46 320	1683	11,2	442

graad-Fries- en Jerseykoeie getoon met hulle totale hoeveelheid melk en bottervet asook die gemiddelde vetgekorrigeerde melk (VGM) per dag van ouderdom en gemiddelde kalfinterval.

Die seleksie van die beste koeie – wat ons nou "elitekoeie" kan noem – behoort geen probleme op te lewer nie; nogtans moet àl vier aspekte in aanmerking geneem word: die totale hoeveelheid melk en bottervet geproduseer, die gemiddelde VGM per dag van ouderdom en die gemiddelde kalfinterval. In Tabel 7 word die vergelyking tussen twee Ayrshirekoeie getref wat dieselfde gemiddelde VGM per dag van ouderdom het maar waar koei 61558 met 'n kleiner totale hoeveelheid melk een bykomende kalf geproduseer het en dus die beste dier is.

Uit hierdie samestelling blyk dit duidelik dat die elitekoeie onder die Suid-Afrikaanse omstandighede met die klein effektiewe teelbevolking opgespoor kan word. Die volgende stap sal dan wees om die koeie spesiaal te selekteer en te gebruik vir embrio-oorpla-

sings. Die ideale situasie sal wees as die diere saamgebring kan word na spesiale plase wat aan K.I.-stasies verbonde is of na proefplase wat aan die Departement van Landbou en Visserye behoort. Op hierdie plase word die superovulasies en die inseminasies met die beste beskikbare sperma uitgevoer. Kort daarna word die embrio-oorplasings deur die spesialiste gedoen en alle bulkalwers volgens bepaalde voorskrifte grootgemaak.

As embrio-oorplasings toegepas word, kan die getal koeie wat as bulmoeders beskou word, drasties verminder word. Weer eens moet na die probleme van embrio-oorplasings verwys word. Daar word gereken dat twee elitekoeie nodig is om in werklikheid een skenkerkoei te verkry vir die produksie van bulkalwers waarvan een finaal die toetsbulfase bereik. In Tabel 8 word 'n voorbeeld van 'n teelprogram gegee. Die totale populasie van 100 000 kan miskien as die Friespopulasie van Natal beskou word, waarvan in werklikheid omtrent 11 500 as effektiewe teelbevolking gereken kan word (30 000 koeie in melkaantekening is nodig om 'n teelprogram volledig in werking te stel).

Tabel 8: VOORBEELD VAN TEELPROGRAM

Grootte van populasie (koeie)		100 000
Aktiewe populasie		30 000
Inseminasie met sperma van beproefde bulle		85 000
Inseminasie met sperma van toetsbulle		15 000
15 % van totale populasie		
50 % van aktiewe populasie		
Toetsbulle per jaar		30
Eerste inseminasie per toetsbul		500
Beproefde bulle		10
Vervanging van beproefde bulle per jaar		5
Eerste inseminasie per beproefde bul per jaar		8 500
Beplande parings nodig vir die produksie van een toetsbul		5
Dragtigheid na gebruik van beste bul	0,60	
manlike kalwers per dragtigheid	0,40	
bulle beskikbaar na grootmaak	0,83	
Elitekoeie nodig vir beplande parings		150
Elitekoeie nodig vir beplande parings na superovulasie		60

Met behulp van superovulasie en oorplasings van embrios is dus net 40% van die elitekoeie nodig, wat 'n groot verskuiwing van die geselekteerde groep na die heel bestes beteken. As dan 'n verbetering van 10% in produksie op hierdie manier per generasie verkry kan word, sal dit die moeite word wees om die beste koeie in die populasie op te spoor en na bepaalde plase te bring.

Die elitekoeie bly op die plase gedurende die tyd van superovulasie en embrio-oorplasing, en minder waardevolle diere word beskikbaar gestel as pleegmoeders. Na die oorplasings gaan die elitekoeie terug na die eienaars, en vergoeding vir melkverlies en transportkoste, ens., word uit die Nasionale Nageslagstoetsskema vir Suiwelrasbulle verhaal. Op dié manier kan die moderne tegnieke van superovulasie en embrio-oorplasing 'n groot bydrae lewer in die Suid-Afrikaanse suiwelraskudde juis omdat net 'n klein gedeelte daarvan aan die effektiewe teelbevolking behoort.

VERWYSINGS

1. Cunningham E P (1976) The use of egg-transfer techniques in genetic improvement. Proc. EEC seminar on "Egg Transfer in Cattle" Cambridge, 345–354
2. Hansen M (1976) Technical and economical aspects in relation to egg transfer in cattle. Proc. EEC seminar on "Egg Transfer in Cattle" Cambridge, 269–376
3. Kräusslich H (1976) Application of superovulation and egg transplantation in A.I. breeding programme for dual purpose cattle. Proc. EEC seminar on "Egg Transfer in Cattle" Cambridge, 333–342
4. Melkaantekening in die Republiek van Suid-Afrika (1977) Leeftydsprestasies van koeie: 1 September 1975 – 31 Augustus 1976. Navorsingsinstituut Vee- en Suiwelkunde, Department van Landbou-tegniese Dienste, 1–419

THE ANATOMY OF THE FEMALE REPRODUCTIVE TRACT OF THE SPRINGBOK *(ANTIDORCAS MARSUPIALIS)*

D.A. ELS

ABSTRACT: Els D.A. **The anatomy of the female reproductive tract of the springbok** *(Antidorcas marsupialis) Journal of the South African Veterinary Association* (1981) **52** No. 1, 29–32 (En) Department of Zoology, University of Pretoria.

The anatomy of the female reproductive tract of the springbok is described, using material of 25 ewes collected at the S.A. Lombard Nature Reserve and 300 ewes collected at the De Beers farm Benfontein. No difference in the length of left and right fallopian tubes (104 ± 24 mm) was observed, and from the funnel to the isthmus it narrows considerably from 15 mm to 1,25 mm. A distinct flexure is formed by the utero-tubal juncture. A complete ovarian bursa with a ventral orifice occurs. The reproductive tract increases in mass from 9,2 g in infants to 38,0 g in adults. The right uterine horn of the bicornuate tract is consistently longer. Caruncles are more numerous in the right (60,2 ± 9,37) than the left (45,8 ± 10,18) uterine horn. The intricate cervical lumen consisting of four to six valves in non-pregnant ewes becomes a simple S-shaped canal with advanced pregnancy.

INTRODUCTION

No reference to the basic anatomy of the reproductive tract of the springbok ewe is available. The terminology for domestic Bovidae[13] has therefore been used to describe the position and gross anatomy.

MATERIAL AND METHODS

The description of the position as well as external appearance of the tract is based on the autopsy of 25 springbok ewes collected from the S.A. Lombard Nature Reserve in the Bloemhof district (approximately 27°36' to 28°50'S and 24°40' to 25°29'E) during 1967 to 1968. From this sample of reproductive tracts as well as 300 reproductive tracts of springbok which were collected during 1970 at the De Beers farm Benfontein in the Kimberley district (approximately 28°40' – 50'S and 24°40' to 49'E) mass measurements were obtained with a Mettler toploading balance and linear measurements with vernier callipers.

RESULTS

The fallopian tubes are extremely coiled. No apparent difference in length of the left and right oviduct was noted in 45 specimens which were dissected free of their membranes. Their length varied from 75 to 155 mm (mean 104 ± 24 mm). The infundibulum has a flattened funnel shape. At its widest point the diameter of this funnel measures approximately 15 mm and it narrows within a distance of 5 mm to a diameter of 2 mm. For the greater part of its length the ampulla (Plate 1) maintains the same diameter. About 50 mm from the ostium fallopi the uterine tube gradually narrows reaching a diameter of approximately 1,25 mm where it forms the isthmus. This is followed by the utero-tubal junction which characteristically shows a distinct flexure (Plate 2, Fig. 2). In non-pregnant ewes and ewes in early pregnancy the flattened infundibulum forms part of the tubal membranes which together with the mesometrium and mesosalpinx form a complete bursa (Plate 2, Fig. 1). This bursa encloses the dorsal and cranial portion of the ovary and has a ventral orifice of 30 × 10 mm (largest diameters at right angles). The tubal membrane, which is part of the bursa and associated with the extensively coiled oviduct, varies considerably in width. It is a few mm wide at the isthmus and up to 15 mm wide at the ampulla and infundibulum.

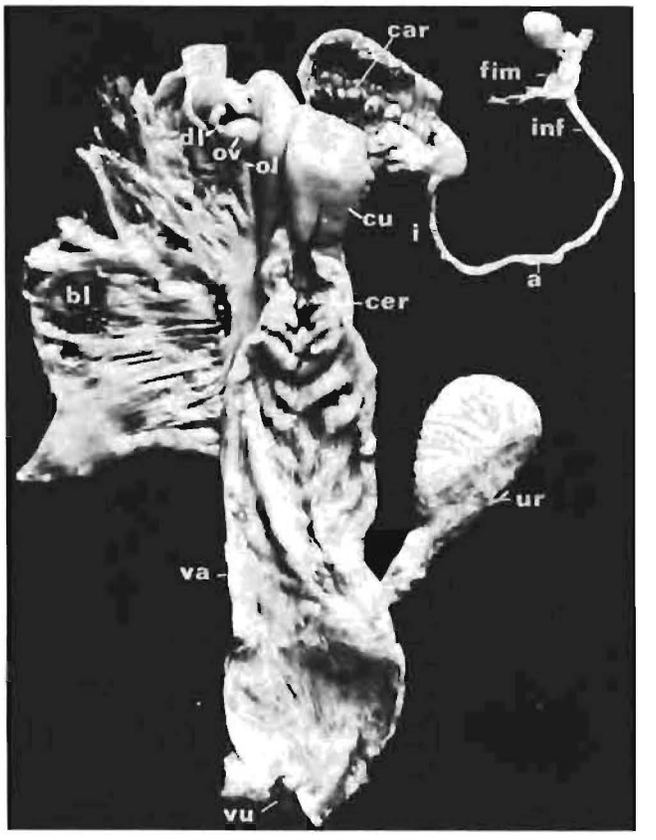


Plate 1. Anatomy of the springbok reproductive tract: the tract is opened up to show the internal anatomy of the vagina, cervix and uterus:
a. – ampulla; b.l. – broad ligament; cer. – cervix; car. – caruncle; c.u. – corpus uteri; d.l. – diaphragmatic ligament; fim. – fimbriae; i. – isthmus; inf. – infundibulum; o.l. – ovarian ligament; ov. – ovary; ur. – ureter; va. – vagina; vu. – vulva.

Through Age Groups I to VI the examined reproductive tracts (uterine tubes, ovaries and cervix) increase in mass from 9,2 to 38,0 g. Though the caudal portion of the bicornuate uterine cornua shares a common peritoneal covering (ligamentum intercornualia), this does not prevent the cornua from being clearly distinguishable up to the point at which they fuse to form the uterine corpus. The latter uterine portion is relatively short, varying in length between 5 and 25 mm (compared with the coiled uterine cornua which measured 60 to 100 mm). The right uterine horn was consistently

0038-2809/81/01/0029–0032

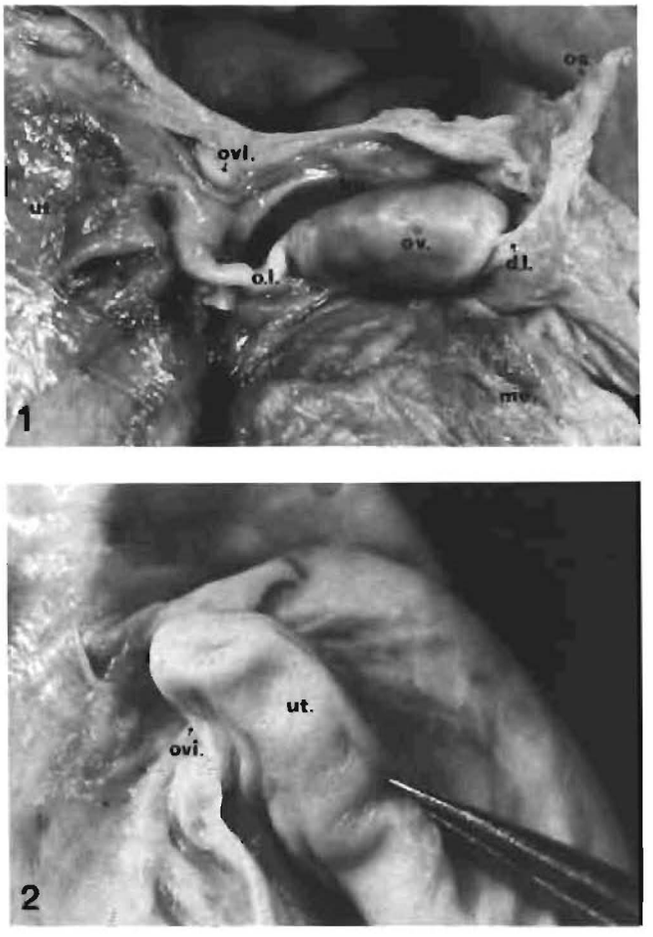


Plate 2. Anatomy of the ovarian bursa and the utero-tubal junction of the springbok. Ventral view.
Figure 1: The complete ovarian bursa showing the positions of the oviduct and the ostium fallopius as well as the ligaments of the ovary.
Figure 2: The utero-tubal flexure typically displayed by all the female reproductive tracts.
bu. – bursa; d.l. – diaphragmatic ligament; me. – mesovarium; o.l. – ovarian ligament; os. – ostium fallopius; ov. – ovarium; ovi. – oviduct; ut. – uterus.

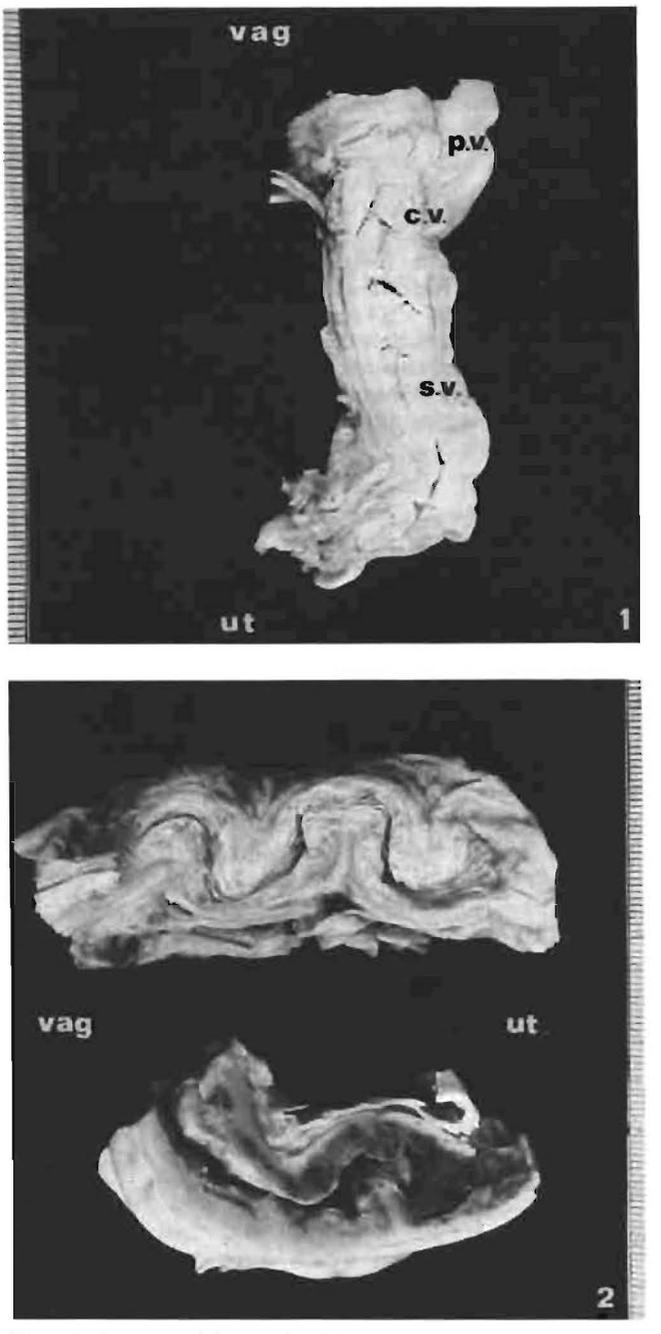


Plate 3. Anatomy of the cervix of the springbok.
Figure 1: Cervix of a young non-pregnant ewe with four cupshaped proximal valves (c.v.) and one simple distal valve (s.v.). The positions of the portio vaginalis (p.v.), uterus (ut.) and vagina (vag.) are indicated.
Figure 2: Cervices of pregnant ewes showing alteration in the position of the cervical lumen. The cervix at the bottom shows a typical haemorrhagic condition and the one at the top a very distinct alternation of the position of the cervical lumen. The positions of the uterus (ut.) and vagina (vag.) are indicated.

longer than the left one. This is true even of young ewes prior to their first conception. A difference in uterine horn length of up to 5 mm in young ewes and 25 mm in adult ewes is not uncommon.

In a 145-day foetus the right uterine horn was also slightly larger than the left horn. The uterine horns are associated with the cranial free border of the broad ligament and curl ventro-laterally, caudal and dorsally at this border giving them a C- or spiral shape. The uterine areas adjacent to the utero-tubal junction were dilated in some of the animals studied and often contained a mucous-like substance. This phenomenon was also observed in pregnant animals where it was associated with blind vesicle-like extremes of the placental membranes. In these areas the membranes contained no cotyledons and the fluids in them often had a milky appearance. The latter phenomenon manifested itself most clearly during early pregnancy. Characteristically the uterine lumen is lined with neat rows of caruncles which may in some adults have pigmented apices (Plate 1). During pregnancy ovoid cotyledons are attached to the spongy uterine caruncles which are more numerous in the right (60,2 ± 9,37 in 29 individuals) than in the left uterine horn (45,8 ± 10,18 in 29 individuals).

The relatively narrow, thick-walled cervix is a distinct part of the reproductive tract with clearly defined cranial and caudal borders. In niliparous animals the cervix narrows towards the uterine border while the opposite is true for parous animals (Table 1). Except for cervices of near-term animals or animals which have recently lambed, the cervix typically has a distinct caudoventral portio vaginalis which projects into the vaginal cavity and the intricate lumen is composed of four to six valves. Most of the caudal valves are cupshaped (Plate 3, Fig. 1) while the most cranial valve is a simple constriction. Comparing the same valve in different

Table 1: MEASUREMENTS OF THE SPRINGBOK CERVIX

Status	Mass (g)	Linear measurements (mm)				Valves number
		Length	Width at:			
			Vagina	Middle	Uterus	
Non-pregnant subadult:						
no.	5	13	10	10	10	13
mean ± S.D.	3,8 ± 0,75	29 ± 2,8	10 ± 3	9 ± 2,6	8 ± 3	51 ± 4,7
Non-pregnant adult:						
no.	6	8	4	4	4	8
mean ± S.D.	10,9 ± 2,27	37 ± 8,8	24 ± 5,4	25 ± 6,3	29 ± 6,1	44 ± 7
Recent parturition:						
no.	3	3	3	3	3	3
mean ± S.D.	32,1 ± 5,14	48 ± 12	24 ± 3,3	30 ± 0	33 ± 1,7	30 ± 8,2
Pregnant:						
no.	9	12	8	8	8	13
mean ± S.D.	22,9 ± 9,41	50 ± 13,3	23 ± 5,9	22 ± 6,5	22 ± 8,4	45 ± 7,5
Total:						
no.	31	39	28	28	27	40
mean ± S.D.	14,6 ± 13,9	40 ± 14	19 ± 8,4	19 ± 9,6	20 ± 11,2	46 ± 8,4

cervices it was evident that the exact route of the canal varies considerably between individuals. The intricate shape of the cervical lumen changes during pregnancy to a simple canal with one or two S-shaped bends (Plate 3, Fig. 2). It is possible to determine the relative positions of these valves by palpating the cervix from the outside (without bisecting it).

A number of large transverse ridges occurred in the cranial half of some of the vaginae studied. Closer to the orificium externum uteri (portio vaginalis) these ridges where progressively bigger. In non-pregnant subadult ewes the ridges are inconspicuous or absent. The urethra opens into the vagina about 30 to 40 mm from the vulva. The vulva has wrinkled labia with an acute ventral commissure formed by the two wrinkled labia of the vulva. Associated with this commissure and the ventral longitudinal axis of the vagina is the clitoris which has a cylindrical shape. It is approximately 10 mm long and 5 mm in diameter. The point of the glans is visible in the ventral commissure of the vulva. The vaginae had an average length of 95 ± 12 mm in the adult ewe.

Medio-lateral to the curled uterine horns and ventral to the broad ligament, the ovaries are suspended in the abdominal cavity by two ligaments (Plate 1 and Plate 2, Fig. 1) and the mesovarium. Of the former two ligaments, one is attached at the ovary's uterine pole (ovarian ligament), connecting the ovary to the ventrolateral uterine surface. This ligament is approximately 10 mm long. From the alternative ovarian pole (tubal pole) a similar ligament (diaphragmatic ligament) projects which gradually merges with the anterior free border of the mesosalpinx immediately dorsal to the coiled uterine tubes. This ligament is approximately the same size as the ovarian ligament, but it is much more difficult to measure its length due to its gradual merging with the mesosalpinx. The ovarian ligament and the diaphragmatic ligament indicate the cranial and caudal borders of both the ovary and its peritoneal mesenterium, the mesovarium.

DISCUSSION

The overall reproductive tract shape as well as the type of attachment to the dorsal body wall and the position of the tract in the pelvic girdle of the springbok resemble that of domestic Bovidae[13], the impala *Aepyceros melampus*[7] and that of the pygmy goat, *Capra hircus*[1]. The springbok tract (especially the ovaries) is slightly smaller than that of sheep *(Ovis aries)* described by Sisson and Grossman[13].

The distinct flexure occurring at the utero-tubal juncture of the springbok is similar to that of the domestic cow *(Bos taurus)* and sheep described by Hafez and Black[5] and to the Type II juncture described by Beck and Boots[1]. The latter authors grouped elk *(Cervus canadensis)*, pygmy goat, antelope, domestic cow and white-tailed deer *(Odocoileus virginianus)* into their Type II juncture. It would seem as if the flexure of the utero-tubal juncture is a typical characteristic of many of the Bovidae. Immediately distal to the utero-tubal juncture the springbok oviduct has a narrow isthmus which gradually widens to form a more or less uniformly wider ampulla. The oviduct eventually ends in a pre-ampulla which characteristically has a flat funnel-shaped infundibulum forming one of the walls of a partial and sometimes complete ovarian bursa. It is therefore clear that the springbok oviduct could be divided into four sections in accordance with the suggestion by Nilsson and Reinius[11]. The springbok ovarian bursa has a wide opening and in general the characteristics could be grouped with two of the types described by Mossman and Duke[9] and three of the types (Types III – V) described by Beck and Boots[1]. However, most of the springbok bursae resembled Type III of the latter authors which is to a certain extent similar to the type of bursa described for cattle[9,13], the main difference from the latter type of bursa being that the bursa hangs free from the ovary.

The occurrence of a relatively short uterine corpus in *A. marsupialis* agrees well with domestic Bovidae[4,13]. A uterine corpus measurement of 5 to 25 mm for the ewe[4] is the same as the measurement taken for springbok and therefore slightly shorter than the uterine corpus of the impala (30 mm[7]). The uterine cornua of the springbok is slightly shorter (60 to 100 mm) than that of the sheep which is 100 to 120 mm[13] and that of the impala which is 110 mm[7]. As in the cow and ewe[13] as well as impala[7] the occurrence of a ligamentum intercornualia in the springbok gives the false impression that the

corpus uteri is very extensive (if viewed from a ventral position).

Skinner and Van Zyl[14] have mentioned that the right uterine horn shows a greater increase in size than the left horn at 20 to 24 weeks, and the results in the present study for both virgin and parous ewes are in agreement. The earliest manifestation of this phenomenon was observed in a 145-day foetus which is similar in impala where Kayanja[7] noted the enlargement of the right uterine horn of 500 mm foetuses. Mossman and Mossman[10] first observed this phenomenon in adult impala and it has also been noted in the common duiker, *Sylvicapra grimmia*[3] and the Uganda kob, *Adenota kob*[2].

The occurrence of a larger number of placentomes in the enlarged right uterine horn observed during the present investigation of springbok uteri has also been reported for Uganda kob[2] and the impala[7]. The springbok does not, however, show a consistent tendency for the right uterine horn to contain the largest number of placentomes and in this respect probably differs from the Uganda kob. The occurrence of more placentomes in the contralateral uterine horn to the pregnant horn, as observed in a few springbok, was also noted in steenbok, *Raphicerus campestris*[6]. On average the springbok has far more placentomes in both left and right uterine horns than the Uganda kob: the left and right uterine horns of the springbok have 60,2 and 45,8 placentomes, respectively, whereas the Uganda kob has 7,0 to 7,6 placentomes in the left and 14,0 to 15,0 placentomes in the right uterine horn[2]. From this it is clear that apart from a considerable difference in the number of placentomes between the two species, the Uganda kob also shows a more marked difference between the average numbers of placentomes in the two uterine horns. The total number of placentomes in the springbok uterus is in the vicinity of 100 which is the same as the figure given for domestic cattle by Sisson and Grossman[13]. Other aspects in which the present study revealed similarities between these two species are: the round to ovoid shape of the cotyledons, its spongy appearance due to numerous crypts receiving villi of the chorion and the tendency for cotyledons to be arranged in rows. As in impala[7], the occurrence of the latter phenomenon has been noted in cattle[4 13], but the cotyledons may also be scattered over the uterine surface. Though a scattered effect was observed in springbok uteri, it was mainly due to the enlargement during pregnancy of a number of smaller cotyledons in between rows as well as the consequent distortion of rows. Pigment is only present in the apices of springbok caruncles and does not occur in the intercotyledonary areas as reported for sheep[4].

As in other Bovidae[4 7 8 13], the springbok cervix is a very distinct feature of the reproductive tract and is macroscopically visible as a narrowed tube between the much widened uterus and vagina. In sheep[4], white-tailed deer[8] and springbok the cervical valves are thick-walled and closely spaced and project their conical apices caudally. The cervical valves of the impala described by Kayanja[7] apparently have a similar appearance. In both white-tailed deer[8] and springbok the anterior valves are more simple and the cervical lumen is situated more centrally. All the bovid cervices mentioned above have a conical sphincter (portio vaginalis) which projects caudally into the vagina. As in white-tailed deer[8] the shape of the portio vaginalis of the springbok varies considerably and its only relatively typical characteristic is its ventral position. An average of four to five cervical valves occur in the springbok cervix. The same average number of valves was recorded in the cervix of white-tailed deer[8] and impala[7]. In the springbok the cervical lumen forms a zig-zagging canal, most clearly observed at the end of pregnancy, which is similar to that of white-tailed deer[8] but differs from that in the cow[4] and impala[7] which are described as being spiralled.

The springbok vagina is narrower but almost as long as the sheep vagina (10 mm)[4] and impala vagina (100 mm)[7]. Some springbok vaginae have large transverse ridges as in humans[12]. However, neither the vaginae nor the external genitals show any outstanding features.

REFERENCES

1 Beck L R, and Boots L R 1974 The comparative anatomy, histology and morphology of the mammalian oviduct. In: *The oviduct and its functions.* Ed. Johnson A D, and Foley C W Academic Press, New York. 369 pp.

2 Buechner H K, 1961 Unilateral implantation in the Uganda kob. *Nature, Lond.* 190: 738.

3 Child G, and Mossman A S 1965 Right horn implantation in the common duiker. *Science, N.Y.* 149: 1265–1266.

4 Eckstein P, and Zuckerman S 1956 Oestrous cycle in the Mammalia. In: *Marshall's physiology of reproduction.* Vol. I. Part I. Ed. Parkes A S Longmans Green, London. 688 pp.

5 Hafez E S E, and Black D L 1969 The mammalian uterotubal junction. In: *The mammalian oviduct.* Ed. Hafez E S E, and Blandau R J The University of Chicago Press. 546 pp.

6 Hofmeyr J.M., and Skinner J.D. 1969 A note on ovulation and implantation in the steenbok and the impala. *Proceedings of the South African Society of Animal Production.* 8: 175.

7 Kayanja F I B 1972 *Reproduction in antelopes. Reproduction in the female impala* (Aepyceros melampus, 1812). East African Literature Bureau, Nairobi. 111 pp.

8 Morris J E 1964 Gross structure of the cervical region of the uterus of white-tailed deer. *Iowa Academy of Science.* 71: 241–247.

9 Mossman H W, and Duke L D 1973 *Comparative morphology of the mammalian ovary.* Univ. of Wisconsin Press, Madison. 461 pp.

10 Mossman A S, and Mossman H W 1962 Ovulation, implantation and fetal sex ratio in impala. *Science, N.Y.* 137: 869.

11 Nilsson O, and Reinius S 1969 Light and electron microscopic structure of the oviduct. In: *The mammalian oviduct.* Ed. Hafez E S E, and Blandau R J The University of Chicago Press. 546 pp.

12 Robinson A 1934 *Cunningham's manual of practical anatomy.* Vol. II. Oxford University Press. 524 pp.

13 Sisson S, and Grossman J D 1940 *The anatomy of the domestic animals.* Saunders W B, Philadelphia. 972 pp.

14 Skinner J D, and Van Zyl J H M 1970 A study of growth of springbok ewes. *African Wild Life.* 24: 149–154.

THE EPIZOOTIOLOGY OF NEMATODE PARASITES OF SHEEP IN A HIGH-RAINFALL AREA OF ZIMBABWE

J.L. GRANT

ABSTRACT: Grant J.L. **The epizootiology of nematode parasites of sheep in a high-rainfall area of Zimbabwe.** *Journal of the South African Veterinary Association* (1981) **52** No 1, 33–37 (En) Grasslands Research Station, P.B. 701, Marandellas, Zimbabwe.

Fifty-two untreated lambs from a contaminated flock were slaughtered at regular intervals throughout the year, and their gastro-intestinal tracts were examined for the presence of nematode parasites.

Haemonchus contortus and *Oesophagostomum columbianum* were found to be of major importance. The incidence of *Haemonchus* rose to a peak and remained at a high level throughout the winter though the fourth larval stages of the parasite predominated at this stage.

Oesophagostomum columbianum was recovered in numbers unusually high for this species, the incidence of which remained at a relatively high level from March until October.

The minor genera recovered were *Trichostrongylus* spp., *Cooperia* spp., *Strongyloides papillosus* and *Trichuris ovis.*

INTRODUCTION

There is little doubt that helminthiasis is one of the major causes of loss of animal production and is widespread in all the countries of the world[23]. The onset of parasitic disease is generally insidious; its effects may be long-lasting, and growth and development of the host may be seriously retarded before clinical signs of disease become apparent.

Outbreak of such disease in domestic livestock is limited by an environment hostile to the development of the free-living stages of the parasites. When the environment becomes favourable, in the absence of the controlling influence of anthelmintic treatment, parasitic disease will occur. This is evidenced by:

I. reduction in appetite;
II. impaired digestive efficiency; and
III. pathogenic effects[15].

Though the reasons for these effects are obscure, they may be due to:

I. the possible secretion by parasites of toxins which cause a pathological condition in the host;
II. removal of blood by blood-sucking parasites which may cause a severe anaemia as in the case of acute haemonchosis; and
III. the production of enzyme inhibitory substances by parasites which may reduce digestive efficiency and induce malnutrition[19].

In order to assist in the control of parasitic disease, it is necessary to know something about the seasonal distribution of parasites. Since information on this aspect was lacking in Zimbabwe, a study was carried out at Grasslands Research Station, Marandellas. This Station is situated at latitude 18′ 10° south, longitude 31′ 30° east, at an altitude of 1 646 m above sea level. The average rainfall experienced at the Station is 935 mm distributed mainly between November and March, and the climate because of the altitude is cool. Temperatures vary between a mean minimum of 6 °C in July and a mean maximum of 27 °C in October.

MATERIALS AND METHODS

Mutton Merino lambs, with some Hampshire and Suffolk crosses, were used in the survey. The ewes with lambs at foot (born in October) were lightly stocked on fertilized Star grass *(Cynodon aethiopicus)* and Love grass *(Eragrostis curvula)* pastures which had been grazed by both sheep and cattle in previous years. The ewes were treated with the anthelmintic thiabendazole shortly before lambing and at 4 to 6 weekly intervals throughout summer. The experimental lambs received no anthelmintic treatment. From mid-November and every 28 days thereafter, 4 lambs were removed from pasture and placed in clean concrete pens. The lambs were slaughtered on 4 consecutive days, after 24 h of starvation, and their gastro-intestinal tracts were removed. Each tract was immediately tied with a double ligature between the omasum and abomasum, the pyloric region of the abomasum, the small intestine just proximal to the caecum, and the anus. The different sections of the tract were separated by cutting between the double ligatures and processed in a water-bath at 38 °C[18 21]. Similarly, the cleaned sections of the gut wall were scraped with a glass slide, the mucosa and muscular layers removed and digested in the water-bath for 12 hours in 3% HCℓ, 1% pepsin solution.

The filtrates and digesta were concentrated through a 400 mesh sieve (aperture diameter 37 microns) under a water jet, an equal volume of formal saline solution added (1 part 40% formaldehyde, 9 parts 0,85% saline solution), stored and labelled for microscopic examination. The ingesta were examined a few mℓ at a time under a strong light in a white enamel tray for any nematodes which failed to migrate through the nylon mesh. The mesh was examined similarly and all trapped nematodes were identified and counted *in situ.*

A representative sample was removed and stored for microscopic examination. The filtrates and digesta were examined, a few mℓ at a time, under a dissecting microscope and all worms were removed, identified and counted. Detection of worms was aided by the addition of concentrated iodine solution. Sexually mature males were identified on a specific basis, while females and the larval stages were identified on a generic basis.

RESULTS

Ten species of nematodes, from 6 genera, were recovered from the 52 necropsies. These were, in order of predominance, numerically:

Haemonchus contortus
Trichostrongylus axei
Trichostrongylus colubriformis

0038-2809/81/01/0033–0037

Trichostrongylus falculatus
Oesophagostomum columbianum
Cooperia pectinata
Cooperia punctata
Cooperia spatulata
Strongyloides papillosus
Trichuris ovis

Haemonchus contortus

This species was by far the predominant nematode parasite of sheep encountered in the survey, and this is probably true of the whole of the higher-rainfall area of the country. The distribution is given in Fig. 1. The incidence of adult *H. contortus* rose steadily to a peak in May, was relatively low during the winter months of June to August and rose again in September. It is of interest to note that the fourth larval stages of the parasite predominated over the winter months. Third larval stages, though recovered in small numbers, occurred in the animals between March and July and may have been picked up on pasture[8 9 17] or may have been acquired some months before and failed to develop.

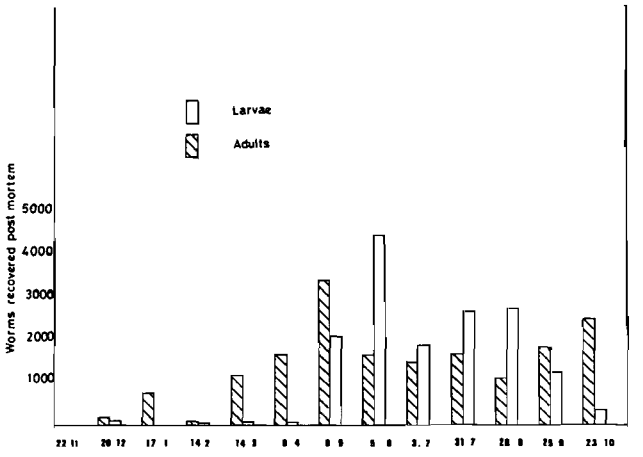


Fig. 1.

Trichostrongylus spp.

This parasite, though second in predominance, is of little pathogenic significance because this genus was present in small numbers. The seasonal incidence is illustrated in Fig. 2. The parasite was recovered from lambs slaughtered throughout the year but occurred most frequently in June. Relatively few larval stages were recovered and these were found mostly between March and June. *T. axei,* the abomasal species, was the most common of the three.

Oesophagostomum columbianum

This species was recovered in fairly large numbers for this parasite, mainly between March and October (Fig. 3). During this period individual animals harboured heavy burdens, the largest number recovered being 721 adult specimens from a lamb slaughtered in April. Prior to March very few specimens were seen.

Fourth larval stages were recovered in moderate numbers between March and August, after which they largely disappeared.

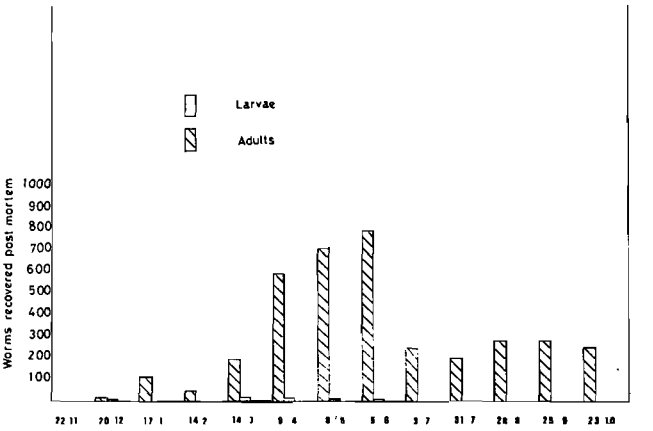


Fig. 2.

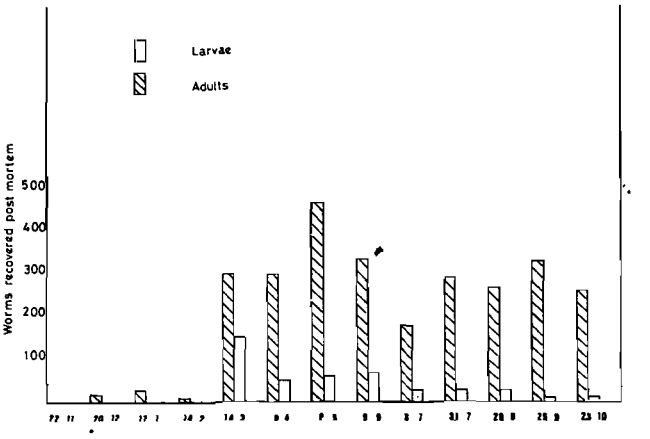


Fig. 3.

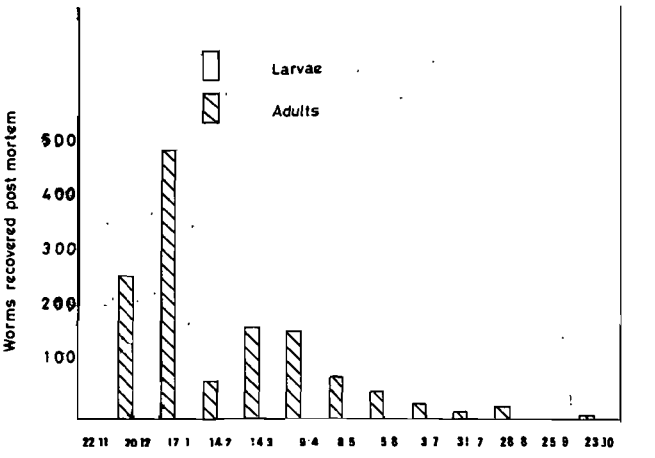


Fig. 4.

Cooperia spp.

The incidence of this parasite is of interest in that it was recovered in moderate numbers from lambs slaughtered in the early part of the year, from December to April, after which few worms were recovered and they disappeared entirely after August (Fig. 4). Three species of the genus (possibly a fourth) were identified: *C. pectinata, C. punctata* and *C. spatula. C. pectinata* was recovered most frequently between December and April, while *C. punctata* persisted in small numbers until August.

Fourth-stage larvae, though recovered in small numbers, occurred between December and July.

Strongyloides papillosus

This species is of little pathogenic significance in sheep at pasture in this region, as it occurs in small numbers. Nevertheless, it was found throughout the year (Fig. 5) and was seen in all but 5 lambs examined. The largest number recorded was 704 adult specimens from an animal slaughtered in August.

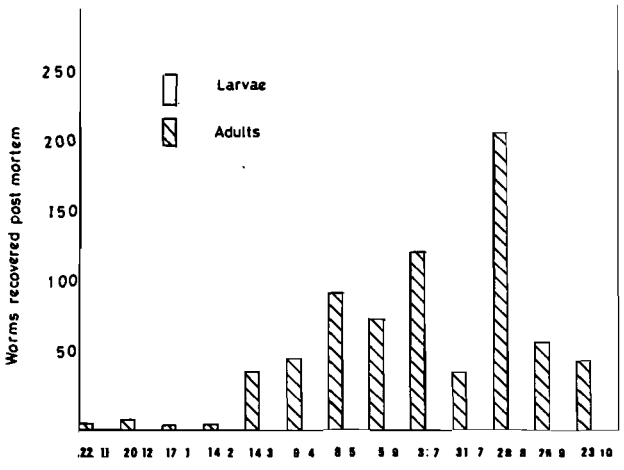


Fig. 5.

Trichuris ovis

This species occurred in insignificant numbers intermittently throughout the year. It is of little consequence as only 27 adult specimens were recovered in all.

Cestodes and Trematodes

More than half of the lambs examined harboured one or more of the tape worm, *Moniezia expansa,* which were present throughout the year. No specimens of the liver tape worm *Stilesia hepatica* were seen in any of the experimental lambs and, because of the isolation of pastures from natural water sources, flukes were also absent.

CLIMATIC DATA

The total rainfall recorded during the experimental period was 474 mm, less than half the normal experienced at the station. This may have adversely affected the development and survival of the free-living stages of the parasites on pasture. Though unlikely to have altered the species composition, the low rainfall, coupled with increased hours of sunshine because of reduced cloud cover, would have to some extent inhibited the development of *H. contortus* and *O. columbianum* in particular, both of which thrive in warm moist conditions. This may have accounted for the lower than expected (16%) mortality from verminosis in the untreated experimental flock.

DISCUSSION

H. contortus is an abomasal parasite which is an avid blood-sucker. It is prolific, has a relatively short generation interval, is able therefore to take advantage rapidly of favourable environmental conditions, and heavy infestations are common in the warm, wet summers.

Generally, conditions are favourable for the development of the free-living stages when mean maximum temperatures exceed 18 °C[5] diurnal fluctuation is between 23,3 °C and 11,6 °C[6] and mean monthly rainfall exceeds 50 mm[10]. These conditions are satisfied in most parts of this country between November and April, and outbreaks of haemonchosis may occur during at least 6 months of the year and when unseasonal rains occur the period may be extended. The numbers of *H. contortus* recovered in the present survey did not reach significant proportions until the season was well advanced. This may be explained by three factors:

I. The age of the animals slaughtered in the early part of the season may well have precluded them from harbouring heavy burdens as, not being weaned, their dependence on pasture was limited.
II. The ewes were regularly treated with anthelmintics and the initial rate of pasture contamination with worm eggs would therefore have been reduced.
III. The reduced rainfall and increased hours of sunshine are likely to have inhibited the development and survival of the free-living stages.

The rise in the incidence of the parasite corresponded to weaning in early February, a time of natural stress to the animals and a time when their total dependence on pasture for food would result in a greater ingestion of infective larvae.

The increased incidence of fourth-stage larvae from April was marked. That these predominated over the winter months is in agreement with the findings of others[12 16 17 20 24]. This retarded development of the fourth stage is probably a result of host resistance[2 17] or may be a natural phase in the life-cycle of the parasite[16] ensuring its survival and regeneration from one season to the next. The rise in the incidence of adults from August is due to the development of latent larvae, which diminished in number from this period. It is unlikely to be due to the acquisition of infective larvae from pasture as conditions during winter are unsuitable for the development of the free-living stages of this species. This confirms the observations of Condy[3] and Condy and Hanham[4] at this station, and Horak and Louw[11] under similar climatic conditions on the Transvaal Highveld.

This phenomenon is probably responsible for the 'spring-rise' in worm egg counts and is triggered by physiological stress, particularly pregnancy, in the host animal[1 2 7]. The result is an increased contamination rate of pasture at a time when there is an influx of susceptible hosts in the new lamb crop.

Trichostrongylus spp. are generally cool-season parasites thriving when mean monthly termperatures range from 14 to 18 °C and disappearing when they exceed 20 °C. The parasite is of considerable importance in the winter and non-seasonal rainfall areas of South Africa[16] where infective larvae are common on pasture during the winter months and may disappear during the summer. The markedly seasonal rainfall experienced in Zimbabwe, with warm, wet summers and cool, dry winters, does not favour the development of this parasite. The incidence is therefore low with peak burdens in May and June after which the numbers recovered *post mortem* were small.

O. columbianum is one of the more pathogenic parasites of sheep and is therefore of considerable ecological importance. The average burden in 5–7 month old weaners was 300–470 adult worms per lamb from March to May, while it has been indicated that 200 worms in adults and 80–90 in young sheep would constitute a severe infestation[10]. The warm, moist summers experienced in this region are well suited to the development and survival of the free-living stages of this species. They show little resistance to desiccation and would be unable to survive the long dry winters[2 13]. This, however, is of little consequence because the species can have a long prepatent period in which the larvae cause the formation of nodular lesions on the intestinal and caecal walls in which they can successfully hibernate within the host.

Cooperia spp. is an intestinal parasite considered to be not particularly pathogenic, although in heavy infestations clinical signs may be similar to those encountered in trichostrongylosis.[14].

There are 22 recognised species of the genus[22] although information on them is lacking. Warm, moist weather conditions offer the most favourable environment for development and survival of the free-living stages, and distribution of the parasite is widespread.

There is some controversy about their host specificity. The genus appears to be primarily a parasite of bovines though some species, particulary *C. curticei* and *C. oncophora,* occur in ovines. Neither of these was recovered in the present survey.

Apart from the three species recovered, *C. punctata, C. pectinata* and *C. spatulata,* a fourth species was noted of which only 2 male specimens were seen. These were unlike any of the other species noted but resembled *Paracooperia;* (Prudhoe. S. 1970 Department of Zoology, British Museum of Natural History, personal communication). However, insufficient material was recovered for accurate identification.

S. papillosus

This parasite is of little pathogenic importance in this region although it was recovered in moderate numbers from lambs slaughtered throughout the year.

The infective larvae have no sheath and have therefore little resistance to desiccation. However, they thrive under warm, moist conditions and, since they can penetrate the skin of their host and reach the small intestine via the bloodstream, lungs and trachea, frequent heavy infestations are seen in lambs and kids that are kraaled at night in summer. Infection may also occur through oral ingestion or via the ewes' milk which may contain third-stage larvae.

T. ovis is a parasite of the large intestine and is of little significance in sheep in this region as it is rare. It is relatively non-pathogenic and only 27 specimens were recovered in all.

Of the 10 species of nematodes recorded from the 52 necropsies described above, only 2 are of any real significance. These are *H. contortus* and *O. columbianum,* both of which thrive during the warm, wet summers experienced in Zimbabwe. The genus *Trichostrongylus* is primarily a cool-season parasite and does not thrive in the warm summers, while the winters are too dry to support development of the free-living stages. *Cooperia* spp. are mainly parasites of bovines and occur infrequently in ovines. *S. papillosus* occurs in numbers too few to be of pathogenic significance and *T. ovis* is rare. No specimen of the hookworms *Gaigeria pachyscelis* and *Bunostomum triginocephalum* were encountered in the survey though both do occur in Zimbabwe.

The incidence of *Moniezia expansa* is widespread in lambs at pasture and, surprisingly, *Stilesia hepatica* was not seen in the experimental sheep.

This study, because of the laborious nature of this type of work, was of necessity confined to one area, albeit an area fairly representative of a vast agricultural region. Further work is needed on a country-wide basis to gain a fuller understanding of the distribution of nematode parasites of livestock in Zimbabwe.

Information hitherto available is sketchy and is largely dependent upon the personal observations of veterinarians and animal workers in the field.

REFERENCES

1 Crofton H D 1954 Nematode parasite populations in sheep on Lowland farms 1. Worm-egg counts in ewes. Parasitology 44: 465–477

2 Crofton H D 1963 Nematode parasite population in sheep and on pasture. Technical Communication of the Commonwealth Bureau of Helminthology No 35

3 Condy J B 1961 Seasonal fluctuations in the worm egg counts of sheep in Southern Rhodesia. British Veterinary Journal 117: 152–157

4 Condy J B, Hanham D P 1966 Nematode infestation of sheep in a high-rainfall area of Rhodesia. Rhodesia Zambia Malawi Journal of Agricultural Research 4: 39–44

5 Dinaburg A G 1944 Development and survival under outdoor conditions of eggs and larvae of the common ruminant stomach worm *Haemonchus contortus* Journal of Agricultural Research 69: 421–423

6 Dinnik J A, Dinnik N N 1958 Observations on the development of *Haemonchus contortus* larvae under field conditions in the Kenya Highlands. Bulletin of Epizootic Diseases of Africa 6: 11–21

7 Dunsmore J D 1965 *Ostertagia* spp. in lambs and pregnant ewes Journal of Helminthology 39: 159–184

8 Durie P H 1961 Parasitic gastro-enteritis of cattle: The distribution and survival of infective strongyle larvae on pasture. Australian Journal of Agricultural Research 12: 1200–1211

9 Durie P H 1962 Parasitic gastro-enteritis of cattle: Seasonal fluctuations in populations of strongyle larvae on a calf pasture and their significance in the infestation of the grazing animal. Australian Journal of Agricultural Research 13: 767–777

10 Gordon H Mcl 1950 Some aspects of parasitic gastro-enteritis of sheep. Australian Veterinary Journal 26: 14–28, 46–52, 93–98

11 Horak I G, Louw J P 1977 Parasites of domestic and wild animals in South Africa. IV. Helminths in sheep on irrigated pastures on the Tranvaal highveld. Onderstepoort Journal of Veterinary Research 44: 261–270

12 Horak I G 1978 Parasites of domestic and wild animals in South Africa. V. Helminths in sheep on dryland pasture on the Transvaal Highveld. Onderstepoort Journal of Veterinary Research 45: 1–6

13 Kates K C 1950 Survival on pasture of free-living stages of some common gastro-intestinal nematodes of sheep. Proceedings of the Helminthological Society of Washington 17: 39–58

14 Lapage G 1962 Mönnigs Veterinary Helminthology and Entomology. 5th Ed. Bailliere, Tindall and Cox, London.

15 Muller G L 1964 The relationship between helminth infestation production and nutrition. Journal of the South African Veterinary Medical Association 35: 535–539

16 Muller G L 1968 The epizootiology of helminth infestation in sheep in the South Western districts of the Cape. Onderstepoort Journal of Veterinary Research 35: 159–194

17 Reinecke R K 1964 Epizootiology and control of nematode parasites in sheep. Journal of the South African Veterinary Medical Association 35: 603–608

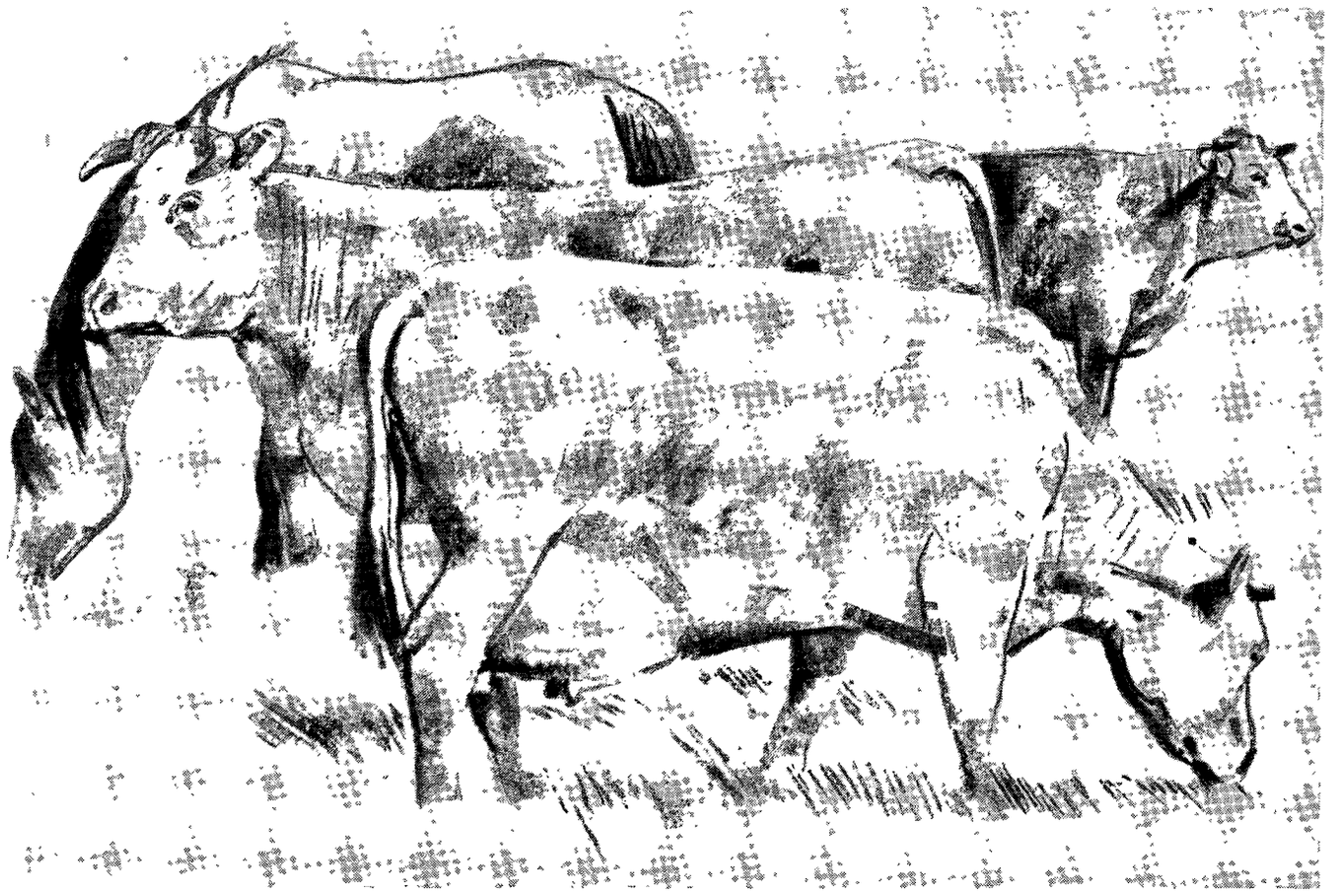
18 Reinecke R K 1967 Improved methods for the recovery of parasitic nematodes at autopsy Onderstepoort Journal of Veterinary Research 34: 547–562

19 Reveron A E, Topps J H 1970 Nutrition and gastro-intestinal

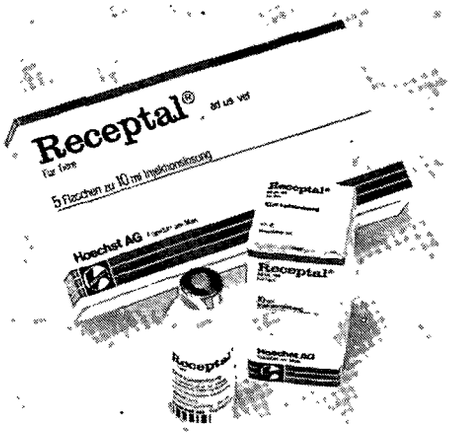
parasitism in ruminants. Outlook on Agriculture 6: 131–136
20 Rossiter L W 1964 The epizootiology of nematode parasites of sheep in the Coastal area of the Eastern Province. Onderstepoort Journal of Veterinary Research 31: 147–150
21 Shone D K, Philip J R 1967 Anthelmintic and toxicity studies with Tetramisole. 1. Anthelmintic efficacy. Journal of the South African Veterinary Medical Association 38: 165–176
22. Skrjabin K I, Shikliobalova N P, Shul'ts R S 1954 Essentials of Nematodology. Volume III: 1. The Academy of Sciences of the USSR. Moscow (English Translation).
23. Soulsby E J L 1963 The evaluation of anthelmintics. Proceedings of the lst International Conference of the World Association for Advancement in Veterinary Parasitology. August 22–23 Hanover, Germany.
24 Viljoen J H 1964 The epizootiology of nematode parasites of sheep in the Karoo. Onderstepoort Journal of Veterinary Research 31: 133–142.

Receptal®

(Synthetic releasing hormone for the release of both lutenising and follicle—stimulating hormones)



YOUR 12 POINT PLAN TO TREAT FOLLICULAR CYSTS



* RECEPTAL® contains the synthetic releasing hormone Buserelin This is equivalent to the natural LH/FSH releasing hormone in its chemical structure and in its action
* The only effect observed is the release of the gonadotrophin LH and FSH
* Thus RECEPTAL® has a physiological mode of action
* There is no risk of hormonal imbalance
* Receptal is synthetically produced and there is no variation in its biological activity
* Low dosage volume
* No adverse systemic or local effects have been observed following the use of RECEPTAL®
* No immunological response to repeated injected has been observed
* Manual rupture of cysts is unnecessary
* Ready to use solution for muscular injection
* No residue restriction period on the use of meat or milk
* RECEPTAL® has a short half-life of 5—10 minutes

Hoechst

Hoechst Pharmaceuticals (Pty) Ltd
3 Caxton Street Industria
P.O. Box 8692 Johannesburg 2000

Veterinary Division

THE EFFICACY OF FENBENDAZOLE AT A DOSAGE RATE OF 5 mg/kg AGAINST NEMATODE INFESTATIONS IN CATTLE

F.S. MALAN

ABSTRACT: Malan, F.S. **The efficacy of fenbendazole at a dosage rate of 5 mg/kg against nematode infestations in cattle.** *Journal of the South African Veterinary Association* (1981) **52** No. 1, 39–44 (En) Hoechst Research Station, P.O. Box 124, Malelane, 1320, Republic of South Africa.

The anthelmintic efficacy of fenbendazole, dosed to artificially infested cattle at 5 mg/kg live mass was determined against immature and adult *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp., *Bunostomum phlebotomum* and *Oesophagostomum radiatum.* At this dosage rate, fenbendazole was more than 80% effective in more than 80% of the animals treated against the abovementioned nematodes. The only exception was against the third-stage larvae of *B. phlebotomum* when it fell to more than 60% effective in more than 60% of the animals treated.

INTRODUCTION

Fenbendazole: methyl 5-(phenyl-thio)-2-benzimidazole-carbamate (Loewe and Urbanietz[10]) is registered in the Republic of South Africa as an anthelmintic for cattle at a dosage rate of 10 mg/kg live mass.

Registration trials with fenbendazole at a dosage rate of 7,5 mg/kg live mass were done and at this dosage rate, fenbendazole was more than 80% effective in more than 80% of the animals treated against *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp., *Bunostomum phlebotomum* and *Oesophagostomum radiatum*[11].

The use of fenbendazole at a dosage rate of 5 mg/kg live mass is reported in the literature. In *Trichostrongylus* spp. efficacy was 100% against adult parasites[1, 3, 4, 5, 6, 16, 17, 18]. In *Cooperia* spp. efficacy was>99% against immatures and >99% against adults[1 3–7 17 18]. Against *Haemonchus* spp efficacy was >96% against immatures and >99% in adults[1 5-8 17–19]. Against *Ostertagia ostertagi* results were >94% in immatures and >99% in adults[1 3–8 16–18] In *Oesophagostomum* spp. efficacy was 100% in both immature and adult stages[1 3–7 16 17 19]. Against immature and adult *Bunostomum* spp. a 100% efficacy was obtained[7, 16]. Against adult and immature *Dictyocaulus viviparus* an efficacy of >99% was obtained[2–7 14 15].

The present paper reports the results of anthelmintic trials conducted according to the modified non-parametric method of Groeneveld & Reinecke[9]. Cattle were artificially infested with *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp., *Bunostomum phlebotomum* and *Oesophagostomum radiatum* and treated with fenbendazole at a dosage rate of 5 mg/kg live mass when the worms were in either the third or fourth larval or adult stages.

MATERIALS AND METHODS

Sixty, three-month-old crossbred beef calves were housed in concrete-floored cattle pens. The calves were bought from farmers in the Vrede district of the Orange Free State and were unweaned at that stage. Weaning took place in the pens. They were fed a calf concentrate twice daily and worm-free hay was available *ad lib.*

The animals were treated on two occasions with a broad-sprectrum anthelmintic for nematodes and cestodes and were vaccinated against *Clostridium chauvoei, Pasteurellosis, Chlamydiosis* and *Clostridium botulinum.*

The calves were divided into three groups, each of which was dosed orally on several occasions with infective larvae of *Haemonchus placei, Ostertagia ostertagi, Cooperia* spp. (*C.pectinata* and *c.punctata*) and *Oesophagostomum radiatum. Bunostomum phlebotomum* was infected percutaneously.

These infestations were so planned that at the time of treatment the worms present would be in the third larval stage in the one group, the fourth in the next and adult in the third group.

Treatment with fenbendazole at 5 mg/kg live mass was administered *per os* with a syringe. A 10% suspension of FBZ was used and its concentration was confirmed before it was dosed.

Autopsies were conducted according to the methods described by Reinecke[12, 13]. Worm recovery in all cases was achieved by washing the gastro-intestinal contents on a 150 μm aperture sieve held over a large sieve of 38 μm apertures.

All digest samples were examined microscopically. A 1/10 aliquot was drawn from each specimen in order to recover and identify larvae which may have been retarded in their development. All macroscopic counts were made by means of a magnifying sorting lamp.

In those animals in which the total worm count of a particular species was estimated to exceed 1 000 worms, three 1/10 aliquots were examined. One of the aliquots was examined by means of a stereoscopic microscope and the other two were examined macroscopically. Specimens were recounted to avoid any possible error in finding the median value[13].

Specimens were also recounted where any doubt existed as to the efficacy of the drug[13].

Worm recoveries from control and treated calves were subjected to statistical analysis by the method described by Groeneveld & Reinecke[9].

RESULTS

The results of the larval anthelmintic tests have been summarized in Table 1.

Haemonchus placei

Third-stage larvae: The median burden of the seven control calves was 1861 with a variation between 336 and 3 327 worms. The range in worm recovery from the treated animals was 0–3.

Fourth-stage larvae: Worm recoveries from the controls varied from 462 to 975 with a median value of 752. Worm recovery from the treated animals was 0–1.

0038-2809/81/01/0039–0044

Fifth-stage and adults: The median burden of the control was 1 288 with a variation between 759 and 1 659 worms. The range in worm recovery from the treated animals was 0–3.

On statistical analysis, Class A was obtained against each of the developmental stages.

Ostertagia ostergi

Third-stage larvae: The median burden of the seven control calves was 2 582 with a variation between 1 201 and 2 790 worms. The range in worm recovery from the treated animals was 10–228 worms.

Fouth-stage larvae: Worm recoveries from the controls varied from 1 005 to 3 690 with a median value of 2 125. Worm recovery from the treated animals was from 12 to 572.

Fifth-stage and adults: The median burden of the controls was 1 095 with a variation between 847 and 1 866 worms. The burdens recovered from the treated animals ranged from 0 to 11 worms.

Class A efficiency rating was obtained against each of the developmental stages.

Cooperia spp.

Third-stage larvae: The median burden of the seven control calves was 2 018 with a variation between 696 and 3 560 worms. The range in worm recovery from the treated animals was 0–41 worms.

Fourth-stage larvae: Worm recoveries from the controls varied from 120 to 2 061 with a median value of 497. No worms were found in the treated group.

Fifth-stage and adults: The median burden of the controls was 369 with a variation between 2 and 1 139 worms. The highest count in the treated animals was 1.

Class A was obtained against each of the developmental stages.

Bunostomum phlebotomum

Third-stage larvae: The median burden of the seven control calves was 45 with a variation between 5 and 262. The burdens recovered from the treated animals ranged from 0 to 36.

Fourth-stage larvae: Worm recoveries from the controls varied from 72 to 307 with a median value of 139. No worms were recovered from the treated calves.

Fifth-stage and adults: The median burden of the controls was 222 with a variation between 118 and 392. Only one worm was found in the treated group.

On statistical analysis, a B efficiency rating was obtained against third-stage larvae. An A efficiency rating was obtained against fourth-stage larvae and against adults.

Oesophagostomum radiatum

Third-stage larvae: The median burden of the seven control calves was 316 with a variation between 199 and 601 worms. The variation in worm recovery from the treated animals was from 0 to 15.

Fourth-stage larvae: Worm recoveries from the controls varied from 113 to 432 with a median value of 236. Only one worm was found in the treated group.

Fifth-stage and adults: The median burden of the control calves was 257 with a variation between 66 and 551 worms. No worms were found in the treated group.

Fenbendazole has now been tested at 3 dosage levels in cattle, i.e. 10 mg/kg, 7,5 mg/kg and 5 mg/kg body mass, and a summary of these results are given in Tables 3, 4, 5, 6, and 7.

DISCUSSION

With the exception of L_3 of *B.phlebotomum* when efficacy fell to 88,9% at 5 mg/kg, FBZ at 5, 7,5 and 10 mg/kg exceeded 90% efficacy against all stages of development of *H. placei, O.ostertagi, O.radiatum* and *Cooperia* spp. When the dosage was 7,5 or 10 mg/kg, FBZ rose to exceed 98% against L_3, L_4, fifth and adult *B.phlebotomum.*

This lower efficacy against L_3 *B.phlebotomum* might be due to the following:

Firstly, infestation rate in the trial with L_3 of *B.phlebotomum* was lower than it was in the trials on the other developmental stages.

Secondly, the L_3 of *B.phlebotomum* are in the lungs and the concentration of FBZ in the lung circulation might be too low to kill all the larvae.

The number of adults recovered at necropsy is expressed as a percentage of the infective larvae dosed in Table 2.

In the previous trial where FBZ was dosed at 7,5 mg/kg live mass, larvae were dosed in such a manner that only one species was present in a specific organ in the gastro-intestinal tract, i.e. in one trial infective larvae of *O.ostertagi, Cooperia* spp. and *O.radiatum* were dosed, and in the other trial *B.phlebotomum* and *H. placei* were dosed.

In Table 2 the two methods of infestation are compared.

O.ostertagi and *H. placei* were present in higher percentages in animals than when both species were dosed simultaneously.

Infective larvae of *B.phlebotomum* and *Cooperia* spp. were dosed at an interval of three days and *Cooperia* spp. seemed to have depressed the establishment of *B.phlebotomum.*

In the fourth-stage and adult trial *B.phlebotomum* had a depressing effect on *Cooperia* spp. The time that elapsed between dosing infective larvae of *B.phlebotomum* and those of *Cooperia* spp. was 12 days in the L_4 trial and 29 days in the adult trial. It seems that the longer *B.phlebotomum* had time to establish itself before *Cooperia* spp., the stronger was the depressing effect.

A factor which should also be taken into consideration is that in the third-stage larval trial, *Cooperia* spp. infestation was given over a 3-day period with 2 000 larvae per dose. In the fourth-stage larval trial *Cooperia* spp. infestation was given over a 5-day period with 1 200 larvae per dose. In the fifth and adult-stage trial, *Cooperia* spp. was given over a 12-day period with 500 larvae per dose. In comparison although there was also a trial dose of 3 000 infective larvae of *B.phlebotomum* dosed to each calf, this was given a single dose.

Retardation was only seen during the adult trial, where smaller doses of *Cooperia* spp. were given over a longer period to the same calves that had been infested with *B.phlebotomum* 29 days prior to the first dose of infective larvae of *Cooperia* spp. It is suggested that both genera were adversely affected with regard to their ability to develop to adult worms, which may be

Table 1: LARVAL DOSE, RANGE IN NUMBER OF WORMS RECOVERED FROM 7 UNTREATED CALVES AND 11 CALVES GIVEN 5 mg/kg FBZ, PERCENTAGE REDUCTION IN WORM BURDENS AND EFFICACY CLASSIFICATION

	L_3		L_4		5th and Adult	
(a) *Haemonchus placei*						
Larval dose	5 000 (2 × 2 500)		5 040 (12 × 420)		4 992 (16 × 312)	
	Control	Treated	Control	Treated	Control	Treated
Range of worm burdens	336–3 327	0–3	462–975	0–1	759–1 659	0–3
Median	1 861		752		1 288	
Group mean	1 866	0,3	765	0,2	1 210	0,3
Group mean reduction		99,9%		99,9%		99,9%
Control median × 0,25	465	0/11>465	188	0/11>188	322	0/11>322
Efficacy classification		A		A		A
(b) *Ostertagia ostertagi*	L_3		L_4		5th and Adult	
Larval dose	3 999 (3 × 1 333)		4 000 (8 × 500)		3 995 (17 × 235)	
	Control	Treated	Control	Treated	Control	Treated
Range of worm burdens	1 201–2 790	10–228	1 005–3 690	12–572	847–1 866	0–11
Median	2 582		2 125		1 095	
Group mean	2 202	105	2 202	217	1 241	1,5
Group mean reduction		95,2%		90,1%		99,9%
Control median × 0,25	645	0/11>645	531	1/11>531	274	0/11>274
Efficacy classification		A		A		A
(c) *Bunostomum phlebotomum*	L_3		L_4		5th and Adult	
Larval dose	3 000 (1 × 3 000)		3 000 (1 × 3 000)		3 000 (1 × 3 000)	
	Control	Treated	Control	Treated	Control	Treated
Range of worm burdens	5–262	0–36	72–307	0	118–392	0–1
Median	45		139		222	
Group mean	72	8	160	0	249	0,1
Group mean reduction		88,9%		100%		99,9%
Control median × 0,25	11	3/11>11	35	0/11>35	56	0/11>56
Control median × 0,4	17	1/11>17				
Efficacy classification		B		A		A
(d) *Cooperia pectinata* and *Cooperia punctata*	L_3		L_4		5th and Adult	
Larval dose	6 000 (3 × 2 000)		6 000 (5 × 1 200)		6 000 (12 × 500)	
	Control	Treated	Control	Treated	Control	Treated
Range of worm burdens	696–3 560	0–41	120–2 061	0	2–1 139	0–1
Median	2 018		497		369	
Group mean	2 181	13	806	0	475	0,2
Group mean reduction		99,1%		100%		99,9%
Control median × 0,25	505	0/11>505	124	0/11>124	92	0/11>92
Efficacy classification		A		A		A
(e) *Oesophagostomum radiatum*	L_3		L_4		5th and Adult	
Larval dose	2 500 (10 × 250)		2 500 (10 × 250)		2 500 (20 × 125)	
	Control	Treated	Control	Treated	Control	Treated
Range of worm burdens	199–602	0–15	133–432	0–1	66–551	0
Median	316		236		257	
Group mean	336	3	273	0,1	320	0
Group mean reduction		99,1%		99,9%		100%
Control median × 0,25	79	0/11>79	59	0/11>59	64	0/11>64
Efficacy classification		A		A		A

Table 2: MEAN TAKE OF INFECTIVE LARVAE WHEN EITHER DOSED AS ONE OR TWO SPECIES PER ORGAN

Trial	*H. placei*		*O. ostertagi*		*Cooperia* spp.			*B. phlebotomum*			*O. radiatum*		
	S_A	T_A	S_A	T_A	S_A	T_{L_4}	T_A	S_A	T_{L_4}	T_A	S_A	T_{L_4}	T_A
	%	%	%	%	%	%	%	%	%	%	%	%	%
$^+L_3$	8,8	37,3	17,8	55	18,6	0	36,3	2,9	0	2,4	17,9	0	13,4
$^+L_4$	14,0	15,3	36,7	55	48,1	0	13,4	2,6	0	5,3	21,3	0	10,8
^-A	4,6	24,2	29,3	31	60,5	4,1	7,9	1,5	2	8,3	15,2	1	12,7

KEY: $^+L_3$ = Third-stage larvae trial
$^+L_4$ = Fourth-stage larvae trial
^+A = Adult-worm trial
T = More than one species per organ
L_4 = Fourth-stage larvae
A = Adult nematodes
S = Single species per organ

Table 3: A SUMMARY OF THE RESULTS OBTAINED WITH THE NON-PARAMETRIC METHOD (NPM) BY USING FENBENDAZOLE AT DOSAGE RATES OF 10 mg/kg, 7,5 mg/kg AND 5 mg/kg LIVE MASS IN CATTLE AGAINST *HAEMONCHUS PLACEI*

Worm species	Developmental stage		Dosage rate mg/kg live mass		
			10 mg/kg	7,5 mg/kg	5 mg/kg
Haemonchus placei	L_3	Median worm burden of 7 controls	127	209	1 861
		Variation of worm burden in 7 controls	33–681	87–1 160	336–3 327
		Variation of worm burden in 11 treated calves	0–6	0–2	0–3
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	98,6	99,9	99,9
	L_4	Median worm burden of 7 controls	294	668	752
		Variation of worm burden in 7 controls	95–695	39–1 504	462–975
		Variation of worm burden in 11 treated calves	0–7	0–2	0–1
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	98,4	99,9	99,9
	L_5 and A	Median worm burden of 7 controls	267	256	1 288
		Variation of worm burden in 7 controls	78–498	61–389	759–1 659
		Variation of worm burden in 11 treated calves	0–3	0	0–3
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,8	100	99,9

Table 4: A SUMMARY OF THE RESULTS OBTAINED WITH THE NON-PARAMETRIC METHOD (NPM) BY USING FENBENDAZOLE AT DOSAGE RATES OF 10 mg/kg, 7,5 mg/kg AND 5 mg/kg LIVE MASS IN CATTLE AGAINST *OSTERTAGIA OSTERTAGI*

Worm species	Developmental stage		Dosage rate mg/kg live mass		
			10 mg/kg	7,5 mg/kg	5 mg/kg
Ostertagia ostertagi	L_3	Median worm burden of 7 controls	145	609	2 582
		Variation of worm burden in 7 controls	11–463	0–1 427	1 201–2 790
		Variation of worm burden in 11 treated calves	0–9	0–82	10–228
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	97,8	95,1	95,2
	L_4	Median worm burden of 7 controls	724	1 262	2 125
		Variation of worm burden in 7 controls	188–1 042	745–2 351	1 005–3 690
		Variation of worm burden in 11 treated calves	0–6	20–81	12–572
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	98,3	95,7	90,1
	L_5 and A	Median worm burden of 7 controls	431	1 175	1 095
		Variation of worm burden in 7 controls	227–967	336–2 067	847–1 866
		Variation of worm burden in 11 treated calves	0–4	3–16	0–11
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,8	99,7	99,9

Table 5: A SUMMARY OF THE RESULTS OBTAINED WITH THE NON-PARAMETRIC METHOD (NPM) BY USING FENBENDAZOLE AT DOSAGE RATES OF 10 mg/kg, 7,5 mg/kg AND 5 mg/kg LIVE MASS IN CATTLE AGAINST *BUNOSTOMUM PHLEBOTOMUM*

Worm species	Developmental stage		Dosage rate mg/kg live mass		
			10 mg/kg	7,5 mg/kg	5 mg/kg
Bunostomum phlebotomum	L_3	Median worm burden of 7 controls	428	82	45
		Variation of worm burden in 7 controls	72–1 016	23–164	5–262
		Variation of worm burden in 11 treated calves	0–9	1	0–36
		Efficacy rating – Classification	A	A	B
		Efficacy rating – Percentage	98,5	99	88,9
	L_4	Median worm burden of 7 controls	110	27	139
		Variation of worm burden in 7 controls	76–368	13–297	72–307
		Variation of worm burden in 11 treated calves	0–2	0	0
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,7	100	100
	L_5 and A	Median worm burden of 7 controls	106	37	222
		Variation of worm burden in 7 controls	57–971	7–102	118–392
		Variation of worm burden in 11 treated calves	0–6	0	0–1
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,7	100	99,9

Table 6: A SUMMARY OF THE RESULTS OBTAINED WITH THE NON-PARAMETRIC METHOD (NPM) BY USING FENBENDAZOLE AT DOSAGE RATES OF 10 mg/kg, 7,5 mg/kg AND 5 mg/kg LIVE MASS IN CATTLE AGAINST *COOPERIA PUNCTATA* AND *COOPERIA PECTINATA*

Worm species	Developmental stage		Dosage rate mg/kg live mass		
			10 mg/kg	7,5 mg/kg	5 mg/kg
Cooperia spp	L_3	Median worm burden of 7 controls	1 149	1 076	2 018
		Variation of worm burden in 7 controls	604–2 497	37–2 236	696–3 560
		Variation of worm burden in 11 treated calves	0–21	0–13	0–41
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	95,8	99,5	99,1
	L_4	Median worm burden of 7 controls	1 284	2 312	497
		Variation of worm burden in 7 controls	989–1 609	1 425–4 752	120–2 061
		Variation of worm burden in 11 treated calves	0–2	0–3	0
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,6	99,7	100
	L_5 and A	Median worm burden of 7 controls	1 345	3 315	369
		Variation of worm burden in 7 controls	972–2 679	1 458–5 223	2–1 139
		Variation of worm burden in 11 treated calves	0–21	0–1	0–1
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,7	99,9	99,9

Table 7: A SUMMARY OF THE RESULTS OBTAINED WITH THE NON-PARAMETRIC METHOD (NPM) BY USING FENBENDAZOLE AT DOSAGE RATES OF 10 mg/kg, 7,5 mg/kg AND 5 mg/kg LIVE MASS IN CATTLE AGAINST *OESOPHAGOSTOMUM RADIATUM*

Worm species	Developmental stage		Dosage rate mg/kg live mass		
			10 mg/kg	7,5 mg/kg	5 mg/kg
Oesophagostomum radiatum	L_3	Median worm burden of 7 controls	251	383	316
		Variation of worm burden in 7 controls	72–706	181–823	199–602
		Variation of worm burden in 11 treated calves	0–17	0–9	0–15
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	98	99,3	99,1
	L_4	Median worm burden of 7 controls	272	540	236
		Variation of worm burden in 7 controls	63–462	130–918	113–432
		Variation of worm burden in 11 treated calves	0–2	0–6	0–1
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,2	99,7	99,9
	L_5 and A	Median worm burden of 7 controls	179	373	257
		Variation of worm burden in 7 controls	78–644	41–593	66–551
		Variation of worm burden in 11 treated calves	0–8	0–1	0
		Efficacy rating – Classification	A	A	A
		Efficacy rating – Percentage	99,2	99,9	100

Table 8

Worm species	3rd-stage larvae	4th-stage larvae	Adult worms
Haemonchus placei	A	A	A
Ostertagia ostertagi	A	A	A
Bunostomum phlebotomum	B	A	A
Cooperia spp. *(C. pectinata* and *C. punctata)*	A	A	A
Oesophagostomum radiatum	A	A	A

Key:

Class	Definition
A	More than 80% effective in more than 80% of the treated herd
B	More than 60% effective in more than 60% of the treated herd.
C	More than 50% effective in more than 50% of the treated herd.
X	Ineffective.

due to prolonged dosing with small numbers of infective larvae of *Cooperia* spp.

If everything is taken into account, one comes to the conclusion that there is no advantage in dosing one nematode which inhabits a specific part of the digestive tract. The economic and time-saving factors obtained by dosing all nematodes to one calf proved to be superior. An important reservation is that young calves should be used with no previous experience of infestation with nematode parasites.

CONCLUSIONS

In the light of the above findings the anthelmintic efficacy of fenbendazole at 5 mg/kg live mass can be classified in terms of the requirements of the Registering Officer (Act 36 of 1947, Republic of South Africa) as is indicated in Table 8.

REFERENCES

1. Benz G W & Ernst J V 1977 Anthelmintic activities of fenbendazole in calves. Abstracts Meeting American Association of Veterinary Parasitologists Atlanta / Ga. – USA. July 11, 1977.
2. Cheney J M, Rubin R & de Ganahl A 1975 Efficacy of fenbendazole against *Dictyocaulus viviparous* in cattle. American Society of Parasitologists 50th Annual Meeting, New Orleans / La, – USA, April, 1975, p. 53.
3. Chroust K & Dyk V 1975 Zur Wirksamkeit von Fenbendazol, thiabendazol und Tetramisol auf Magen und Darmnematoden der Wiederkäuer. Deutsche Tierärztliche Wochenschrift 82: 487–491
4. Chroust K The efficacy of fenbendazole and other anthelmintics in ruminants. Proceedings 3rd International Congress of Parasitology, Munich/Germany *3*: 1400
5. Corba J, Reisz T, Krupicer I, Pacenovsky J & Breza M Ucinnost fenbendazolu (Panacur) u dobytka Invadovaneho gestrointestinalnymi a Plucnymi Nematodami (The Effectiveness of fenbendazole (Panacur) in cattle invaded by gastrointestinal and pulmonary nematodes) Veterinarni Medicina (Praha) *22*: 201–206
6. Crowley J W, Foreyt W J, Bliss D H & Todd A C 1977 Further controlled evaluations of fenbendazole as a bovine anthelmintic. Journal of American Veterinary Research *38*: 689–692
7. Düwel D & Kirsch R 1975 Experimentelle Untersuchungen zur Wirksamkeit von Fenbendazol auf Magen-, Darm – und Lungenwurmbefall des Rindes. Zeitschrift für Parasitenkunde *46*: 83–90
8. Duwel D 1976. Panacur – die Entwicklung eines neuen Breitband – anthelminthikums. Die Blauen Hefte *55*: 189–203
9. Groeneveld H T & Reinecke R K 1969 A statistical method for comparing worm burdens in two groups of sheep. Onderstepoort Journal of Veterinary Research 36: 285
10. Loewe H & Urbanietz J 1974 Proceedings of the Third International Congress of Parasitology 1306.
11. Malan F S 1979 The efficacy of fenbendazole at a dosage rate of 7,5 mg/kg against nematode infestation in cattle. Journal of the South African Veterinary Association 50: 161–163
12. Reinecke R K 1972 An anthelmintic test for the gastrointestinal nematodes of cattle. Onderstepoort Journal of Veterinary Research 36: 285.
13. Reinecke R K 1973 The larval anthelmintic test in ruminants Technical communication 106, Department of Agricultural Technical Services, Republic of South Africa
14. Saad M B E 1976 The efficacy of fenbendazole against lungworms in cattle. Thesis, Fort Collins/Co-USA
15. Saad M B E 1977 Efficacy of fenbendazole against adult *Dictyocaulus viviparus* in experimentally infected calves. American Journal of Veterinary Research 38: 1427–1428
16. Tiefenbach B 1976 Panacur – weltweite klinische Prüfung eines neuen Breitband – Anthelminthikums. Die blauen Hefte *55*: 204–218
17. Todd A C, Bliss D, Scholl Ph. And Crowley J W 1976. Controlled evaluation of fenbendazole as a bovine anthelmintic. The American Journal of Veterinary Research 37: 439–441
18. Wikerhauser T, Zuković M, Kuticić V, Meići A & Tabkovic R 1974 Studies of the efficacy of fenbendazole against gastro-intestinal nematodes of cattle. I.A preliminary study and a comparison with thiabendazole. Acta Parasitologica Jugoslavica *5*: 79–81
19. Wikerhauser T, Brglez J, Kuticic V, and Campa F 1974 Studies of the efficacy of fenbendazole against gastro-intestinal nematodes of cattle 11. Further trials and a comparison with tetramisole. Acta Parasitologica Jugoslavica *5*: 83–85.

LAPAROSCOPIC EXAMINATION TO DETERMINE SEX IN MONOMORPHIC AVIAN SPECIES

E.W. BURR*, F.W. HUCHZERMEYER† and A.E. RILEY‡

ABSTRACT: Burr E.W., Huchzermeyer F.W., Riley A.E. **Laparoscopic examination to determine sex in monomorphic avian species,** *Journal of the South African Veterinary Association* (1981) **52** No 1, 45–47 (En) A small animal otoscope was used as a laparoscope to determine the sex of several species of monomorphic birds. This instrument provided an inexpensive and accurate method for sexing birds.

INTRODUCTION

Mammalian and avian laparoscopy in the United States is well established[2 3 8] and remarkable success has been achieved. In South Africa, sexing of birds by laparoscopy is still a new field. Many practitioners have been loath to apply the technique due to the high cost of the equipment required. Our technique utilizes an otoscope that is readily available and inexpensive (Fig. 2.).

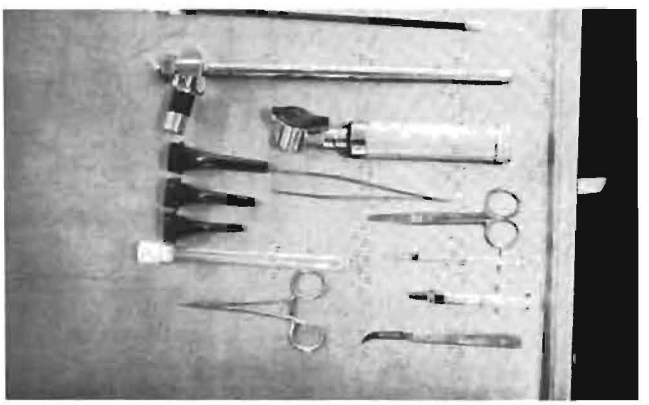


Fig. 2 Instrumentation required to perform laparoscopy in birds.

In recent years the high market value of psittacines has led many fanciers to breeding their own birds. In monomorphic birds like some species of parrots, pairing is sometimes impossible. Many fanciers try pairing off birds that have been seen in courtship display, in the act of mutual preening or during copulation. However, more often than not, 2 females or 2 males may be seen mating or finding mutual satisfaction in each other. In these cases and where birds show no signs of pairing, the need for an inexpensive and efficient method of sexing arises. In expensive birds such as the larger psittacines, a mistake in sexing could be costly because of a loss of one or more breeding seasons. Accurate sex determination before pairing will save the owner both time and money.

MATERIALS AND METHODS

Rhea, flamingo, chicken, pigeon, African Grey parrot, cockatiels, budgies and others were sexed under general anesthesia. Several of the specimens used were post mortem cases which were viewed by laparoscopy before dissection. This allowed confirmation of laparoscopic results and taking of photographs. Ketamine hydrochloride ("Ketaset")* at a dose of 50 mg/kg or metomidate ("Hypnodil")† at a dose rate of 10 mg/kg was injected into the pectoral muscles using a tuberculin syringe and a 26 gauge needle. Hypnodil was found to produce a better plane of anesthesia with a faster recovery time. The anaesthetised birds were placed on their right side to expose the left side for surgery. Feathers were plucked from an area just cranial and ventral to the greater trochanter of the left femur (Fig. 1). As a general rule, the penetration site was in the last

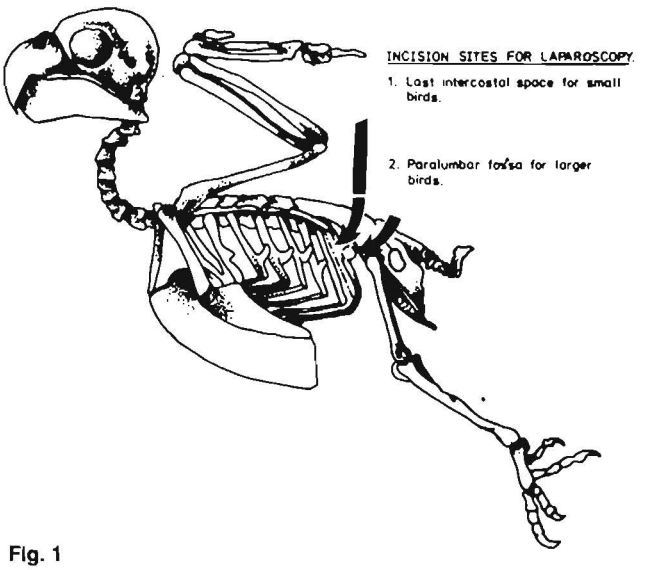


Fig. 1

Fig. 3 Surgical area is prepared and incision is made in the last intercostal space (post mortem case).

*Present address: 5 Larkey Rd., Oxford, Connecticut 06483, U.S.A.
†Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110.
‡Small Animal Clinic, Brooklyn Circle, Pretoria.

*Bristol Labs.
†Janssen Pharmaceutica.

0038-2809/81/01/0045–0047

intercostal space for conures and smaller birds and through the paralumbar fossa in larger birds[5]. The site was surgically prepared and a small incision (10–30 mm) long was made (Fig. 3). Gentle caudal traction on the legs resulted in tension over the area and allowed easier penetration and blunt dissection of the muscles. By blunt dissection the tissue was separated down to the abdominal air sac. The gonads were viewed through a small animal fibre-optic otoscope with various size heads depending on the size of bird to be examined (Fig. 4, 5 & 6). In some birds the air sac membrane was transparent enough to allow examination of the genital organs through the intact membrane. In larger birds the membrane had to be punctured. The gonads can be better viewed if an aquarium pump is used to deliver air through the otoscope head as this keeps the otoscope free of fluids and clears the field of vision[4]. A membrane filter* placed at the output of the air pump allows filtering out of debris and bacteria[4]. This lowers the possibility of bacteria and foreign particles entering the abdominal cavity.

Dorsally in the body cavity the testes or ovary can be identified at the cranial pole of the kidney adjacent to the adrenal gland. The testes are characterized by the presence of numerous vessels and by their elongated smooth appearance (Fig. 7). They can be pink to dark grey or black in colour. The ovaries, on the other hand, have numerous follicles of varying sizes which are transparent to dark yellow in colour.

Fig. 4 Laparoscopy examination with an otoscope on an African Grey parrot (post mortem case).

Fig. 5 Laparoscopy examination with an otoscope on a cockatiel (experimental case).

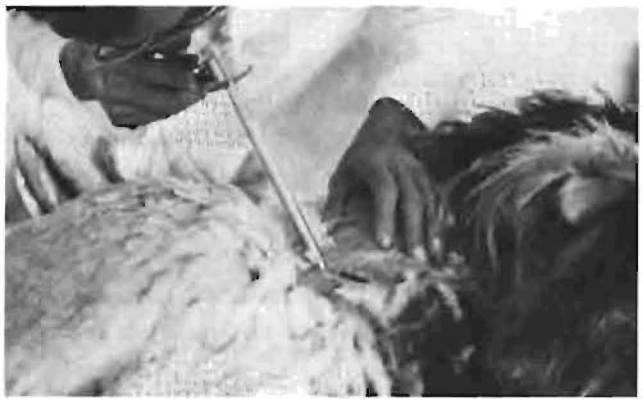


Fig. 6 Laparoscopy examination with an otoscope on a rhea (post mortem case).

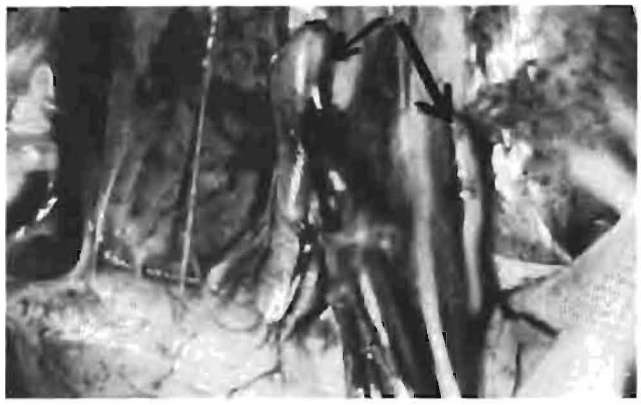


Fig. 7 Testes of the rhea.

Following surgery, the skin is sutured with 2–0 to 4–0 catgut using one or two horizontal mattress sutures (Fig. 11). This is mainly for aesthetic purposes in large and expensive psittacine birds. Post-operative care includes placing the birds in a heated cage (19–32°C or 85–90°F) and administration of 50–100 mg/kg ampicillin†.

Fig. 8 Lobulated adrenal glands in a flamingo resembling active ovaries.

*Millipore Filter Corp., Bedford, Mass. 01730
†Penbritin, Beecham

RESULTS AND DISCUSSION

The use of a small animal otoscope with various size heads has proved to be an inexpensive and effective way of sexing birds for the practitioner. The expensive and larger units[1,2,3,8], may allow for clearer viewing but certainly do not produce more accurate results. However, irrespective of the intrument used, the technique is not foolproof[1]. In obese birds, fat can be streaked with vessels and resemble testes. In obese birds, fat can be lobulated in such a way as to appear as ovarian follicles (Fig. 8). Intestines, lymph nodes, kidneys and adrenals need to be identified before positive identification of gonad tissues can be made.

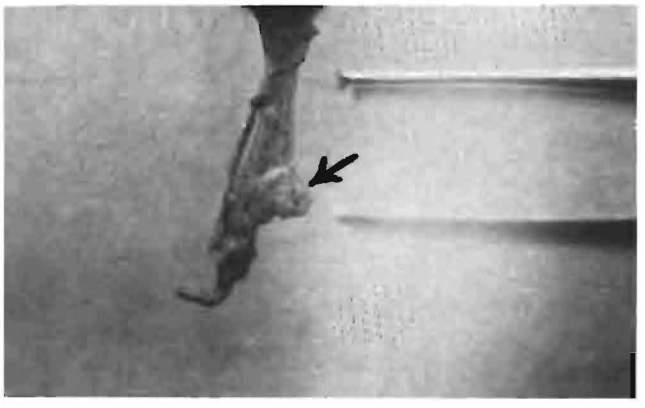


Fig. 9 Inactive ovary at the tip of the forceps in a flamingo.

Fig. 10 Normal active ovarian follicules in a pigeon.

Fig. 11 Simple interrupted suture is placed in the skin to close the incision site (Experimental case).

One of the other problems that may occur is haemorrhage from the intercostal or abdominal vessels. Insufflation with air may help in these cases to clear the field of blood if haemorrhage is slight. Administration of anticoagulants may also help. The size of the gonads is another consideration that must be anticipated before the bird is sexed. A juvenile rhea had testes a good 50 mm long, whereas in budgies the testes were 5 mm long or less. Size and development of testes and ovaries vary greatly with age and time of breeding season. The breeding season for most birds would be spring – in South Africa, August to November. The flamingo when sexed off-season showed ovarian follicles no bigger than pinpoints that would be difficult or impossible to detect by laparoscopy (Fig. 9). However, in the breeding season the follicles would no doubt be well developed and large enough to view. Only the left ovary of birds is developed so that a search for paired ovaries is fruitless. In pigeons, the breeding season extends throughout the year and the ovarian follicles are well developed and easily identified (Fig. 10). In pigeons and doves the anaesthetic should be injected close to the keel bone due to the presence of a pectoral venous sinus.

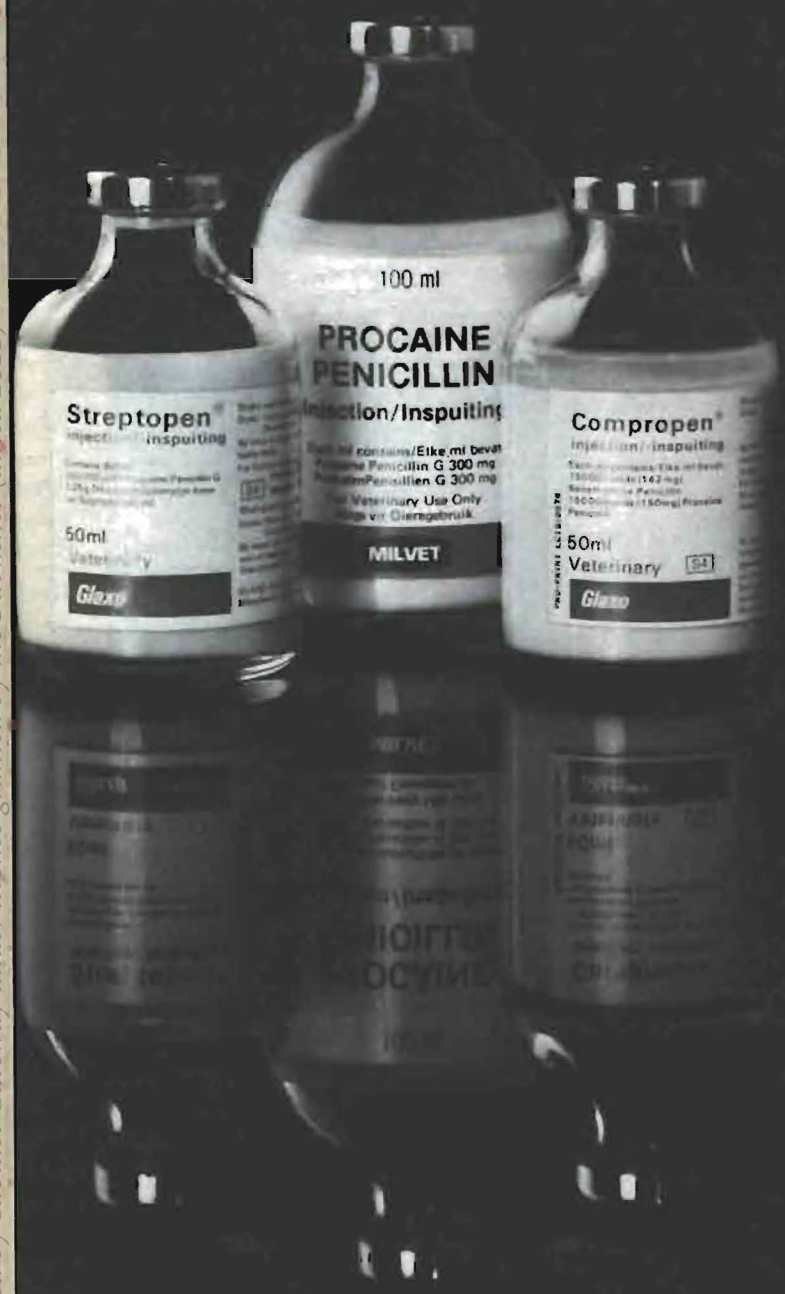
The sexing technique should be learned on chickens, pigeons and other inexpensive birds before turning to valuable exotic birds, but with practice and patience, the sex of monomorphic birds can be accurately determined using this technique.

REFERENCES

1. Billet R 1980 Sexing birds. Farmers Weekly. Feb. 20: 14–16
2. Bush M, Kennedy S, Wildt D E, Seager S W J 1978 Sexing birds by laparoscopy. International Yearbook 18: 197–198.
3. Bush M, Wildt D E, Kennedy S, Seager S W J, 1978 Laparoscopy in zoological medicine; Journal American Veterinary Medical Association 173 (9): 1081–1087.
4. Button C, 1980 (Personal Communication) Faculty of Veterinary Science, Dept. of Pharmacology & Toxicology, University of Pretoria.
5. Harrison G J, 1978 Endoscopic examination of avian gonadal tissues. Veterinary Medicine/Small Animal Clinician 73 (4): 479–484.
6. Harrison R M and Wildt D E, 1980 Editors, Animal Laparoscopy. Williams & Wilkins, Baltimore, MD.
7. Ingram K A 1978 Laparotomy technique for sex determination of Psittacine birds. Journal American Veterinary Medical Association. 173 (9): 1244–1246
8. Wildt D E, Bush M, Whitlock B S, Seager S W J, 1978 Laparoscopy: A method for direct examination of internal organs in zoo veterinary medicine and research. International Zoo Yearbook 18: 194–197.

. . . advances a step further

The Milvet range of Antibiotics . . .



New from Milvet

The introduction of Dihydro-streptomycin to our tried and trusted range of antibiotics for veterinary use represents a significant step forward in the fight against infection.

Dihydrostreptomycin offers a high level of antibacterial activity in the effective treatment of many Gram-negative and Gram-positive infections.

Furthermore, Dihydrostreptomycin is rapidly absorbed which results in high blood levels and fast action in reaching the site of infection.

Administered intramuscularly, the dosage is 5 mg/kg (1 ml/50 kg) every 8 hours regardless of species.

Presentation
10 x 50 ml Economy Pack

Dihydrostreptomycin

for antibacterial impact, when you need it

MILVET

DIAPHRAGMATIC HERNIA IN BOVINES

J. VAN HEERDEN AND W.A.S. BRÜMMER†

ABSTRACT: Van Heerden, J., Brümmer, W.A.S. **Diaphragmatic hernia in bovines.** *Journal of the South African Veterinary Association* (1981) **52** No 1, 49–50 (En) Dept. Medicine, Univ. Pretoria, Box 12580, Onderstepoort 0110, Rep. of South Africa.

Three cases of diaphragmatic hernia in bovines were encountered over a period of 5 years. The successful surgical repair of one case is reported.

INTRODUCTION

Diaphragmatic hernia in bovines has been described in bulls[2 6 9] cows, heifers[3 6 8 9] and calves[5 9 11]. In the latter the condition is congenital and may be responsible for death soon after birth[5] or the animal may survive for several months[9 11]. It has also been reported in male and female buffaloes *Bubalus bubalis*[1 4 6 7 8]. Clinical diagnosis is often difficult and surgical repair often unrewarding, although successful surgical repair has been described[3 9 20].

The condition has not been reported in the Republic of South Africa. Three cases were seen over a period of 5 years from 1971 to 1975. Two cases were presented as post mortem cases with a history of chronic intermittent bloat. The other case, a mature Friesland cow, was presented as a clinical case. The following report deals with the successful diagnosis and surgical repair of a diaphragmatic hernia in this cow.

CLINICAL FINDINGS AND SURGICAL TECHNIQUE

The cow showed chronic intermittent bloat and progressive mass loss and decline in milk production. The exact duration of the condition could, however, not be determined. Clinical examination revealed the left paralumbar fossa to be moderately distended with gas. Heart sounds were very clear on both sides of the chest. An intermittent pericardial friction rub was heard. Disappearance of this sound was associated with ruminal movements. Intestinal sounds could be heard cranial to the sixth rib. The bloating could be relieved by passing a stomach tube.

An exploratory laparotomy and rumenotomy revealed the following: mildly frothy ingesta, adhesions between the rumino-reticulum and body-wall, adhesions between the reticulum and diaphragm, and the presence of the reticulum in the thoracic cavity. The latter finding was evaluated by holding part of the ruminoreticular wall from the inside (as far cranially as was possible) and then pulling on it. It could be slightly withdrawn and on release it was immediately "sucked" back.

Based on these findings, a tentative diagnosis of diaphragmatic hernia was made.

One week following the exploratory rumenotomy a paramedian laparotomy was performed. The cow was prepared for surgery in the usual way. Anaesthesia was induced with intravenously administered chloral hydrate, the animal was intubated and anaesthesia was maintained with halothane (*Fluothane*, I.C.I.) and oxygen. Positive pressure anaesthesia was maintained when needed by manual compression of a rebreathing bag.

The patient was positioned in dorsal recumbency and an incision was made paramedially in the left paracostal space, just caudal to the xiphoid cartilage. The abdominal cavity was thus opened and the herniation of the reticulum into the thoracic cavity could be palpated just to the right of the median plane. Adhesions between the reticular wall and diaphragm were broken down by blunt dissection. Almost the entire reticulum was in the thoracic cavity and this was reduced manually by pulling on an umbilical tape purse-string suture positioned around the reticulum. The reticulum and rumen were pulled posteriorly and away from the operating area. The diaphragmatic gap was subsequently sutured together in a criss-cross pattern with through-and-through sutures using pliable orthopaedic steel wire. Each suture included a fair amount of tissue in every bite. Before the final sutures were secured, the lungs were maximally inflated with oxygen. Placing the sutures through the diaphragm resulted in mild haemorrhage which terminated on completion of suturing of the diaphragm. The reticulum was then returned to the abdominal cavity and sutured to the ventral abdominal wall. The abdominal incision was sutured in the usual manner.

During and after the operation, supportive treatment in the form of polyionic electrolyte solutions* and corticosteroids** were given. Antibiotics‡ were administered for one week.

For a couple of days following the operation, bloat was relieved by passing a stomach tube whenever it was necessary.

Rumenotorics in the form of sugar, vinegar, dried yeast and water mixture were also given.

DISCUSSION

The aetiology and pathogenesis of diaphragmatic hernia in bovines are not clear. It has been proposed that it might occur due to congenital or acquired causes such as traumatic reticuloperitonitis. The presence of a congenitally weak diaphragm prone to tear has also been proposed. Factors such as pregnancy, a fall or dystocia which cause an increase in intra-abdominal pressure might then trigger off a diaphragmatic hernia[8]. Improper embryologic fusion of the septum transversum and pleuroperitoneal folds might lead to a diaphragmatic defect[9].

Clinical diagnosis of the condition is difficult because of the wide range of symptoms manifested[6 9]. Chronic recurrent bloating, although not specific for the condition, was present in all the cases that we encountered and seems to be one of the most common symptoms. Auscultation of intestinal (reticular) sounds beyond the

†Box 368, Welkom

*Plasmolyte B. Baxter
**_Betsolan_ Glaxo
‡_Compropen_ Glaxo (Milvet)

0038-2809/81/01/0049–0050

6–7th intercostal space is taken by some authors to be pathognomonic for diaphragmatic hernia[6].

Radiographic examination might be useful in younger animals[9 11].

In our clinical case, final diagnosis was based on the results of an exploratory laparo-rumenotomy. This is in accordance with the findings of other authors[3 6]. The fact that bloat still occurred after the operation, can be ascribed to the after-affects of surgery, anorexia and ruminal stasis.

Different surgical approaches (via a thoracotomy or a paramedian laparotomy) have been used in correcting diaphragmatic hernias[3 9 10]. The defect has also on occasion been repaired by the incorporation of nylon mesh[9].

Our case made an uneventful recovery and was later sold in good physical condition.

REFERENCES

1. Angelo S J, Joshi B P 1969 Diaphragmatic hernia in a buffalo bullock. Indian Veterinary Journal 46: 828–829
2. Clatworthy R, Grant D G A 1971 Chronic bloat associated with diaphragmatic hernia in a bull. Rhodesian Veterinary Journal 2: 21–23
3. De Moor A, Verschooten F, Desmet P 1969 Thoracic repair of diaphragmatic hernia in a heifer. Veterinary Record 85: 87–89
4. Iyer G R 1969 Diaphragmatic hernia in buffaloes. Indian Veterinary Journal 46: 918
5. Lewis A M 1974 Congenital diaphragmatic hernia in the calf. Veterinary Record 2: 102
6. Mally K V, Jayadevappa S M 1974 Diaphragmatic hernia in bovines. Mysore Journal of Agricultural Science 8: 252–264
7. Naik P V, Mehendale S S 1969 A case of diaphragmatic hernia in a buffalo. Indian Veterinary Journal 46: 625–627
8. Seshadri S J, Subba Sastry G N, Rajasekharan D, Mohiyuddeen S 1971 Diaphragmatic hernia in bovines. Mysore Journal of Agricultural Science 5: 365–370
9. Troutt H F, Fessler J F, Page E H, Amstutz H E 1967 Diaphragmatic defects in cattle. Journal of the American Veterinary Medical Association 151: 1421–1429
10. Vig M M 1972 Studies on the diagnosis and repair of experimentally induced diaphragmatic hernia in buffaloes *(Bubalus bubalis)*. Indian Veterinary Journal 49: 1269–1271
11. Wagenaar G 1961 Hernia diaphragmatica bij een kalf. Tijdschrift voor Diergeneeskunde 86: 1359–1366

CURRENT POSITION OF THE F&DA REGARDING VETERINARY DRUG AND FEED ADDITIVE REGISTRATION IN THE USA

W.H.D. LEANING*

BACKGROUND

Programs and products intended to control diseases of domestic animals in order to increase their productivity have made tremendous advances. However, the development and sale of new drugs with genuine merit with regard to efficacy and safety also gave an opportunity to unscrupulous individuals to market some products of dubious efficacy or safety. To remedy this situation, the Pure Food and Drug Act was signed into law on June 23, 1906, by President Theodore Roosevelt. For 32 years this Act, which was amended several times during that period to meet other specific needs, was used as an efficient tool for the protection of the American consumer. In 1931, a new organization, the Food and Drug Administration (F&DA), was created to take over law enforcement functions that had been carried out by different organizations since January 1, 1907, when the Food and Drug Act of 1906 became effective. The authority of the F&DA was strengthened by the Food, Drug and Cosmetic (FD&C) Act which was signed into law by President Franklin Roosevelt on June 25, 1938. This new law increased penalties and gave the F&DA a new weapon, the court injunction, as an enforcement tool. The law also provided the F&DA with authority for pre-clearance review of safety for new drugs for use in man and animals. Although the Act prohibited the addition of poisonous or deleterious substances to food, it did provide for exemptions and safe tolerances for substances that were necessary in production or unavoidable. Since 1938 numerous amendments to the Food, Drug and Cosmetic Act have evolved. These amendments include the Insulin and Antibiotic Certification Amendment of December 22, 1941, which required batch certification of safety and efficacy of insulin and certain antibiotics. The Durham-Humphrey Amendment became effective on October 26, 1951, and required that drugs that cannot be safely used without medical supervision be dispensed only upon prescription. The Factory Inspection Amendment of August 7, 1953, clearly re-established the right of the F&DA to inspect at reasonable times and in a reasonable manner factories in which foods, drugs and cosmetics are manufactured. The Federal Insecticide, Fungicide and Rodenticide Act of 1947 gave the U.S. Department of Agriculture the responsibility for registering pesticides on proof of safety. The responsibility for establishing safety of these substances in or on raw agriculture commodities was transferred to the F&DA by the Miller Amendment to the FD&C Act which became effective on July 22, 1954. Today, the federal regulation of pesticides is the responsibility of the Environmental Protection Agency (EPA). The Food Additive Amendment to the FD&C Act became effective on September 6, 1958. It recognized the need for additives but also required that the manufacturer prove that the food additive was safe for man and animals under the conditions of its intended use. During the debate in the House of Representatives about the bill, a very well-known amendment sponsored by Congressman Delaney was included in the Act at the last minute. The amendment prohibited the use of a food additive if it was found to induce cancer in animals or man. On July 12, 1960, the Color Additive Amendment became effective and this Amendment also contains the Delaney Clause. With this new law, all colors used in foods, drugs and cosmetics were subject to the determination of their safety before certification for use.

On October 10, 1962, part of the Kefauver-Harris amendments became effective. These amendments require among others that new drugs be shown to be not only safe but also effective before approval for sale. These amendments also require that after a drug has been approved, the sponsor keep records and make periodic reports to the F&DA concerning data relating to clinical experience, adverse reactions, unexpected reactions, label mixups, instability, etc. The amendments also provided relief from the Delaney Clause by providing that animal drugs that are suspect carcinogens could be added to animal feed, provided they did not cause cancer in the target species and there was no residue of the drug in the edible tissues of treated animals. Good Manufacturing Practice Regulations were promulgated in 1963 under one of the provisions of the Kafauver-Harris Amendments, which allow F&DA to declare that a drug not manufactured according to the regulations is adulterated without needing proof of the adulteration of the product itself. On July 4, 1967, the Freedom of Information Act (FOIA) became effective. In 1976, in line with this Act, F&DA promulgated regulations to implement it. F&DA's interpretation of it requires that a summary of the safety and effectiveness data to support the approval of the application be made available to the public when the approval of an original or supplemental New Animal Drug Application (NADA) is published in the *Federal Register*.

On July 13, 1968, the President signed into law the Animal Drug Amendments which consolidated into one section of the Food, Drug and Cosmetic Act all aspects of the requirements for pre-market clearance of animal drugs. These requirements previously had been located in three different sections of the Act. Following passage of the National Environmental Policy Act (NEPA) of 1969, an environmental impact analysis report is now an integral part of the NADA. An analysis of the environmental impact of the manufacturing process itself as well as the fate of the drug from animal excreta and its effect on the ecology must be supplied. This Act was passed as a declaration of a national policy to encourage enjoyable harmony between man and the environment and to establish a Council on Environmental Quality (CEQ). The CEQ must report yearly to the President on the progress toward a cleaner environment. Good Manufacturing Practice (GMP)

*Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065 U.S.A.

0038-2809/81/01/0051–0056

Regulations were also published to assure adequate controls in plants manufacturing medicated feed and medicated premixes much in the same manner as discussed above for other drugs. Effective June 20, 1979, the F&DA adopted new regulations for Good Laboratory Practice (GLP) for non-clinical (or safety) studies. These new regulations with emphasis on regular internal inspection of testing facilities placed an even greater cost on the pre-registration development of animal health drugs. The GLP regulations require the establishment of a Quality Assurance Unit (QAU) consisting of auditors who must be completely independent of any involvement in studies with the test drugs, and who must inspect each critical phase of on-going tests. Specific requirements are designated regarding animal care, facilities, handling of test and control drugs, specimen and data storage. Emphasis is given throughout to written Standard Operating Procedures for all repetitive operations of an experimental facility. Protocol formats are quite specific and retention times for records are clearly spelled out. Non-compliance with the GLP regulations can result in varying degrees of disqualification from an individual study right through to the disqualification of all data from a testing facility. In addition to the GLPs for safety studies, two new regulations have also been proposed that will apply to clinical investigations. The proposed regulations are: "Obligations of Sponsors and Monitors of Clinical Investigations" proposed September 27, 1977, and "Obligations of Clinical Investigators" proposed August 8, 1978. Final regulations have not yet issued but are expected during 1980. F&DA claims that the need for the new regulations on clinical practices parallels those of non-clinical practices; namely that, based on federal inspections, they found that existing requirements either were not being followed or were subject to varying interpretations. With respect to data generated in overseas facilities and used in support of an NADA, the F&DA has the right to reject such data unless it complies with GLPs and presumably the proposed Good Clinical Practices also. Effective May 10, 1979, a Memorandum of Agreement was signed by both the U.S. and Canadian governments to allow the conduct of GLP inspections in both countries. These inspections, the memo said, will be designed to promote mutual understanding of the respective inspection programs and consistency of inspection practices and assessments. Neither country will initiate an inspection in the other country without having first obtained the consent of the national authority of that country. Joint inspections shall be conducted under the auspices of the host country. The aim is to establish substantially consistent GLP standards and guidelines between the respective countries and to provide for reciprocal recognition of non-clinical laboratory inspection programs and reports. A Memorandum of Understanding between Sweden and the United States has also been signed, the agreements being identical to those of Canada and the U.S.A. A group of experts representing the Organization for Economic Cooperation and Development (OECD) met in Washington during 1979 to draft a set of Good Laboratory Practice Principles. At the Washington meeting were representatives of 11 nations including a representative of the European Economic Community (EEC). The draft agreement was reportedly going to be reviewed by the OECD group in October 1979 and then adopted by the member countries. It is expected that the OECD Good Lab Practice Principles will reflect the scientific principles contained in the F&DA GLPs. It is also understood that the governments of Belgium and Great Britain have drafted GLP guidelines.

It is felt that the various GLPs or Memoranda of Understanding between national governments will provide for adequate inspection programs by national authorities with inspection approximately every 2 years of all non-clinical laboratories conducting studies intended to be submitted to national authorities. Inspections shall include an assessment of laboratory procedures and operations and also, where appropriate, audits of data from completed studies. It is understood that procedures will be included in these agreements by which either party can request the other to conduct an inspection or a data audit.

The safety assessment of new drugs is a dynamic area of new product development both because of changing technology; e.g. sensitivity of residue assays, but also changing regulatory requirements. The recent F&DA proposal and probably the one likely to have the greatest economic impact on new product development is the proposed Sensitivity of Method (SOM) regulation, dated March 20, 1979, to establish the *"Criteria and procedures for evaluating assays for carcinogenic drug residues in food animals."* A threshold assessment of all new animal drugs for clearance in food animals must be made to assure that edible animal tissue presents no risk of human carcinogenesis. This threshold assessment is based on three factors: 1. frequency and extent of use, 2. amount of residues of toxicological concern, and 3. potential toxicological significance of structure and mutagenic assessment. The Sensitivity of Method (SOM) was originally proposed in July 1973 and was promulgated as a final order in February 1977. The Animal Health Institute filed suit against the F&DA alleging that the final order included substantial changes from the original proposal and therefore should have been reissued as a proposal. The AHI won this court battle and F&DA has re-proposed the regulation and revoked the final order in May 1978 as ordered by the court. The SOM was re-proposed on March 20, 1979, and public hearings on it were held in June of 1979. The agency extended to September 4, 1979, time for submitting any comments on the proposal. The Delaney Clause of the Federal Food, Drug and Cosmetic Act as mentioned previously prohibits the use of carcinogenic or suspect carcinogenic compounds in food-producing animals unless there is no residue of the compound found in the edible portion of the slaughtered animal by an assay method approved by the Secretary. The proposed SOM regulation presents principles to guide the experimental work that is necessary: 1. to determine whether or not a compound is carcinogenic, and 2. to establish the requirements for residue-testing methods to ensure that the compounds used in food-producing animals will satisfy the proviso of the Delaney Clause of the Act. The SOM regulation applies to drugs used in food-producing animals which have the potential for:

1. leaving residues in food, the consumption of which puts humans at risk to cancer, or
2. increasing the normal levels of carcinogenic and potentially carcinogenic substances endogenous to the target animals.

Existing chemical, biochemical, physiological and toxicological data from the sponsor's own studies or from the scientific literature and the compound's proposed pattern of use will provide the bases for determining carcinogenic potential. Sponsors of these compounds will be required to follow an Agency-monitored sequential procedure of data collection and evaluation whose objective is to ensure that the veterinary use of such compounds does not cause a significant human risk of cancer. The procedure is designed to assure a human life-time cancer risk no greater than 1 in 1 million for each drug. It is hoped that F&DA will accept an industry proposal that if at any point in the sequential process the evaluation of the data shows that there is no human cancer risk in the proposed uses of a compound, the F&DA will consider the approval of such compound under the general safety provisions of the Act rather than under SOM.

The stepwise procedure specified by the SOM includes the following:

1. A metabolic study in the target animals designed to identify edible tissue residues of carcinogenic concern.
2. Metabolic studies of the sponsored compound in laboratory test animals designed to aid in selecting the test animal species to be used in chronic toxicity bioassays and in evaluating the carcinogenic potential of residues that cannot practically be tested individually (intractable residues).
3. Chronic feeding studies in the laboratory test animals (chosen as suitable surrogates to man on the basis of the comparative metabolic studies) to assess the carcinogenic potential of residues and to furnish data suitable for statistical treatment so that the "no residue" requirement of the Act can be applied and implemented.
4. A detailed metabolic study of the sponsored compound in target animals designed to identify a specific residue (marker residue) and a specific tissue (target tissue) that serve as indicators to determine whether the "no residue" requirement of the Act is satisfied.
5. Development of a practical and reliable regulatory assay to measure the marker residue in the target tissue with an assay sensitivity at or above the level established in No. 3 above.
6. Establishment of the withdrawal period for use of the sponsored compound.

Another proposal with significant economic consequences on currently marketed animal health and feed additive products is the proposed F&DA *cyclical review*. The F&DA is now in the process of compiling a list of all approved veterinary compounds and establishing criteria for review of previously submitted data. It is premature to estimate precisely how many of these would meet the proposed threshold assessment of the SOM, but it is felt that most would not pass the initial screen and thus would at least require two-year chronic toxicity studies. It is assumed that priorities for the selection of marketed products for review will fall into several classes in decreasing order of toxicity with suspect carcinogens being in the first group to be reviewed. The cost of bringing old products up to new standards with respect to the SOM requirements has been estimated to be US $3-$4 million. In many instances the size of the market will preclude any further expenditure on such products and the withdrawal of these products would be the obvious outcome. Actual guidelines for cyclical review are expected within the near future.

F&DA PROCESS FOR THE APPROVAL OF NEW ANIMAL DRUGS

To provide a perspective on the present position of the F&DA with regard to veterinary specialties and feed additives, it is necessary to review the process of having a new animal drug approved by the Bureau of Veterinary Medicine (BVM) of the F&DA.

INVESTIGATIONAL NEW ANIMAL DRUG (INAD)

As the first step in having a new animal drug approved by the BVM for marketing, the sponsor must submit an Investigational New Animal Drug (INAD) application. With the submission, the sponsor will provide the F&DA with preliminary information on the new animal drug and request permission for a quantity of the product to be used by specific investigators for testing purposes. If the drug is intended for use in food animals, the data provided with the INAD must support evidence that the proposed drug use is consistent with the public interest.

An INAD must include the following information:

1. Identification of the new animal drug.
2. Adequate labeling.
3. Name and address of each clinical investigator.
4. Approximate number of animals to be treated.

If the new animal drug is to be given to food-producing animals, a commitment must be made by the sponsor that the edible products from such animals shall not be used for food without prior authorization from the F&DA. The INAD will permit the applicant to conduct field trials and under certain conditions to recover some of the costs of the test animals by permitting their slaughter and sale for human consumption. Two types of data should be provided to the F&DA in order to make a decision on the withdrawal time necessary for investigational trials:

1. Subchronic studies (90-d) for the parent drug in one rodent (usually the rat) and one non-rodent (usually the dog) species; and
2. measurement of the total residues in the edible tissues of the target animal and residue depletion during withdrawal of the drug.

The F&DA recommends that these data be obtained by the use of appropriate radioactive-labelled drug in the target animals.

NEW ANIMAL DRUG APPLICATION (NADA)

When a sponsor feels that as a result of an exhaustive development program sufficient data are available to support the claim that the new animal drug is safe and effective, then an NADA is filed with the BVM of the F&DA. In evaluating the NADA, the experts of the BVM will confer with the Bureau of Foods on those aspects of the NADA covering metabolism, toxicology and tissue residues.

A New Animal Drug Application must include the following information:

1. Identification – trade and chemical names of the new animal drug.
2. Table of Contents and Summary.
3. Labeling.
4. Components and composition – a complete list of all articles used for production of the new animal drug.
5. Manufacturing methods, facilities and controls.
6. Samples.
7. Analytical methods for residues – a description of practical methods for determining the quantity, if any, of such drug in or on food and any substance found in or on food because of its use and the proposed tolerance or withdrawal period or other use restrictions required in order to assure that the proposed use of such drug will be safe.
8. Evidence to establish effectiveness and safety – the NADA must contain full reports of adequate tests by all methods reasonably applicable to show whether or not the new animal drug is safe and effective for use in the target animal/bird as suggested in the proposed labeling. The effectiveness data must include substantial evidence based on "adequate and well-controlled" investigations, including field investigations by experts qualified by scientific training and experience to evaluate the effectiveness of the new animal drug involved.

   The application must include detailed reports of all the safety investigations including studies made on laboratory animals in which the purpose, methods and results obtained are clearly set forth for the acute, subchronic and chronic toxicity studies. All of these data must be obtained following the GLP regulations.
9. Environmental Impact Analysis Report – the F&DA requires that the sponsor submit an Environmental Impact Analysis Report analyzing the environmental impact of the manufacturing process and the effect of drug excreta from treated animals on the ecology.
10. Freedom of Information Summary – the F&DA requires that the sponsor submit a summary of relevant safety and efficacy data obtained for the compound for release to the public if so requested.

The F&DA has 180 d within which to review and approve or disapprove the NADA. If the F&DA approves the NADA, the clearance will be published in the *Federal Register,* and only then is the sponsor authorized to market the product. Alternatively and more frequently, the F&DA may consider the NADA incomplete and require additional information or even decline to approve the drug for the proposed use. Upon resubmission of the requested additional information, the F&DA has a further 180 d within which to review and reply. These continuing 6-month extensions can considerably prolong the approval process.

SAFETY ASSESSMENT OF NEW ANIMAL DRUGS

A guide to the toxicity data for an NADA cannot provide rigid requirements for target animal toxicity. Among the items precluding specific recommendations regarding the required margin of safety and the numbers of test animals are:

1. The drug product characteristics including undesirable side effects
2. Specific target animal species characteristics and responses
3. Route and duration of administration
4. Individual versus group medication
5. Over-the-counter versus prescription use
6. Environmental influences.

In principle, studies should be well-conducted, carefully-controlled and large enough to be meaningful.

The evaluation of toxicity of a new animal drug must include the following types of studies:

1. *Acute toxicity*

a. LD_{50} determination in laboratory animals.
b. Minimal lethal dose determination in the target animal by the proposed routes of administration.
c. The therapeutic index in the target animal – in large animals it may be more feasible to elucidate the potential toxicity in the target species with acute toxicity studies employing dose levels that produce toxic signs. An approximation on the safety index can be made by calculating the ratio between the observed toxic dosage and the effective dosage.
d. The evaluation of these studies should include clinical observations, signs of intoxication, clinical pathology, and histopathology.

2. *Subchronic Toxicity*

The F&DA requires that a parent drug be tested both in a rodent and a non-rodent mammalian species by daily oral administration for at least 90 d. In general, the rat and the dog are the preferred species because of the large base of reference data available for comparison. The F&DA is now recommending that the rodent study follow an expanded protocol that includes exposure *in utero,* exposure during suckling through the mother's milk and 90-d exposure after weaning which is during the period of rapid growth. In each toxicity study, it is required that 3 treatment levels be used – a high level to produce some toxicity, an intermediate level to assess a dose-response relationship and a low level to demonstrate no adverse effect. An untreated group should be kept as controls. The F&DA recommends that 20 to 25 animals per sex per dose level be used for the rodent test and about 4 animals per sex per dose level for the dog study. The subchronic toxicity studies are of great importance since they will provide the maximum no-effect dose levels and the mininum toxic dose and establish the nature of the toxic effects of the test drug. In addition, the subchronic toxicity tests will provide a great deal of information on the biological effects elicited by a compound, as well as data to estimate the no-effect dose levels for the lifetime studies if needed.

3. *Chronic Toxicity Studies*

At the present time, if a compound is not considered to be a suspect carcinogen by the threshold assessment calculation, no special chronic studies are required by the F&DA. However, if a compound or any of its residue in food is a suspect carcinogen or the residue levels are high enough, then chronic toxicity studies are required. In this respect the proposed SOM regulations will take effect to some degree.

With regard to the life-time studies, the F&DA recommends that the protocol of one rodent study be combined with a reproduction study using one of the

litters from the first generation offspring for the chronic study to provide exposure beginning *in utero*. Selection of the dose levels for these studies is based on the results of the subchronic studies. The high dose level should stress the animals and produce some toxic response; however, it should not be high enough to cause early death. The lowest dose should not produce adverse effects compared with the unmedicated control group. A dose level between these 2 extremes should be given to a third group.

Oral administration should be used for all routine testing of food additives and the life-time studies should include at least 50 animals per sex per dose level. Termination of each test group is recommended when only 20% of the starting group survives. If in a rat study the 80% mortality is not reached at 30 months, termination is recommended at that time.

SPECIFIC TOXICITY CONSIDERATIONS

Additional investigations on the safety of new animal drugs are required according to the characteristics and the intended use of the product. Teratogenicity and reproduction studies may be requested in support of human safety for drugs intended to be used in food-producing animals. If a compound is structurally related to known teratogens, teratogenic studies are required in at least 2 species. If abnormal effects are observed in subchronic studies or teratology studies, then three-generation reproductive studies are also required.

At the present time the F&DA does not specifically require tests for mutagenicity. It has been suggested, however, that the sponsor company test for mutagenicity early in the development stage of a new compound, in order to gain information to plan for additional toxicity studies. At present, the F&DA is suggesting the use of the Ames *in vitro* bacterial mutagenicity test for early evaluation of new products. When more information on the reliability of other *in vitro* and *in vivo* mutagenicity tests is available, the F&DA may require that a battery of bacterial and mammalian system mutagenicity tests be conducted on new compounds.

FEED ADDITIVES

Feed additives usually refer to drugs approved for use in animals feeds at low levels for growth promotion, for disease control and prevention and/or for therapeutic purposes. In the U.S.A. before any feed manufacturer may mix any drug, including low-level antibiotics, into a feed or repackage a medicated feed, he must register as a drug manufacturer with the F&DA. Also, in order to use an approved drug premix, the feed manufacturer must submit a medicated feed application or a form FD-1800 requesting approval to use the drug premix in manufacturing medicated feeds.

MANUFACTURING PRACTICES FOR MEDICATED FEEDS AND PREMIXES

As previously noted, the F&DA has published regulations called Current Good Manufacturing Practices for the Production of Medicated Feed and Medicated Premixes. According to the F&DA, *medicated feed* is any animal feed that contains one or more drugs at any level and includes:

(a) Medicated concentrates, which are mixed with other feed materials to make either a supplement or a complete feed before being offered to the intended animal;

(b) Medicated supplements, which are safe for direct consumption by the intended animal; these can be offered in a free-choice feeding plan;

(c) Medicated complete feed, which is intended to be the sole ration for an animal.

Medicated premixes are intended for use in the production of either medicated concentrates, medicated supplements or medicated complete feeds. They contain one or more drugs that are not safe for consumption by the intended animals at that level. These levels cannot be legally fed to animals without further dilution.

The GMP regulations have given the F&DA the power to inspect and control feed manufacturing operations with regard to buildings, equipment, personnel, feed ingredients, records, feed manufacturing, packaging and labeling, laboratory controls and distribution records.

ANTIBIOTICS AS ANIMAL FEED ADDITIVES AND THEIR IMPORTANCE IN HUMAN AND ANIMAL SAFETY

Some 25 years ago, it was found that the continuous addition of antibacterial agents to the diet improved the productivity of chickens, pigs and other species. In 1969, the Swann Committee in the U.K. recommended that antibiotics that were used for human medicine be not used at subtherapeutic levels in animal feeds (used for improved rate of growth and feed efficiency or for disease prophylaxis). This recommendation stemmed from the concern that the use of these antibiotics might lead to the emergence of strains of resistant bacteria which may cause illness in man.

As a result, today in the U.K. antibacterial agents that are for "animal use only" can be purchased without veterinary prescription, while any antibiotic or antibacterial agent that is used in human medicine is restricted by requiring a prescription by a veterinarian for any animal use.

Because of the concern about the development of antibiotic-resistant bacteria, the F&DA appointed a task force on the use of antibiotics in animal feeds in April 1970. On January 31, 1972, the F&DA released the recommendations of this antibiotic task force. The majority report of the task force expressed concern with regard to the possibility that some antibacterial drugs may cause:

1. An increase in the reservoir of *Salmonella* spp. in animals
2. An increase in the antibiotic resistance of coliform bacteria in animals, with a consequent influence on the health of man and animals
3. An impairment of the therapeutic efficacy of antibacterial drugs commonly used in feed at subtherapeutic levels when subsequently employed in the treatment of clinical diseases in animals.

There was, however, a minority report issued by 7 of the 15 task force members that stressed the absence of persuasive evidence linking the increased number of

resistant organisms in animals with any human health problem.

On April 20, 1973, the F&DA published a statement of policy and interpretation regarding antibacterial animal drugs and medicated feeds and requested that drug companies marketing antibacterial drugs for use at subtherapeutic levels in animals submit information on human and animal safety. Specific criteria were developed by the F&DA on the evaluation of antibiotics with regard to animal health, human health and drug effectiveness. Numerous studies sponsored by the animal health industry, academia and the F&DA on *Salmonella* reservoirs, resistant coliforms and the impact of the use of subtherapeutic antibiotics in animal feeds on therapy of animal diseases have been completed since 1973. Some manufacturers, however, did not comply and in February 1976 the approval to market their particular product was withdrawn by the F&DA. In April 1977, the F&DA Commissioner, after a review of the recommendations submitted by F&DA's National Advisory Food and Drug Committee, announced that the F&DA would withdraw approval of the NADAs for low-level use of penicillin and the tetracyclines. These 2 announcements were published in the *Federal Register* of August 30 and October 21, 1977, respectively. The reaction of the animal producers and pharmaceutical manufacturers was strong and unanimous and as a consequence the F&DA was asked to hold public hearings on the question.

The F&DA concluded that there was a potential hazard to man from antibacterials when used in animal feeds. Yet, in many quarters it is felt that the extent of the hazards to man cannot be adequately estimated or evaluated from existing data and that regulatory action should only be initiated when answers to certain questions are known. These questions are:

1. The minimum infective dose required for colonization in man by non-R-factor-bearing coliforms;
2. The minimum infective dose for colonization in man by R-factor-bearing coliforms from animals;
3. Is colonization a necessary first requirement in the transfer process of R-factors from animals to man;
4. The length of time it takes for significant levels of R-factor transfer to occur;
5. The vigor of R-plasmid-bearing bacteria versus the non-R-plasmid types;
6. The need for antibiotics to be in the gut for R-plasmid-bearing bacteria to survive in competition with non-plasmid-bearing bacteria; and
7. From what source do the R-factors found in coliform bacteria in normal humans originate?

During March and April of 1978, 3 public hearings were conducted with the participation of the F&DA Commissioner. During these hearings most of the witnesses contested the need for the restrictions since there was no evidence that the use of antibiotics in animal feeds constitute a hazard to man. On the other hand, the cost of animal-derived food could rise substantially if these products were removed from the market. On August 8, 1978, the F&DA announced its intention to postpone final action on its January 20, 1978 proposal, which would have required veterinarian orders to dispense low-level uses of tetracyclines and penicillin. Final action on the proposal will be postponed until evidentiary hearings on the safety of penicillin and tetracyclines are held. Numerous reasons are considered responsible for this postponement; these include:

1. The unavailability of veterinarians for issuing prescriptions in many farming regions;
2. Severe economic disadvantage for the smaller producers resulting from competition with the large feedlots and farms in obtaining continuous veterinary services; and
3. A particular shortage of veterinarians trained in poultry disease.

There is no question that the use of antibiotics in feed can increase the prevalence of R-factor bacteria in animals and that the meat products of those animals can contain R-factor bacteria of animal origin. There is, however, some doubt that R-factor bacteria from animal sources can colonize man or pass R-factors on to human flora. This doubt remains in spite of the fact that colonization usually precedes infection in man and that infection due to R-factor organisms is a significant clinical problem. All the argument appears to center on the question as to whether R-factor bacteria of animal origin can colonize and pass R-factor on to the human host flora. It is obvious, however, that the last word on the issue of antibiotics and potential human hazards has not yet been said.

THE EFFECT OF NEW F&DA REGULATIONS ON THE COST OF DEVELOPING NEW ANIMAL DRUGS

There are a number of estimates on the cost of developing new animal drugs and the individual sources differ somewhat in the actual amounts. Since an animal *Health Institute survey in 1974 showed that* approximately 50% of the research and development expenses were devoted to the safety aspects, both environmental and human, of new product development, we have recently reassessed the particular cost impact of the two major regulations, Good Laboratory Practices (GLP) and the proposed Sensitivity of Method (SOM) regulations. A development profile of an animal health product in 1979 shows that approximately 60% of the cost of R & D lies in the safety assessment areas of toxicology, tissue residue, metabolism and environmental toxicology. The clinical program, even though increasing in total cost through inflation, is now shrinking to something of the order of 40% of the total development effort. If we consider an animal health product for therapeutic use, it would currently have an estimated development cost of about $10 M. More than $3 M of this has been estimated as being an added expense in response to the recent GLP and SOM regulations. Estimates made for prophylactic use of a product, for example a feed additive, are almost $11 M. Again, more than $3 M is estimated as the increase due to GLP/SOM regulations. Spiraling research and development costs, mainly due to more stringent regulatory requirements but also including inflation, is certainly slowing down new product market introduction.

It is obvious that common registration requirements between major international markets and the recognition of "good scientific data" from any source would go a long way towards stemming the recent extraordinary escalation in proposed new regulations. Perhaps the recent sharing between the F&DA and several major countries of Memoranda of Understanding with respect to good laboratory practices is the start of a trend towards similar regulatory requirements. I sincerely hope this is the case.

CORRELATION OF CHANGES IN BLOOD CHEMISTRY WITH PATHOLOGICAL CHANGES IN THE ANIMAL'S BODY: I SERUM NUTRIENTS AND PROTEINS

LEA STOGDALE*

ABSTRACT: Stogdale L. **Correlation of changes in blood chemistry with pathological changes in the animal's body: I Serum nutrients and proteins.** *Journal of the South African Veterinary Association* (1981) **52** No. 1, 57–63 (En) Department Medicine, Faculty Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110, Rep. of South Africa.

The usefulness of laboratory tests is discussed, with emphasis placed on the correlation of the results with the clinical findings. The advantages and disadvantages of clinical chemistry and the precautions necessary when collecting the blood sample are delineated. Brief consideration is given to selecting the laboratory, the normal range, and the knowledge essential for interpretation of the results. The concentrations of the blood constituents are considered relative to the rate of release from cells, the method of transport in the blood, and the rate of removal from the blood. The physiological and pathological alterations in these factors dictate blood chemistry results.

The physiologic influences on the concentrations of blood glucose, serum cholesterol, and plasma free fatty acids are considered. The causes of physiologic and pathologic increases and decreases in these blood constituents are discussed. Serum triglyceride concentrations are usually performed for an objective assessment of intestinal fat absorption. The principal causes of inadequate absorption are mentioned.

The changes in the concentrations of total serum protein, albumin, the various globulin fractions, fibrinogen, and prothrombin are non-specific but are frequently valuable diagnostic aids. The physiological and pathological influences on these parameters are described.

INTRODUCTION

"The most important responsibility of the practicing veterinarian is to establish a diagnosis"[10]. An accurate aetiological diagnosis is essential before the veterinarian can perform any of his other duties, namely prognostication, treatment, and disease prevention. Clinical pathology is the application of laboratory methods to animal specimens, in order to aid in the diagnosis of disease conditions in the living animal[11]. The results obtained must be interpreted and correlated with the history and clinical findings of the animal. If the laboratory results do not agree with the physical findings, they should be rechecked: human or technical error is possible. While a complete history and a thorough clinical examination can frequently result in a diagnosis, it is often essential to utilize further investigative procedures. Such procedures may be necessary to clarify which organs are affected, and to indicate the extent of the pathology (mild, severe, localized, diffuse), the type of pathological process which is occurring (degenerative, necrotic, inflammatory, neoplastic), and its level of activity (chronic, acute, active). In addition, special tests are extremely useful in confirming a suspected diagnosis, in convincing a client that a diagnosis is correct, in aiding the clinician in prognostication, in deciding on the rational treatment, and in gauging the response to therapy. The clinical pathology findings from a blood specimen are only a very small part of the information from any patient available to the veterinary clinician. However, due to the ease of collecting blood samples, the increasing availability of the common tests, and the numerous organ systems that can be examined by the various tests, they are frequently performed. It must be stressed that the results of any special examination *must* be correlated with the clinical findings: a test result in isolation is meaningless.

*Present address: Department of Veterinary Clinical Studies, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

Advantages of clinical chemistry include the speed with which the tests can be performed, and hence the rapid application of the result to the patient. The tests can be repeated, so allowing for confirmation of an unexpected result, or showing the progression of the disease process. Many of the tests indicate biochemical changes, which may be of paramount importance to the animal, but which are not evident in histological or even electron-microscopic examination of the patient's tissues. A series of clinical pathology results gives an indication of the dynamic biochemical changes that are occurring in the animal.

Disadvantages of clinical chemistry include the cost of the various tests, and the tendency for inexperienced practitioners to rely on laboratory results for a diagnosis, sometimes to the exclusion of a thorough client interview or patient examination. The age-old medical dictum remains true, despite the proliferation and ready availability of sophisticated tests: when a diagnosis is not evident, examine the patient. In addition, there can be problems of test selection and interpretation. The clinician must have a reasonably good level of knowledge, or must seek the advice of a clinical pathologist if he does not. The random selection of tests, along with uninformed attempts at interpretation are time-consuming, costly, unrewarding, and frustrating.

Precautions in collecting and handling the blood samples must be adhered to, so that the results may be accurate and meaningful. It is preferable to collect blood samples early in the morning (between 08h00 and 10h00), so that consistent results may be obtained. This is particularly relevant for those parameters which show a diurnal rhythm, especially hormones. This also

0038-2809/81/01/0057–0063

enables the laboratory to receive the samples early and to provide the results on the same day.

The state of the animal at the time of blood collection can dramatically influence the results. The animal should be resting, unexcited, unstressed, and fasting. Exercise, excitement and stress raise the concentration of blood cells, and of several serum parameters. Eating influences many of the blood chemistry tests, apart from the obvious examples of glucose and cholesterol. Dehydration causes an increase in the concentration of all the blood constituents, and should either be corrected prior to sampling or, less satisfactorily, taken into account when the results are being interpreted. Many drugs affect the blood parameters. In particular, tranquilizers and anaesthetics can raise the blood glucose concentration, sometimes dramatically.

The blood must be collected carefully so as to avoid haemolysis, which changes the concentration of many of the blood constituents and interferes with many of the photometric readings. Jaundice, hyperlipidaemia, and certain dyes, such as bromsulphthalein (BSP) and methylene blue, also cause photometric interference.

The blood, or the serum sample, must reach the laboratory rapidly. Tests such as blood glucose and serum inorganic phosphate must be performed immediately, unless a special preservative is added. The sample must not sit in the clinician's pocket for hours, or in the mail for days, if accurate results are to be obtained. The blood should be centrifuged soon after collection and the serum harvested. If there is to be a delay in performing the tests, such as getting the sample to the laboratory, serum should be stored at 4°C but not frozen. Obviously, any contamination of the sample must be avoided.

The laboratory selected to perform the tests must be able to give accurate and repeatable results. This requires trained and competent technicians, supervised by an experienced clinical pathologist. They must frequently check their methods by testing quality control sera; between 5 and 10% of all samples tested should be quality control sera. Many laboratories utilize international monitoring of their test results to ensure a consistently high standard[12 15]. If a medical laboratory is used for veterinary samples, the staff must be aware of the different requirements of animal's blood for various tests. Some human test methods give inaccurately high or low results when applied to animals, while other tests don't work at all. The accuracy of any test result depends upon the quality of the sample provided and the expertise of the laboratory.

Test results vary between laboratories, technicians, and the method used. Hence, it is essential to establish a normal range of values for each animal species in the laboratory utilized. The author does not consider knowledge of the numerous normal values to be essential to the practitioner; these can easily be listed and kept in a convenient place for reference. However, understanding the biochemical, physiological, and pathological mechanisms which cause changes in the blood constituents is essential to the interpretation of the results. In addition, it is important to know the limitations of any test method and its precision. When any clinical chemistry data is being evaluated, it is important to consider the three basic factors which control the level of metabolites or enzymes in blood, plasma, or serum. These factors are: the rate of release from cells; the mechanism of transportation in the blood; and the rate of removal from the blood, either by cellular uptake and metabolism, or by secretion or excretion[2].

The aim of this paper and its sequel is to correlate the changes in the blood chemistry with the general pathological conditions that affect the body organs. The meaning and significance of normal values are briefly considered. Only those tests which are in common veterinary use will be discussed. Specific hormone assays are not dealt with in these papers. Serum tests have been divided into six major groupings: nutrients and proteins will be discussed in this paper; electrolytes, kidney function tests, enzymes (hepatic, pancreatic and miscellaneous), and the liver function tests will be considered in the second paper. All the tests are discussed with reference to the factors affecting their levels in health and disease. Any relevant information with respect to species differences is provided.

NORMAL VALUES

Normal values are essential if any sense is to be made of laboratory data. Normal values are obtained by collecting blood samples from a series of healthy animals (usually a minimum of 100 is considered necessary), and having the tests performed by the laboratory that will be doing the practitioner's work. If the method used for a particular test is changed, a new range of normal values must be obtained. It is essential to establish normal values for the population of animals involved, eg domestic dogs, or racehorses in training; the conditions under which the samples will be collected, eg on an out-patients' basis, or in stables; and for the laboratory and specific test method that will be utilized.

Although ranges of normal values are usually quoted, a more accurate method, if less convenient, is to state the mean and the standard deviation. As 95% of the population will fall within two standard deviations of the mean, a value outside this range is usually considered abnormal[12]. It must be noted that the concentrations of many of the blood constituents remain remarkably constant in any one animal, as long as the samples are collected, and the animal is maintained, under similar conditions. It is the variations between individuals and groups of animals that result in the wide normal ranges that are quoted in the veterinary literature. Blackmore[1] monitored the changes in numerous blood parameters from thoroughbred racehorses over a ten-month period. He found that the concentrations of most compounds varied by as little as 1,2 standard deviations. This was considerably less than the variations exhibited by the group as a whole or by the normal ranges in certain situations, such as racehorse practice. It may be extremely useful to establish reference values for each individual. Later, test results can be compared with the reference values rather than with the normal range for the species.

SERUM NUTRIENTS

Serum or blood glucose concentration indicates the nutritional state, the emotional condition, and the hormonal balance of the animal. In the normal animal, the blood glucose concentration is maintained at a fairly constant level, principally by the liver, and to a lesser extent by

the kidneys, under the influence of many hormones. Glucose enters the blood from the gastrointestinal tract following food digestion, and from the liver stores. Somatotropin (growth hormone), glucagon, adrenaline, and the glucocorticoids are the most important hormones which maintain an adequate blood glucose concentration. However, all the anterior pituitary, thyroid and sex hormones have some hyperglycaemic effects. Glucose travels in the blood in solution. The red blood cells of dogs and cats contain nearly as much glucose as the plasma, but the erythrocytes of adult ruminants and horses contain much less[9]. Insulin and anoxia are the main physiological factors which cause glucose to leave the blood stream and enter cells. Glucose can be lost through the gastrointestinal tract in severe enteritis and helminthiasis, through the kidneys in interstitial nephritis with tubular damage, and through burn wounds[3].

Physiological hyperglycaemia of a mild degree occurs during excitement and stress (particularly in cats), and after carbohydrate ingestion in monogastric animals. A transient hyperglycaemia frequently occurs during attacks of acute or chronic pancreatitis; during hypocalcaemia (milk fever or eclampsia); and during convulsions due to adrenaline release[3].

The most common cause of persistent marked hyperglycaemia is diabetes mellitus[6]. Diabetes mellitus occurs most frequently in dogs, but has been recorded in all the domestic species and in many other animals. The cause of diabetes mellitus is either an insulin deficiency due to inadequate secretion from the ß-cells of the pancreatic islets of Langerhans, or a relative inadequacy of activity due to an excess of glucagon, antibody inactivation, or target receptor abnormality. In animals, damage to the pancreatic ß-cells with subsequent insulin secretion deficiency is the most common cause. In dogs, recurrent pancreatitis, with necrosis and fibrosis affecting all the cells of the pancreas, is probably responsible for most cases of diabetes mellitus. Other causes include hydropic degeneration of the ß-cells, hyalinization or amyloidosis of the pancreas (especially in cats), and complete disappearance of the ß-cell granules, probably due to hyperglycaemic-induced exhaustion[8 14].

Persistent hyperglycaemia occurs with some functional pituitary tumours in dogs and horses. These tumours, usually adenomas of the acidophil or chromophobe cells, probably secrete either somatotropin (growth hormone) which causes marked hyperglycaemia and acromegaly, or corticotropin (adrenocorticotropic hormone, ACTH) which results in secondary hyperadrenocorticism. In horses, hyperadrenocorticism is sometimes accompanied by hyperglycaemia[9] (L Stogdale 1979 unpublished work, presented at the South African National and International Veterinary Congress, Johannesburg, 5 September, 1979). An excess of glucocorticoids only rarely causes hyperglycaemia in dogs owing to the efficiency of the pancreatic β-cells in secreting insulin[6]. However, a few cases of prolonged hyperglycaemia and diabetes mellitus have been recorded with primary hyperadrenocorticism owing to adenomas or carcinomas of the cells of the zona fasciculata in the adrenal cortex; secondary hyperadrenocorticism due to an acidophil or chromophobe adenoma of the anterior or intermediate lobes of the hypophysis; and with prolonged glucocorticoid therapy[9 14].

Physiologic hypoglycaemia occurs with starvation, prolonged muscle exertion, especially during clonic convulsions, and in heavily pregnant or lactating animals. Severe hypoglycaemia occurs in numerous disease conditions. Cows with ketosis (acetonaemia) develop hypoglycaemia because of an inadequate carbohydrate intake which is insufficient to maintain the blood glucose level while milk production continues. The same situation occurs in pregnancy toxaemia of sheep with twins or a single large foetus. The aetiology in both of these metabolic disorders is a net energy deficiency. In primary ketosis the cause is either a low carbohydrate diet or a high energy output in the form of milk. In secondary ketosis the cause is anorexia because of some other disease. Hypoglycaemia results in mobilization of the animal's fat reserves, with the subsequent development of ketonaemia, hyperlipidaemia, and fatty infiltration into the liver and kidneys[3 14].

Neonatal hypoglycaemia occurs in many newborn animals, especially piglets. The principal predisposing causes are a cold environment and insufficient carbohydrate intake[3]. In the piglet, gluconeogenesis only begins at 6–7 days of age, and so the maintenance of the blood glucose concentration is dependent on food intake. In neonatal hypoglycaemia there are no significant pathological lesions evident[8].

Functional pancreatic β-cell tumours (insulinomas) occur occasionally in the dog. They cause hypoglycaemia by their excessive production of insulin. They may be either adenomas or carcinomas, unifocal and well demarcated, or multicentric and dispersed throughout the pancreatic tissue. Some tumours are microscopic in size[8].

Hypoadrenocorticism (Addison's Disease) is consistently accompanied by mild persistent hypoglycaemia because of the absence of glucocorticoids[6]. The hypoglycaemia in this condition is neither severe nor life-threatening. The causes of hypoadrenocorticism are either primary or secondary. Primary lesions include adrenocortical degeneration of unknown origin (idiopathic), involving all 3 zones, or more rarely, bilateral tuberculosis granulomas, coccidiodomycosis, histoplasmosis, toxoplasmosis, or amyloidosis. Secondary hypoadrenocorticism is due to destruction of the corticotropin (ACTH) secreting cells (chromophobes) of the pituitary gland. In animals this is usually caused by an expansive non-functional adenoma of the hypophysis. This only results in glucocorticoid deficiency and not in any interference with mineralocorticoid secretion[8 14].

Hypoglycaemia occurs occasionally when there is severe generalized damage to the liver[6 9]. Any widespread damage to the hepatocytes will result in hypoglycaemia, but the animal is much more likely to die of liver insufficiency than from the effects of the hypoglycaemia, which usually only occurs pre-terminally[6]. Glycogen storage diseases, which predominantly affect the liver, have been recorded in young puppies. The cause is an inherited deficiency of one of the many enzymes involved in glycogenolysis; the puppies die from hypoglycaemia. There are no gross changes in the liver, but microscopically the hepatocytes are distinctly vacuolated (the glycogen being dissolved by the aqueous fixative)[14].

Death from hypoglycaemia is due to cessation of function of the brain neurones, resulting in cardiac and respiratory failure. The neurones depend on a continuous supply of glucose for their energy requirements.

Acute hypoglycaemia does not produce any cerebral lesions. Prolonged hypoglycaemia causes non-specific neuronal changes, indistinguishable from autolysis. Occasionally severe neuronal degeneration is seen. This is characterized by rapid and complete chromatolysis, pyknosis and fading of the nucleus, and fading of the cytoplasm and cell membranes[8]. Usually there are no specific lesions in the heart (which is dilated), lungs or liver. Rarely, mild degenerative changes in the myocardial fibres may be seen microscopically.

A glucose tolerance test, either by intravenous or oral administration of glucose, is occasionally indicated. The intravenous test should be performed when mild fasting hyperglycaemia is found. A diagnosis of subclinical or overt diabetes mellitus is confirmed when the fall in the blood glucose level is delayed. The oral glucose tolerance test is indicated when intestinal absorption or liver funcion is in question.

Cholesterol is present in varying amounts in all tissues and cells. It is synthesized in all tissues, but the most important sources are the liver, intestine, and skin. The serum cholesterol is derived from intestinal absorption of free cholesterol, and from its release from all body tissues. However, as most of the cholesterol in the serum is esterified, and as this process is carried out mainly in the hepatocytes, most of the serum cholesterol is derived from the liver. The esterified cholesterol is transported in the blood bound to α- and β-globulins; the non-esterified portion, which has a rapid turnover, is bound to albumin[9]. In addition to synthesis and esterification, the liver is also responsible for the degradation of cholesterol and the excretion of the metabolic breakdown products in the bile as bile acids. The level of cholesterol in the blood is also influenced but not controlled by the level of fat mobilization and by many hormones[3 9].

Serum cholesterol concentrations are altered in numerous physiological states and disease conditions, but this is virtually always a secondary response in animals[3]. Thus, the assay of the total serum cholesterol concentration does not have a primary diagnostic value: it is only an indication of the fat metabolism status in the animal[6]. The serum cholesterol level is raised in normal animals, by stress and by feeding a diet high in animal fats, especially table scraps[3].

High serum cholesterol concentrations are consistently found in diabetes mellitus and in hyperadrenocorticism (Cushing's Disease)[3 6]. In diabetes mellitus, the probable cause of the hypercholesterolaemia is the high rate of lipid mobilization from the adipose tissue which occurs in response to the intracellular energy deficiency. In hyperadrenocorticism, the hypercholesterolaemia results directly from the lipolytic action of glucocorticoids.

Hypercholesterolaemia may also occur with hypothyroidism (in approximately 60% of cases in dogs)[3 4]. The cause of the high serum cholesterol level in this endocrinopathy is not clear. The cause of primary hypothyroidism in dogs is usually unknown and is therefore labelled idiopathic. Microscopically, the follicles are small and contain little colloid. Other causes, which occur rarely, are lymphocytic thyroiditis (Hashimoto's disease of man), haemorrhage, necrosis, amyloidosis, metastatic infections, and destruction by connective tissue tumours (sarcomas). The result is fibrosis and a decrease in the available active tissue. In cats, iodine deficiency occurs on an all-meat diet and results in hyperplasia and goitre. Congenital hypothyroidism and goitre occurs in calves and foals in iodine deficient areas, or when the dam has been fed on goitrogenic substances, or in congenital inherited (autosomal recessive) goitre of Africaner cattle, sheep, goats and pigs. Secondary hypothyroidism, resulting from destruction of the anterior pituitary basophils, occurs very rarely. This is usually a result of an expanding hypophyseal adenoma. However, the basophils are very resistant to destruction, the other adenohypophyseal cells being affected first[8 14].

Advanced nephrosis, especially the nephrotic syndrome, results in hypercholesterolaemia in association with retention of neutral fat and phospholipids in the blood. The pathogenesis of the rise in blood lipid concentrations is not known[3]. The nephrotic syndrome results from any diffuse glomerular disease resulting in albumin loss in the urine. Causes include membranous glomerulonephritis (an autoimmune reaction with antibody-antigen complexes deposited on the basement membrane), proliferative glomerulonephritis, amyloidosis, and nodular glomerulosclerosis[14].

Primary hyperlipidaemia has been recorded in Shetland and pony mares, either heavily pregnant or recently foaled. The cause of the hyperlipidaemia is mobilization of fat, secondary to anorexia. The aetiology of the anorexia is not always evident, and interestingly, ketosis is not present. The total serum cholesterol concentrations are extremely high, and the post-mortem lesions are fatty infiltration into the liver and kidneys[3 8].

Hypercholesterolaemia also occurs in acute pancreatitis, as a result of lipase release into the peritoneal cavity. This enzyme damages the mesenteric adipose tissue[8].

High serum cholesterol concentrations frequently accompany liver damage[3 4]. The cause of the hypercholesterolaemia in obstructive jaundice is not retention of cholesterol, which is normally excreted in the bile, but probably an overproduction by the liver. The pathogenesis is not delineated[4]. Obstructive jaundice in animals is not common but may be caused by fluke *(Fasciola spp.)* or *Echinococcus* infestation of the liver; cystic hyperplasia of the mucous-producing glands in the gall bladder wall, and mucosal hyperplasia in the large bile ducts, usually as a result of irritation by fluke or chlorinated naphthalene poisoning; cholangiocellular adenomas and carcinomas (predominantly in dogs and cats); primary mesodermal tumours of the gall bladder (seen in dogs and cattle); hepatocellular adenomas or carcinomas; neoplastic metastases to the liver (a rare occurrence in animals) which can cause pressure on the bile ducts, as can tumours of the pancreas or duodenum; gall stones (cholelithiasis), which are also rare in animals; parasites, particularly Ascarids, aberrantly migrating into the bile duct; and cicatricial stenosis of the bile ducts. In animals, biliary obstruction is usually the result of cholangitis[8]. The hypercholesterolaemia seen with acute leptospirosis is probably due to the liver damage. In this disease the liver is enlarged and friable. Microscopically, haemorrhages and centrilobular necrosis are seen. The severe anaemia, the mild icterus, and the other lesions resulting from haemolysis and toxaemia are evident[8 14].

The significance of high-serum total cholesterol concentrations in animals is not well established[9]. In ani-

mals with markedly high-serum cholesterol concentrations for prolonged periods, microangiopathy may occur, primarily affecting the glomerular, retinal and cutaneous arterioles. Hypercholesterolaemia is invariably associated with fatty infiltration of the hepatocytes[8].

Low-serum cholesterol concentrations occur in a variety of liver diseases, both acute and chronic. This results from interference with the esterification process before the liver's ability to degrade and excrete cholesterol is impaired[3 4]. In dogs, low-serum cholesterol values have been found with very low-fat diets, starvation, fatty degeneration of the liver, severe anaemia, acute febrile infections, leptospirosis, advanced nephritis and hyperthyroidism[3]. Hyperthyroidism is very rare in animals and has only been recorded in dogs and cats. It is usually primary, being caused by thyroid adenomas (follicular or papillary) and adenocarcinomas. Secondary hyperthyroidism, due to a thyrotropin (thyroid stimulating hormone, TSH) secreting basophil adenoma of the adenohypophysis, is extremely rare[8 14].

Plasma-free fatty acids are derived from lipids and are a normal constituent of blood. They originate either from intestinal absorption, after the degradation of ingested fat, or from adipose tissue in the body. They are a sensitive indicator of the rate of fat mobilization occurring in the body. They are carried in the blood bound to albumin. Free fatty acids are removed from the blood by all tissues, especially the skeletal muscles, heart, kidney, mammary gland, liver and adipose tissue. Once in the cells, they are utilized for energy or they are conjugated with glycerol to form triglycerides, which are stored as fat, principally in the adipose tissue. Their blood concentration is influenced by the nutritional, emotional and hormonal status of the animal[9].

Increases in the circulating concentration of free fatty acids occur with food deprivation or starvation and with increases in the blood levels of adrenalin, prolactin, glucocorticoids, somatotropin (growth hormone), thyroxine and heparin. Food deprivation causes the release of these hormones, all of which cause catabolism of fat, which decreases the utilization of glucose. Thus, increases in the plasma-free fatty acid levels are seen in the physiological states of stress (adrenaline and glucocorticoid release), and lactation (prolactin release); and in the pathological conditions of hyperadrenocorticism, gigantism and acromegaly, and hyperthyroidism. Heparin stimulates lipoprotein lipase activity in the adipose tissue, so resulting in increased fat mobilization. Increased free fatty acid concentrations also occur in cows with ketosis, in sheep suffering from pregnancy toxaemia, and in animals with diabetes mellitus. In all these conditions there is a high level of fat mobilization. Necrosis and cirrhosis of the liver result in increased concentrations of free fatty acids, probably as a result of impaired hepatocyte uptake. The concentrations of free fatty acids in the plasma is decreased by insulin, which actively increases the rate at which the fatty acids enter cells. Thus, carbohydrate ingestion or glucose administration will lower their concentration[9].

Serum triglyceride concentrations tend to parallel those of plasma-free fatty acids, and the factors influencing the levels are similar. Serum triglyceride estimations are usually performed for an objective assessment of intestinal fat absorption in dogs. Vegetable oil is fed to the animal, and the 2- and 3-hour post-prandial results are compared with the fasting concentration. Inadequate absorption, as indicated by a less than threefold rise in the serum triglyceride concentration, indicates either maldigestion or malabsorption. Maldigestion is nearly always due to exocrine pancreatic enzyme deficiency, which results from either congenital hypoplasia of the pancreas, seen in dogs (especially German Shepherd dogs) and calves, or destruction of the organ from acute pancreatic necrosis or acute pancreatitis (frequently with recurrent attacks). The most common causes of permanent malabsorption are chronic enteritis due to viral, bacterial, fungal, protozoal or helminth infestation, resulting in destruction of the villi and absorptive cells, ie sprue gut; and cellular infiltration into the intestinal wall as seen in eosinophilic enteritis, lymphocytic-plasmocytic enteritis, neutrophilic enteritis, and lymphosarcoma[8 14].

BLOOD PROTEINS

The serum protein concentration depends on the current rates of synthesis and release from cells, the distribution in the body, and the rates of utilization and catabolism. In particular, the rates of synthesis are influenced by the nutritional status of the animal, especially vitamin A and protein intake; and the extent, duration, severity, and primary nature of damage to various organs, particularly the liver and kidneys. The damage to organs is, in turn, influenced by the circulatory, inflammatory, metabolic, degenerative, reparative and regenerative processes occurring at any time[4 6]. Plasma proteins are very closely related to the tissue proteins, and hence the general status of the body's protein metabolism may be obtained from the informed study of this readily available tissue[3 5]. The concentrations of the total serum proteins and the specific protein fractions are non-specific indicators of the sum of the protein intake, metabolism and output occurring in an animal[4].

Serum albumin is synthesized by the hypatocytes and released into the blood stream, where it is responsible for most of the blood's osmotic pressure. Albumin binds many endogenous and exogenous compounds in the blood, including free fatty acids, bilirubin, haematin, approximately 50% of the total serum calcium, various hormones, and numerous drugs. Reported values for the half-life of albumin in the dog vary from 8 to 23 days. Albumin degradation occurs in the liver and in all other tissues where the amino acids are utilized for tissue proteins. No albumin is excreted in the normal animal[3 9]. The factors directly controlling the serum concentration of albumin are not known, but the rate of hepatic synthesis and the balance between protein anabolism and catabolism, as controlled by the glucocorticoid hormones, appear to be very important. The serum albumin concentration is influenced by hepatic function, amino acid availability, the amount lost in disease conditions, and by the water and electrolyte balance of the animal. When nutrition and liver function is normal, 90% of the total plasma protein can be synthesized in a week[3 4 5].

Hyperalbuminaemia is only seen in animals in states of shock and dehydration, from any cause[3 5].

In general, decreases in the serum albumin concentration may be caused by an insufficient intake or absorption of protein, deficient albumin synthesis, excess-

ive breakdown of protein, or loss of albumin from the body. An inadequate intake of protein occurs in anorexia, starvation, and malnutrition. It is occasionally seen during pregnancy and lactation, when the animal's diet is insufficient to meet requirements. Decreased absorption of protein occurs with defective protein digestion, as caused by pancreatic exocrine enzyme deficiency (maldigestion), and in intestinal malabsorption resulting from chronic diarrhoea or cellular infiltration into the small intestinal wall. In these conditions, the amino acids required for albumin synthesis are derived from tissue protein catabolism. Hence, the hypoproteinaemia seen with reduced protein intake or absorption is of a lesser extent than that frequently found with chronic liver disease, catabolic states, or excessive albumin loss[3].

As albumin is almost entirely synthesized by the liver, hypoalbuminaemia is a common finding in many liver diseases. Albumin is synthesized in the liver at a rate of 12% per day in dogs and 5% per day in cattle. Thus, its serum concentration falls in chronic liver conditions such as subacute hepatitis, diffuse fibrosis, and cirrhosis, irrespective of the specific aetiology. Amyloidosis and extensive neoplastic involvement of the liver have a similar effect. In addition, severe anaemia and right-sided congestive heart failure result in hypoalbuminaemia due to hepatocyte hypoxia which causes decreased synthetic function[4 5 7 9 13].

Excessive breakdown of protein occurs in any chronic disease resulting in cachexia, especially neoplasia, prolonged pyrexia, traumatic tissue injury, and congestive heart failure.

Albumin may be lost from the blood into body cavities. Peritoneal and pleural effusions, due to right-sided congestive heart failure, contain significant amounts of albumin (modified transudates). Pleuritis and peritonitis result in a large accumulation of albumin in the exudate. Hypoproteinaemia due to albumin loss from the body can be very severe. Such loss occurs from the gastrointestinal tract in verminosis, particularly in Ancylostomiasis, Trichostrongylosis, Haemonchiasis and Ostertagiasis. Albumin is excreted by the kidneys in acute nephritis, and in nephrosis with glomerular damage. In severe, acute haemolysis much protein in the form of haemoglobin is lost through the kidneys. In addition, the glomerular and tubular damage results in albinuria. A similar situation occurs with azoturia in horses, and capture myopathy in game, where myoglobin is lost in the urine and causes nephrosis. Albumin and all the other blood proteins are lost in haemorrhage, draining wounds, and in extensive partial and full-thickness burns[3 4 8 9 13 14].

The consequences of hypoalbuminaemia is generalized oedema. In particular submandibular and brisket oedema ("bottle jaw") in cattle and sheep, and hydroperitoneum (ascites) in horses, dogs and cats. In severe hypoalbuminaemia, dependent anasarca, hydrothorax, hydropericardium, and lung oedema occur. Hypoproteinaemia associated with liver fibrosis or cirrhosis usually manifests primarily as ascites, but in severe, long-standing cases it also results in oedema of the hind-leg tissues[8 14].

The globulin fraction of the total serum proteins is composed of numerous fractions, the number depending upon the sophistication of the separation procedure used. In dogs, 7 groups are usually distinguishable by paper electrophoresis, but at least 21 distinct fractions can be isolated using advanced chemical techniques[3]. However, the principal groups are alpha (α), beta (β), and gamma (γ), based on their electrical mobility. These can be further subdivided by usual laboratory electrophoretic methods into α_1, α_2, α_3, β_1, β_2, β_3, and γ portions, depending upon the species of animal[3 5]. Hypoglobulinaemia is rare but may be seen in some protein-losing enteropathies and nephropathies[13].

The alpha and beta globulins are synthesized in the liver, and circulate in the blood. They carry various lipids, lipid-soluble hormones and vitamins, and other fat-soluble compounds; these complexes are called lipoproteins[3 6].

The alpha globulins carry, in addition to fat-soluble compounds, carbohydrate metabolites as glycoproteins; copper as ceruloplasmin; and haemoglobin as haptoglobulins. Glycoproteins, the majority of which are carried by the plasma globulins, are produced by the liver. They are released from many tissues that are damaged (inflammatory or degenerative changes), and may be synthesized as a response to tissue injury. Thus an increase in their concentration indicates acute non-specific tissue damages[3]. Alpha globulins also contain the antitrypsin factor, which counteracts the activity of the pancreatic trypsin which continually occurs in the blood stream in low concentrations. The serum concentration of antitrypsin factor may be raised in any acute tissue damage. Thyroxin-binding globulin (binds thyroxin, T_4), transcortin (binds cortisol), and erythropoietin are also alpha globulins. The half-life of alpha globulins in the dog is approximately 13 days[5 9].

Increases in the serum alpha globulin concentrations are seen with many inflammatory or destructive conditions. In particular, α_2 rises in acute bacterial and viral infections, particularly when the liver is affected. This is probably due to a rise in the level of circulating glycoproteins. Decreases in the concentration of alpha globulins are extremely rare[3 4 5 7].

The beta globulins transport fat-soluble compounds as lipoproteins and iron as transferrin or siderophyllin. Complement, which is synthesized in the liver, the macroglobulin IgM, and the local globulin IgA, are all beta globulins. Fibrinogen, prothrombin and plasminogen are also beta globulins, but are obviously not present in serum[3 5]. The half-life of the beta globulins in the dog is 14 – 16 days[9].

Beta globulin concentrations rise in all hyperlipidaemias, whether of primary or secondary aetiology. Increases have also been recorded in nephrosis, probably because of an increase in the lipoprotein fraction, associated with altered function of the renal tubular cells; in hepatitis in dogs and cattle; in biliary obstruction, and in all cases of jaundice (β_1 increases)[3 4 5 7].

The gamma globulins are synthesized by the reticuloendothelial cells of the body, in particular by the β-lymphocytes and plasma cells. They are principally IgG antibodies[3]. The half-life of gamma globulin in the dog is approximately 20 days[9].

Hypergammaglobulinaemia occurs in response to antigenic stimulation, viral (particularly in feline infectious peritonitis), rickettsial (marked increase in Ehrli-

chiosis), bacterial, protozoal (including anaplasmosis and babesiosis), and parasitic; liver disease, especially in acute hepatitis, cholangitis, portal fibrosis, and in all jaundiced animals; some autoimmune diseases; and in some lymphosarcomas. These conditions all cause a polyclonal increase in gamma globulin. Monoclonal gammopathies occur with plasma cell myelomas (multiple myelomas) of the bone marrow[3 4 5 6 7]. The liver diseases which result in increases in the gamma globulin concentration are usually due to infective organisms and so probably represent an antibody response to exogenous antigen. Toxic liver damage due to chemicals or plants, even those causing severe degeneration and necrosis, such as carbon tetrachloride, phosphorus or Seneciosis, rarely result in a hypergammaglobulinaemia[4].

Hypogammaglobulinaemia occurs in cows with the onset of colostrum formation, due to migration of antibodies into the mammary gland[5]. It may also occur in malnutrition, debilitating diseases, particularly neoplasia, and in cachexia. Low γ-globulin concentrations indicate decreased antibody formation or increased loss, and impaired resistance to infection[3].

Fibrinogen is a globulin plasma protein, which is synthesized in the liver. It circulates in the blood and is primarily involved in the clotting mechanism. The turnover rate of fibrinogen is very rapid; the half-life is the shortest of all the plasma proteins, 3–5 days. This short half-life may be due to fibrin coating of the vascular endothelium[3 7].

Increased plasma fibrinogen concentrations occur in pregnancy; in mild liver injury (cloudy swelling, hydropic degeneration of fatty infiltration); in some cases of liver cirrhosis; slight cellular destruction in any tissue, due to inflammation, suppuration, trauma or neoplasia; most acute bacteraemias and septicaemias; nephrosis; and in multiple myeloma (originating from the myeloblasts or myelocytes of the bone marrow).

The causes of low concentrations of plasma fibrinogen are not well documented in animals, except for disseminated intravascular coagulation. This occurs most commonly in acute conditions such as babesiosis; gastric torsion; obstetrical emergencies, particulary those involving foetal death, and subsequent maternal toxaemia; shock; major surgery; and in severe burns[3 4 8].

Prothrombin is synthesized exclusively by the liver. The synthesis requires adequate amounts of fat-soluble vitamin K. Prothrombin deficiency may occur in diffuse hepatic fibrosis or cirrhosis as a result of an inadequate functional amount of liver tissue (less than 30% functional hepatocytes), and an insufficient output of bile salts into the duodenum. Bile salts are essential for the emulsification of ingested fat, prior to its lipolysis and absorption. Prothrombin deficiency also occurs in cattle with fatty infiltration of the hepatocytes in the early lactation period; in acute viral hepatitis in dogs resulting from infectious canine hepatitis; in bile duct obstruction; and in coumarin poisoning from sweet clover hay, or as dicoumarol in rat poisons. It does not appear to be associated with pancreatic exocrine enzyme deficiency[3 7].

REFERENCES

1. Blackmore D J 1976 A New Concept of Normal Values. Modern Veterinary Practice 57: 732
2. Coffman J R 1974 Clinical Chemistry in Equine Practice. Modern Veterinary Practice 55: 559
3. Coles E H 1967 Blood Chemistry. In: Veterinary Clinical Pathology. Saunders W.B. Company, Philadelphia
4. Cornelius C E 1970 Liver Function. In: Kaneko J J, Cornelius C.E. (eds) Clinical biochemistry of Domestic Animals 2nd end. Academic Press, New York
5. Dimopoullos G T 1970 Plasma Proteins. In: Kaneko J J, Cornelius C E (eds) Clinical Biochemistry of Domestic Animals 2nd end. Academic Press, New York
6. Hardy R M, Stevens J B 1978 The use and Interpretation of Clinical Laboratory Data. Lecture notes, Veterinary Clinician's Group, South African Veterinary Association
7. Hoe C 1969 Liver Function Tests. In: Medway W, Prier J E, Wilkinson J S (eds) A Textbook of Veterinary Clinical Pathology. The Williams & Wilkins Co, Baltimore
8. Jubb K V F, Kennedy P C 1963 Pathology of Domestic Animals. Academic Press, New York
9. Kronfeld D S, Medway W 1969 Blood Chemistry. In: Medway W, Prier J E, Wilkinson J S (eds) A Textbook of Veterinary Clinical Pathology. The Williams & Wilkins Co, Baltimore
10. Low D G, Osborne C A, Finco D R 1975 The Pillars of Diagnosis: History and Physical Examination. In: Ettinger S J (ed) Textbook of Veterinary Internal Medicine: Diseases of Dogs and Cats. Saunders W B Company, Philadelphia
11. McSherry B J 1969 Forward. In Medway W, Prier J E, Wilkinson J S (eds) A Textbook of Veterinary Clinical Pathology. The Williams & Wilkins Co, Baltimore
12. Rich L J 1976 The Commercial Laboratory. Veterinary Clinics of North America 6: 539
13. Schall W D 1976 Laboratory Diagnosis of Hepatic Disease. Veterinary Clinics of North America 6: 679
14. Smith H A, Jones T C, Hunt R D 1972 Veterinary Pathology. Lea & Febiger, Philadelphia
15. Tasker J B 1976 The Veterinary Hospital Laboratory. Veterinary Clinics of North America 6: 523

FIBROTIC MYOPATHY, HAEMATOMAS AND SCAR TISSUE IN THE GASKIN AREA OF THE THOROUGHBRED

D.H.G. IRWIN & D.W. HOWELL

ABSTRACT: Irwin D.H.G.; Howell D.W.: **Fibrotic myopathy, haematomas and scar tissue in the gaskin area of the Thoroughbred** *Journal of the South African Veterinary Association* (1981) **52** No. 1, 65–66 (En) Box 4107, Alrode 1451, Rep. of South Africa. An easy diagnostic technique for recognising fibrotic myopathy and scarring in the caudal popliteal area is described. A simplified corrective surgical technique is offered and discussed briefly. Some aspects of haematomas are reviewed.

INTRODUCTION

The aim of this short communication is to add a little to what has been written by Hammel & Raker[1] on fibrotic myopathy. Although we had not recognised this condition for 9 years in equine practice, we have diagnosed 7 cases of the disease in the last 7 months. Curiously 4 of the cases were in grey horses. The easiest way to recognise the disease, is to watch a string of horses walking in a ring with either tannin bark or sand under foot. Normally, when the hind foot of the horse strikes the ground under these conditions, a little puff of debris and dust is displaced in an anterior direction. When a limb is affected with fibrotic myopathy or scarring, however, the hind limb so affected is noted to displace no debris in a forward direction; if anything is displaced, it is in a caudal direction. The shortened stride is a goose step which can be demonstrated to less observant owners by a simple test, as follows: rake over or draw a sack over soft sand, and allow the horse to walk across this: one can pin-point the imprints of the 4 feet. The affected hind limb is shorter at striding in relation to the forehoof imprint than the normal limb on the other side. Another way to demonstrate the diagnosis is to walk the horse on concrete, as for example down the passage of the hospital barn. A loud sound is quite distinct as the hoof of the affected limb strikes the concrete. Careful palpation of the caudal aspect of the hind limb on the level of the stifle joint and a little above and below this level will reveal subcutaneous scarring. If the skin of the area is shaved of hair, one can sometimes see a scar in the skin. In fact, this scar is sometimes raised so that in the shaving process a line of redness is produced by the razor. In some cases a dimple can be seen in the limb. If this is the case, it helps in deciding upon the exact site for the operation which we currently employ, which is described below.

PATHOGENESIS

It would appear that the aetiology is either external (kick) or internal (muscle tear) trauma with subsequent haematoma formation. In the course of healing, the resultant scar prevents normal protraction of the affected limb. The scar tissue appears to become orientated in a vertical plane and acquires the appearance and texture of perimuscular and intramuscular aponeurosis.

TREATMENT

In the first case we diagnosed, we followed the operation as described by the above-mentioned authors and sutured the wound with quill sutures because we anticipated quite a lot of tension. On the fifth day, however, the wound broke down and it was allowed to heal by granulation and epitheliazation. In the first operation the incision was about 200 mm long. In the second case a smaller wound was made and in the third, which was also done under general anaesthesia, we again made the incision smaller. All 3 of these wounds burst open and, in fact, the first one produced such a large wound when the sutures broke down that we referred to it as our "hand-grenade" case! In all subsequent cases the operation was performed under local anaesthetic in the standing position with the horse in the stocks and tranquilized.

The operation which we now do is far less radical than that described by Hammel & Raker. After making a vertical 30 or 40 mm incision over the scarred area, we separate the skin from the subcutaneous fascia. The fascia is then slit longitudinally, and any tight vertical bands are nibbled across with the scissors. In all but 2 cases we were able to free the tight bands, which we take to be responsible for the disease, through one incision as described. In 2 cases, however, it was elected to make a second incision 50 mm lateral and in another 50 mm medial to the first so that other bands could be nibbled through without traumatizing and stretching the tissues as much as would have to be necessary, had the second incision not been made.

If, in the course of the operation, we feel that we have completed the procedure, we let the horse out of the stocks and allow it to walk along a passage. If there is significant improvement in the gait, we are satisfied. Note, however, that the final caudal snap may not be overcome till 10–14 d after surgery.

If the limb is drawn forward in extension by an assistant in the course of the operation, the surgeon can very readily detect the tense bands which limit anterior movement with one exploring finger in the wound. If the limb is in the vertical standing position, such bands cannot be palpated or identified and insufficient fascia or scar bands are severed.

The horse is returned to the stock and the final exploratory finger is inserted into the wound, and if no more turgid longitudinal bands can be palpated, we plug the wound with a gauze bandage soaked in acrifla-

0038-2809/81/01/0065–0066

vine and glycerine. On 2 occasions it was necessary to enlarge the wound in order to seek a small artery which could not be ligated without a further exposure. The wounds are allowed to heal by granulation. Walking exercise is given for 7–10 d, then trotting. One g of phenyl butazone is administered daily. The assumption is that the scar is formed in extension and is less strong than would obtain in the absence of the anti-inflammatory drug.

The commonest haematoma encountered occurs in the caudal aspect of the hind limb on a level close to the stifle joint. Mostly, these are treated by lancing on the 8th day after appropriate surgical preparation, including protection against tetanus, has been effected.

In one case, a pregnant mare, incision of a very large haematoma was delayed until the 14th day after occurrence. Following drainage of the haematoma, it was clear that with the loss of back pressure, haemorrhage recurred. It was only after extensive packing and suturing of the wound that the blood flow was staunched but not before considerable apprehension had been generated. In view of this case we have decided that when an animal presents a large haematoma, it will be prepared starved as in preparation for surgery and if, after drainage in the original way, all appears to be well 4 or 5 hours later, the animal will be fed.

If, on the other hand, haemorrhage recurrs, the patient is anaesthetised, the site adequately exposed and the offending vessel ligated. If the animal had not been starved, one might prejudice important options, namely general anaesthesia and adequate exposure.

Although we have no conclusive case history evidence, it appears that horses which have suffered from haematomas in this area are likely to develop fibrotic myopathy as discussed above.

DISCUSSION

The first three cases of the form of fibrotic myopathy described here were diagnosed and treated within a period of 6 weeks. They were admitted to the clinic at intervals of roughly 2 weeks and it was interesting to note that all 3 of the wounds healed finally at about the same time. Subsequent cases have healed in still shorter time (10–14 d) when using the less radical operation described here. All horses treated so far have acquired normal gait within 10 to 14 days of the operation. If the conservative operation described here proves ineffective in any horse, the bolder more radical operation could be done later.

REFERENCE

1. Hammel, Elaine P, Raker C W 1972 Myopathies. In Equine Medicine and Surgery Eds Catcott E J & Smithcors J F 2nd ed. P 585. American Veterinary Publications Inc., Wheaton, Illinois

BONE ABNORMALITIES IN THE CAPE VULTURE *(GYPS COPROTHERES)*

L.B. EVANS AND S. PIPER†

ABSTRACT: Evans L.G., Piper S.E. **Bone Abnormalities in the Cape Vulture** *(Gyps coprotheres). Journal of the South African Veterinary Association* (1981) **52** No 1, 67–68 (En) 15 Village Road, Kloof 3600, Natal, Rep. of South Africa. Bone abnormalities found in free-living juvenile Cape vultures are described. The possible aetiology of this nutritional osteodystrophy syndrome is discussed.

INTRODUCTION

The Cape vulture *(Gyps coprotheres)* is the commonest vulture in South Africa. It is distributed generally throughout South Africa except in the central Kalahari and northern parts of the country[5].

During the period 1976–1978 the Vulture Study Group affiliated to the Endangered Wildlife Trust undertook a survey of 4 major colonies of Cape vultures in order to attempt to determine the apparent reason or reasons for the declining number of this bird species in South Africa.

Six juvenile birds approximately 6–8 weeks of age were removed under permit from their nests during the period 1976–1978 and presented for veterinary examination. Of these 6 birds, 4 were found to have severe to very severe skeletal abnormalities. X-rays were taken and the following observations made:

1. bone cortices were thin
2. marked bending of the long bones was clearly shown
3. folding fractures were present in some birds' wings
4. each bird had several healing and/or healed fractures present in the long bones (radius & ulna) of the wings (Fig. 1).

A diagnosis of juvenile osteoporosis[2] or, more correctly, nutritional osteodystrophy was made[3]. (The term nutritional secondary hyperparathyroidism is also sometimes used for this syndrome[4].)

DISCUSSION

Nutritional osteodystrophy is a bone disease commonly recognised in small domestic animals, resulting from a nutritional imbalance of the required calcium and phosphorous ratio. Animals fed on an all-meat diet will have a low calcium and high phosphorous intake, i.e. the Ca: P ratio will be approximately 1:30 instead of the required 1,5:1.

The low dietary calcium intake results in a low serum calcium level. This in turn stimulates the parathyroid glands to increase parathormone production and calcium is resorbed from the bones in an attempt to correct the hypocalcaemia. The withdrawal of calcium from the bones results in inadequate mineralization and the bones are softer than normal. Consequently, bowing and other deviations occur in the long axes of the bones. Folding fractures as well as complete fractures are found and these fractures heal poorly, if at all[1,3,4].

†Dept. of Land Surveying, Univ. of Natal, Durban.

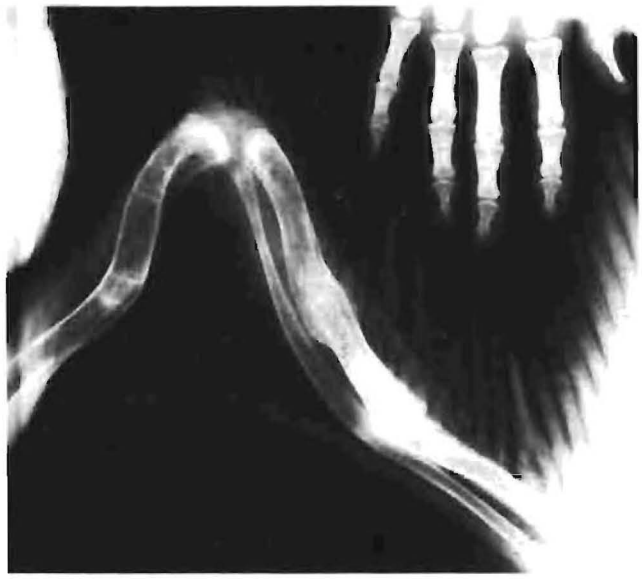


Fig. 1 X-ray of vulture wing showing the following bone abnormalities: thin bone cortices, bowing of the long bones and 2 poorly healed fractures.

This syndrome, as described above, was found to be present in 4 out of the 6 vulture chicks that were examined and thus a diagnosis of nutritional osteodystrophy was made.

For normal bone development, 3 factors are required, namely calcium, phosphorus and vitamin D. In the wild state, vultures normally will nest on cliffs or similarly exposed places and are therefore exposed to a source of Vitamin D in the form of sunlight. Ingested fat also provides another source of the vitamin. The calcium and phosphorus needs are supplied to the chicks by the parents which feed them on scavenged meat, offal and bone fragments from the carcases they find in the environment. In view of the bone problems recently found, it must therefore be concluded that although meat and offal, and therefore protein, are in sufficient supply to rear the young, there is a deficiency of calcium, probably as a result of an inadequate supply of bone fragments and the increasing tendency of the adult vultures erroneously to pick up fragments of plastic, porcelain and similar materials in place of bone and feed them to their chicks. This latter observation has been made by examination of vulture faeces and the discovery of the presence of these materials in it.

The inadequacy of the supply of bone fragments is probably due to fewer wild and domestic carcases available to scavengers, and also to the fact that more vig-

0038-2809/81/01/0067–0068

ilant farmers are removing what carcases are found and burying them before the birds have had an opportunity to remove enough pieces of bone. This hypothesis is supported by the fact that of the 4 major colonies of Cape vultures studied, bone abnormalities were found in birds of 3 of them but not in birds taken from the colony occurring in the Transkei area. This area is less intensively farmed and local population is more scattered and less likely to detect and remove carcases with the same rapidity that might occur at the other 3 sites.

Apart from naturally occurring phenomena such as infertility or chick and adult deaths resulting from infectious, toxic or accidental causes, the number of chicks affected by bone disease becomes increasingly significant when one takes into account the fact that a vulture pair only lays one egg per year. Hence the loss of young birds due to flight disability following bone deformities and/or fractures also becomes more significant. It is estimated that some 20% of all chicks are affected by nutritional bone disease. The importance of this figure with regard to the already recognized fact that the numbers of Cape vulture are steadily decreasing is thus self-evident.

ACKNOWLEDGEMENT

I wish to acknowledge the assistance given by Mr. P. Massie in reproducing the X-ray of the one bird's wing.

REFERENCES

1. Cooper J E (1975). Osteodystrophy in birds of prey. Veterinary Record 97:307
2. Douglas S W, Williamson H D 1970 Veterinary Radiological Interpretation. Heineman, London
3. Doxey D L 1971 Veterinary Clinical Pathology. Tindall, London
4. Kirk R W 1971 Current Veterinary Therapy IV. Saunders, Philadelphia
5. McLachlan G R, Liversage R 1971 Roberts' Birds of South Africa. Central News Agency, Cape Town

KORT MEDEDELINGS OOR GEVALLE VAN DIE OOG, LARINKS, TRAGEA EN VEL

S.W. PETRICK

ABSTRACT: Petrick S.W. **Short communications on cases of the eye, larynx, trachea and skin.** *Journal South African Veterinary Association* (1981) **52** No 1, 69–70 (Afr) Department Surgery, Faculty of Veterinary Science, University of Pretoria. Box 12580, Onderstepoort 0110, Rep. South Africa. A freefloating iris cyst in a dog, the congenitally attached apex of the epiglottis in a cat, spontaneous healing of *Filaroides osleri* in a dog and dermoid sinus in a Ridgeback crossbreed are discussed.

INLEIDING

Irissiste in honde is skaars, dikwels van kongenitale oorsprong, laat gewoonlik lig deur, kan losraak en vrydrywend wees in die voorste kamer en mag selfs groter word.[1,4,6]

Geen literatuur kon opgespoor word oor die band tussen die apeks van die strotklep en die basis van die tong nie.

Die spontane verdwyning van *Filaroides osleri* in honde na afsondering[5] is nie algemeen bekend nie.

Dermoïed-sinusse in Rifrug-honde en-kruise kom voor in die nekgebied, voorste torakale en sakrokoksigeale gebied maar nie in die gebied van die rif nie.[2,3]

GEVALLEBESPREKING

Geval I. Vrydrywende irissist in 'n hond

Pasiënt: 'n 10-jaar oue Spaniel-kruisteef.
Geskiedenis: Verwys met 'n verdagte melanoom van die iris in die linker-oog (Fig 1). Dit het volgens die einaar groter geword.

Fig. 1 Vrydrywende irissist in 'n hond.

Diagnose: Met beweging van die kop is vasgestel dat dit vrydrywend was en dus onwaarskynlik 'n melanoom sou wees. 'n Corpora nigra of irissist is voorlopig gediagnoseer. Die oog was verder normaal.
Behandeling: Dit is chirurgies verwyder en omdat dit daarna ook saamgeval het, blyk dit dus 'n irissist te gewees het.

Geval II: Kongenitale hegting van die apeks van die strotklep aan die basis van die tong in 'n kat

Pasiënt: 'n Huiskatwyfie, 5 maande oud.
Geskiedenis: Verwys met 'n chroniese hoes en 'n verdagte trageoesofageale fistel.
Diagnose: 'n Radiologiese ondersoek het die vermoede bevestig. Onder algemene narkose is die aanhegting van die apeks van die strotklep aan die basis van die tong met laringoskopie waargeneem terwyl die esofagoskopie niks opgelewer het nie.
Nadoodse ondersoek: Voordat die band chirurgies gesny kon word, is die kat dood a.g.v. 'n vreemdevoorwerplongontsteking wat nadoods vasgestel is en ook die aanhegting bevestig het.
Bespreking: Weens genoemde band kon die strottehoofspleet nooit behoorlik sluit tydens die slukproses nie.

Geval III: Spontane genesing van *Filaroides osleri* in 'n hond

Pasiënt: Schipperke-kruisreun, 6 maande oud.
Geskiedenis: Die dier is opgeneem met 'n chroniese hoes en 'n verdagte vreemde voorwerp in die tragea.

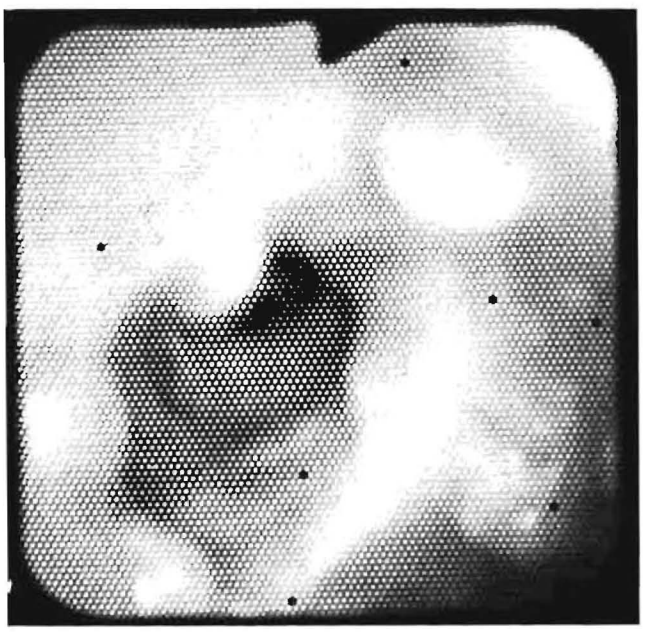


Fig. 2 *Filaroides osleri* knoppe in die tragea van 'n hond.

0038-2809/81/01/0069–0070

Diagnose: Verdikking van die trageale slymvlies met klein knoppe is met 'n trageoskopie vasgestel. Dit is ook radiologies bevestig. 'n Diagnose van *Filaroides osleri* is gemaak en die dier opgeneem vir observasie en moontlike behandeling.
Verloop: Na 2 weke was die trageaknoppe groter (Fig 2). Na 'n verdere 4 weke was die knoppe minder en kleiner en na nog 'n verdere 7 weke was die tragea skoon.
Behandeling: Die *Filaroides osleri*-besmetting het spontaan genees. 3 weke na die laaste ondersoek is 5 *Spirocerca lupi*-granulomata chirurgies uit die slukderm verwyder. Hulle is ook tydens die eerste ondersoek gediagnoseer en was met elke daaropeenvolgende ondersoek groter.

Geval IV: Dermoïed-sinusse in 'n Rifrug-kruis

Pasiënt: 'n Chow X Rifrug-reun wat lyk soos 'n Chow en sonder 'n teken van 'n rif.
Geskiedenis: Die hond is behandel vir dermatitis in die dorsale nekgebied. Nadat dit skoongeskeer is, is 'n aantal dermoïed-sinusse gevind.
Behandeling: Onder algemene narkose is 5 afsonderlike dermoïed-sinusse chirurgies verwyder. 7 weke later is 'n verdere 6 sinusse chirurgies verwyder waarvan 2 in die torakolumbale gebied was en 4 net voor die stertwortel. Fig. 3 toon die 11 dermoïed-sinusse.
Bespreking: Dit blyk dat in Rifrug-kruise sonder die teenwoordigheid van 'n rif die dermoïed-sinusse ook kan voorkom in die gebied waar so 'n rif normaalweg teenwoordig is en waar daar dan geen sinusse normaalweg gevind word nie.

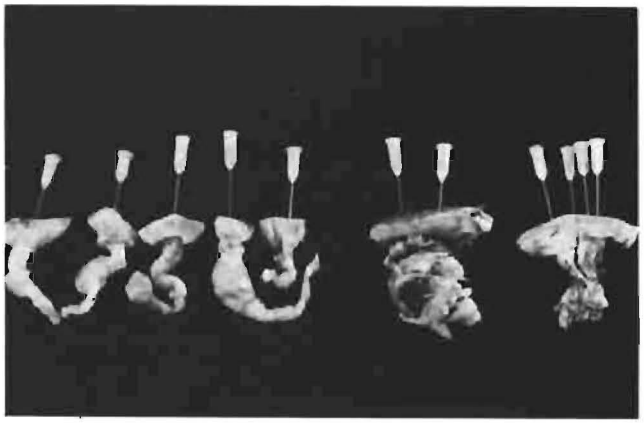


Fig. 3 Dermoïed-sinusse van 'n hond.

VERWYSINGS

1. Bellhorn R W 1969 Removal of ciliary body adenoma. Modern Veterinary Practice June: 47.
2. Hofmeyr C F B 1966 Dermoid sinus in the Ridgeback dog. Journal of Small Animal Practice 4: 5.
3. Mann G E 1966 Dermoid sinus in the Rhodesian Ridgeback. Journal of Small Animal Practice 7: 631.
4. Peiffer R L, Gwin R M, Gelatt K N, Jackson W F, Williams L W & Hill C W 1978 Ciliary body epithelial tumours in four dogs. Journal of the American Veterinary Medical Association 172, 5: 578.
5. Pillers A W N 1935 Some remarks on the clinical aspect of chronic tracheobronchitis of the dog due to *Oslerus osleri* (Cobbold, 1879) 15, 3: 62.
6. Roberts S 1959 Iris cysten beim Hund. Wiener Tierärztliche Monatsschrift 46, 1: 20.

OUT OF THE PAST **UIT DIE VERLEDE**

THE ERADICATION OF EAST COAST FEVER IN SOUTH AFRICA*

ABSTRACT: Anon. **The eradication of East Coast fever in South Africa.** *Journal of the South African Veterinary Association* 1981 **52** No 1, 71–73 (En) Reprints: Head, Protozoology Section, Veterinary Research Institute, Onderstepoort 0110, South Africa.

An epizootic of East Coast fever followed the introduction of a highly fatal strain of *Theileria parva parva* into South Africa in 1902. This is a brief historical review of the campaign to control and finally eradicate the disease through tick control, quarantine and the slaughter of exposed cattle. The disease was responsible for an estimated 5,5 million deaths before its final eradication in 1954.

East Coast fever derived its name from the fact that it was first recognized by Koch on the east coast of Africa in 1897. Later, Theiler described the parasite and its transmission. In 1901, after infected cattle had been shipped from Tanganyika (Tanzania) and landed at Beira and then moved to Umtali and Salisbury, the disease became firmly established in Southern Rhodesia (Zimbabwe). In the same year infected cattle were imported into Lourenco Marques (Maputo) and thus Portuguese East Africa (Mocambique) became infected. The first outbreak of East Coast fever in South Africa took place at Komatipoort (Eastern Transvaal) in 1902. In the same year the disease spread up the Lourenco Marques (Maputo) – Pretoria railway line and along the transport routes as far west as Zeerust. It also spread into Swaziland. In 1903 the Piet Retief district in Eastern Transvaal became infected as well as an area in Northern Natal. The disease also spread from Southern Rhodesia (Zimbabwe) into the Northern Transvaal. In 1906, the scourge swept through Natal, causing very heavy losses. Transkei was next to become infected, namely in 1910. By 1913 the disease had reached the East London area. Between 1910 and 1914 no fewer than 900 000 head of cattle died from East Coast fever in Transkei alone.

The role played by ticks in the transmission of the disease and the necessity for tick control was realised early. In 1901, Joseph Baines built what is apparently the first cattle dip ever to be built in South Africa near Pietermaritzburg. It is still in use today.

When East Coast fever became established in Natal in 1906, tick control became a vital issue, and at the Allerton Laboratory, Pietermaritzburg, Colonel Watkins-Pitchford did important pioneer research into the problem. His findings formed the basis of our dipping practice today.

The brown ear tick *Rhipicephalus appendiculatus* was undoubtedly the most important transmitter of East Coast fever in South Africa. It usually feeds around the eyes and on the ears. The female tick lays about 4 000 eggs which hatch in approximately 4 weeks. The larvae or seed ticks await a host and can, under suitable conditions, survive for several months without feeding. They feed on the animal for 3 to 7 d, then drop off and moult into nymphae which can survive even longer than the larvae without feeding. The nymphae feed for 3 to 7 d, then drop off and moult into adults which can survive without feeding for 18 months or longer. The adults take 4–10 d to engorge, after which the females drop off to lay their eggs. As the brown ear tick feeds on 3 separate animals in its life cycle, it is known as a 3-host tick.

The infection of East Coast fever may be picked up in the larval stage and transmitted by the nymph, in which case the subsequent adult is not infective. The infection may also be acquired by the nymph and transmitted by the adult, but it does not pass through the egg from adult to larva.

The red-legged tick *R. evertsi* also transmits East Coast fever, but the role played by this species appears to be a minor one. The adult ticks are usually found under the tail of the animal. The red-legged tick is a 2-host tick and, since the larval and nymphal stages are completed on the same host, the infection can only be carried from the first host to the second.

When infected ticks feed on an animal, the infection first develops within the tick itself and only enters the host after 68 to 72 h. About 6 d after the tick has attached itself, the lymph node draining the site of infection begins to swell. If, at this stage, gland smears are made, we find on microscopic examination that the parasites introduced by the tick have developed into schizonts or Koch's bodies. The lymphocytes themselves show proliferation while the red cells are not yet affected. The body temperature rises on about the 9th day, and the other lymph nodes of the body begin to swell about 2 d later. A lymph node smear taken at this stage shows that Koch's bodies are breaking up into numerous small parasites which enter the blood cells where they are known as small piroplasms. Only when these infected red cells enter the general circulation can ticks feeding on the animal become infected. This happens usually more than 14 d after original infection took place.

The eradication of ticks was naturally the objective of a campaign against East Coast fever and other tick-borne diseases, and this objective could only be achieved by a regular weekly dipping programme which was compulsory in all potential East Coast fever areas. In many of the tribal reserves the life of the African goes on much as it has done for centuries, almost unaffected by European influence. The cattle are of poor stock but very hardy, and their care and management is exceedingly primitive. Dipping tanks were

*The campaign to control and finally eradicate East Coast fever (*Theileria parva parva* infection) from South Africa after its introduction in 1902 lasted for more than half a century. Details of the final stages of this campaign in particular were never published. This is an edited transcript of the sound track of a film titled "East Coast fever" made by the Department of Agricultural Technical Services towards the end of the campaign. This film was made primarily for the lay public and, although superficial and out of date in many respects, it does give some idea of what was involved during the campaign.

0038-2809/81/01/0071–0073

installed throughout these areas. In the Transkei alone there were 725 tanks for 1,5 million head of cattle. Each stock owner was assigned to a particular tank where dipping took place every 7 d. A wash was used containing arsenite of soda. A simple field test for the strength of the arsenic used was developed and could be carried out at the dipping tanks. It may be mentioned that new synthetic organic dips appeared on the market for controlling the arsenic-resistant blue tick. Investigations were carried out to evaluate spray races with the object of using these dips fresh every dipping day.

Dipping in the tribal areas started with the first light of dawn and often went on well into the day. The rhythmic splash of the cattle taking the plunge, the peculiar acrid smell of the dipwash and the sound of voices calling from the hill-side are all memories which will never fade for those who took part in this work. The cattle of each stock-owner were counted as they entered the collecting kraal of the tank and complete records of them were kept. The movements of the cattle were restricted to the premises and all discrepancies in numbers had to be accounted for. As many as 2 000 head of cattle may congregate at one tank.

All deaths had to be accounted for by a spleen smear. The person responsible brought the spleen of a dead animal to the local tank assistant who made a smear which was later handed to the inspector. Smears were made as soon as possible after death. In cases where the animal had been dead for some time, a blood smear was also made from the tip of the tail, as this part is the last to decompose. Lymphnode smears were also made. The smears, each wrapped in duplicate forms, were forwarded to examining centres giving full particulars and stamped with serial numbers.

The smear was stained and examined microscopically and a diagnosis forwarded by the examining centre. Some of the smears received were unsuitable; for instance, it was impossible to focus on a curved surface such as a bit of broken bottle and large, jagged sheets of thick glass were impossible to handle. Even smears made on mirrors and bits of paper with blood on them were sent in.

Immediately a positive East Coast fever smear was found, the State Veterinarian of the area began an investigation by inspecting the herd for an animal which was feverish and listless. In a case of East Coast fever, prescapular and subparotid lymph nodes are swollen in an affected animal. There is also a nasal discharge and salivation. The eyes are sunken and watery and the ears hang. The muscles quiver, respiration is heavy, and there is severe loss of condition and diarrhoea. The veterinarian then made a lymph node smear, stained it and examined it under his portable microscope. If Koch's bodies were found, the diagnosis was confirmed.

Animals suffering from East Coast fever usually died 10–15 d after the onset of the illness. Over 90% of infected animals succumbed. A post mortem examination would reveal that the lungs were swollen and heavy. The septa stand out prominently, being distended by oedematous fluid. The bronchi contained large quantities of froth which often appeared at the nose shortly before death. An increase of clear fluid was usually present in the heart sac. The most characteristic lesion was found in the kidneys which showed white spots of lymphoid tissue on the surface and in the substance of the organ. Numerous erosions were frequently found in the abomasum. Though the spleen might be normal in size and appearance, it was usually markedly enlarged. The enlarged spleen showed prominent Malphighian corpuscles. The lymph nodes were usually swollen, and had a marrowed appearance. After the post mortem examination the hide, feet and head were burned to prevent the spread of infected ticks.

Dipping on farms infected with East Coast fever and on adjoining farms was carried out on a 5-5-4 d basis in an effort to destroy all infected ticks before they were fully engorged. Ticks in the brush of the tail may escape wetting by the dipping fluid and it was therefore compulsory that hair of the brush be clipped short. As the wash frequently does not penetrate into the ear and under the tail, these parts had to be hand-dressed at each dipping. A careful tab was kept on all cattle present at each dipping, and deaths unaccounted for by a satisfactory smear were suspected of having been due to East Coast fever. The cattle on the infected and frequently also on first and second contact farms were kept in quarantine for at least 18 months after the last case of East Coast fever.

The magnitude of the problem can be visualised with the situation in the Vryheid district of Natal in 1944 when 110 outbreaks of East Coast fever occurred. This is mainly a cattle farming area and the minimum quarantine period of 18 months imposed on infected farms created particular problems for the farmers. When first- and often second-contact farms were placed under quarantine, more than 800 farms engaged in stock farming were affected. This also meant that a large governmental staff had to be employed; during this crisis period there were more than 140 stock and assistant stock inspectors in the Vryheid area alone.

Although this policy succeeded in reducing the number of outbreaks, it failed to eradicate East Coast fever in some areas. The areas where East Coast fever gave the most trouble are all broken hilly country, such as one finds in the Umkomaas Valley in Natal. Some factor in the biology of the tick would seem to account for this phenomenon. In this type of country, East Coast fever frequently reappeared some years after it had apparently been eradicated. Whether this reappearance is due to some unknown factor such as a carrier stage or whether it was simply due to cases being missed is not known. As can be imagined, it was exceedingly difficult to keep track of every animal in these deep valleys and wooded ravines.

For these reasons the policy since 1948 was to clear all cattle from infected farms and to keep them clear for 18 months. This involved official supervision of fences after removal of the animals. Before sunrise on the morning the cattle were to be removed, the temperature of each animal was taken and any showing fever were destroyed. They were dipped and carefully handdressed to ensure that no infected ticks would drop off en route. The animals were then conveyed by the quickest possible means to the nearest abattoir. The cattle were conveyed by the Road Motor Service of the S.A. Railways to the nearest railway station, whence they were trucked.

Stock other than cattle were allowed to graze on these farms as it was known that infected ticks feeding on non-susceptible animals lose their infection.

A comparatively small compensation was paid for

the slaughtered animals. The State Veterinarian was guided only by current market prices when arriving at his estimates, and provision was made for any possible condemnation for measles, bruising, etc.

The Division of Veterinary Services worked in close collaboration with the stock owners. Regional advisory committees, elected by the farmers, met officers of the Division regularly to discuss local problems. Only by frank and open discussion could the essential co-operation between official and farmer be brought about.

Up to 1920 very little progress was made in the reduction of the incidence of East Coast fever, although the number of deaths per outbreak had been greatly reduced by intensive dipping. Even in 1935, East Coast fever was still widespread along our eastern borders but, largely due to the adoption of the policy of clearing infected farms of cattle, the incidence was greatly reduced in recent years. In 1948 there were only 4 infected areas.

As a result of the slaughter-out policy, East Coast fever was eradicated in South Africa between the years 1948 to 1954 after the country had been ravaged for 52 years. It is estimated that this scourge was responsible for 5,5 million deaths, besides the very high cost of control. It has been conservatively estimated that East Coast fever cost this country R100 million.

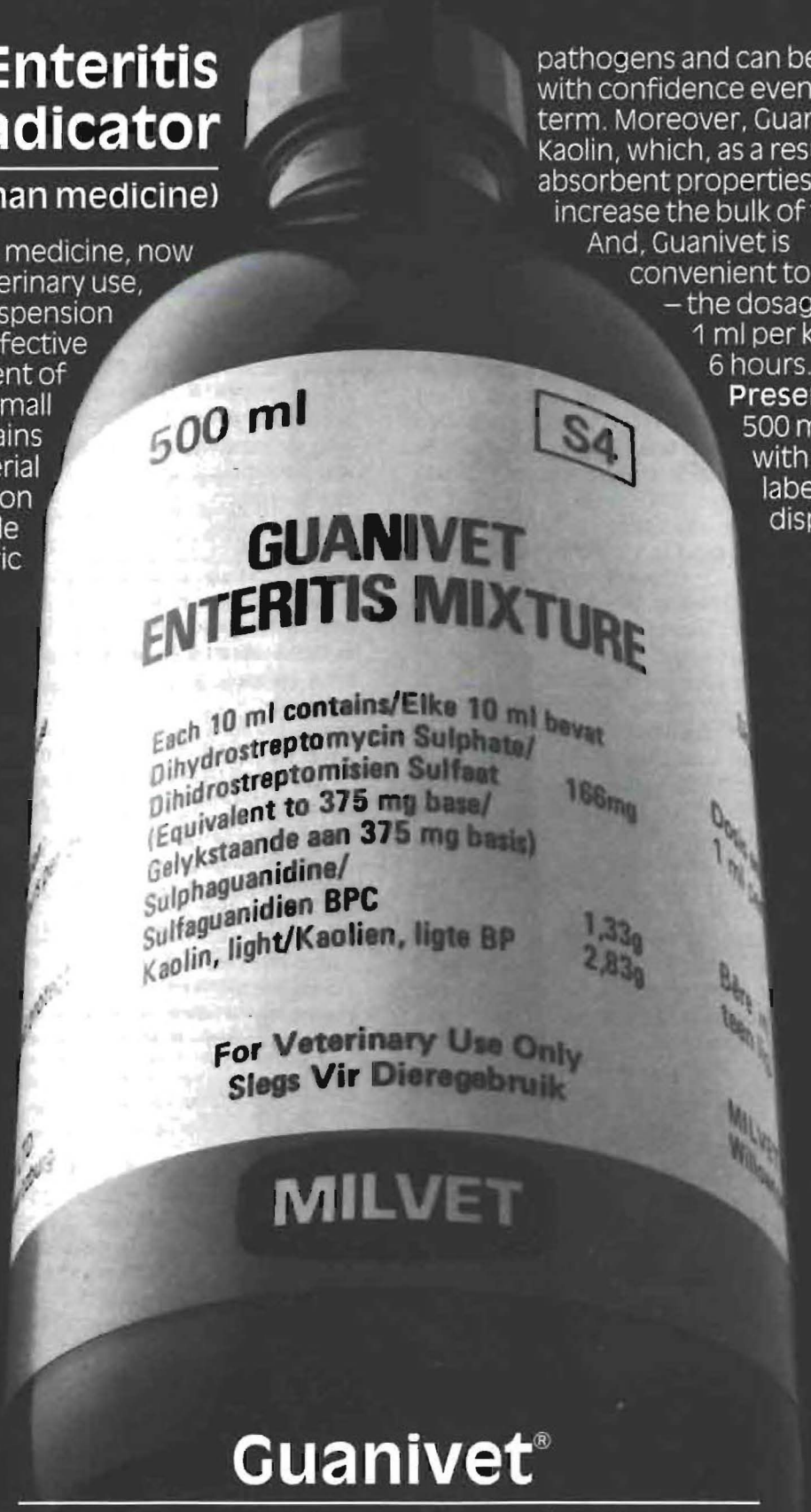
The Enteritis Eradicator

(proven in human medicine)

Proven in human medicine, now available for veterinary use, new Guanivet suspension is safe and effective in the treatment of enteritis in small animals. Guanivet contains two proven anti-bacterial agents for reliable action against a wide spectrum of enteric pathogens and can be used with confidence even in the long term. Moreover, Guanivet contains Kaolin, which, as a result of its absorbent properties, helps to increase the bulk of the faeces.

And, Guanivet is convenient to administer – the dosage is simply 1 ml per kg every 6 hours.

Presentation
500 ml Glass bottle with 5 gummed labels for easy dispensing



Guanivet®

gets the better of enteritis, fast

MILVET

ABSTRACTS OF PAPERS DELIVERED AT THE PROCEEDINGS OF THE VETERINARY PATHOLOGY GROUP OF THE SOUTH AFRICAN VETERINARY ASSOCIATION ON 8 SEPTEMBER 1980

ASPECTS OF EQUINE LEUKOENCEPHALOMALACIA

N.P.J. KRIEK*, W.F.O. MARASAS† and T.S. KELLERMAN‡

Toxigenic strains of *Fusarium verticillioides* (syn. *F. moniliforme*) occur throughout the RSA. Strains of this fungus cause field outbreaks of equine leukoencephalomalacia, a condition which can be reproduced at will experimentally. Depending on the dosage rate, either leukoencephalomalacia, a toxic hepatosis or a combination of the two, may be induced in horses.

There is a prominent interspecies difference in respect of manifestation of the lesions, target organ and susceptibility. Rats and baboons are much more resistant and manifest a chronic hepatosis and cirrhosis. Rats, additionally manifest widespread proliferative endothelial changes and thrombosis of the larger vessels and the heart. It appears as if the basis for at least some of the changes in both the horse and rat is a marked endotheliotoxicity of the associated toxins.

*Dept. of Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110.
†National Research Institute for Nutritional Diseases of the South African Medical Research Council, Tygerberg.
‡Section of Toxicology, Veterinary Research Institute, Onderstepoort.

SYMPTOMS, PATHOLOGY AND DIAGNOSIS OF EPIZOOTIC DIARRHOEA IN INFANT MICE (EDIM)

J.C. AUSTIN and E.S. GROSSMAN

A diarrhoeal disease of suckling mice is prevalent in two major breeding colonies in Johannesburg. The disease is characterised by the onset of a profuse diarrhoea about one week after birth with the passage of yellow mucoid faeces which soils the hair coat and the tail, hindlegs and perineum of affected animals. Morbidity and mortality amongst the newborn mice are low and clinical symptoms of the disease fluctuate between 5 and 20% of litters. The mice in affected litters continue to suckle and spontaneous recovery occurs prior to weaning at 21 days. Recovered animals are undersized or grossly stunted. Deaths are usually caused by rectal obstruction arising from the adherence of a dried plug of faecal material to the perineum.

Three murine enteric viral conditions associated with diarrhoea with or without mortality are now known. These are Reovirus 3 infection, Epizootic Diarrhoea in Infant Mice and the intestinal form of Mouse Hepatitis Virus infection. Although serological tests have been developed for differentiating these 3 diseases, they are difficult to use and are not currently available in South Africa. The causative agents can, however, be morphologically differentiated by electron microscopy. Mice from infected litters were, therefore, killed to provide tissues for gross and histopathological examination. Segments of mouse jejunum were excised and fixed in formol saline and cacodylate buffered glutaraldehyde and were then sectioned and stained for examination by light microscopy and T E M. Material from jejunal impression smears was negatively stained with phosphotungstic acid and mounted on carbon collodion grids and examined by T E M for viral particles.

Histopathological examination of jejunal sections revealed the characteristic lesions of EDIM. Intestinal pathology was mainly limited to the tips of the villi. These were bulbous and congested. Clear pathological changes were evident in the epithelial cells which showed marked cytoplasmic vacuolation, disintegration and sloughing. Characteristic rotavirus particles were identified by T E M in the negatively stained mucosal scrapings and in sectioned epithelial cells. A diagnosis of EDIM was made on the basis of virus morphology and the presence of rotavirus particles in the cytoplasm of infected intestinal epithelial cells and gut contents.

Dental Research Institute, School of Dentistry, Univ. of Witwatersrand, 1 Jan Smuts Avenue, Johannesburg 2001.

PARBENDAZOLE AS A CAUSE OF PARALYSED LAMBS

L. PROZESKY* and J.P.J. JOUBERT†

Since 1968 complaints of paralysed lambs were received intermittently from various parts of South Africa. Investigation of this phenomenon revealed that on some of these farms in addition to paralysed animals, lambs were born with various skeletal malformations. Paralysed lambs were unable to stand although no skeletal malformations were present. An experiment was undertaken to determine whether an overdose of parbendazole could cause paralysis in lambs.

0038-2809/81/01/0075

From a group of 250 treated ewes, a total of 68 lambs were born, 5 of which showed the paralysed lamb syndrome, 5 skeletal deformities and 2 were ataxic while 1 foetus was aborted. Cerebral hypoplasia was observed in 2 of the paralysed lambs while 2 others showed internal hydrocephalus. Various histopathological lesions were observed in the affected animals.

*Pathology Section, Veterinary Research Institute, Onderstepoort, 0110
†Toxicology Section, Veterinary Research Institute, Onderstepoort, 0110

CANINE CONGENITAL CEREBELLAR ATROPHY

J.W. NESBIT and HANNELIE UECKERMANN

Congenital neurological dysfunction is being recognised with increasing frequency in domestic animals. Not least amongst these disturbances are those of cerebellar origin. Hypoxia, mutant genes, viral infections and toxic agents may be included in the list of possible aetiological factors. The present paper records a case of congenital cerebellar atrophy in a puppy. The condition was attended by intractable cerebellar ataxia and characterised by a selective degeneration of the Purkinje cells of the cerebellar cortex.

Dept. Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110.

ENCEPHALOMYOCARDITIS VIRUS INFECTION

M.C. WILLIAMS

An outbreak of disease in pigs on 2 adjacent farms in the Natal Midlands, characterized by sporadic sudden deaths in young pigs over a period of months in the winter of 1979, is reported. The most outstanding macroscopic lesions at autopsy were found in the heart and consisted of pale, yellow-brown foci and streaks of varying size in the myocardium, particularly involving the subepicardial cardiac muscle of the right ventricle. In a number of cases severe pulmonary oedema and hepatic congestion were observed. Microscopic examination of the heart revealed that the pale foci and streaks were caused by lysis of muscle fibres, lymphocytic, plasmacytic and macrophage cell infiltration and, in some cases, calcification of the necrotic muscle fibres. In the liver varying degrees of centrilobular degeneration and necrosis, together with congestion, were seen.

The disease was reproduced in mice and pigs by parenteral inoculation of tissue homogenates from a pig which had died of the disease and the virus was reisolated from the experimental mice and pigs. This outbreak represents the first cases of deaths in pigs due to EMC virus infection in South Africa.

Dept. of Pathology, Faculty of Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110

PANEL DISCUSSION: PARVOVIRUS INFECTION IN DOGS

I.B.J. VAN RENSBURG*; F. REYERS*; V.A. LIEBMANN†; P. HOWELL* and J.M. PLETCHER‡

The clinical, clinical-pathological, virological and gross and histopathological changes of parvovirus infection in dogs were discussed. Two syndromes resembling those encountered elsewhere in the world occur: a non-purulent myocarditis resulting in acute death and congestive heart failure as result of a consequent myocardial fibrosis, and an enteric form which closely mimicks feline panleukopaenia.

*Faculty of Veterinary Science, University of Pretoria, Box 12580, Onderstepoort 0110.
†Private practitioner, Bramley, Johannesburg.
‡Pathology Section, Veterinary Research Institute, Onderstepoort.

THE CLINICAL AND PATHOLOGICAL CORRELATION OF CERTAIN SKIN CONDITIONS IN DOGS

B.C. WESSELS* and W.S. BOTHA†

The canine itchy skin is a most exasperating dermatological problem and remains a diagnostic challenge. A multidisciplinary approach to the problem is suggested where both clinical and pathological features are evaluated for diagnostic purposes.

Ectoparasitic allergic dermatitis which includes flea, sarcoptic and demodectic dermatitis is probably the most common pruritic condition and may play some part in the development of neurodermatitis. Rhabditic dermatitis (saprophytic nematode larvae) or even hookworm dermatitis must be differentiated from the other forms of parasitic allergic dermatitis. Treatment of these conditions include insecticidal baths, collars and sprays.

Contact allergic dermatitis must be differentiated from atopic or inhalent allergic dermatitis. This may be possible by careful evaluation of the history, clinical signs. histopathology and provocative testing.

Food induced hypersensitivity conditions are difficult of interpretation. The acute cases usually present as urticaria. The chronic cases present with parakeratotic lesions and are therefore difficult to differentiate from other chronic dermatoses.

Bacterial hypersensitivity leading to an allergic dermatitis is a much more common entity than generally accepted. Histopathologic changes and bacteriological examination, however, are usually diagnostic.

The following suspected autoimmune skin diseases have been encountered: pemphigus vulgaris; pemphigus foliaceus; subcorneal pustular dermatitis; bullous pemphigoid and toxic epidermal necrolysis. Histopathology proved to be a valuable diagnostic tool in these cases.

*Private practitioner, 43 Union Lane, Pinetown 3600, Natal.
†Dept. Pathology, Faculty of Veterinary Science, Onderstepoort 0110

ANATRICHOSOMA SP. INFESTATION IN THE FOOTPADS OF A CAT

LANGE A. LUCIA, VERSTER ANNA, VAN AMSTEL S.R., and DE LA REY R. 1980

Journal of the South African Veterinary Association 51 No. 4. 227–229.

FIELD OUTBREAKS OF HYPEROESTROGENISM (VULVO-VAGINITIS) IN PIGS CONSUMING MAIZE INFECTED BY *FUSARIUM GRAMINEARUM* AND CONTAMINATED WITH ZEARALENONE

AUCOCK H.W., MARASAS W.F.O., MEYER C.J. and CHALMERS P. 1980

Journal of the South African Veterinary Association 51: 163–166.

BOOK REVIEW **BOEKRESENSIE**

THE BASIC PRINCIPLES OF INSECT POPULATION SUPPRESSION AND MANAGEMENT

E.F. KNIPLING

U.S. Department of Agriculture, Agriculture Handbook No 512 1979, pp IX + 659, Figs 38, Tabs 106. Price not stated.

Most of this book is devoted to the control of insects which are important crop pests in the United States of America. However, because the subject matter deals largely with basic principles of insect-population suppression rather than with specific controls for individual species, many of these principles are probably also applicable to the control of vertebrate insect pests.

The book is divided into 14 chapters which deal succintly with the full range of strategies for controlling insect pest populations. These include: insects and their relationship to man and the environment, the dynamics of insect populations, regulation of insect-pest numbers in the natural environment by parasites and predators, insect-population suppression by augmentation of the number of parasites and predators in the ecosystem, insect suppression by means of microbial agents, the development of insect-resistant plant varieties, the use of chemical insecticides for insect suppression, chemical sterilization of insects, autocidal control, cultural and environmental control methods, the use of insect attractants and combined strategies for insect control.

Of particular interest to veterinarians are sections dealing with the use of insecticides to suppress vectors of animal diseases, the use of chemosterilants to control vertebrate insect-pest populations, screw worm suppression and eradication and the control of mosquitoes and blood-sucking flies.

M.C.Williams

BOOK REVIEW **BOEKRESENSIE**

ISOTOPE AND RADIATION RESEARCH ON ANIMAL DISEASES AND THEIR VECTORS

International Atomic Energy Agency p p XI + 468 Figs 70 Tabs 71 Published price Austrian Schillings 680

This is a report of the International Symposium held in Vienna 7–11 May 1979 to discuss the host pathogen relationships and the environmental impact of control procedures on trypanosomiasis and other parasitic diseases. The use of isotopes for research and control of these diseases is discussed. The Symposium was organized jointly by the International Atomic Energy Agency and the Food and Agricultural Organization of the United Nations. There are 37 papers and discussions which vary from review type articles to reports on experimental results.

The emphasis of this symposium is on n'gana but papers are also included on babesiosis (6), anaplasmosis (2), leishmania (2), pesticides (3) and the sterile insect technique (5) as a means of control. A number of papers discuss the ecology, behaviour and rearing of tsetse flies (11).

This book is highly recommended for parasitologists and for those interested in the study and control of vector borne diseases.

C.G.Stewart

TOEKENNING **AWARD**

TYDENS 'N BUITENGEWONE GRADEPLEGTIGHEID HET DIE UNIVERSITEIT VAN PRETORIA OP 10 OKTOBER 1980 DIE DVSc HONORIS CAUSA TOEGEKEN AAN

PROF. EMERITUS DOUW G. STEYN

en wel op grond van die volgende motivering:

"Douw Gerbrand Steyn het in 1916 aan die Steynsburgse Hoërskool gematrikuleer en drie jaar later die BSc-graad aan die Universiteit van Stellenbosch verwerf, waarna hy na Europa vertrek vir sy veeartsenykundige opleiding. Hy studeer eers aan die Universiteit van Utrecht en slaag daarna in die Staatseksamen in Veeartsenykunde aan die Universiteit van Weenen in 1924. 'n Jaar later behaal hy ook die graad DrMedVet in Farmakologie aan die Universiteit van Weenen, waarna hy na Suid-Afrika terugkeer en diens by die Navorsingsinstituut vir Veeartsenykunde op Onderstepoort aanvaar. In 1933 ken die Universiteit van Pretoria die DVSc-graad aan hom toe op grond van 'n proefskrif getiteld: "The toxicology of plants in South Africa".

As navorsingsbeampte op Onderstepoort word hy ook aangestel as deeltydse dosent in Farmakologie en Toksikologie en word hy in 1934 bevorder tot professor in en hoof van die Departement Farmakologie en Toksikologie in die Fakulteit Veeartsenykunde, Universiteit van Pretoria. Hierdie pos beklee hy tot 1946 toe hy aangestel word as professor in en hoof van die Departement Farmakologie in die nuutgestigte Fakulteit Geneeskunde, Universiteit van Pretoria. Hy tree met pensioen in Junie 1963 af. Gedurende sy ampstermyn op Onderstepoort het hy sistematies elke moontlike verdagte giftige plant in Suid-Afrika getoets asook die siektetekens en nadoodse letsels beskryf. Sy bevindings is aangeteken in 'n groot aantal wetenskaplike publikasies en twee boeke (1934 en 1949) het ook oor dié onderwerp uit sy pen verskyn. Laasgenoemde boek word nog steeds as standaardwerk gebruik. Daar kan van prof Steyn gesê word dat hy 'n braak veld betree het en dat hy deur doelgerigte harde werk die probleme een na die ander ontrafel het. Hy het die geleenthede en die uitdagings wat hom gebied is, na die beste benut.

Nadat hy sy taak by die Fakulteit Geneeskunde in 1946 aangepak het, is hy gou erken as die deskundige op die gebied van die menslike toksikologie en het hy in 'n menigte ondersoekkomitees oor geneesmiddels en die aptekerswese gedien.

Soos van iemand met soveel belangstellings verwag kan word, was prof Steyn ook lid van 'n stuk of sestien wetenskaplike verenigings, waaronder volle lid van die Suid-Afrikaanse Akademie vir Wetenskap en Kuns. Deur verskeie van die verenigings is hy tot ere- of lewenslid verkies en het hy ook by verskeie geleenthede as voorsitter of raadslid gedien. Sy sin vir korrekte en etiese optrede blyk ook duidelik uit sy verhouding tot die verenigings waaraan hy behoort. Selfs vandag nog is hy seker dié lid wat die getrouste vooraf verskoning aanteken wanneer hy 'n vergadering of byeenkoms nie kan bywoon nie.

Erkenning vir sy werk het ook nie agterweë gebly nie, en hy is onder andere met die volgende vier hoog-aangeskrewe toekennings bekroon:

- Die Senior Kaptein Scott-medalje, toegeken deur die Suid-Afrikaanse Biologiese Vereniging vir sy navorsingwerk op die gebied van die Farmakologie en Toksikologie, 1941
- Havengaprys vir Geneeskunde, toegeken deur die Suid-Afrikaanse Akademie vir Wetenskap en Kuns, 1954
- Goue medalje vir uitmuntende diens aan die veeartsenykundige professie, toegeken deur die Suid-Afrikaanse Veterinêre Vereniging, 1975
- Eredoktorsgraad, Universiteit van Weenen, 1977.

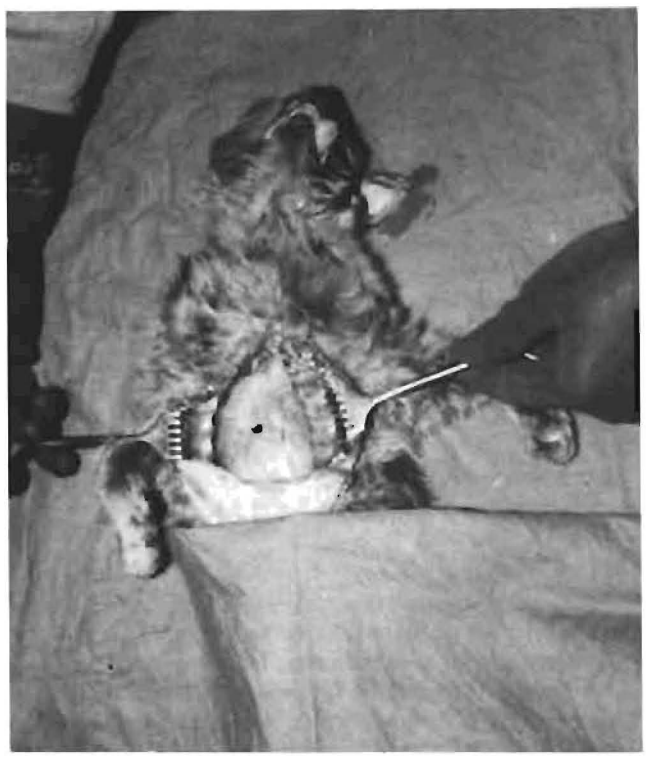
Benewens die meer as 200 wetenskaplike artikels en pamflette het prof. Steyn ook vier boeke oor sy vakgebied die lig laat sien. Ook het hy in verskeie wetenskaplike komitees van ondersoek gedien. Prof Steyn is veral bekend vir sy omvattende werk oor die Suid-Afrikaanse gifplante, as deskundige konsultant by gevalle van vergiftiging of oordosering met geneesmiddels en in later jare ook oor die fluoridering van drinkwater.

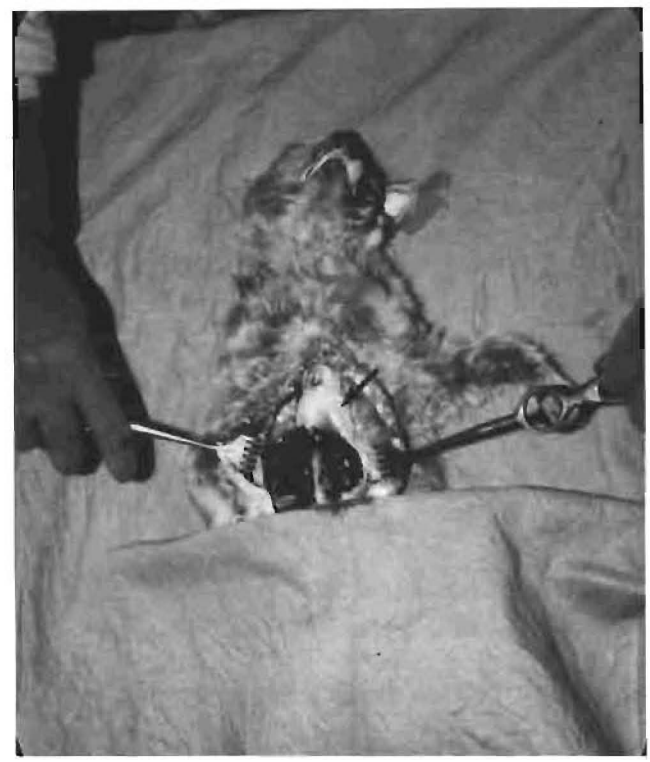
Prof Steyn het internasionale erkenning geniet. Hy het by verskeie geleenthede opgetree as deskundige konsultant in sake met betrekking tot vergiftiging en die fluoridering van drinkwater, hier en in die buiteland. Hy is ook uit die buiteland geraadpleeg in verband met die talidomiedtragedie in 1967. Op uitnodiging is hy aangestel in die Raad van die "Association Francais pour l'advancement des Sciences de l'Homo" van Lyon, Frankryk.

Prof Steyn is 'n persoon wat eer aan sy land en sy werkgewer, die Universiteit van Pretoria, laat toekom het. 'n Man met 'n aangenamer persoonlikheid, voorbeeldiger karakter en vaster beginsels sou kwalik gevind kan word. Prof D G Steyn, professor emeritus aan die Universiteit van Pretoria, is een van die uitsonderlike wetenskaplikes wat ondanks hul hoë ouderdom nog steeds met groot entoesiasme nuwe take aanpak. Na 'n lang aktiewe en vrugbare loopbaan is hy nog steeds op hoogte van die nuutste vakliteratuur en besoek hy nog gereeld navorsingsinstitute hier en selfs in die buiteland. Eers op tagtigjarige ouderdom het hy finaal uit voltydse diens getree."

Die S.A.V.V. maak hierdie geleentheid met groot genoeë bekend en wens hom daarmee veels geluk!!

Redakteur





CONGENITAL DIAPHRAGMATIC HERNIA IN A CAT

Fig. 1 shows the post-mortem examination on a 5-month old female kitten. The thorax has been exposed revealing how the pericardium almost completely fills the entire thorax. The lungs are not visible. In Fig. 2 of the same case the pericardium has been incised, thus exposing portion of the liver, the gall bladder and the apex of the heart (arrow). The liver developed in the pericardium as a result of a congenital diaphragmatic hernia. While alive the kitten showed dyspnoea and eventually died. On post mortem, the lungs were compressed against the wall of the thorax.

According to Saperstein et al[1] the condition is described as follows: Agenesis of part of or the whole of the diaphragm with displacement of a portion of the abdominal contents into the thorax with concomitant enlargement of the pericardial cavity. The condition occurs as a result of a simple autosomal recessive gene. The incidence is between 1: 500 and 1: 1 500 births.

Reference 1: Saperstein G., Harris S., Leipold H.W. 1976. Congenital Defects in Domestic Cats. Feline Practice.

I wish to express my appreciation to the sponsors who enabled the colour photographs to be printed:

Byk Gulden	Louw's Vet.
Centaur Labs.	SmithKline
Ciba-Geigy	Upjohn

Photographs: Miss N Venter
Submitted by Dr J.S.J. Odendaal, 152 Benade Ave., Bloemfontein.

KONGENITALE DIAFRAGMATIESE BREUK IN DIE KAT

Fig. 1 toon 'n post mortem ondersoek op 'n 5 maande oue wyfiekatjie waarvan die toraks oopgerek word. Die foto toon hoe die perikardium die hele toraks vul en dat geen longe sigbaar is nie. In Fig. 2 van dieselfde post mortem is die perikardium oopgesny en vertoon 'n deel van die lewer, die galblaas en die punt van die hart (pyltjie). Die lewer het in die perikardium ontwikkel a.g.v. 'n kongenitale diafragmatiese hernia. Voordoods het die katjie dispnee getoon en gesterf. Die post mortem het getoon dat die longe dun en platgedruk teen die torakswand was.

Volgens Saperstein et al[1] word die kondisie as volg beskryf. Agonese van die hele of gedeeltelike diafragma, met verplasing van 'n deel van die abdominale inhoud in die toraks, en gepaardgaande vergroting van die perikardiale holte. Die toestand kom voor a.g.v. 'n eenvoudige outosomale resessiewe geen. Die voorkoms is tussen 1: 500 en 1: 1 500 geboortes.

Verwysing 1: Saperstein G., Harris S., Leipold H.W. 1976 Congenital Defects in Domestic Cats. Feline Practice.

My hartlike dank word betuig aan die volgende borge wat die plasing van die kleurfotos moontlik gemaak het:

Byk Gulden.	Louw's Vet.
Centaur Labs.	Smith Kline.
Ciba-Geigy.	Upjohn.

Fotos: Mej. N. Venter.
Ingestuur deur: Dr J.S.J. Odendaal, Benaderylaan 152, Bloemfontein.

0038-2809/81/01/0080