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Jl S. Afr. vet. med. Ass. 41 (4): 257-261 1970

REFRESHER COURSES IN PHARMACOLOGY*

I. The Movement of Drugs across Biological Membranes

W. L. JENKINS**

INTRODUCTION

A drug is generally administered to an animal in order to produce a characteristic pharmacodynamic or chemotherapeutic effect. To achieve the desired response, adequate concentrations of the drug must be available in the biophase at the sites of action. These may be a specific enzyme system or the microsomal enzymes; most often, however, they are constituted by ill-defined drug receptors which are associated with cellular and subcellular macromolecular components.

Whatever the route of administration, it is usually essential for a compound to traverse a number of membrane barriers before it reaches a site where it may react with a receptor. The principles which govern the movement of drug molecules across cellular membranes will be reviewed here.

GENERAL STRUCTURE AND COMPOSITION OF CELLULAR MEMBRANES

The structure of the plasma membrane has been studied for nearly seventy-five years, but doubt still exists regarding its fundamental architecture. The original membrane model, described in 1935, was conceived as having a lipid core with the polar ends of the lipid molecules pointing outward and covered on each side by a monomolecular film of protein linked to the lipid surfaces by ionic bonds. Upon direct observation with the electron microscope, the cell membrane appeared as a thin band, approximately 75 to 100 Å thick, consisting of two dark lines, each about 25 to 30 Å thick, on either side of a light zone. This ubiquitous trilaminar structure has been taken as strong support for the early models, which were thus accepted as being essentially correct, although more refined modifications were suggested.

In recent years disagreement about the basic structure has become evident and today there is a growing tendency to seek alternatives to the lipid-bilayer hypothesis. There is now evidence available which clearly shows that when both the quantity and geometrical shape of a membrane are taken into consideration, there is insufficient protein to cover the two surfaces of repeating bimolecular lipid leaflets. The recent findings suggest that α-helical sections of the membrane protein may be buried in a relatively hydrophobic region and that the lipid-protein bonds are nonionic. The most modern concepts propose that biological membranes are always formed by the association of repeating proteolipid structural units.

It has also been established that there is a wide variation in the chemical composition of diverse membranes. Nevertheless, lipids account for 25 to 50 per cent of the dry weight of membrane preparations. Less is known about the protein fractions but it does seem that the monomeric species generally have a molecular weight in the region of 22,000.

The early membrane model did not explain the rapid cellular penetration of small, lipid-insoluble molecules such as urea, acetamide and water. It was then suggested that the lipid film was not continuous but was frequently interrupted by small water-filled channels or pores and this proposal has become known as the "lipoid-sieve hypothesis". There is as yet, no finality regarding the presence and form of such pores in bio-

* This is the first of a series of articles in which the more important concepts of basic pharmacology will be reviewed. The emphasis will primarily be on the clinical applications of these fundamental principles.

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logical membranes: what is surmized at present has been deduced mostly from kinetic data. The evidence for the existence of pores, through which water flux may occur, is based on the dependence of diffusion rates on the molecular diameter of most hydrophilic molecules and the fact that the behaviour of so many molecules can be predicted satisfactorily on the basis of the lipid-pore theory. Measurements of the pore radii in various cells have been carried out: these dimensions vary from 4 Å for intestinal mucosal cells to 7.4 Å for the canine red cell. It is noteworthy that the presence of large aqueous pores in the interstices of epithelial membranes, such as the epidermis or ruminal epithelium, may allow a solute to bypass a series of membranes.

Thus the pertinent features as regards the structure of body membranes revolve around the basic concept of a lipo-protein plasma membrane through which pass a small but significant number of aqueous channels.

PROCESSES BY WHICH MOLECULES CROSS CELLULAR MEMBRANES

A membrane can play any of several roles during the process of transfer of a solute. These may be grouped into two general categories, namely:

1. passive transfer, in which the membrane behaves as an inert, lipid-pore boundary and solutes traverse this barrier either by diffusing through the lipo-protein regions or by crossing through the aqueous pores;
2. specialized transfer, in which the membrane makes an active contribution to the transfer of the solute and this transport occurs in a manner which cannot be simply ascribed to the structure or physical properties of the membrane.

PASSIVE TRANSFER PROCESSES

Simple diffusion

In simple diffusion the force moving a solute across a membrane is the concentration gradient of the solute and the rate of passage is a linear function of this concentration difference. In the case of lipid diffusion, lipid-soluble substances dissolve in the lipid phase of the membrane and diffuse down their concentration gradients to the aqueous phase on the other side of the barrier. Thus the ability of a compound to traverse a membrane by simple lipid diffusion is a function of its degree of lipid solubility. This is the major mechanism by which drugs cross biological membranes and will therefore be explained in some detail.

Most agents of pharmacological interest are weak organic electrolytes. At physiological hydrogen ion concentrations, these weak acids or bases are present partly in the dissociated (ionized) and partly in the undissociated (nonionized) form. The concentration of the nonionized compound depends on both its dissociation constant* and the pH of the solution in which it is dissolved. Compounds that penetrate a biological membrane by lipid diffusion become distributed across the membranes according to their degree of ionization, the charge of their ionized form and the extent to which they are bound to proteins or other macromolecules in the solutions bathing the membrane. The explanation for this behaviour is that the cell membrane is much more permeable to the undissociated molecule than to the ionized form, especially when the nonionized form is lipid soluble. This latter facet is an important consideration: although a compound may be nonionized, it may be so poorly lipid soluble that it will only penetrate a membrane to a very limited extent.

A partly ionized, unbound compound may distribute unequally, either because of a Donnan type of ionic distribution or because of a difference in hydrogen ion concentration on the two sides of the membrane. This difference in pH affects the distribution of a partly ionized substance because of the preferential permeability of membranes to the lipid-soluble, nonionized form of the compound. Under such conditions, at steady-state, the concentration of the nonionized solute is the same on both

* In this series the dissociation constant which will be recorded will be the negative logarithm of the acid dissociation constant or pKa. The pKa is defined by the Henderson-Hasselbalch equation:—

for acids: $pKa = pH + \log \frac{\text{nonionized acid}}{\text{ionized acid}}$;

for bases: $pKa = pH + \log \frac{\text{ionized base}}{\text{nonionized base}}$.

sides of a membrane but the concentration of the ionized solute will be unequal. Accordingly, the total concentration of the solute in both solutions will be a function of the pH of the two fluids and the dissociation constant of the solute. It is important to note that pH differential as small as 0.1 unit, such as that existing between plasma and cerebrospinal fluid, can significantly affect the distribution of drugs between the two fluids. Even more striking effects are seen with larger pH differences.

It is now supposed that aqueous pores exist in lipo-proteinaceous biological membranes. Lipid-insoluble compounds diffuse through these pores at rates which depend on their molecular weights and their concentration gradients. With ions, however, the speed of transfer may be determined more by the charge than by the size. Aqueous diffusion also contributes to the passage of lipid-soluble compounds of low molecular weight.

Filtration, hydrodynamic flow and solvent drag

When a hydrostatic or osmotic pressure difference exists across a membrane, water will flow and this bulk fluid movement will carry or "drag" solute molecules through the pores in the moving stream. The dimensions of these solute molecules must obviously be less than those of the aqueous channels.

SPECIALIZED TRANSFER PROCESSES

The passive transfer processes described above adequately explain the passage of many substances across biological membranes but they do not explain the transport of certain organic ions and certain large, lipid-insoluble compounds. A number of specialized transfer processes have now been recognized which account for these observations.

Carrier transport

There are at least three distinct types of carrier-mediated transport recognized at this time: active transport, facilitated diffusion and exchange diffusion.

Active transport: The term "active transport" has often been used to designate any process that appeared to be inconsistent with the laws of simple diffusion. In recent years, however, an attempt is being made to restrict the term to those transfer processes, which are mediated by a carrier and in which a solute is moved from a phase of lower to a phase of higher electro-chemical potential; cellular metabolism supplies the necessary chemical energy for the work to be done.

This form of membrane transfer is a well-defined process, characterized by the following important criteria:

1) The movement of a solute against a concentration or electro-chemical gradient.
2) The depression of the rate of transfer by non-competitive inhibition of energy-yielding cellular reactions.
3) The competitive inhibition of the transport system with similar compounds that have a common pathway.
4) Saturation of the transfer mechanism when the concentration of the solute becomes sufficiently high.
5) Specificity of the process for a particular type of chemical structure.
6) The inhibition of the transport mechanism at low temperatures.

Facilitated diffusion: The term "facilitated diffusion" was coined to describe a carrier-mediated transport system in which the rate of attainment of diffusion equilibrium is greatly accelerated although no direct expenditure of energy is required. The fundamental distinction between this process and active transport is the lack of any movement against a concentration or electrochemical gradient.

Exchange diffusion: This is a mechanism by which a compound is transported bi-directionally by a carrier transfer system.

Facilitated diffusion not associated with membrane carriers

Transport processes, which apparently are not dependent on carrier-mediated transfer systems but which do result in accelerated passage of a solute across a membrane, have been suggested.

Augmented diffusion

Recent detailed kinetic studies involving intestinal absorption of several therapeutic agents have produced results which are not entirely consistent with the concept of drug transfer by only passive diffusion. The compounds involved in these investigations were mostly quaternary ammonium compounds. The simultaneous administration of a phosphatido-peptide fraction of intestine

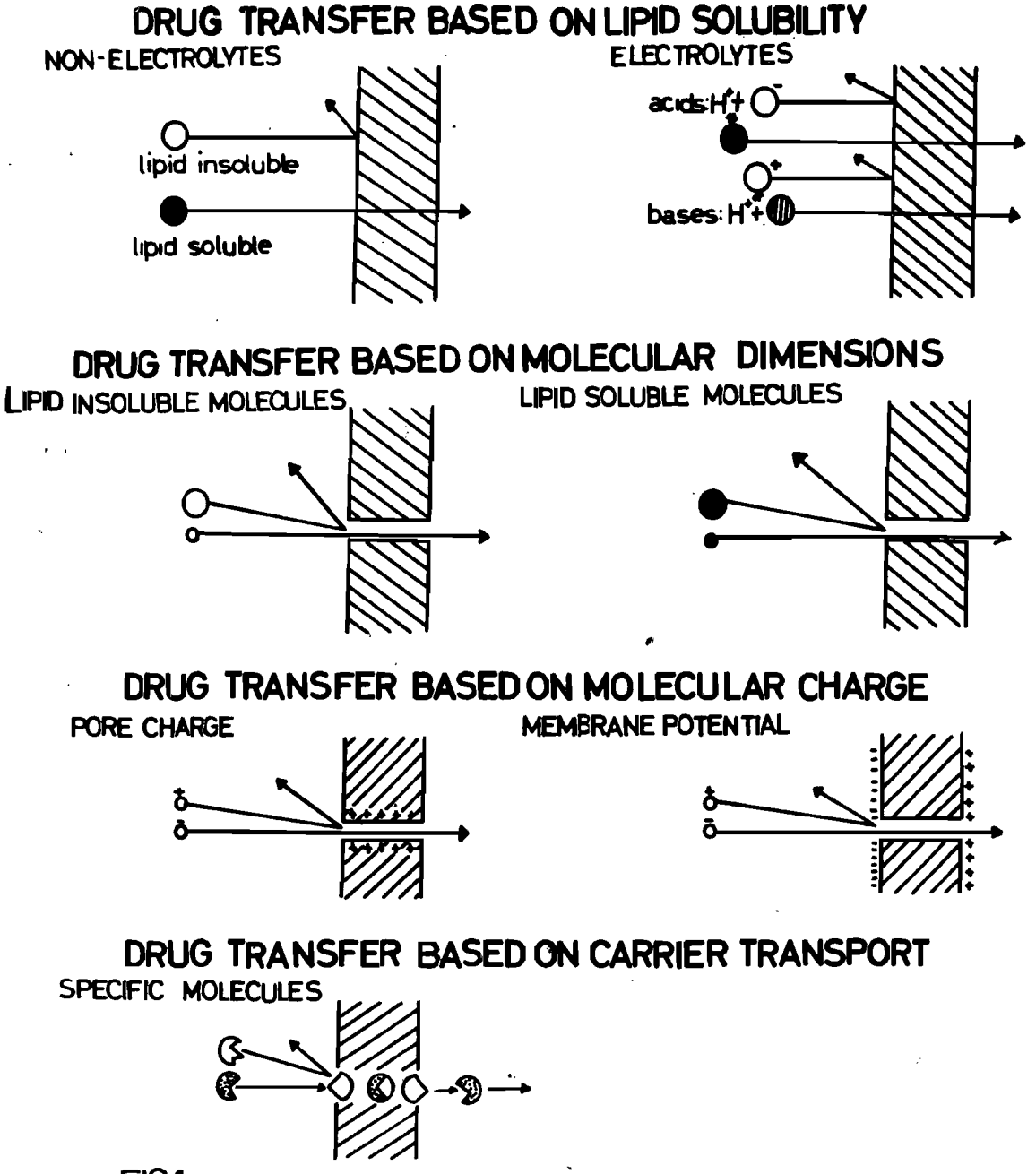

FIG.1. SCHEMATIC ILLUSTRATION OF THE MORE IMPORTANT MECHANISMS BY WHICH DRUG MOLECULES CROSS BODY MEMBRANES

augmented the absorption of a number of these quaternary ammonium compounds.

Pinocytosis

This well-described phenomenon is commonly described in amoebas but is now being recognized as an important process in mammalian cells, particularly intestinal epithelial cells. Drugs with large molecular weights, or which exist in solution in molecular aggregates, may possibly be taken up by pinocytosis.

The most important processes by which drug molecules may cross cellular membranes are diagramatically illustrated in Fig. 1 in order to summarize the preceding section.

SUGGESTED READING

Brodie, B.B. & Hogben, C.A.M. 1957 Some physico-chemical factors in drug action. *J. Pharm. Pharmacol.* 9 : 345

Shanker, L.S. 1964 Physiological transport of drugs. In N.J. Harper and A.B. Simmonds (Eds.): *Advances in Drug Research.* Vol. 1. New York: Academic Press pp. 71 - 106

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BOOK REVIEW

THE VETERINARY ANNUAL

Edited by C. S. GRUNSELL

10th Issue. John Wright and Sons Ltd., Bristol 1970 pp XX + 336, Figs 7, Publ. Price £3.25.

The publication of this 10th issue has been delayed by the untimely death of Mr. W. A. Fool, the creator, not only of this Annual, but the distinguished pioneer of comprehensive documentation of veterinary literature and responsible for originating the *Index Veterinarius* and *The Veterinary Bulletin.*

The new editor has maintained the previous broad division of the Annual into two sections, viz. Current Developments and Reviews of Current Literature. The specially contributed articles on Current Developments include authoritative discussions on laboratory investigations in the 1967/8 foot and mouth disease outbreak in Great Britain, intervertebral disk protrusion in the dog, Marek's disease, lymphosarcoma in the cat, preventive medicine in pig practice, preventive medicine in intensive and semi-intensive beef units and recent developments in veterinary vaccines. Among the longer reviews of current literature may be found papers on mycotic abortion, therapy of bovine mastitis, trichinosis, ovum transplantations, equine pediatrics, role of weather in animal disease problems and physiology and pathology of reproduction.

The inestimable value of this publication to all veterinarians may be gauged from the publisher's statement that the contributors discuss about 3 000 references — after sifting through several thousand more. Useful lists of new drugs, appliances and publications are included, together with a detailed subject index.

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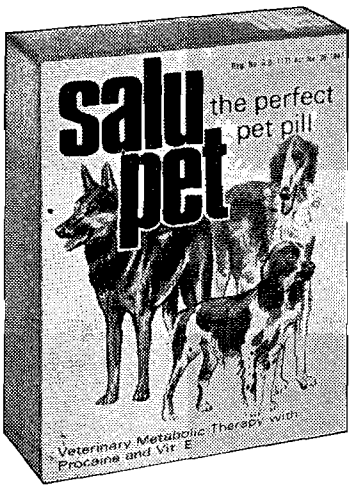

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Jl S. Afr. vet. med. Ass. 41 (4): 263-272 1970

SOME PUBLIC HEALTH ASPECTS OF BILTONG*

L. W. VAN DEN HEEVER**

SUMMARY

This report concerns the results of a survey of the chemical and microbiological status of 60 samples of commercial biltong as well as some laboratory findings.

Survey mean values ($\bar{x}$) and ranges (R) were as follows: *Moisture,* 25.2 and 41.9%; *NaCl content,* 6.6 and 8.6%; % NaCl/WP, 30.2 and 75.6; *Water activity,* 0.742 and 0.628; *pH,* 5.8 and 1.0. In addition, 9(15%) samples contained nitrites (max. 103 ppm $NaNO_2$), 25% contained nitrates, and 25% contained boron ($\bar{x} = 3.35$ and $R = 5.1$ ppm). No samples yielded *Staphylococcus aureus,* 3.3% yielded *Salmonella,* 45% contained *Escherichia coli* I and 98.3% yielded faecal streptococci, mainly *Sc. faecalis,* whilst 68.3% were contaminated with yeasts and fungi. Total counts ranged from 1200/g to over 500 million /g ($\bar{x} = 68.98$ million). No samples were mislabelled as far as species of meat animal is concerned.

Laboratory findings confirm that faecal streptococci survive better in low a_w and high salt concentrations than *E. coli* I; that fresh and salted meat as well as 'green' and dry biltong may readily be contaminated with *Salmonella* and *S.aureus* and that such contamination may persist for some time; that endogenous contamination of meat with *Salmonella* results in particularly extended periods of survival in biltong; and that *Cysticercus bovis* is rendered inactive by controlled conversion of infested beef to biltong containing at least 22% NaCl/WP.

The implications of these findings are discussed with particular reference to existing and proposed standards for ready-to-eat meat products.

INTRODUCTION

Earliest written reference to 'biltong' dates to 1851[1], the word being derived from Dutch ('bil', posterior thigh and 'tong', tongue-shaped). It is an air-dried, salted raw meat product resembling South American 'charque' or 'x a r q u e', North American 'jerky' or jerked beef, and similar products of dry climates. Once essential to early pioneers on trek, commando and hunting expeditions, and made of beef or game meat, it is today a highly prized product produced commercially in excess of 650 000 kg per annum[2]. It is sold in 'tongues' or sticks, or sliced, as cubes, and in the ground or pulverized form. Biltong may be purchased loose, paper-wrapped or packed in perforated, vacuum sealed or gasfilled pouches or glass containers.

Biltong is not defined in South African legislation and specific standards for the product do not exist. It is however a 'processed meat product'[3], for which there are legal requirements, and like all foods, must be 'clean, sound, wholesome and free from disease, infection and contamination'[4]. Proposed specifications for open pack ready-to-eat meat products would require biltong to contain fewer than 10^5 organisms/g, no more than 10 of the coli-aerogenes group/g, and no *Escherichia coli, Salmonella, Shigella* or *Staphylococcus aureus*[5].

Military interest in biltong during World War II resulted in work which identified those muscles suitable for biltong, yield of biltong meat per carcass, dehydration losses, suitable climatic conditions, etc.[6]. Although air-dried foods are generally considered so contaminated that they can only be rendered safe by cooking[7], only four incidents of biltong-induced food poisoning, including one fatality and all caused by a salmonella,

* Presented at the 64th Scientific Congress of the S.A.V.M.A. at Cape Town, Sept. 1969, and extracted from a treatise presented in May 1970 to the University of Pretoria in partial fulfilment of the requirements for the degree M. Med. Vet. (Hyg.) in the Dept. of Pathology, Faculty of Veterinary Science.

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have been reported[8-11]. In South Africa food poisoning is not notifiable.

After finding *E. coli* I in 28.1 per cent and *S. poona* in one of 120 samples, Bokkenheuser considered biltong to be unhygienic[10]. He and others[9] emphazied the significance of endogenous or *in vivo* infection of meat used for biltong after *S. newport* was found to survive in such biltong for two years. Van den Heever[12] confirmed survival of *S. dublin* for at least 6 months in biltong produced from the meat of a case of experimental salmonellosis. The same author[12] reported that exogenous contamination of meat with *S. typhimurium* and *S. dublin* resulted in survival in the biltong for 12 and 45 days respectively, the former organism also surviving for 8 days after surface contamination of dry biltong. Van den Heever[12] also showed that processing of infested meat to biltong containing at least 22.2 percent NaCl in the aqueous phase rendered *Cysticercus bovis* non-viable.

Organoleptic identification of the species derivation of biltong meat is unreliable. Van den Heever[13] has reported on differentiation between horse and beef biltong by means of immunoprecipitin agar gel diffusion tests.

This report deals with the results of a study of commercial biltong relative to compliance with labelling requirements, the microbiological, physical and chemical status of such biltong, the interrelationship between these parameters, and the results of some specific laboratory experiments.

MATERIALS AND METHODS

I Survey

Sixty randomly purchased samples of commercial biltong from various parts of the Republic were transferred to the laboratory as sold and examined on arrival, all possible precautions being taken to prevent contamination. Aliquots consisted of central cross sections of 'sticks' and randomly selected portions of ground or chipped biltong.

Using standard techniques, each sample was examined for nitrite (as $NaNo_2$)[5], NaCl[14], pH-value[14], nitrate[14], moisture[15], and boron compounds[15]. Food animal derivation was assessed by double diffusion immune precipitin tests in layers of 1% special Noble agar (Difco)[16-20], using aqueous extracts of biltong against mixed rabbit anti-bovine serum obtained from Mr. I. Ward-Cox, Blood Group Laboratory, Veterinary Research Institute, Onderstepoort.

Microbiological examination was based on 11 g of sample homogenised in Na-K buffer solution[21]. Total viable counts/g were established according to the Miles and Misra drop method[22] on tryptone-glucose-yeast-extract agar (TGY) incubated at 37°C for 48 h. Salt-tolerant organism counts were similarly established on TGY-agar containing 6.5 and 10.0 percent NaCl v/w respectively. Yeast and fungal counts were likewise established by inoculation of Rose Bengal-streptomycin -agar (RBS)[22] incubated at 30°C for 5 days and counted daily. The most probable number (MPN) of faecal streptococci/g was established by inoculation of serial dilutions of sample homogenate into KF broth[23], incubation at 37°C for 48 h and estimation according to standard tables[21]. The MPN of *E. coli* I was similarly established after inoculation of MacConkey's broth and incubation at 44.0° ±0.5°C for 48 h[21, 22].

Samples were examined for *S. aureus* by inoculation of primary homogenate into Chapman-Stone (C.S.)-agar[24] and incubating for 48 h at 37°C. To detect *Salmonella* 20 g of sample was homogenised in 200 ml of Na-K buffer solution at pH 6.5, diluted to one litre, and shaken periodically during 6 h of incubation at 37°C[25]. After standing, 25 ml of sediment was transferred to 200 ml of brilliant green-selenite F enrichment broth[26] which was incubated at 37°C. From this 4x0.01 ml quantities were transferred to Salmonella-Shigella(SS) agar[22] after 24, 48 and 72 hours of enrichment. Non-lactose fermenting colonies were sought after incubation of SS agar at 37°C for 24 hours and transferred to triple-sugar-iron-agar slants[25], peptone water and urea broth[22], and suspect *Salmonella*[27] were subsequently examined serologically by Dr. C. M. Cameron, Section Bacteriology, Veterinary Research Institute, Onderstepoort.

The percentage NaCl in the watery phase (% NaCl/WP) and the water activity (a_w) was established from the NaCl and moisture values according to published data[28].

II Experimental

a) A 'tongue' of *M. longissimus dorsi* was aseptically removed from a chilled beef carcase and momentarily immersed into a saline suspension of *S. aureus* (FP strain, No. 62/10312, Centr. P. H. Lab., U.K.), *S. typhi-murium* (Culture 2656, Vet. Res. Inst., Onderstepoort) and *E. coli* I and *Sc. faecalis* strains isolated from biltong. The organisms were obtained from 18-hour broth cultures,

equated to Brown's No. 2 turbidity tube and further diluted to 1:1000 in saline. After one hour at room temperature the meat was treated with salt at the rate of 1:24 of meat, w/w, stacked overnight and hung to dry. Samples were taken and examined as described in part I (Survey).

b) A saline suspension containing $27x10^6$ *S. aureus*, $96x10^6$ *S. typhi-murium*, $7x10^6$ *E. coli* I and $225x10^6$ *Sc. faecalis*/ml was prepared as in a) above. One tenth ml quantities were transferred to 10% serum-broth tubes containing 3.0 to 40 percent w/v concentrations of NaCl. After incubation at 37°C for 24 h, 0.01 ml quantities of salt-serum-broth were transferred to solid and liquid media in an effort to recover the organisms.

c) A 'tongue' of meat was removed and processed to biltong as described in a). The meat was immersed into a saline suspension of *S. aureus* [see a)] containing $12x10^6$ colony forming units/ml before and after salting. 'Green' and dry biltong was contaminated by one minute contact with 6 mm filter paper discs holding 0.024 g of the bacterial suspension (ca 300 000 organisms).

d) Twenty five isolates of *Sc. faecalis* from commercial biltong were incubated in KF-broth at 37°C for 48 h and thereafter 0.01 ml quantities were transferred to 10% serum-broth tubes containing various concentrations of NaCl. Subsequent to incubation at 37°C for 24 h, 0.01 ml of salt-serum-broth was transferred back to KF-broth which was examined for typical growth after 48 h at 37°C. Attempts at recovery of *Sc. faecalis* from the salt-serum-broths were repeated after they had stood at room temperature for one week.

e) Biltong was prepared in the standard manner [see a)] and also sprinkled with vinegar (1ml to 34g meat) before stacking in enamelled and galvanised metal trays. Survey as well as experimental biltong samples were examined spectrophotometrically for zinc by the Section Biochemistry of the Veterinary Research Institute, Onderstepoort.

RESULTS

I *Survey*

The results of the survey are summarized in Tables 1 and 2.

Table 1: SUMMARY OF RESULTS OF PHYSICO-CHEMICAL EXAMINATION OF 60 COMMERCIAL BILTONG SAMPLES

	pH-value	% Moisture w/w	% NaCl w/w	% NaCl/WP w/w	a_w value
Stick					
R	0.7	37.7	7.0	32.0	0.274
$\bar{x}$	5.9	27.3	6.2	24.4	0.790
S	0.17	7.93	1.57	8.73	0.073
Sliced					
R	1.0	30.8	8.2	75.3	0.624
$\bar{x}$	5.9	25.1	6.8	31.8	0.729
S	0.22	9.60	1.92	16.71	0.139
Ground					
R	0.6	10.7	3.6	37.3	0.302
$\bar{x}$	5.8	16.3	7.4	47.9	0.592
S	0.48	4.08	1.20	14.11	0.105
Total					
R	1.0	41.9	8.6	75.6	0.628
$\bar{x}$	5.88	25.2	6.6	30.2	0.742
S	0.20	8.97	1.73	14.99	0.125

(R = range, $\bar{x}$ = mean, S = Std. deviation).

No. of samples positive for:

Nitrites: 9 (15%), $\bar{x}$ = 38.83 ppm $NaNo_2$, R = 103.0.
Nitrates: 15 (25%).
Boron compounds: 15 (25%), x = 3.35 ppm B, R = 5.1.
Meat animal spp. other than as labelled or implied: 0.
Meat animal spp. other than beef: 1 (Ostrich).

Table 2: SUMMARY OF RESULTS OF MICROBIOLOGICAL EXAMINATION OF 60 COMMERCIAL BILTONG SAMPLES

	COUNTS x10⁵ / g OF BILTONG					
	Total at 37°C	In 10.0% NaCl	In 6.5% NaCl	Yeasts and fungi	MPN F. strep.	MPN E. coli I
Sticks						
R	4895	4319.9	5351.9	43.2	2.4	0.009
$\bar{x}$	952.1	299.7	316.2	3.307	.5887	0.0009
S	1594	859.7	1085.3	9.246	.9414	0.002
Sliced						
R	3480	3120	2184	1440	2.4	2.4
$\bar{x}$	608.6	414.1	278.9	62.97	0.76	0.09
S	873.2	731.2	540.9	266.3	1.05	0.45
Ground						
R	240	28.8	26.4	0.38	2.4	2.4
$\bar{x}$	66.5	10.2	7.6	0.07	0.85	0.4
S	88.0	12.4	9.4	0.14	0.90	0.89
Total						
R	3896	4320	5352	1440	2.4	2.4
$\bar{x}$	689.8	322.9	265.1	30.8	0.69	0.08
S	1227.5	762.7	795.7	184.5	0.99	0.43

No. of samples positive:

For **S. aureus**: None.
For **Salmonella**: 2 (3.3%).
S. blukwa and **S. woodstock.**
For Faecal streptococci: 59 (98.3%).
For **E. coli** I: 27 (45.0%).

Figs. 1 and 2 illustrate the distribution of moisture and NaCl content values, whilst Fig. 3 gives details of the ratio of NaCl to moisture in the samples examined.

Fig. 1 Frequency distribution of moisture content in 60 biltong samples

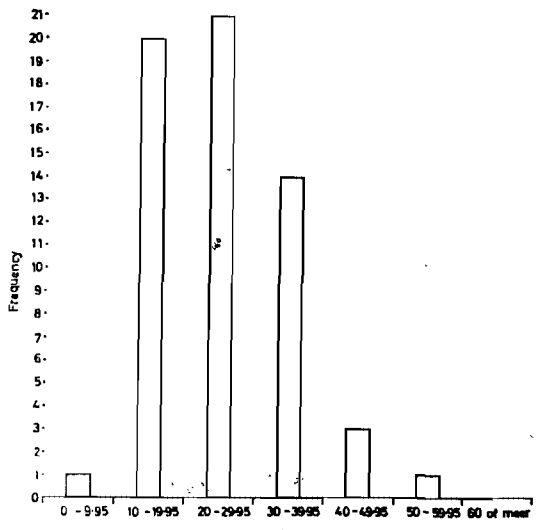


Fig. 3 Die verhouding van NaCl : Vog in 60 biltongmonsters.
The ratio of NaCl : Moisture in 60 biltong samples.

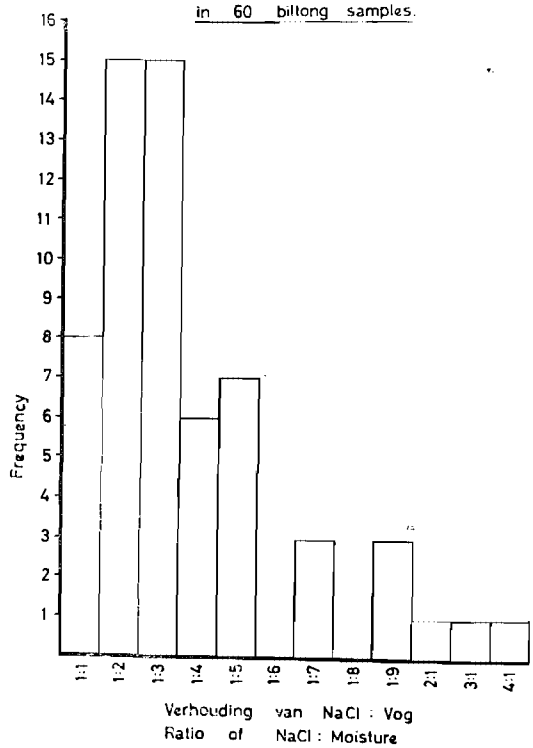
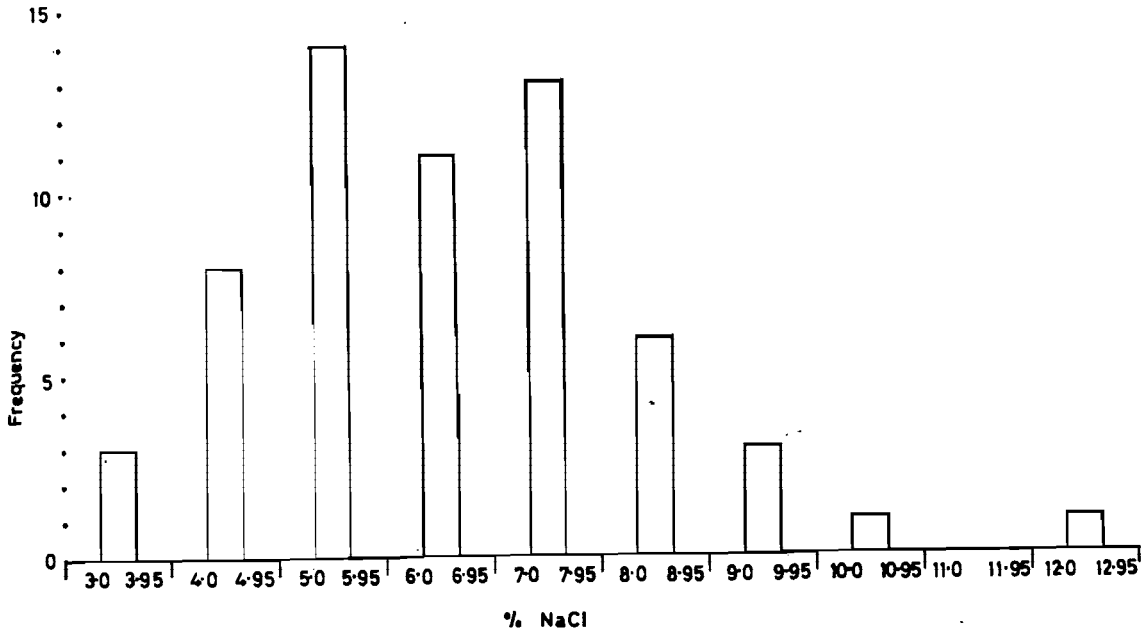


Fig. 2 Frequency distribution of NaCl content in 60 biltong samples (% w/w)



In Fig. 4 the pH values of the samples are shown, and Fig. 5 demonstrates histogrammatically the frequency distribution of % NaCl/WP in the survey samples.

Fig. 4. Frequency distribution of pH values in 60 biltong samples.

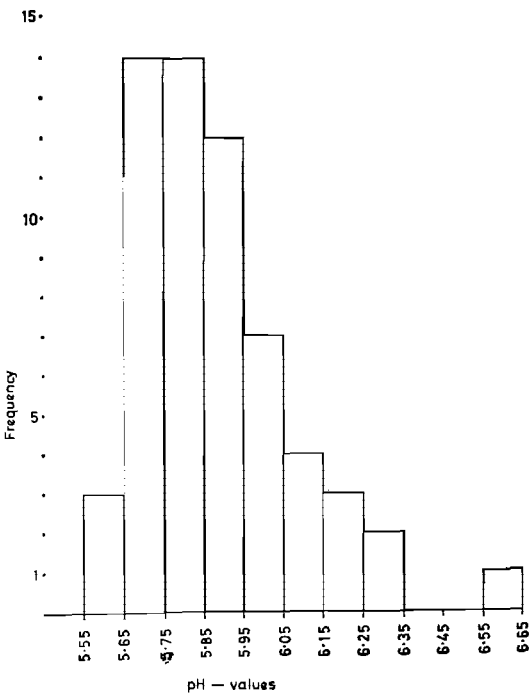


Fig. 5. Frequency distribution of NaCl content in the aqueous phase of 60 biltong samples.

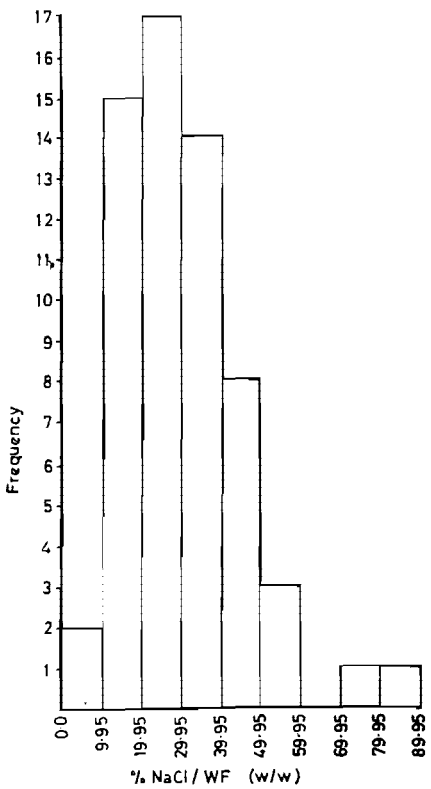
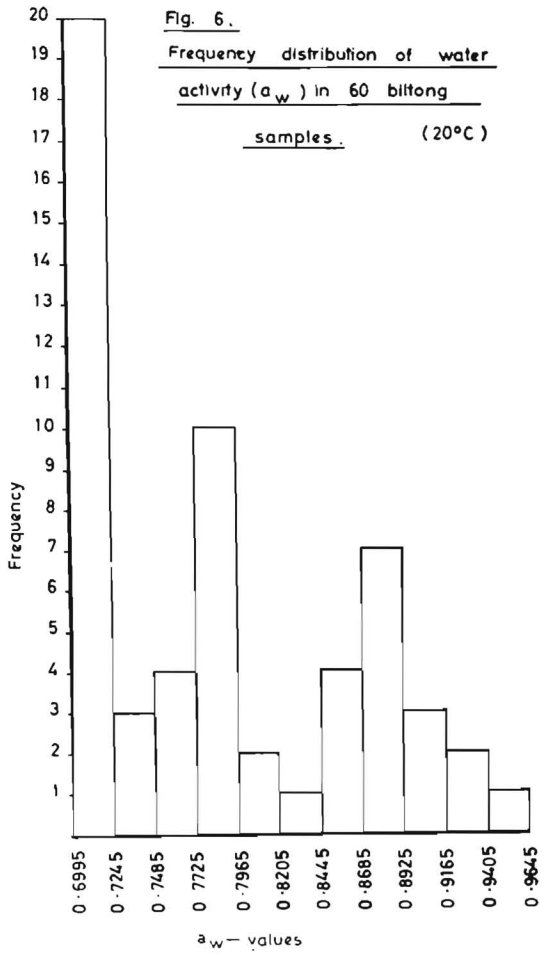


Fig. 6 illustrates the frequency distribution of a_w values in the survey of biltong.



The precipitin reaction between rabbit anti-bovine serum (RAB) and extracts of biltong made from the meat of various species is shown in Fig. 7.

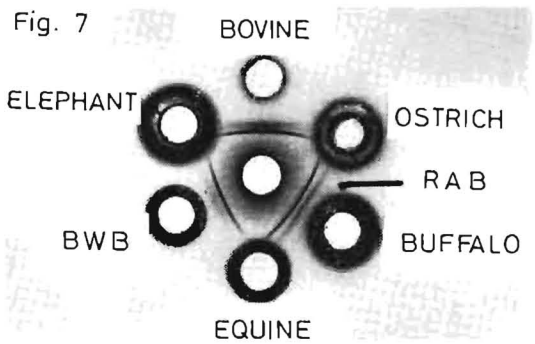


FIG. 7 Immune precipitin reaction by double diffusion in agar gel between rabbit anti-bovine serum (RAB) and extracts of biltong from various species. (BWB = blue wildebeest).

The precipitin reaction lines between RAB and extracts of survey samples (nos. 43 to 47) are shown in Fig. 8. Sample no. 45 was labelled "Ostrich biltong".

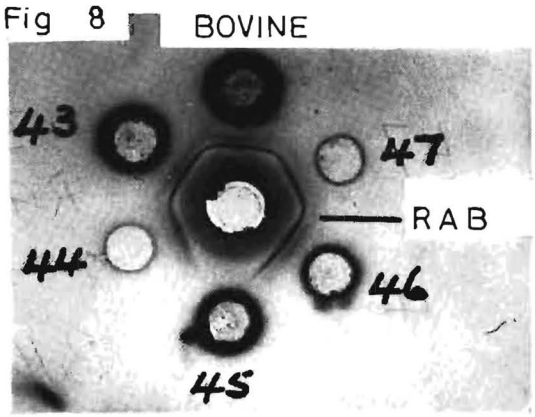


FIG. 8 Immune precipitin reaction by double diffusion in agar gel between rabbit antibovine serum (RAB) and extracts of survey samples.

II Experimental

a) Over a period of 64 days after salting the meat, the number of *S. aureus*/g of biltong remained essentially constant (380-550x10^2), whilst *S. typhi-murium* counts dropped from 53 000 to 200/g. *E. coli* I counts dropped from 54 000 to 30/g, whilst *Sc. faecalis* remained ca constant at 350-2400x10^2/g.

b) After incubation at 37°C for 24 h all organisms were recoverable from 9.0% NaCl-serum-broth. In broth ranging from 10.5 to 40% NaCl only *S. aureus* and *Sc. faecalis* were recovered.
After storage at room temperature for 7 days, *Sc. faecalis* survived in 40% NaCl-serum-broth, *S. aureus in 25%* NaCl-serum-broth, and *S. typhi-murium* and *E. coli* I at 9.0% NaCl. After 72 days all survived in 6.0% NaCl-serum-broth but only *S. aureus* survived in serum-broth containing up to 17.5% NaCl.

c) After focal surface contamination of biltong, *S. aureus* was less readily recovered from biltong hung in the sunlight. There was no marked difference in the number of organisms recovered from biltong contaminated 1, 5, 10 and 20 days after salting, and even 40 days after contamination some 240 colony forming units of *S. aureus* could be recovered from one g of surface biltong.
After immersion contamination, *S. aureus* could be recovered in large numbers

(240/g) from biltong, i.e. up to 40 days after post-salting contamination and up to 105 days after pre-salting exposure to the organism.

d) Of the 35 isolates of *Sc. faecalis,* twelve could be recovered from serum-broth containing 20% NaCl and 13 from 12% NaCl-serum-broth incubated at 37°C for 48 h. No recovery was attempted from broth containing more than 20% NaCl.

e) Fresh beef was found to contain 43.0 to 47.0 ppm Zn. Commercial biltong, standardised to 30 percent moisture, contained from 71 to 123 ppm. Experimental biltong, similarly standardised, contained 367 ppm Zn when prepared with vinegar in a galvanised tray, 225 ppm when stacked in a galvanised tray but without the use of vinegar, 95 ppm using vinegar and an enamelled tray, and 72 using no vinegar and an enamelled container.

DISCUSSION

Economic considerations dictate that biltong should contain at least ca 30 per cent of *moisture* when sold (trade opinion), whilst others consider 20 to 30 percent as ideal[6]. Inhibitory levels should be reached soon after processing commences to prevent interim microbial growth. Dehydrated meat contains from 3 to 10 percent[29,30], and enzymes are inactive below 20 percent[31]. Whereas the survey mean of 25.2 percent appears satisfactory, reference to Fig. 1 indicates that numerous samples were too wet or too dry.

Processors use from 1:20 to 1:100 ratio (w/w) of salt to meat. This and factors such as meat pH, temperature, stacking time and dehydration, influence the *salt content* of the final product. The survey mean of 6.6 percent (wet basis) should be viewed in the light of the wide range (R = 8.6) and Fig. 2.

Rhodesian game biltong regulations require that the *ratio of salt to residual moisture* should not lie below 1:4. Fig. 3 indicates that many commercial biltong samples fall outside this requirement. Reference to Fig. 4 indicates that most survey samples possess *pH values* which correspond to that of normal meat.

Although NaCl, as such, exerts an antimicrobial effect[32], survival of halophilic organisms depends primarily on ability to withstand high osmotic pressures. Meat products containing less than 10 percent NaCl in the aqueous phase are likely to spoil slowly, whereas at levels from 10-20% NaCl/WP only salt tolerant and halophilic organisms survive[25]. Numerous spoilage and pathogenic organisms survive NaCl concentrations of 8 to 25 percent, and there is much unfounded faith in the ability of NaCl to control microbes in meat products[33]. The mean % NaCl/WP of survey samples (30.2) is satisfactory but the wide R and Fig. 5 indicates that numerous samples did not contain an adequate concentration.

Scott's a_w values are obtained by dividing the equilibrium vapour pressure of a solution (i.e. % NaCl/WP in biltong) by that of pure water at the same temperature[30]. This ranges from a theoretical 0.00 to 1.00, and is inversely proportional to the number of solute molecules present. Bacteria and fungi do not grow at a_w levels below 0.75 and 0.62 respectively[30], but bacterial decomposition is practically controlled at 0.85[28]. Most organisms find optimum conditions at 0.950 to 0.990[30], whilst the minimum a_w for *Salmonella* and *S. aureus* is 0.94 and 0.86 respectively[28]. Fig. 6 indicates that no survey samples possessed a_w levels low enough to control yeast and fungal growth whilst many a_w levels would still permit bacterial proliferation. The high microbial counts and the well known trade problem of mouldy biltong, particularly when sliced, support these findings. Halophilics such as *Pseudomonas cutirubra* and *Serratia marcescens* have been recorded as causing discolouration of charque and dried fish[34]; no instances of such discolouration in biltong are on record.

The incorporation of *nitrates* in biltong curing salt for the purpose of achieving a red product is probably responsible for the presence of nitrates in 25 per cent of survey samples, but reduction of nitrate to nitrite does not occur so readily. Direct use of *nitrite* produces a red biltong due to formation of nitrosomyoglobin and -myochromogen. Unbound nitrite is legally limited to 200 ppm in the final product[3], and none of the positive samples exceeded this limit. Higher levels may result in methaemoglobinaemia, especially in children, and there is also the danger of nitrosamines (oncogenic) being formed[35].

Compounds containing *boron* are readily available and once commonly used as meat preservatives. When biltong threatens to

spoil in warm humid weather, the temptation may exist to use boric acid or borates. Legislation prohibits unauthorised preservatives and B is a potent cumulative poison: repeated intake of 0.5 g boric acid (88mg B) leads to chronic poisoning[36]. The presence of B in biltong is foreign and objectionable even though the quantities present in positive survey samples were insignicant as poisons or preservatives. It is suggested that commercial biltong and curing salts be examined periodically for boron compounds.

Although high microbial counts in food are not necessarily harmful as such, and food poisoning may be induced by foods containing small numbers, total counts are generally accepted as indication of the sanitary quality, organoleptic acceptability, safety and usefulness of food[37]. Large numbers of organisms in food represent foreign and unnecessary contaminants and are not acceptable[38]. In the survey samples salt-tolerant organisms constituted a large proportion of the total. The fact that no *S. aureus* were isolated is gratifying. The presence of bacterial indices of faecal contamination, such as *E. coli* I and faecal streptococci[38] is, however, alarming, and the frequency of such contamination makes the finding of *Salmonella* spp. in two (3.3%) of the survey samples not unexpected. These data more than confirm Bokkenheuser's conclusion that commercial biltong is hygienically unsatisfactory[10]. It is also clear that such biltong would not satisfy usual standards for dehydrated and ready-to-eat foods[5, 39]. Since recognition of the oncogenic properties of mycotoxins[40], the isolation of potentially toxigenic fungi such as *Aspergillus flavus* etc. from biltong extends the significance of fungal contaminants beyond that of ordinary spoilage, and this aspect requires further investigation.

Statistical analysis of results by multiple regression determinations (undertaken by Mr. P. J. Becker, Section Biometry, Department Agricultural Technical Services) revealed no linear correlation between a values and the distribution of the various microbial types of biltong. This indicates that the microbial status of the end product is determined by conditions prior to sale and that such conditions frequently support microbial growth.

Experimental evidence of the survival of *S. aureus* after infection of the meat and biltong at various stages of processing is in accordance with known characteristics of the organism; the negative survey samples are gratifying, and it is unlikely that significant quantities of enterotoxin will be produced in infected biltong. The ease of contamination however, emphasizes the need for steps to avoid contamination.

Bokkenheuser[10] suggested that group D streptococci would be a better indicator of faecal contamination than *E. coli* I. *Sc. faecalis* and *S. aureus* have rather similar survival abilities when exposed to low a_w-values, whereas *E. coli* I and *S. typhimurium* are reduced in number to a greater extent than the cocci. The survey and experimental findings confirm Bokkenheuser's contention[10]. It should, however, be borne in mind that both *E. coli* I and faecal streptococci exist extra-enterally with comparative ease and may establish themselves in processing plants.

This study confirms and amplifies other reports to indicate that, whereas endogenously contaminated meat poses a greater threat to the health of the biltong consumer, exogenous infection during processing and handling is established quite easily and steps to prevent such contamination are fully justified.

The fact that biltong contains about twice the amount of Zn than fresh meat and that which is permitted by law, is recorded, but is itself of no significance. Zn is, however, toxic in large amounts, and where galvanised (especially new) containers are used to stack salted meat before the strips are hung out to dry, the Zn levels rise considerably. Acid promotes 'leaching' of the Zn, and the pH of meat ($\bar{x}$=5.88) is therefore significant. The use of vinegar or other organic acids increases the danger of toxic quanitities of Zn accumulating in such biltong. It is justified to issue this warning to householders and manufacturers alike.

CONCLUSIONS

It is considered significant that samples of commercially prepared biltong should show such a wide variation in those parameters which form the basis of preservation thereof, i.e. moisture and salt content and the resultant a_w, the latter frequently being inadequate to control or eliminate spoilage and pathogenic organisms satisfactorily. Evaluation of these values of the end product

provides little evidence of preceding events or situations, as low a_w levels are not necessarily correlated with a satisfactory microbiological status. The rather frequent presence of numerous microorganisms in commercial biltong indicates the existence of opportunities for heavy contamination and/or unhindered proliferation during processing.

The presence of faecal organisms, sometimes in considerable numbers, as well as the occasional finding of a *Salmonella* in a product which is consumed raw, is of considerable concern. It is difficult indeed to see how much of the biltong presently being marketed can ever hope to comply with the requirements of the Public Health Act and the usual specifications for open-pack ready-to-eat meat products.

The survival of endogenous infections, the ease with which exogenous microbiological contaminants establish themselves and survive, the added load of organisms present in unsterilized spices and contaminated immersion brines all indicate that responsibility for the hygenic status of biltong lies with all who partake in the conversion of a live animal's tissues into a food product. The high levels of Zn in biltong prepared in galvanized metal containers, especially with acid additives such as vinegar, and the detection of boron compounds in curing mixtures as well as the final product, emphasized that control of biltong involves materials, utensils and procedures.

In view of what they indicate, assessment of the actual or numerical presence of faecal streptococci in biltong should provide a valuable indication of the hygiene status of biltong. Adulteration of biltong by substitution of species of food animal does not seem to be prevalent but may be detected and controlled by gel-precipitin tests.

Controlled conversion of slightly infested 'measly' beef constitutes a safe and satisfactory way of utilising such meat which cannot be treated by conventional means such as freezing.

In view of the health hazards to which the consumer of biltong may be exposed and the increasing popularity and commercialisation of this traditional food product, it appears timely for steps to be taken to institute standards and control measures which would ensure that biltong is at all times safe and acceptable to society.

ACKNOWLEDGEMENTS

Acknowledgement is gratefully recorded to the Director, Veterinary Research Institute and the Department of Agricultural Technical Services, for facilities to carry out this investigation. I am also indebted to numerous colleagues and friends for help and criticism, to Mr. J. J. van Staden for assistance in the laboratory and to Mr. A. du Bruyn for photography.

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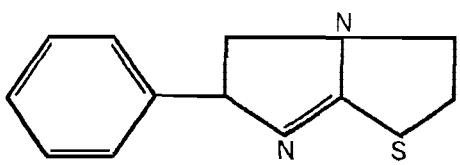
TRAMISOL WM

Nuwe Tramisol WM Breëspektrum Rondewurmmiddel

(L-Tetramisoolhidrochloried)

Beskikbaar in twee samestellings Kies nou self

‚Tramisol' WM (**L**-Tetramisoolhidrochloried) is die linksdraaiende isomeer van rasemiese tetramisool, en is **L**-2, 3, 5, 6, -tetrahidro-6- fenielimidaso (2, L-b) tiasoolhidrochloried. Dit is 'n wit, reuklose poeier wat maklik in water oplosbaar is, en die chemiese struktuur daarvan is soos volg:



l HCl

Die orale formulering van ‚Tramisol' WM is beskikbaar as 'n **L**-tetramisoolhidrochloried-oplossing van 2.5 g/v in verpakkings van 1, 2.25, 10 en 20 liter. Dosisvoorskrifte: Een standaardvoorskrif vir alle diere — skape en bokke — 1 ml per 10 kilo; beeste — 5 ml per 50 kilo. Enige konvensionele veeldosisuitrusting kan gebruik word.

Ons wil u aandag ook vestig op die feit dat die aktiwiteit van ‚Tramisol' WM vir die verskillende maagdermnematodes en longwurms by beeste, skape, bokke en volstruise geregistreer is ooreenkomstig die Nie-Parametriese Evaluasiemetode (Reinecke/Groenewald).

TRAMISOL WM

ICI

Verkrygbaar by:

I.C.I. SOUTH AFRICA (PHARMACEUTICALS) LIMITED

Posbus 11270, Johannesburg.

Posbus 948, Durban.

Posbus 3451, Port Elizabeth.

Posbus 1519, Kaapstad.

Ook by:
MILBOROW & CO.

Jl S. Afr. vet. med. Ass. 41 (4): 275-284 1970

THE HAEMATOLOGY OF EXPERIMENTAL *THEILERIA LAWRENCEI* INFECTION

R. R. H. HILL* AND B. A. MATSON*†

SUMMARY

Blood and bone marrow changes during the course of experimental infection with *Theileria lawrencei* are described. The findings were analogous to the clinical pattern of the disease, encompassing a range of reactions from a mild form, comparable to *Theileria mutans* infection, to the peracute course, similar to *Theileria parva* infection in symptomatology, but with significant differences in the haematological changes produced. In particular, a high terminal peripheral blood lymphocytosis and depression of bone marrow erythropoiesis are reported. The demonstration of peroxidase activity in blood and bone marrow films assisted in the elucidation of the atypical cells that are a characteristic of the theilerioses.

INTRODUCTION

There are four disease entities associated with bovine parasites of the genus *Theileria*[1]. Of these, *T. parva* has been fully studied and the blood and bone marrow changes described[1-5]. The prominent features recorded are a generalized terminal leucopaenia, anaemia without compensatory regeneration, icterus and lymphoid proliferation in tissues, bone marrow and capillaries. *T. annulata* is reported by Sergent *et al.* (*cit.* Neitz[7]) to give haemoglobinuria, icterus and anaemia followed by reticulocytosis. The haematology of *T. mutans* has a poor representation in the literature: de Kock *et al.*[6] report lymphoid hyperplasia and anaemia with possible icterus.

Matson[7] clarified the aetiology of Rhodesian theileriosis, identifying the parasite as *T. lawrencei*. It was associated with a range of pathogenicity encompassing a virulent form, with the features of East Coast fever on one hand and an almost inapparent form, comparable to *T. mutans* infection, on the other. In a later paper[8] indication was given of a marked terminal leucocytosis in fatal cases, with a progressive, uncompensated anaemia. The present paper reports the results of haematological examinations of a number of bovines experimentally infected with *T. lawrencei*. This and previous work was undertaken to ascertain whether outbreaks of theileriosis still occurring in Rhodesia had any relationship to *T. parva* infection.

MATERIALS AND METHODS

For the protozoological and xenodiagnostic methods used in the major experiments, reference should be made to Matson[7] and Matson and Hill[8]. All the haematological techniques were employed according to Dacie and Lewis[9], with the exception of the Graham-Knoll methods for the detection of peroxidase activity in granulocytes and for haemoglobinization of erythroblasts[10]. The van den Berg method was used for the estimation of bilirubin.

Venous blood samples were collected by alternate right and left jugular venepuncture, using sodium sequestrine as anticoagulant. Bone marrow aspirates were obtained from the sternum using the technique indicated by Wilde[11] and the dried films were stained by the May-Grünwald-Giemsa combination of dyes.

RESULTS

Clinical reactions to tick-transmitted, experimental theileriosis varied over a wide range in both intact and splenectomized subjects. Concurrent work indicated that this variation was due to strain differences. It was possible to group those animals examined haematologically into three categories. Four animals (782, 439, 778, 785) underwent a mild form of the disease, ex-

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† Deceased.

periencing only fever, slight anaemia, parasitaemia, and transient lymph node enlargement. One subacute course was studied (494). This is of particular interest, as animals suffering from clinical theileriosis in field outbreaks invariably die. Regular blood checks on this animal were continued for seven weeks, although even then full clinical health was not achieved and the haemoglobin level was only 8.5g/100ml. Nine animals died of acute and peracute theileriosis (432, 455, 484, 774, 779, 803, 804, 805, 812). All suffered from the typical pathology of the disease, death occurring between the 17th and 23rd days after tick infestation. Mean results have not been presented in view of this wide variation in the duration of the disease in different subjects.

HAEMATOLOGY OF MILD THEILERIOSIS

Of the four animals exhibiting this type of reaction, Bovine 785 was typical, and the results are presented in Figs. 1 and 2. Schizonts were present in lymph nodes between the 14th and 21st days, accompanied by a high fever. The four animals lost an average of 2.5g of haemoglobin/100ml of blood during this period. A slight generalized leucopaenia was observed, also during the schizont parasitaemia. Bilirubin was not demonstrated at any time, and reticulocytosis and recovery occurred from Day 22. Endoglobular piroplasms appeared on Day 24 but were not associated with any rise in temperature, nor did they impair haemoglobin recovery. The course of Bovine 778, however, was complicated by *Eperythrozoon wenyoni* infection on Day 26.

HAEMATOLOGY OF SUB-ACTIVE THEILERIOSIS

The significant blood changes in Bovine 494 are presented in Figs. 3 and 4. Schizonts were present between Days 12 and 26, during which time a severe clinical deterioration took place and the haemoglobin dropped to 7.5g/100ml. Bilirubinaemia was demonstrated between Days 24 and 35 with a peak of 4.5mg/100ml on Day 32. There was little change in the total leucocyte count, but on the 18th

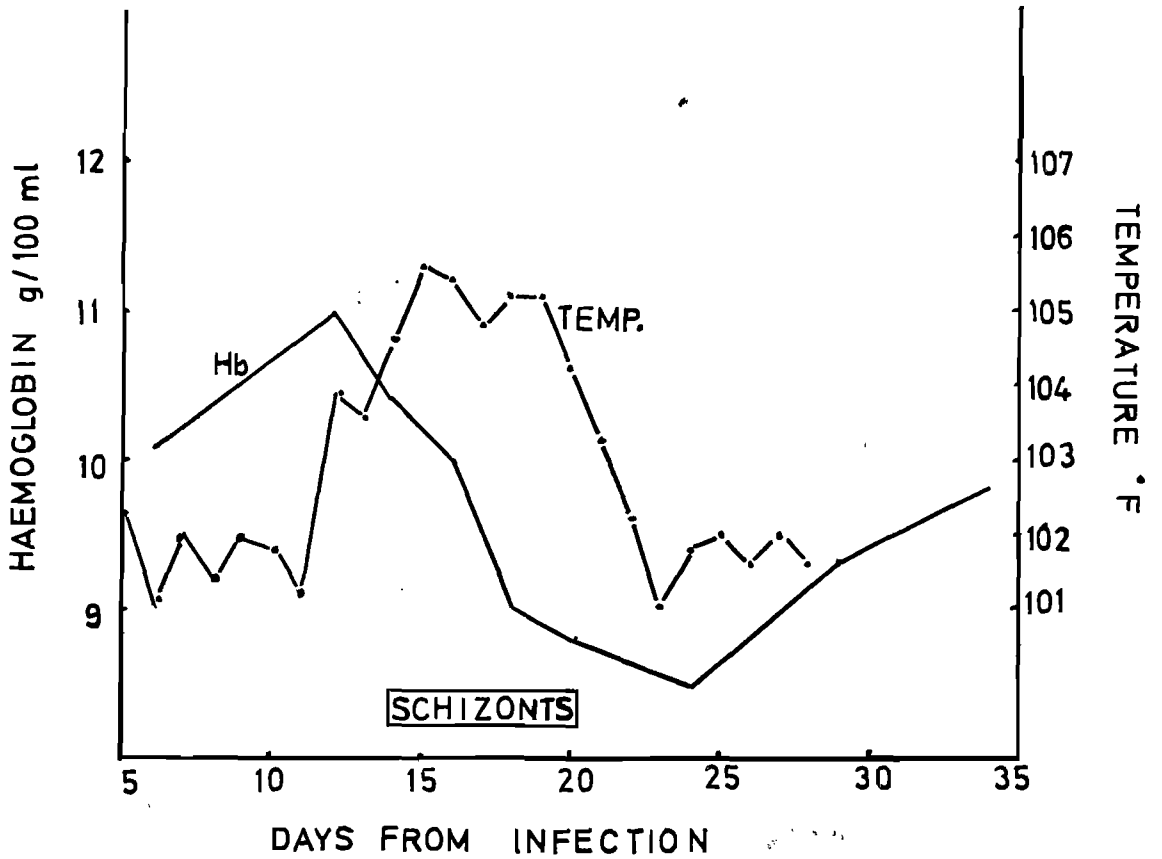

FIG. 1. Haemoglobin and thermogram — Bovine 785.

day, atypical cells appeared and increased in numbers so that by the 20th day the differential count read: atypical cells 3 7000/mm³, lymphocytes 4 300/mm³ and neutrophils 1 500/mm³. The atypical cell type was not seen after the 39th day.

The reaction in capillary blood, however, was dramatic, the total leucocyte count being 54 400/mm³ with 58 percent lymphocytes on Day 18.

HAEMATOLOGY OF ACUTE AND PERACUTE THEILERIOSIS

There was ample opportunity to study the blood changes in fatal cases during the cross-immunity trials in the major experiment. Many animals studied were normal controls exposed to particularly virulent strains, others were premune but did not withstand challenge. Most animals were splenectomized, although this did not appear to influence the course or severity of the disease. The results from the intact animal 774 are presented in Figs. 5 and 6.

Death occurred between the 17th and 23rd days and schizonts appeared between the 11th and 14th days, the duration of the disease being an average of 7 days. The peracute cases died with little opportunity for full clinical symptoms to develop, although blood changes in some were marked. There was an average haemoglobin loss of 3.0g/100ml with a maximum of 5.0g in eight days. Reticulocytosis was not detected at any time. Serum bilirubin was demonstrated in all animals, the results of seven serial estimations being illustrated in Fig. 7. The earliest leucocyte change was a degenerate neutrophil nuclear shift to the left after two or three days of schizont activity. A day or so later, atypical cells were detected, and from then until the termination by death, lymphocytes, atypical cells and immature neutrophils increased in numbers. The

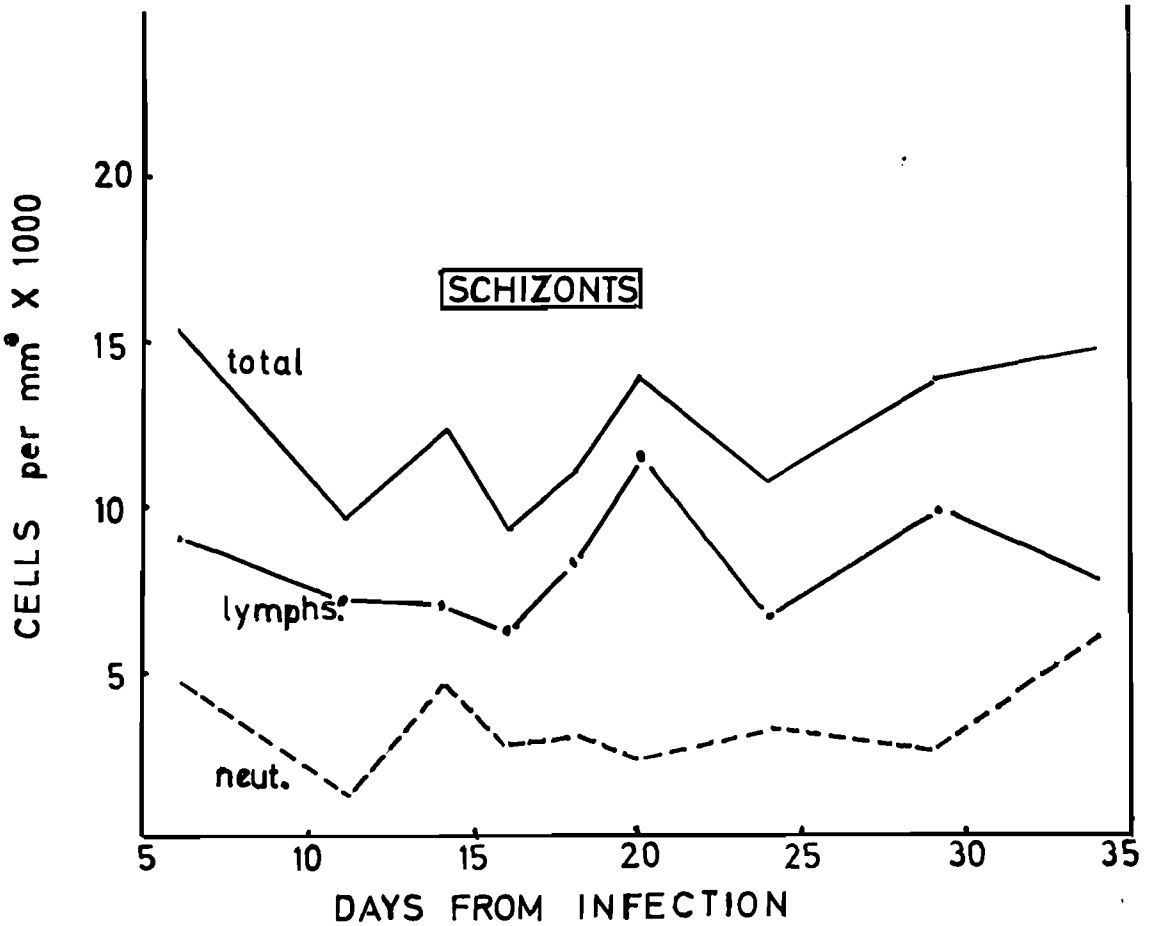

FIG. 2. Leucocytes — Bovine 785.

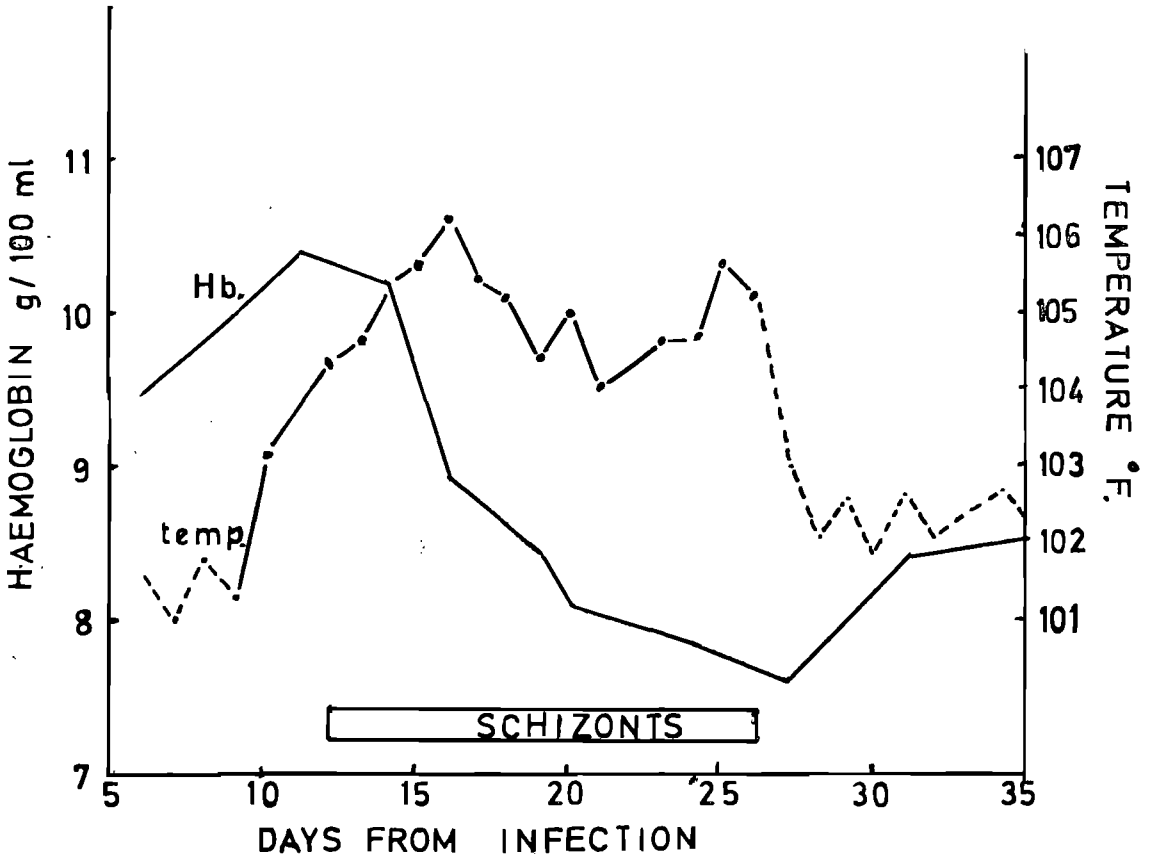

FIG. 3. Haemoglobin and thermogram of subacute case — Bovine 494.

average terminal total count was 35 000/mm³, but Bovine 803 showed a maximum figure of 6 700/mm³, the lymphocyte count being 55 400/mm³. The capillary leucocyte count of this animal on the day before death was 112 400/mm³, the lymphocytes totalling 65 000/mm³.

Blood piroplasms appeared two days after schizonts were first detected in lymph nodes, but it was difficult to assess their influence on the dramatic blood changes that were taking place.

ERYTHROCYTES

There was a loss of haemoglobin in all cases, irrespective of clinical group. Absolute values, mean cell haemoglobin and mean cell volume remained fairly constant, except in the regenerative stages of the mild and subacute cases, in which a reticulocytosis, roughly proportional to the severity of the anaemia, was observed three to four days after the disappearance of schizonts from the blood and lymph nodes.

The serum bilirubin, when demonstrated, was always unconjugated and plasma haemoglobin estimations on two peracute cases (484 and 812) showed little variation from the control average of 2mg/100ml. Direct Coomb's tests on washed erythrocytes from random animals undergoing active haemoglobin loss and bilirubinaemia were consistently negative.

The red cell fragility curves of Bovines 484 and 812 were calculated at regular intervals during the course of the disease. The mean cell fragility was constant, with variation of ± 4 per cent, but the curve on Day 23, illustrated in Fig. 8, shows a shape reflecting a uniform cell population: young, resistant cells were not forthcoming from the bone marrow and the old, fragile cells have been destroyed in the haemolytic process.

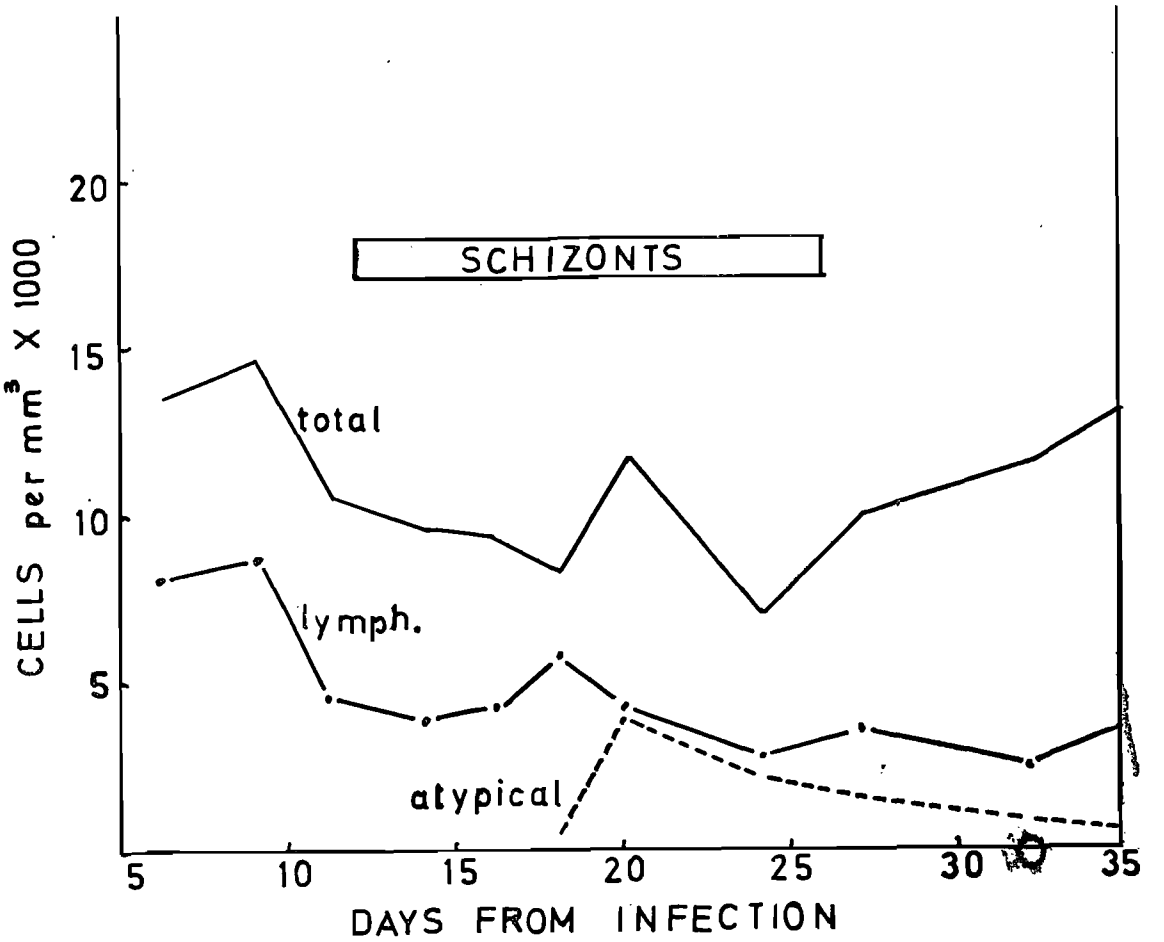

FIG. 4. Leucocytes of subacute case — Bovine 494.

LEUCOCYCTES

The leucocyte response was a complex one, resulting from the interaction of a number of factors. As the disease progressed, correlation between venous and capillary leucocyte counts became less and less; the extreme case of Bovine 803 has already been quoted. As venous samples are technically more consistent, these values have been presented; allowing for error in blood collection, the accumulation of lymphocytes in capillaries is in keeping with the pathology of the other theilerioses[7].

The high terminal venous leucocyte counts reported in this paper were due essentially to increases in the lymphocyte/monocyte cell types. In common with other workers on *T. parva* infection, considerable difficulty was experienced in classification of this series; this was complicated by the appearance of cells bearing characteristics common to both large lymphocytes and young monocytes[5,6]. Wilde[5] indicates that those found in East Coast fever could be atypical lymphocytes, with blast cell origins shared with granulocytes. This suggestion is based on their morphological similarity with early granulocytes, yet being susceptible to schizont infection. In Rhodesian theileriosis it was found that these cells were peroxidase-negative, thereby excluding identity with promyelocytes and more mature members of the granulocyte series. The decision on schizont infection was not conclusive, due in part, to the low level of parasitaemia in *T. lawrencei* infection, and also to the difficulty in clearly defining the cell types. During the course of this work few atypical cells were seen to be infected with schizonts.

BONE MARROW

A composite picture of bone marrow changes was built up from myelograms on six sternal marrow aspirations from four animals at various stages of the disease. These are shown in the table, the day of death being regarded as Day zero. The method for haemoglobin detection[10] enabled one to distinguish between small, pyknotic lymphocytes and early normoblasts. The counts so obtained offered confirmation of the degree of lymphocyte infiltration and depression of erythropoiesis. The data presented in the table do not give a critical reflection of the bone marrow changes, but the figures are sufficient to draw conclusions about the haematology of the circulating blood. Absolute counts on marrow cells were not performed, but as the disease progressed there was a tendency to obtain "bloody taps", with few marrow fragments. We concluded that the relative figures presented in the table are a fair guide to the absolute changes.

Lymphocytes increased early in the disease, reaching terminal counts of over 50 per cent, comprising both large and small mature lymphocytes and prolymphocytes, up to 17 per cent being parasitized. All stages of the erythrocyte series were severely depressed, although in the few remaining cells haemoglobinization appeared to be normal. The granulocyte series was less affected, particularly the young primitive forms, but maturation was retarded: band forms and metamyelocytes had been released into the peripheral blood to give the marked nuclear shift to the left seen in the more severe cases of the disease.

Megakaryocytes were only sligthly affected. Platelets were maintained at normal levels in flood films and no coagulation defects occurred clinically.

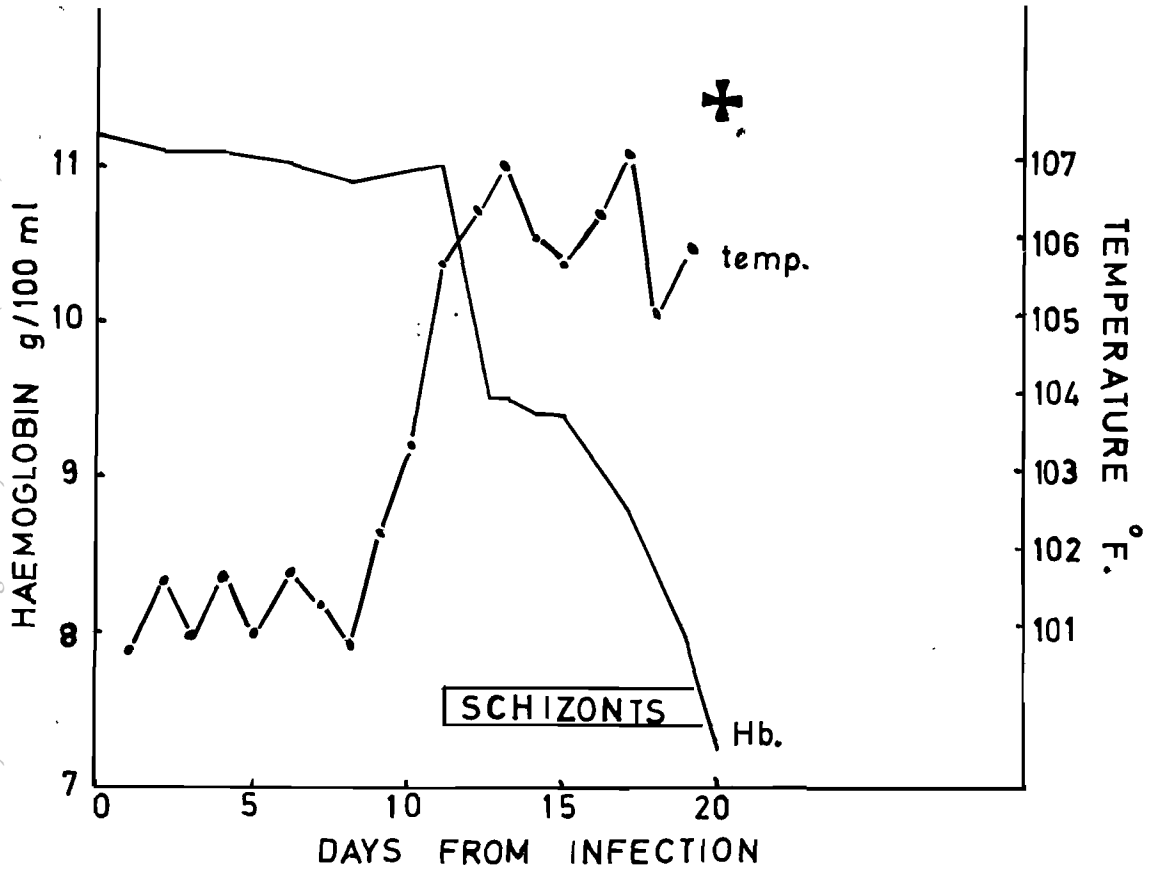

FIG. 5. Haemoglobin and thermogram of peracute case — Bovine 774.

DISCUSSION

The explanation for the haematological changes in *T. lawrencei* infection is to be found in the bone marrow and it is evident that the triggering mechanism lies in the presence of schizonts in the lymphoid centres. Toxin production is the only explanation of the initial depression of erythropoiesis, although the haemolytic process could be due to excessive destruction of red cells by a parasitized spleen or reticuloendothelial system. Lymphoid hyperplasia, with its attendant parasitaemia, further aggravated the position by a crowding-out effect in the marrow. Anaemia was found in all cases examined, the haemoglobin losses being comparable, irrespective of the virulence of the infection; unconjugated bilirubin, of haemolytic origin, was only demonstrated in the severe and fatal cases.

The leucocyte reaction, on the other hand followed the clinical picture, with maximum response seen in cases with peracute symptoms. As in East Coast fever, it is not clear whether the presence of lymphoid cells in the marrow was due to proliferation or infiltration. Although mitoses were seen in peroxidase-negative, non-haemoglobinized cells, none of these was parasitized, thus depriving us of any evidence of

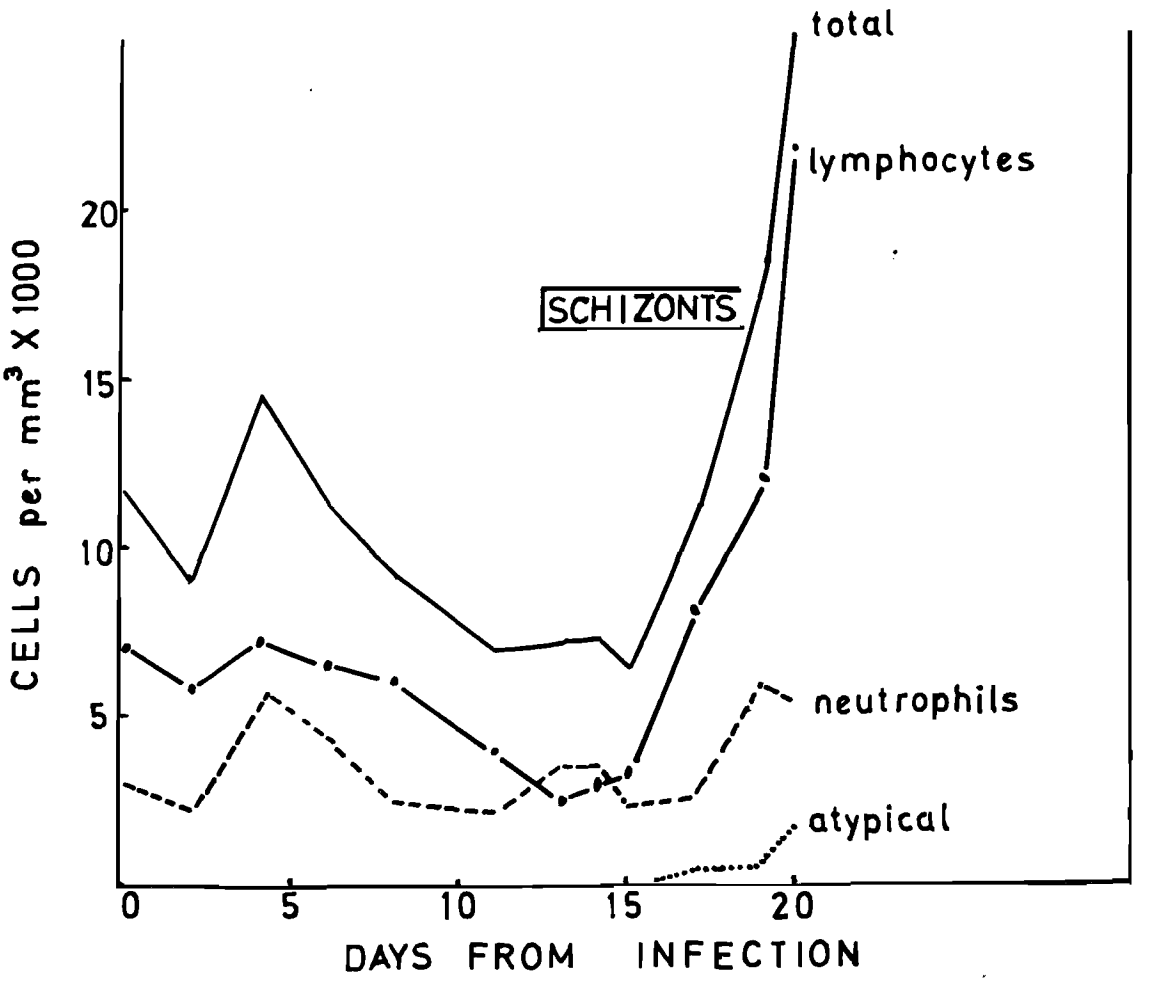

FIG. 6 Leucocytes of peracute case — Bovine 774.

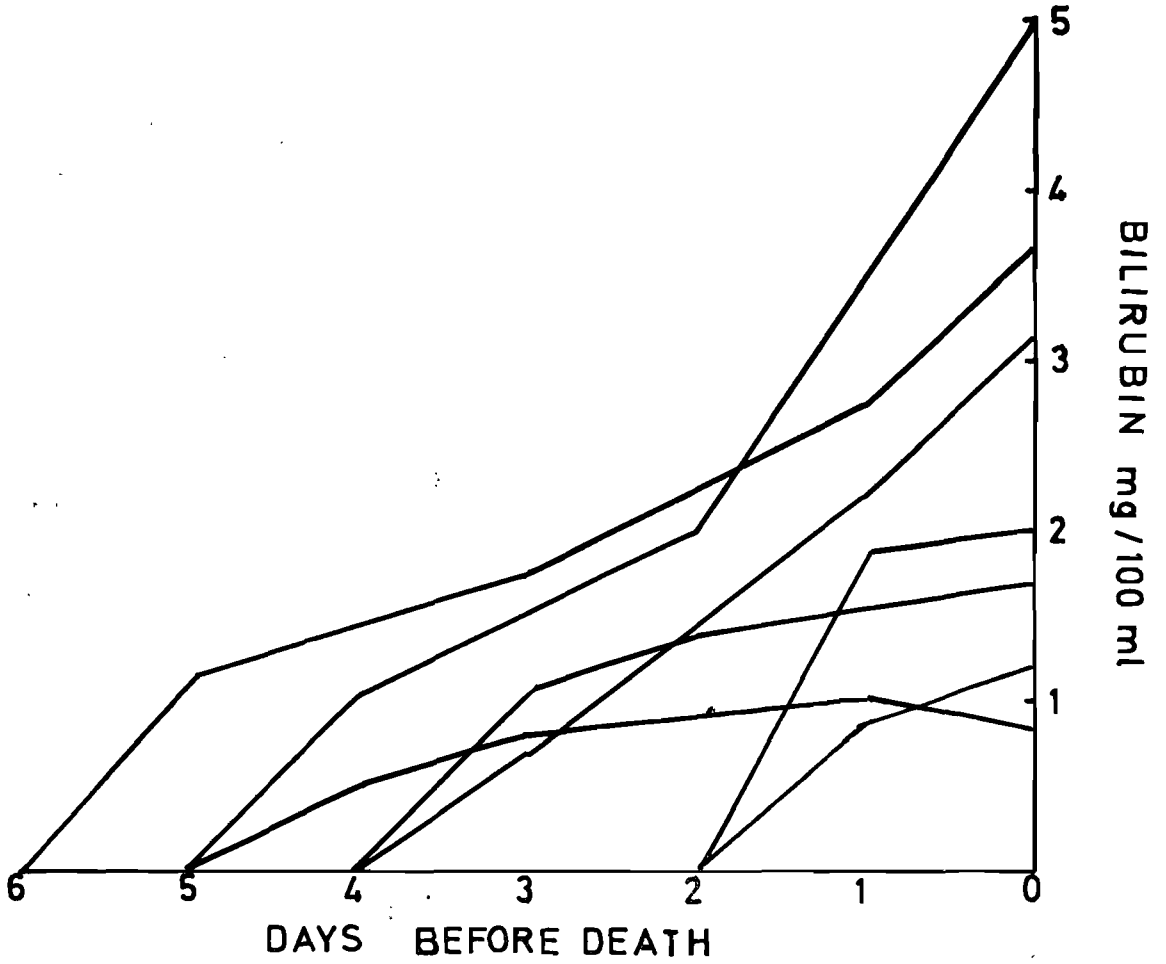

FIG. 7. Serum bilirubin in acute and peracute theileriosis.

lymphocyte proliferation. The lymphoid population in the marrow reached higher levels in our study than in the published reports on East Coast fever, although the schizont infection rate was lower, as is to be expected. Satisfactory criteria were used for detecting the atypical cell type that is characteristic of the disease, although the origin of the cells and their relationship with classical cell series are still an enigma. The lymphocytes are at the epicentre of schizont influence. Hulliger[12] observed the division of schizonts in lymphocyte mitoses in tissue culture, and we have recorded this in films from infected lymph nodes. Such an initimate relationship between cell and

parasite could well be the explanation of the disturbance of cell morphology seen in the atypical cells.

CONCLUSION

Further evidence is offered that *T. lawrencei*, or Rhodesian Theileriosis, has different characteristics from that of the more virulent disease, East Coast fever, particularly with reference to the part played by the lymphocyte in the blood picture. Nevertheless, the results do not offer a method of distinction between the mild *T. lawrencei* syndrome and *T. mutans* infection.

ACKNOWLEDGEMENTS

The staff of the Veterinary Department are thanked for their invaluable help and advice, in particular Dr. J. A. Lawrence for his assistance in bone marrow aspirations, and Miss S. Boucher for typing the manuscript. The paper is published with the permission of the Director of Veterinary Services of Rhodesia.

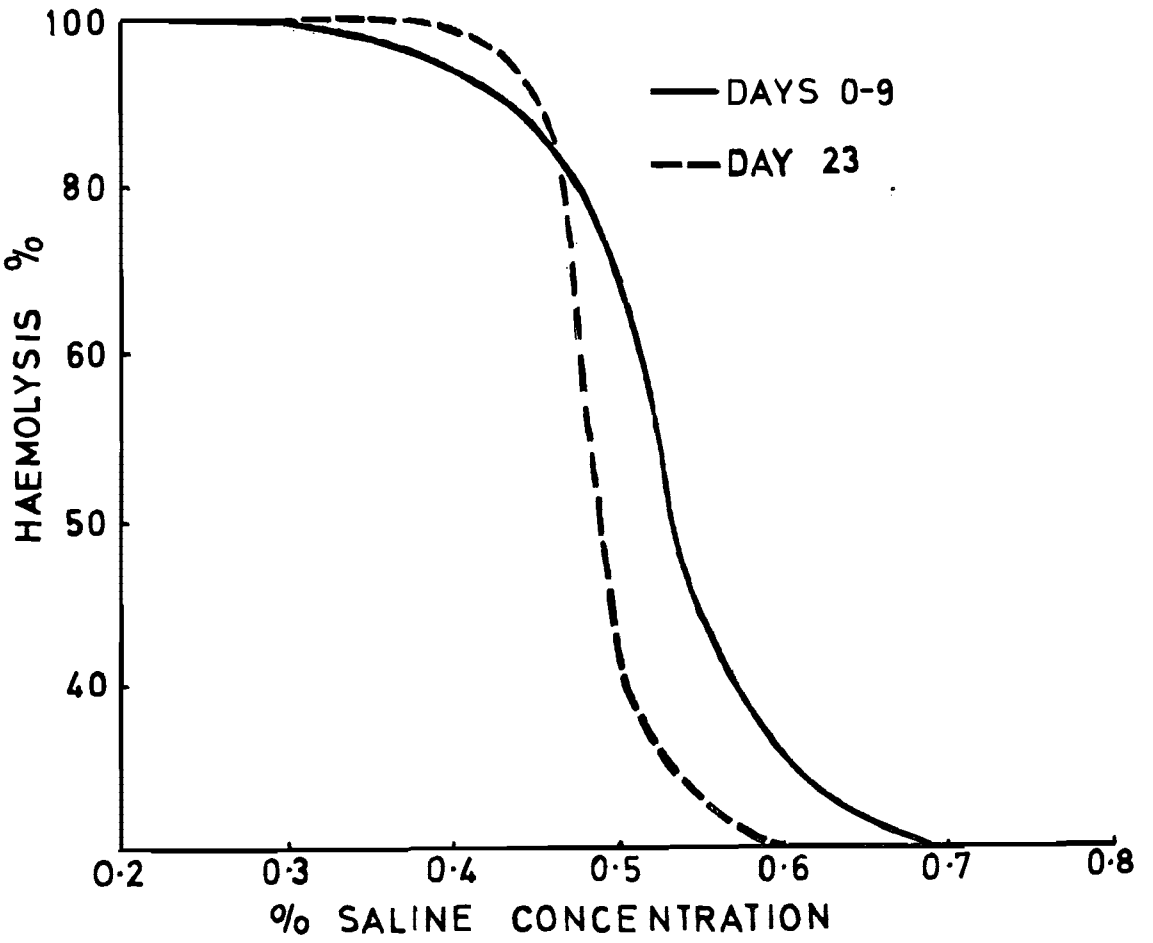

FIG. 8. Mean red cell fragility — Bovines 812 and 484.

Bovine number	812	805	803	812	804	804
Day before death	Pre-infection	4	2	1	0	Post Mortem
Pronormoblasts	3.1	1.6	1.0	1.5	2.1	0.5
Normoblasts	32.5	3.0	5.8	7.5	2.3	1.2
Total erythroid cells	35.6	4.6	6.8	9.0	4.4	1.7
Myeloblasts	1.5	0.3	0.8	1.0	1.0	1.8
Promyelocytes	2.7	2.3	1.7	0.5	2.0	3.1
Neutrophils:—						
Myelocytes	3.5	3.0	0.9	3.0	2.3	3.1
Metamyelocytes	9.1	16.1	4.9	11.5	11.2	8.5
Polymorphonuclears	24.5	4.5	9.8	16.0	8.8	2.0
Eosinophils	6.4	4.9	2.4	1.0	2.2	2.6
Basophils	1.1	Nil	Nil	Nil	0.3	0.3
Total myeloid cells	48.8	31.1	20.5	33.0	27.8	21.4
Prolymphocytes	Nil	1.6	2.5	2.5	2.7	1.8
Lymphocytes	7.8	27.3	50.5	31.0	34.2	46.5
Monocytes	0.4	Nil	Nil	Nil	Nil	Nil
Atypical cells	Nil	4.0	4.6	15.0	7.3	5.8
Parasitized:—						
Prolymphocytes	Nil	Nil	0.7	Nil	0.3	0.1
Lymphocytes	Nil	1.6	7.4	1.0	1.8	8.0
Atypical cells	Nil	Nil	2.2	1.0	0.1	0.1
Damaged	Nil	1.6	0.3	0.5	1.7	3.3
Damaged cells	6.8	27.3	4.0	6.0	19.6	11.3
Plasma cells	0.2	Nil	0.1	0.5	0.1	Nil
Megakaryocytes	0.2	0.3	Present	Present	Present	Present
Mitoses	0.2	0.6	0.4	0.5	Nil	Nil
Total lymphoid cells	8.2	34.5	67.9	50.5	46.4	62.3
Myeloid / Erythroid ratio	1.4 : 1	7.7 : 1	3.4 : 1	3.8 : 1	6.1 : 1	12.4 : 1

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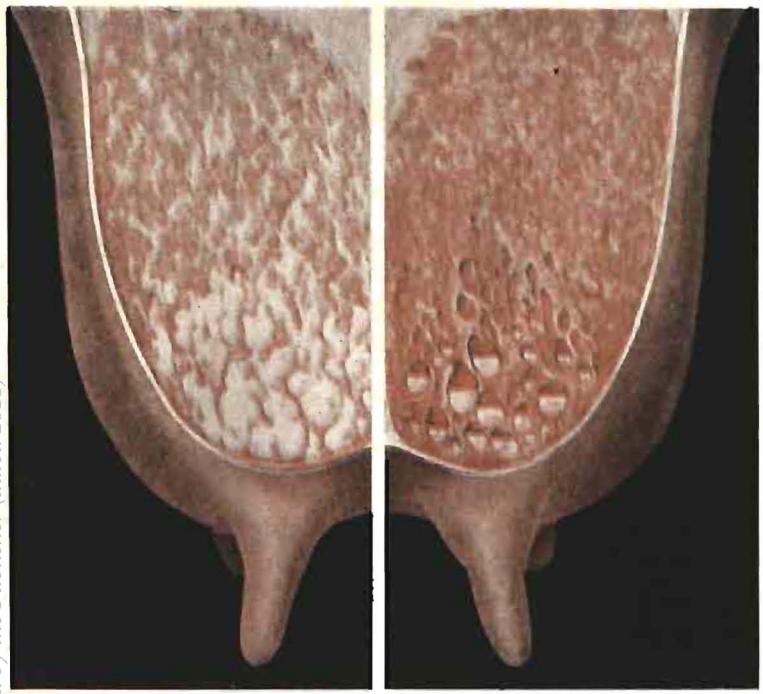
Spring mastitis voor eer u pasiënt nog „Moee" kan sê



Die illustrasie toon aan hoe nuwe Metibiotic-Mastitisskuim, as dit ingespuit word, die uier-kwart volkome vul.

In teëstelling hiermee vul die gewone olierige middels net die speen en die onderste gedeelte van die uier. Hulle kom nooit met die boonste agterkwart in aanraking nie.

Nuwe METIBIOTIC is 'n selfgedrewe skuim. Slegs 'n skuim kan die hele kwart vul. En dan daar bly om sy taak te verrig!

Daar is talle puik antibiotika vir mastitis. Maar konvensionele metodes van inspuiting ná melkery laat die middels nie versprei nie; hulle dryf net in die speen en onderste gedeelte van die uier rond. Die geneesmiddel bereik gewoonlik nie die besmette boonste gedeelte van die uier vir 'n volle melksiklus nie.

Nuwe Metibiotic- aërosol-gedrewe matitisskuim bereik onmiddellik die boonste gedeelte van die uier. Metibiotic bevat ook Tween, 'n verspreidings- en emulgeringsmiddel wat die antibiotikum dra na versamelbuise en alveoli wat voorheen moeilik bereik is.

Geen „uitmelkery" voor behandeling nie. Met konvensionele behandeling word 'n groot hoeveelheid van die geneesmiddel dikwels „uitgemelk" voordat dit die besmette weefsel bereik. Metibiotic se onmiddellike verspreidingswerking laat die volle dosis werk voordat „uitmelkery" kan plaasvind.

Beproefde formule, verminder weefselbeskadiging. Aktiewe bestanddele sluit in: Twee beproefde antibiotika (penisillien-G-prokaeïen 100,000 eenhede, dihidrostreptomisien 300 mg.) en een kortikosteroïde (prednisoonasetaat U.S.P. 4 mg.) om infeksie te beheer en inflammasie te stil. Behandeling is vinniger, weefselbeskadiging minder, koste is laag.

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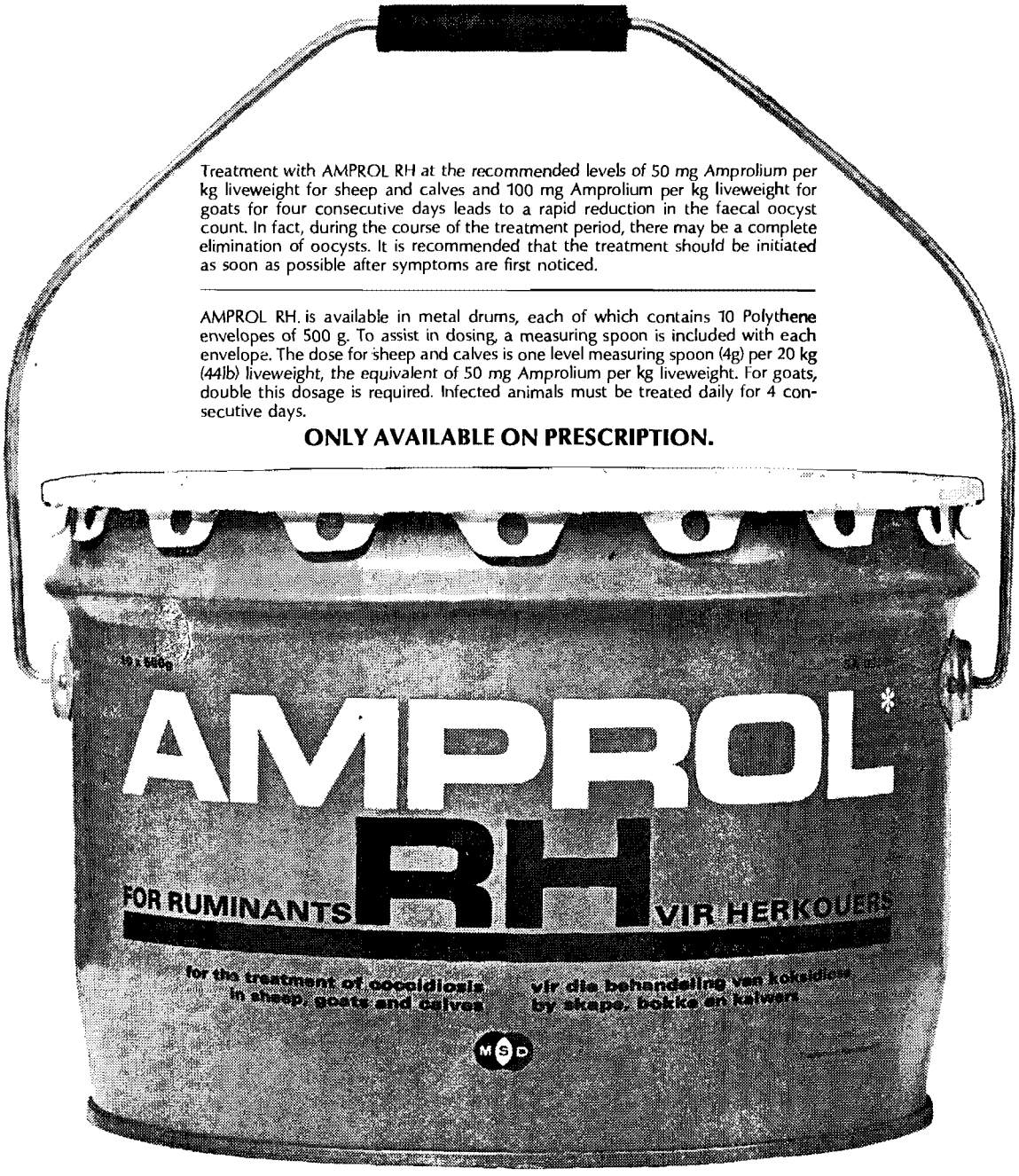

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Jl S. Afr. vet. med. Ass. 41 (4): 287-288 1970

THE ISOLATION OF *ACTINOBACILLUS SEMINIS* FROM BOVINE SEMEN: A PRELIMINARY REPORT

E. M. VAN TONDER AND T. F. W. BOLTON*

SUMMARY

A case of bilateral epididymitis in a young Friesland bull is described. *Actinobacillis seminis* was isolated from the bull's semen. The organism proved to be identical in microscopical, cultural and biochemical characteristics to strains isolated from rams. Serum from this animal, when subjected to a complement fixation test, yielded a suspicious reaction against an antigen prepared from the isolate of a naturally infected ram with clinical epididymitis.

Blood sera from two bulls in the Orange Free State gave positive and suspicious reactions respectively to the same antigen.

INTRODUCTION

Actinobacillus seminis was first isolated from three natural cases of epididymitis in Australia by Baynes & Simmons[1]. Since then it has been reported in the United States of America by Livingston & Hardy[3] and in this country by Worthington & Bosman[5] and Van Tonder & Bolton[4]. In New Zealand, however, Ekdahl, Money & Martin[2] isolated Actinobacillus-like organisms from cases of epididymitis but failed to obtain the identical organism as originally described[1]. In all these instances isolations were made from infected rams only.

This report describes the isolation of *Actinobacillus seminis* from the semen of a bull with bilateral clinical epididymitis. The serological results of the complement fixation test on serum of this animal as well as two other specimens received from the Orange Free State, are also given.

CASE HISTORIES

Two serum specimens were received from the State Veterinarian, Bloemfontein, on the 29th August 1969. One specimen. (No. 1901) was obtained from a bull of unspecified breed and suffering from a bilateral epididymo-orchitis. The other specimen (No. 1902) was taken from an Africander bull with severe unilateral orchitis.

A 16 months old Friesland bull, one of a group of four bulls to be tested for sale purposes, was examined clinically on the 10th September 1969 and found to be suffering from bilateral epididymitis, the heads on both sides being affected. Semen collected by electrical stimulation was subjected to microscopical and bacteriological examination and a serum specimen (No. 2057) to the complement fixation test for *Brucella abortus, Br. ovis* and *Actinobacillus seminis*.

MATERIALS AND METHODS

Apart from the usual semen evaluation, smears were stained in Giemsa solution as well as according to Stamp's modification of the acid fast technique. Semen was also streaked on tryptose-blood-agar (Difco) plates and incubated at 37° in air and in an atmosphere containing 15 per cent CO_2.

Sera tested by the CF test against three different antigens. Standard *Br. abortus* and *Br. ovis* antigens were obtained from the Veterinary Research Institute, Onderstepoort. An *A. seminis* antigen was prepared from an isolate of a naturally infected Merino ram (ram No. 70.64) with clinical epididymitis, according to the method described by Worthington & Bosman[5]. The CF test was performed as described by Worthington & Mulders[6].

RESULTS

The ejaculate was approximately 10 ml in volume and had a cloudy appearance. No motility or live spermatozoa could be demonstrated. Smear examination revealed only few spermatozoa but abundant neutrophils and Gram-negative pleomorphic organisms. These organisms were lying free or situated within the neutrophils. They were not stained by Stamp's method.

* Regional Veterinary Investigation Centre, P.O. Agrikollege, Middelburg, Cape Province.

An almost pure and abundant growth, consisting of small dewdrop-like colonies, was obtained on both culture plates. Subcultures were subjected to the usual range of bacteriological and biochemical tests. The results of these tests were identical to those of the ram isolate.

The results of the CF tests are given below:

Serum	ANTIGEN Br. abortus	Br. ovis	A. seminis
Ram 70.64	–	–	+(1 : 80)
1901 (O.F.S. bull)	–	–	+(1 : 20)
1902 (O.F.S. bull)	–	–	+(1 : 40)
2057 (Friesland bull)	–	–	+(1 : 20)

DISCUSSION

The clinical lesions in the three bulls were similar to those found in rams infected with *A. seminis*. These lesions may vary from acute to chronic uni- or bilateral epididymitis, orchitis or epididymoorchitis.

The semen picture was also identical to that usually found in rams with clinical lesions due to infection with *A. seminis*.

Furthermore, a comparative bacteriological examination on semen isolates from the bull as well as ram 70.64 revealed that these organisms were indeed identical as regards cultural, morphological and biochemical characteristics.

The fact that two bulls gave suspicious and one a positive titre against *A. seminis*, whereas they were all negative to *Br. abortus* and *Br. ovis*, furthermore indicates infection due to the first-named organism.

Further work is in progress.

ACKNOWLEDGEMENTS

We have pleasure in thanking the Director of Veterinary Services for his permission to publish this report, the Director, Veterinary Research Institute, Onderstepoort for supplying the required reagents and Prof. T. F. Adelaar for his assistance in preparing this report.

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BOOK REVIEW

NUTRITION AND DISEASE IN EXPERIMENTAL ANIMALS

Editor: W. D. TAVERNOR

Publisher: Bailliere, Tindall & Cassell. London. 1970. pp 165. Price 50s.

The publication consists of sixteen papers presented at a symposium organized by the British Small Animal Veterinary Association, British Laboratory Animal Veterinary Association and the Laboratory Animal Scientific Association, held in May 1969.

The topics of the papers deal with relationships between human and experimental animal nutrition, protein requirements, trace mineral deficiencies, vitamins, sterilized diets, diet formulations, and the use of animals as dogs, cats and monkeys. A chapter is included on the laws in Britain pertaining to laboratory animal experimentation.

Books handling symposia proceedings do not usually cover entire fields and this one is no exception. It does contain, in several chapters, valuable new information which will serve to increase the volume of available information.

The publication is recommended to persons building up a library of literature on laboratory animals.

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Jl S. Afr. vet. med. Ass. 41 (4): 292-294 1970

A LARGE *BABESIA* SP. AND A *THEILERIA*-LIKE PIROPLASM OF THE SQUARE-LIPPED RHINOCEROS

R. D. BIGALKE*, M. E. KEEP**, PEARL J. KEEP** AND J. H. SCHOEMAN*

SUMMARY

The occurrence of two different species of piroplasms in the square-lipped rhinoceros (*Ceratotherium simum*) in Zululand, South Africa is recorded. The parasites concerned are a large *Babesia* sp. and either a *Theileria* or a small *Babesia* sp.

INTRODUCTION

During routine immobilization of rhinoceroses (*Ceratotherium simum* Burchell, 1817) for the purpose of translocation, between 5th July, 1967 and 7th March, 1969, thin blood films were prepared from apparently healthy animals, stained with Giemsa and examined microscopically. A total number of 106 animals were involved of which 64 were adults, 27 subadults and 15 calves. Individuals still accompanied by an adult cow were regarded as calves; except for one animal, which was about two months old, their ages were probably between nine months and three years. Those considered to be subadults were not accompanied by an adult female, but were not fully grown; they were thought to be between three and seven years of age. Adults were fully grown animals.

OBSERVATIONS

The parasites observed were of two distinct types. One, a large *Babesia* sp., was seen in blood films from two animals only, namely those from a female and from a male calf made on the 21st February and 7th October, 1968, respectively. The large piroplasms were extremely rare. Only two parasitized erythrocytes could be found in the male calf. Each cell contained two pyriform parasites of approximately equal size (Fig. 1). The pale, purple-staining nuclear material was concentrated towards the attenuated ends, where, in one case, the daughter individuals still appeared to be attached to each other after a recent division. The cytoplasm was light blue in colour. The small piroplasms described below were also fairly frequent in this animal.

Two parasitized cells, each harbouring a single large organism, were also found in the female calf. They were respectively round and roughly ovoid in shape. In the round form the nuclear material appeared to be lining the periphery whilst the central portion was unstained. The internal structure of the ovoid form was similar to that of the pear-shaped parasites described above.

The other species was a much smaller piroplasm and was noted more frequently. It was rather pleomorphic in that rod-shaped, comma-shaped, round and oval forms were seen (Fig. 1). In the round and oval forms the nucleus was usually represented by a purple-staining cap extending from one- to three-quarters of the way around the periphery of the parasite. Sometimes the nucleus was a minute granule or even lobed. Occasionally a dividing line appeared to bisect the parasite. The cytoplasm stained a pale blue, if at all. No maltese cross forms could be found. It was not possible to decide whether a *Theileria* or a small *Babesia* was involved, but the former seems to be more likely in view of the presence of rod-shaped forms.

Small piroplasms were recorded in smears from 34 animals (Table). The degree of infection was arbitrarily classified as either fairly frequent or rare. In the most heavily infected animal, an immature male, 3.3% of the erythrocytes contained one, sometimes two and very occasionally three of these parasites. They were rare in preparations from 25 animals and fairly frequent in those from another nine. No small piroplasms could be found in smears from the remaining 72 rhinos but the possibility that they were present in very small numbers cannot be excluded. Positive films were

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** Natal Parks, Game and Fish Preservation Board.

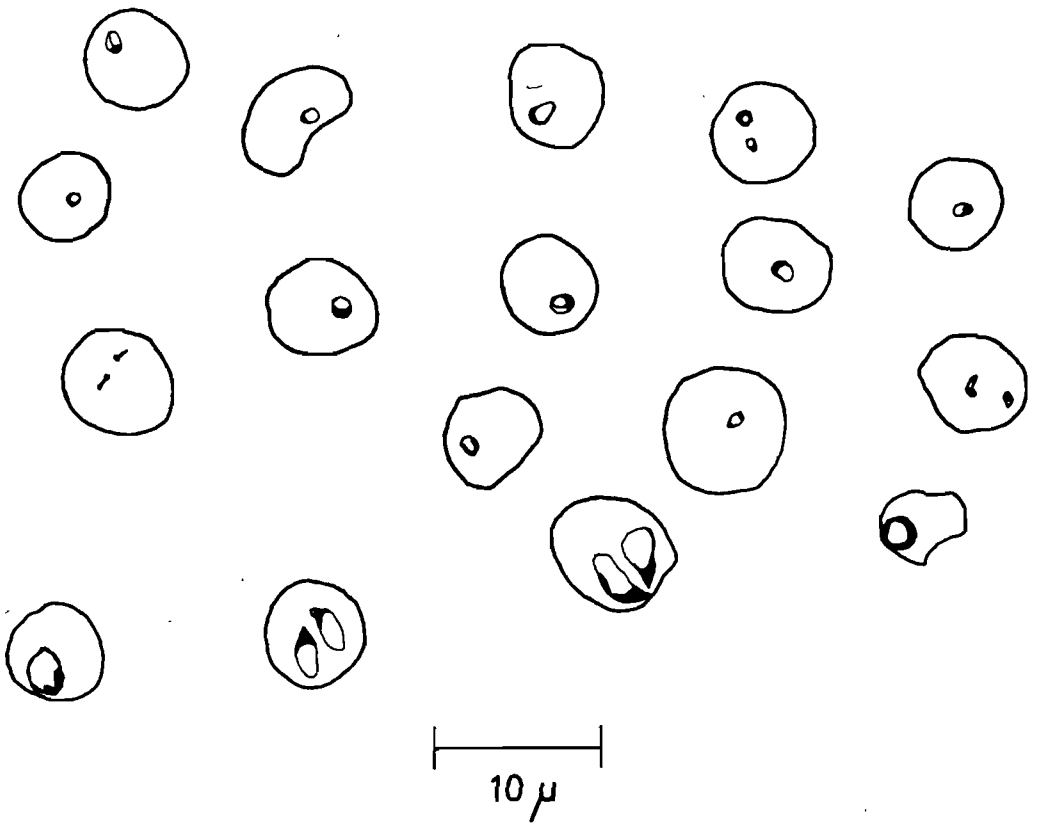


FIG. 1. The erythrocytic parasites, illustrated with the aid of a drawing tube, represent the large **Babesia** sp. in the bottom row and the **Theileria**-like parasite above.

Table: THE INCIDENCE OF PARASITIZATION OF DIFFERENT AGE GROUPS WITH **THEILERIA**-LIKE ORGANISMS

	No. examined	No. Pos.	% pos.
Adults	64	16	23.9%
Subadults	27	12	44.4%
Calves	15	6	40.0%
Total juveniles	42	18	42.8%
Total	106	34	32.1%

recorded from 32% of the 62 males and from 30% of the 47 females. Young animals were more often parasitized (42.8%) than adults (23.9%; Table). The incidence of infection calculated on a monthly basis in terms of the smears made is illustrated in Fig. 2. No definite seasonal trend is noticeable.

DISCUSSION

Small piroplasms have been seen in the square-lipped as well as the black rhinoceros (*Diceros bicornis* [Linnaeus, 1758]) in Zululand by Neitz and he has also observed a *Babesia* sp. in the latter host (personal communication, 1970). Large as well as small piroplasms have also been recorded in the black rhinoceros by Brocklesby[1] in Kenya, who refers to earlier reports on the large parasite by Jarrett, Jennings, Murray and Harthoorn (1964) and Brocklesby and Vidler (1965).

As far as we can determine this is the first record of a large *Babesia* in the square-lipped rhinoceros. Whether it is specific for this particular host or perhaps also occurs in the black rhinoceros remains to be determined. The number of parasites seen was too small to warrant a comparison with those of the black rhinoceros at this stage.

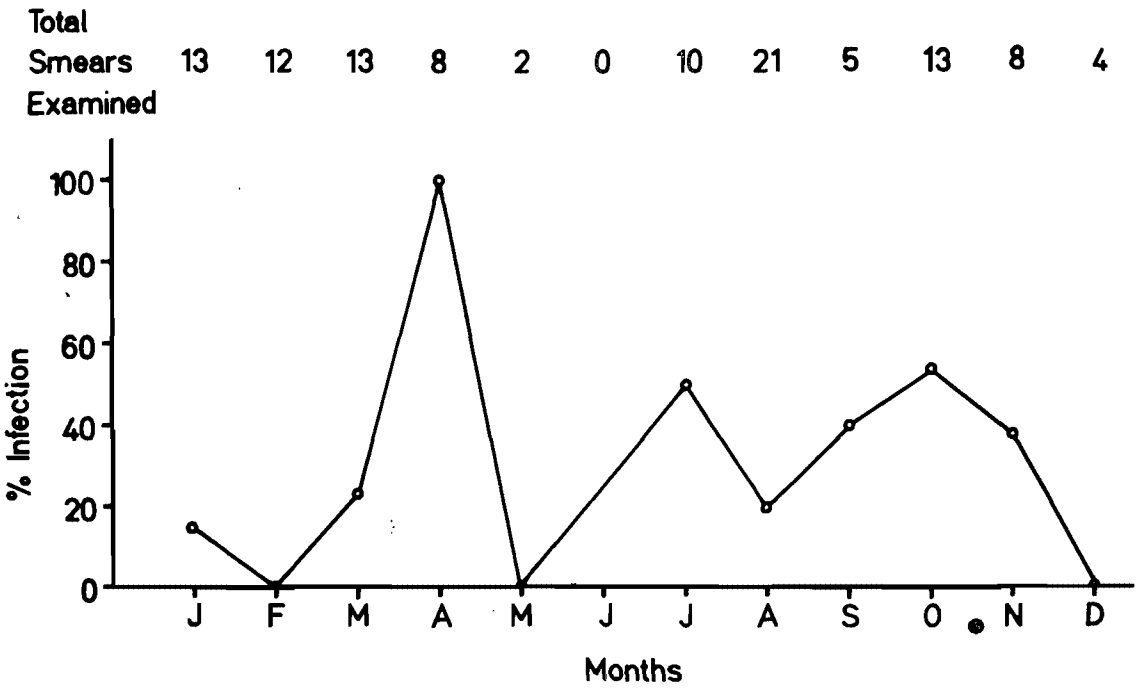
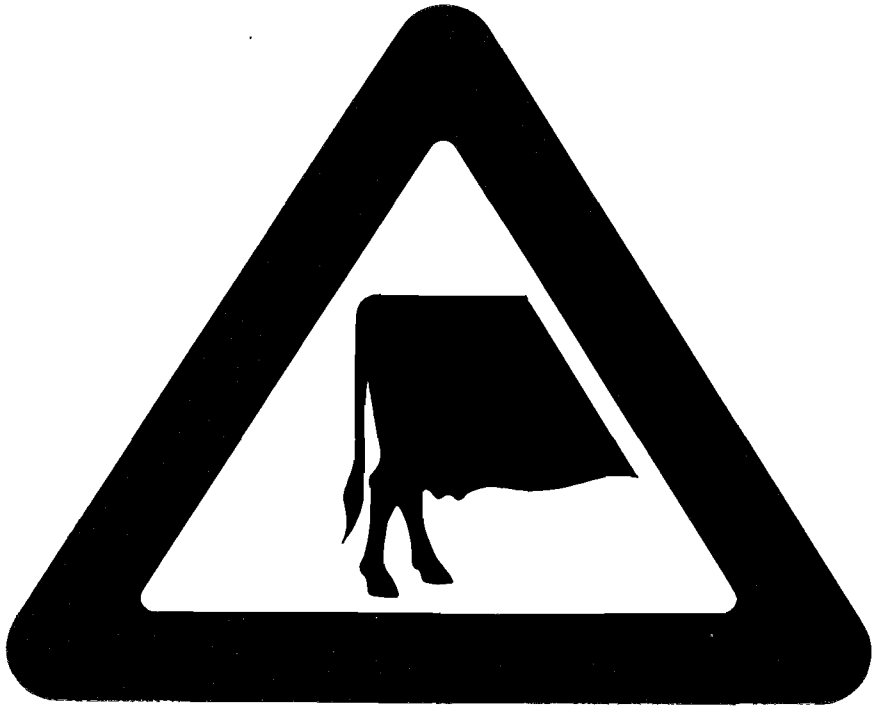


FIG. 2. Graph illustrating the monthly incidence of infection as a percentage of the smears during the month concerned.

REFERENCE

1. Brocklesby D.W. 1967 *Vet. Rec.* 80 : 484



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TREATMENT OF *SCHISTOSOMA MATTHEEI* INFESTATION IN SHEEP: FURTHER OBSERVATIONS

J. A. LAWRENCE* AND R. L. MCKENZIE**

SUMMARY

Investigations into the treatment of sheep experimentally infested with *Schistosoma mattheei* revealed that lucanthone, administered orally in doses of 50 mg/kg, 40 mg/kg and 30 mg/kg on three successive days was moderately effective in two of three sheep and free from severe side effects. This treatment has proved useful in natural outbreaks of clinical schistosomiasis. Hycanthone administerd intramuscularly as a single dose of 3 mg/kg was moderately effective in four of five sheep, and as a single dose of 6 mg/kg was highly effective in two sheep. A strain of *Klebsiella pneumoniae* administered intravenously had no curative effect in three sheep. A marked lung shift was observed in a number of animals after treatment, and the mechanism and implications of this are discussed.

INTRODUCTION

Previous investigations into the treatment of sheep naturally infested with *Schistosoma mattheei* revealed that lucanthone at 40 mg/kg daily for three days was satisfactory in four out of six sheep treated[1]. Subsequent to this work, trials of possible remedies were continued, using sheep infested experimentally with a reasonably uniform parasite burden, in preference to animals originating from natural outbreaks. The parasites were recovered by perfusion of the mesenteric veins in preference to attempts at assessing their number *in situ*. At the same time the opportunity was taken to study the fate of the parasites after treatment by reverse perfusion of the intrahepatic portal veins and the pulmonary arteries to recover parasites which might have lodged in these vessels.

Lucanthone has been shown to be reasonably effective but side effects, at a dosage rate of 40 mg/kg daily for three days, are quite severe. It was suggested by Standen[2] that the side effects might be lessened without reducing the efficacy by giving an initial high loading dose followed by progressively smaller doses on the two following days, as recommended for human treatment.

Hycanthone, a microbiological oxidation derivative of lucanthone, has been shown to be highly effective against *S. mansoni* and *S. haematobium* in man when given as one dose by intramuscular injection[3]. The ease of administration of a remedy is of great importance in the flock treatment of sheep and this drug was considered worthy of trial.

A new departure in the field of antischistosome treatment is the use of bacterial cultures inoculated into the host to kill the parasite[4]. A strain of *Klebsiella pneumoniae* previously used with good effect against *S. mansoni* in mice, hamsters and monkeys, and against *S. mattheei* in mice was obtained for investigation.

MATERIALS

Animals

The sheep used were adult Dorper ewes, which had been experimentally infested with 1 000 cercariae of a bovine strain of *S. mattheei*[5]. Their average weight was 31 kg. The animals were stabled permanently.

Remedies

Lucanthone Tablets B.P. ('Nilodin'—Burroughs Wellcome) were administered orally or by oesophageal tube. The total dose previously shown to be effective, 120 mg/kg, was divided and administered in single daily doses on three successive days: 50mg/kg on the first day, 40 mg/kg on the second day, and 30 mg/kg on the third day.

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Hycanthone ('Etrenol' — Winthrop) drug was administered by intramuscular injection into the thigh as a single dose of 3 mg/kg or 6 mg/kg at a dilution of 100 mg/ml.

Strain EZ 164 of *Klebsiella pneumoniae* was obtained in freeze dried form. The average number of viable organisms in three vials was determined by plate count on nutrient agar. For the treatment of each sheep, a fresh vial was reconstituted with sterile distilled water and the dose was estimated on the basis of the average viability count of the batch. At the same time a plate count was performed on the reconstituted contents so that the number of organisms administered could be accurately estimated retrospectively. The material was given by intravenous injection, as a single dose ranging from 1.1×10^8 to 9.4×10^8 organisms.

METHODS

A total of 17 sheep was used in this trial, as follows:

Treatment	Dosage	No. of Sheep
Lucanthone	50/40/30 mg/kg	3
Hycanthone	3 mg/kg	6
Hycanthone	6 mg/kg	2
Hycanthone	3 mg/kg and 6 mg/kg	1
K. pneumoniae EZ 164	$1.1 - 9.4 \times 10^8$	3
Control	—	2

The animals were observed clinically during the period between treatment and slaughter. In the case of animals treated with EZ 164, blood samples and rectal swabs were cultured for *Klebsiellae* at intervals after treatment, and rectal temperatures were recorded daily. In two sheep treated with lucanthone and one treated with EZ 164 daily blood samples were checked for packed cell volume, total and differential white cell count, serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) during the first nine days after treatment.

Sheep were slaughtered at various intervals after infestation and therapy by stunning and bleeding five minutes after the intravenous administration of 4 000 units of heparin. Schistosomes were collected from the mesenteric veins, the intrahepatic portal veins, and the pulmonary arteries by the perfusion method of McCully & Kruger[6]. The efficiency of recovery of the parasites from the mesenteric veins was very variable. A number of parasites was lodged in the terminal branches, completely occluding them, and by obstructing the flow of saline through the vessels they were probably protected from the effects of the formalin. These remaining parasites were counted *in situ*.

From some animals, blocks of liver, lung, kidney, heart and spleen were fixed in 10% formol-saline, sectioned and stained with haematoxylin and eosin for histological examination.

RESULTS

The number of parasites present in mesentery, liver and lungs at slaughter in each sheep is recorded in Table 1.

Parasites were recorded as a) pairs of adults, pairing being taken as the criterion of the adult state; b) single adults, of the same size as the paired parasites; and c) single small parasites, significantly smaller than the adults in the same animal. No detailed record was kept of the ratio of males and females among the unpaired parasites. When the mesentery and intestine was examined after perfusion, each remaining parasite detected was assumed to be a pair. The absence of a larger proportion of single parasites of either sex recovered on perfusion was taken as justification for this assumption.

Control sheep No. 373 was the last animal to be examined in the trial. The parasite burden is considered to have been much below the average for the group. The untreated control and the five sheep, Nos. 311, 413, 341, 338 and 305, in which treatment appears to have been unsuccessful, all had more than 236 pairs of adult parasites in the mesentery. In the remaining sheep, in which the pairs of parasites numbered less than 236, there was evidence of a disturbance in the parasite burden as a result of treatment, in the form of an increased number of parasites in either liver or lung or both, or the presence of lesions in these organs resulting from lodging of parasites in the smaller blood vessels. For the purpose of calculation of schistosomicidal activity, we have assumed therefore, a minimum parasite burden of 240 pairs before treatment in all animals.

Efficacy of Treatment

A percentage cure has been calculated on the formula: $240 - x \div 240 \times 100$, where x is the number of pairs of parasites in the mesenteric veins after treatment if less than 240. The percentage cure rate for each animal is recorded in Table 2.

Table 1: EFFECT OF TREATMENT

Sheep No.	Treatment	Days between completion of treatment and slaughter	Months between infestation and slaughter	Parasites present		
				Mesentery	Liver	Lung
327	Control	—	8	P 280	N/C	2
				A 0		1
				S 0		22
373 (1)	Control	—	13	P 73	N/C	0
				A 3		0
				S 0		3
311 (2)	EZ 164 1.1×10^8	14	7	P 236	3	0
				A 6	0	0
				S 0	0	68
413 (2)	EZ 164 3.8×10^8	7	7	P 341	0	4
				A 9	0	2
				S 0	0	68
341	EZ 164 9.4×10^8	8	7	P 239	1	0
				A 12	5	1
				S 9	10	20
338	Lucanthone 50, 40, 30 mg/kg	7	7	P 273	11	2
				A 1	0	0
				S 0	0	2
322	—do—	14	7	P 8	18	N/C
				A 2	30	
				S 0	10	
404	—do—	16	7	P 86	14	39
				A 2	2	60
				S 0	0	78
305	Hycanthone 3 mg/kg	2	11	P 251	0	0
				A 3	0	1
				S 0	0	25
304	—do—	15	11	P 71	10	86
				A 8	3	57
				S 0	0	10
335	—do—	23	12	P 201	7	5
				A 13	2	16
				S 0	0	3
321	—do—	32	11	P 98	0	0
				A 16	1	0
				S 0	0	8
332	—do—	34	11	P 33	1	0
				A 11	5	0
				S 0	0	0
330	—do—	55	12	P 92	0	14
				A 1	0	2
				S 0	0	41
333 (3)	Hycanthone 3 mg/kg 6 mg/kg	75 8	12	P 62	3	93
				A 10	0	54
				S 0	0	0
337 (3)	Hycanthone 6 mg/kg	13	13	P 6	4	2
				A 0	3	1
				S 0	0	0
336 (3)	—do—	20	13	P 0	0	7
				A 1	8	54
				S 0	0	0

P = pairs of adults, A = single adults, S = single small parasites, N/C = not-counted.

Footnotes to Table 1

(1) This is considered to be much below the average level of infestation of the group. See text.

(2) Adult schistosomes at least 50% larger than normal.

(3) Adult schistosomes about half normal size.

Table 2: PERCENTAGE CURES

Sheep No.	Treatment	Days between completion of treatment and slaughter	Percentage cure
311	EZ 164	14	0
413	,,	7	0
341	,,	8	0
338	Lucanthone	7	0
322	,,	14	97
404	,,	16	64
305	Hycanthone 3 mg/kg	2	0
304	,,	15	70
335	,,	23	16
321	,,	32	59
332	,,	34	86
330	,,	55	62
333	Hycanthone, 3 and 6 mg/kg	75 and 8	74
337	Hycanthone 6 mg/kg	13	98
336	,,	20	100

Side Effects of Treatment

K. pneumoniae EZ 164: A moderate febrile reaction persisted for seven days in the animal receiving 1.1×10^8 organisms, and until slaughter at seven and eight days in the animals treated with the larger doses. There was slight depression and loss of appetite at the height of the reaction, but no other clinical signs were noted. *Klebsiellae* were recovered from the blood at 1½ and 3 hours after treatment respectively in two animals, but not at 24 hours in any of the three animals. No *Klebsiellae* were recovered from rectal swabs taken four and eight days after treatment in Sheep No. 311.

No histological abnormalities attributable to treatment were noted in liver, kidney, heart, lung and spleen of Sheep Nos. 311 and 413. No bacterial cultures were made from these organs. Sheep No. 341, which received 9.4×10^8 organisms, had a few small foci of necrosis with polymorph infiltration in the liver, and *Klebsiellae* were recovered on culture. There were no histologically detectable lesions in kidney, heart and lung.

Lucanthone: No clinical side effects were noted after treatment. No significant alteration in total and differential white cell counts or SGOT and SGPT were noted during the first nine days after the commencement of treatment in either Sheep No. 404, in which treatment was partially effective, or in Sheep No. 338, in which it was ineffective. These factors were not studied in Sheep No. 322.

At *post mortem* examination liver and/or lung lesions associated with the lodging of schistosomes in small blood vessels were noted in Sheep Nos. 404 and 322, in which a partial cure was effected, but not in Sheep No. 338.

Hycanthone: No clinical side effects were noted after treatment with 3 mg/kg or 6 mg/kg. There was no significant reaction at the site of injection when 3 mg/kg was used, but after 6 mg/kg a large nodular lesion with a necrotic centre was detected *post mortem* in sheep examined 13 and 20 days after treatment. No obvious lameness resulted from this lesion.

At necropsy liver and/or lung lesions associated with the lodging of schistosomes in small blood vessels were noted in all animals except Sheep No. 305, which was slaughtered before the drug had had any detectable effect on the parasites. In Sheep Nos. 337 and 336, slaughtered 13 and 20 days respectively after treatment with 6 mg/kg, there were no histological changes in liver, kidney, heart and lung attributable to the drug itself.

The liver lesions resulting from lodging of parasites in the terminal branches of the portal veins were irregular, white or yellow nodules, about 2 mm in diameter and of variable length. The number of lesions varied, 60 being counted in one animal. The nodules were raised above the surface and were most commonly found near the thin free margin of the organ. If a branch of the portal vein was opened along its length until it disappeared into the nodule, a parasite could be found embedded in the lumen of the vein within the lesion. Liver lesions from four sheep slaughtered 13 to 23 days after treatment were examined histologically. The parasite could be seen in the vein surrounded by degenerating eosinophils. The wall of the vein was largely necrotic and replaced by an inner zone of epitheliod and giant cells, surrounded by an extensive granulomatous reaction in which numerous eosinophils, lymphocytes and macrophages were involved. This reaction extended for some distance into the adjacent smaller portal tracts. In one of the livers examined, there was some parenchymal necrosis surrounding the nodule, but in none was there evidence of infarction.

The lung lesions resulting from lodging of the parasites in the terminal branches of

the pulmonary artery took the form of solid, slightly raised, red foci, more or less sharply defined, up to 1 cm in diameter. Two hundred of these lesions were counted in the lungs of one animal. Lungs from six animals slaughtered 8 to 55 days after treatment were examined histologically. In each case schistosomes were found embedded in branches of the pulmonary artery and were surrounded by a reaction, similar to that seen in the hepatic portal veins, which extended some distance into the adjoining interstitial tissue. At 8 days there was marked oedema of the alveoli surrounding the affected artery. The oedema was still marked at 13 days, and discrete foci of haemorrhage and necrosis, i.e. infarcts, were seen in the vicinity of the affected arteries. At 16 days also oedema and infarction were apparent. At 20 and 23 days fibroblasts and alveolar epithelial cells had begun to proliferate and invade the infarcted tissue. By 55 days the oedema and cellular infiltration of the surrounding alveoli had dispersed, but fibrosis marked the site of previous infarction.

Fate of Parasites after Treatment

In Table 3 is recorded the percentage distribution of parasites recovered from mesentery, liver and lung in those animals in which all three sites were examined.

No distinction has been made between parasites of different sizes. Reference to Table 1 will show that in Sheep Nos. 404, 304, 335 and 330 there was a significant number of full-sized adult schistosomes, many in pairs, present in the pulmonary arteries. In an attempt to ascertain how these worms reached the pulmonary artery from the mesenteric vein, further investigations were carried out on two sheep.

In Sheep No. 333, the parasites were collected from the pulmonary artery by perfusion. They were all much smaller than normal, the males being approximately 5mm in length. Saline was passed through the liver from the portal vein to the caudal vena cava under pressure from a head of approximately 75 cm. After confirmation that there were no parasites in the caudal vena cava, 41 pairs and 10 single adult schistosomes were introduced into the portal vein. Four pairs and four singles were recovered from the caudal vena cava within a few seconds. The flow was then reversed gently to redistribute the remaining parasites within the hepatic vessels, and perfusion restarted. A further four pairs were recovered. This result confirmed that small schistosomes, even when paired, could pass through the liver with ease.

No further sheep from which significant numbers of larger schistosomes could be recovered to repeat this experiment became available during the trial. When the last sheep in the trial, Control No. 373, was slaughtered, an attempt was made to force normal-sized schistosomes from the portal venous system into the caudal vena cava via intrahepatic or extrahepatic routes. After heparinization and slaughter, the external and circumflex iliac arteries and veins were tied off, but the internal iliacs were left intact, thus isolating the hind limbs and abdominal wall from the perfusion circuit without excluding the pelvic cavity. The caudal vena cava was tied off just caudal to the liver. Saline was introduced at a

Table 3: PERCENTAGE RECOVERY OF PARASITES ACCORDING TO SITE

Sheep No.	Treatment	Days between completion of treatment and slaughter	Total	Parasites recovered % of total Mesentery	Liver	Lung
311+	EZ 164	14	552	86.6	1.1	12.3
413+	,,	7	769	89.9	0	10.1
341+	,,	8	537	92.9	3.2	3.9
338+	Lucanthone	7	575	95.2	3.8	1.0
404	,,	16	420	41.4	7.1	51.5
305+	Hycanthone, 3 mg/kg	2	531	95.1	0	4.9
304	,,	15	412	36.4	5.6	58.0
335	,,	23	460	90.2	3.5	6.3
321	,,	32	221	95.9	0.5	3.6
332	,,	34	84	91.7	8.3	0
330	,,	55	256	72.3	0	27.7
333	Hycanthone, 3 and 6 mg/kg	75 and 8	380	35.3	1.6	63.1
337	Hycanthone 6 mg/kg	13	28	42.9	39.3	17.8
336	,,	20	77	1.3	10.4	88.3

+No cure achieved.

pressure of 1 lb/sq in into the thoracic aorta by a canula, and an outflow canula was inserted in the thoracic portion of the caudal vena cava. When all the blood had cleared from the circuit, 5 ml of 40% formalin were introduced into the input canula, in order to cause the schistosomes in the mesenteric veins to release their hold. The pressure was gradually increased to 4 lbs/sq in, at which stage some distention of the liver was noted. No schistosomes were recovered. This indicated that parasites had failed to pass through the hepatic circulation or extrahepatic porto-caval connections in the diaphragmatic region.

The direction of perfusion was reversed for one minute, to clear the intrahepatic portal veins of parasites. The ligature on the caudal vena cava caudal to the liver was removed, the thoracic aorta was tied off, the input canula was inserted into the main extrahepatic portal trunk, directed caudally, and the outflow was collected from the thoracic caudal vena cava. The only route available for the perfusion fluid was thus via extrahepatic porto-caval connections in the abdominal and pelvic cavities. Pressure was gradually increased from 1 to 5 lb/sq in. There was very little flow except when massage was applied in the diaphragmatic region and within the pelvic cavity. No schistosomes were recovered. The portal vein was then perfused in the normal manner and 34 pairs and three single adults of normal size were recovered. In this instance, therefore, it appeared that it was not possible for normal adult schistosomes, either single or paired, to pass from the portal veins to the caudal vena cava and thence to the pulmonary arteries.

DISCUSSION

Efficacy and Safety of Treatment

EZ 164 of *K. pneumoniae* had no apparent curative effect at a dosage rate of up to 9.4×10^8 organisms. Larger doses might have been more effective, but 1×10^{10} organisms have been shown to cause death in sheep after 24 hours, and 1×10^9 organisms cause a severe pyrexia[7,8].

Lucanthone in three daily doses of 50, 40 and 30 mg/kg gave an almost complete cure in one sheep and a partial cure in a second, but was ineffective in a third. The variable efficacy of this drug reported previously is thus confirmed. It is unlikely that the failure of the drug in Sheep No. 338 was only apparent due to the fact that it was slaughtered only seven days after the completion of treatment, a week earlier than the other two: Dickerson[9] and Hewitt & Gill[10] found the liver shift in mice well established at seven and ten days respectively after treatment. Side effects with this dosage regime were negligible. This drug does have practical value in the treatment of field outbreaks. Approximately 30 sheep have been treated on two farms where natural outbreaks of schistosomiasis were occurring. The progress of the animals was not followed closely; the owners reported a definite clinical improvement. Three sheep that were in a very advanced stage of emaciation and weakness died following treatment, but excessive side effects were not reported in the remainder. Because sheep find the taste very unpleasant, administration by oesophageal tube is recommended to ensure ingestion of the complete dose.

Hycanthone at 3 mg/kg gave an average cure of 59% in the five sheep slaughtered 15 days or more after treatment. In only one animal was the percentage removal of worms unlikely to have been of much clinical benefit. It thus has an efficacy comparable to that of lucanthone, with the considerable advantage of greater ease of administration, and the disadvantage, at present, of higher price. The absence of a liver shift in Sheep No. 305, slaughtered two days after treatment, is not surprising. Khayyal *et al.* [11], when treating *S. mansoni* in hamsters with a single dose of the drug, found no liver shift at 24 and 48 hours after treatment, but a marked shift one week after treatment.

Hycanthone at 6 mg/kg was almost completely effective in the two sheep slaughtered 13 days or more after treatment. Clinical side effects were negligible. Due to the nodular lesion remaining at the site of injection, the drug should be administered in the muscles of the neck, where subsequent possible condemnation of meat at slaughter would be less costly.

A danger inherent in the efficient removal of a heavy schistosome infestation lies in the damage to liver and lung caused by parasites dislodged from the mesenteric veins. In this trial, the animals were in good condition, although suffering from a variable degree of anaemia. In untreated animals,

liver damage was negligible and there was no evidence of secondary pneumonia. No clinically detectable ill effects resulted from the parasite shift after treatment. The situation might differ in clinically more severely affected animals, particularly those with secondary pneumonia, a common complication of schistosomiasis in the sheep. In such animals, lung infarcts might provide an ideal site for invasion of bacterial pathogens already established in the lung. It is impossible to predict what the incidence of such ill effects might be. In man, severe toxic effects and occasional mortality do follow treatment for bilharzia, but there is apparently no definite evidence that this is due to the destruction of parasites rather than to the drug itself, apart from a few exceptional cases of fatal verminous pneumonia reported by Kenawy[12] and Mousa[13].

Fate of Parasites after Treatment

An interesting feature of this investigation was the occurrence of a significant shift of parasites to the lungs after treatment in some animals. Such a shift has been recorded previously by Le Roux[14] in sheep after treatment or after a long period of decubitus prior to death.

In laboratory animals, parasites, dislodged from their site in the mesenteric veins as a result of treatment, usually lodge in the smaller portal veins in the liver. However, a 'lung shift', in which a proportion of the parasites is found in the terminal branches of the pulmonary arteries, has been described in mice by Hewitt & Gill[10] and others. The proportion varies considerably from one individual to another, and the occurrence of a lung shift is directly related to the duration of infestation at the time that treatment is commenced, as found by Geake[15], Hewitt & Gill[16] and others. Hewitt & Gill[16] suggest that the lung shift occurs via porto-caval collateral vessels which develop as a result of liver lesions caused by the infestation. The degree of collateral development would, in part, be dependant on the weight of infestation in the animal; the number of parasites dislodged by treatment would also have an influence on the likelihood of a significant number of parasites being carried through the collaterals. There is some evidence of a lung shift occurring in man after treatment[17, 18] but the position is by no means proved.

Although Day[17] and Hewitt & Gill[10] considered that the lung shift probably occurred via intrahepatic collaterals, to our knowledge this assumption has not been proved previously. We were able to show that both paired and single parasites, reduced in size presumably as a result of treatment, would pass through the liver from portal vein to caudal vena cava with ease. Recent studies provide evidence of the route taken by the parasites. What evidence there is, suggests that the diameter of the liver sinusoids does not exceed 35μ[19, 20]. However, Prinzmetal *et al.*[21] showed that glass beads with a diameter of up to 180μ injected into the portal vein of the normal rabbit would rapidly appear in the lung, indicating that larger porto-caval anastomoses exist. Such anastomoses were demonstrated anatomically in the normal rabbit and guinea pig liver by Del Rio Lozano & Andrews[22]. They produced vascular casts by retrograde injection into the hepatic vein and demonstrated the presence of translobular veins which ran directly from the hepatic veins to the peribiliary veins, which in turn opened into the portal vein. The translobular veins measured about 150μ in diameter, except for a constriction at the point of entry into the hepatic vein. Vessels of this diameter would not permit the passage of schistosomes, but there is evidence of dilation of the peribiliary plexus in pathological conditions, e.g. in cirrhosis in man[23], in acute *S. mansoni* infestation in mice[24], and following ligation of the common bile duct of the rabbit[25]. In the latter condition there is also enlargement of the translobular veins. Cheever & Warren[26] and Del Rio Lozano & Andrews[25] suggest that the peribiliary plexus enlarges to act as a shunt, bypassing obstructed portal veins, and enlarged translobular veins may carry an increased flow of portal blood directly into the hepatic vein. Vessels enlarged in this way probably provide the pathway for the lung shift of schistosomes. Hales *et al.*[23] also demonstrated dilation of the umbilical vein and the development of paraumbilical collateral vessels in cirrhosis, which could also act as intrahepatic porto-caval shunts. Whether the intrahepatic portal phlebitis resulting from the lodgement of schistosome ova alone is sufficient to cause the development of porto-caval shunts large enough to accommodate the parasites is not known, although in mice the appearance of parasites in the lungs one hour after treatment sug-

gests that it is[9]. Certainly the additional obstruction of portal veins by parasites dislodged by treatment must also have an effect on the development of the shunts.

None of the authors quoted has commented on the size of the parasites found in the lungs, other than Le Roux[14], who noted that those found there after treatment were very much reduced in size. Parasites *in extremis* moving to the lungs were not reduced in size; in our trials we found normal-sized parasites in the pulmonary arteries of four sheep. The fact that in one sheep we were unable to pass normal-sized schistosomes from the portal vein to the caudal vena cava, does not mean that parasites can only pass when diminished in size. The animal in question was only lightly infested, and, as Hewitt & Gill[16] suggest, this could be expected to reduce the possibility of any lung shift occurring. It is probable that normal-sized parasites will pass through portocaval shunts in the same way as small ones.

The life-span of schistosomes in the pulmonary arteries is not known. Most authorities working with mice have found that the majority of parasites shifting to the lungs were dead from seven or fifteen days after treatment, but Hewitt & Gill[10] noted that some were still alive at 30 days; Geake[15] found up to 50% of the worms alive at 31 days after treatment. In this trial we found substantial numbers of live, normal paired parasites in the pulmonary arteries of one sheep at 55 days after treatment. Apparently these parasites had been affected by treatment sufficiently to dislodge them permanently from their natural site without killing them. If parasites can maintain themselves in this situation and lay eggs for any lenght of time, it could lead to severe pulmonary complications.

The finding of a few live schistosomes, of both normal and reduced size, in the intrahepatic portal veins and pulmonary arteries of untreated or unsuccessfully treated sheep, confirms McCully & Kruger's[6] observations. Similar findings have been made in experimental animals and man. These parasites probably represent individuals which have matured and remained in sites other than the mesenteric veins, or which have been dislodged from the mesenteric veins as a result of ageing processes or due to the action of the host's defences.

Size of Parasites

Detailed records of size of parasites recovered from the mesenteric veins were not kept. It was noted, however, that in two sheep treated with hycanthone at 6 mg/kg, the parasites were about half the normal size. This could be attributed to the effects of the drug. It was also noted that in two sheep treated with strain EZ 164 of *K. pneumoniae*, the parasites were 50% larger than normal. This was attributed to the effects of the treatment. This may be so, but Dickerson[7] has reported a similar increase in size of parasites in one untreated sheep. The size of the parasites may be an indication of their vigour and is a criterion worth recording in investigations into schistosomiasis.

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THE ANTHELMINTIC EFFICACY OF FEED MASH OR PELLETS MEDICATED WITH THIABENDAZOLE*

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SUMMARY

Experiments are described in which the anthelmintic efficacy of feed pellets or mash medicated with thiabendazole was determined in naturally infested sheep and goats.

Efficacy was determined in one trial in which treated and untreated sheep were slaughtered, and in four experiments based on the percentage reduction in faecal worm egg counts and the number of animals excreting nematode eggs.

The administration and efficacy of feed pellets, medicated to supply a daily low level of thiabendazole, are also described.

INTRODUCTION

Anthelmintics for ruminants are usually administered orally as individual drenches. This procedure wastes time and labour, as it necessitates the collection and handling of the animals to be drenched. It is necessary, however, where animals are grazed extensively and are unaccustomed to supplementary concentrate feeding. Where supplement-concentrate feeding is practised, the addition of an anthelmintic to the feed would eliminate both collection and drenching of animals.

The following experiments describe the anthelmintic efficacy of feed mash and pellets, medicated with thiabendazole, against various nematodes in naturally infested sheep and goats.

MATERIALS AND METHODS

Experiment 1

Twenty young Dorper sheep, with worm egg counts ranging from 700 to 16 000 eggs per gram (epg) of faeces, were purchased in the Tierpoort district of the Transvaal and transported to the laboratory where they were housed in a concrete-floored indoor pen. They were fed lucerne hay and, in addition, were accustomed to a proprietary concentrate mash*** for a period of nine days.

On the tenth day three sheep were slaughtered as indicator controls and processed for worm recovery.

The remaining 17 sheep were divided into two groups. One group, consisting of eight sheep, was fed 1.8 kg of mash containing 0.552 per cent thiabendazole. This was calculated to provide 1.25 g of thiabendazole per 22.7 kg liveweight. The other nine sheep received 2.0 kg of non-medicated mash. Faecal worm egg counts[1] were done on several occasions prior to and after treatment. The 17 sheep were slaughtered and processed for worm recovery[2,3] two to seven days after treatment.

Total macroscopic counts were made of adult *Haemonchus contortus*, *Oesophagostomum columbianum* and *Trichuris* spp. Two one-tenth or one-twentieth aliquots of the abomasum, contents from large and small intestine and their digests were examined microscopically for the presence of worms.

Anthelmintic efficacy was calculated by a comparison of the worm burdens in the control and treated sheep.

Experiment 2

A flock of Dorper sheep in the Hennops River district of the Transvaal, which was kraaled at night, was accustomed to non-medicated feed pellets fed on hessian bags in the kraal over a period of six days. Four days later the flock was fed sufficient medicated pellets to ensure that sheep under 22.7 kg liveweight would receive on the average 1.25 g thiabendazole and those over 22.7 kg liveweight 2.5 g thiabendazole. A group of

* Thibenzole, registered trade mark of Merck & Co. Inc., Rahway, N.J., U.S.A., contains 2-(4' thiazolyl)-benzimidazole.

** MSD Research Centre, Hennops River, P.O. Box 7748, Johannesburg.

*** Containing 14.02% crude protein, 6.00% fibre, 1.42% Ca and 0.62%P.

controls was separated from the flock and fed non-medicated pellets. Faecal worm egg counts were done on the day of treatment and four days later.

Anthelmintic efficacy was calculated from the percentage reduction between the average pre- and post-treatment faecal worm egg counts and in the number of sheep excreting worm eggs.

Experiment 3

In the Broederstroom district of the Transvaal, a flock of ewes and lambs, penned continuously and fed green oats and maize cob meal, and a flock of goats and kids, penned only at night, were given non-medicated feed pellets over a period of five days. On the sixth day the animals were fed medicated pellets calculated to give the same average anthelmintic dosage levels as in the previous experiment. A group of sheep and goats were kept as untreated controls. Faecal worm egg counts were done on the day of treatment and four days later.

Experiment 4

A flock of Merino stud lambs in the Beaufort West district of the Cape Province, grazing poor quality natural pasture and fed 13mm diameter lucerne and maize meal cubes in addition, was found to be excreting *Nematodirus* spp. eggs on faecal examination. The lambs weighed 24 to 30 kg and were divided into two groups. One group of 28 lambs was fed medicated pellets, of which they consumed only sufficient to supply an average of 1.48 g of thiabendazole per lamb, instead of the required 2.5 g for lambs of their weight. The other group of 20 lambs was kept as untreated controls.

Faecal worm egg counts were done on the day of treatment and four days later.

Experiment 5

Ten months after the completion of Experiment 2 the same flock of Dorper sheep was again divided into two groups. One of the groups was fed non-medicated pellets for one day and two days later was fed medicated pellets from the same batch as used 10 months previously, calculated at the same rate as then. The other group was kept as untreated controls and fed no pellets whatsoever.

Faecal worm egg counts were done at treatment and seven days later.

Experiment 6

A flock of 112 Merino ewes treated on one occasion in April 1968 with thiabendazole at 88 mg/kg liveweight was thereafter grazed on artificial pastures during the day and separated into two numerically equal flocks which were penned indoors in the evening. In the pens one flock received non-medicated pellets daily at the rate 230 g per ewe while the other flock received non-medicated pellets mixed with medicated pellets to give 230 g of pellets per ewe. The addition of medicated pellets to the non-medicated pellets was calculated to supply each ewe with thiabendazole at a daily rate of 7.5 mg/kg liveweight.

To ensure that infestation was available on the pasture, a flock of naturally infested sheep was grazed with the experimental flocks during the day.

Faecal worm egg counts were done at fortnightly intervals on approximately 20 ewes from each of the experimental flocks and 10 sheep from the naturally infested flock.

RESULTS

Experiment 1

The average faecal worm egg counts of of the untreated control and treated sheep before and after treatment are summarized in Table 1.

Table 1: Experiment 1: FAECAL WORM EGG COUNTS IN THE CONTROL AND TREATED GROUPS

Group	Average faecal worm egg count (epg) on days before or after treatment						
	−10	−4	0	1	2	4	7
Control	4233	4272	5381	4772	5233	4000	5100
Treated	5338	4850	3290	8200	200	0	0

Throughout the experiment the average faecal worm egg count in the control group remained fairly constant between 4 000 and 5 381 epg.

In the treated group, which had similar average egg counts to the controls prior to treatment, a marked rise was noted the day after treatment, due largely to one sheep which had an increase from 3 600 to 18 200 epg. Only two sheep had egg counts two days after treatment, while all were negative four and seven days after treatment. On the latter two days the average egg counts were calculated from decreased numbers in both groups as some sheep had already been slaughtered.

The worm burdens of the control and treated sheep and the anthelmintic efficacy of the medicated mash are summarized in Table 2.

Immature and adult *Haemonchus contortus, Trichostrongylus* spp. consisting of *T. axei, T. colubriformis* and *T. falculatus, Strongyloides papillosus* and *Oesophagostomum columbianum* and adult *Cooperia punctata, Bunostomum trigonocephalum* and *Trichuris* spp. were recovered from the sheep.

The average anthelmintic efficacy of the mash medicated with thiabendazole was greater than 95 per cent against immature and adult *H. contortus* and *Trichostrongylus* spp., and adult *S. papillosus* and *O. columbianum*. It was 61.8 per cent effective against fourth stage *O. columbianum* and had no effect on adult *Trichuris* spp.

Two untreated sheep each harboured fourth stage *S. papillosus,* while one control and one treated sheep had adult *C. punctata* and two control sheep each had one adult *B. trigonocephalum.* No reliable efficacy figures could be calculated from these small numbers.

The results of experiments 2 to 5 are summarized in Table 3.

Experiment 2

The average faecal worm egg count in the treated sheep was reduced by 97.6 per cent, while the number of animals with faecal worm egg counts was reduced by 95.5 per cent. At the same time, however, the average faecal worm egg count in the controls decreased by 68.5 per cent and the number of sheep excreting nematode eggs by 44.4 per cent.

Worm counts, which were done *post mortem* in some of the untreated sheep, revealed that they were infested with *H. contortus, T. colubriformis, S. papillosus, O. columbianum* and *Trichuris* spp.

Experiment 3

The average faecal worm egg counts in the treated sheep and goats were reduced by

Table 2: Experiment 1: AVERAGE WORM BURDENS IN THE CONTROL AND TREATED GROUP

Number of sheep	Average number of worms recovered at autopsy							
	H. contortus		Trichostrongylus spp.		S. papillosus	O. columbianum		Trichuris
	4th stage	Adult	4th stage	Adult	Adult	4th stage	Adult	Adult
INDICATOR CONTROLS 3.	2433	843	20	580	1447	87	29	2
CONTROLS 9	1863	217	9	529	1950	173	74	5
TREATED SHEEP 8	57	2	0	12	31	66	<1	6
% EFFICACY	96.9	99.1	100.0	97.7	98.4	61.8	99.8	0.0

Table 3: Experiments 2 to 5: FAECAL WORM EGG COUNTS IN THE TREATED AND CONTROL GROUPS

Group	No. of animals	Average epg		% Reduction	No. positive		% Reduction
		Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
Experiment 2							
Treated sheep	98	1037	25	97.6	89	4	95.5
Controls	19	1121	353	68.5	18	10	44.4
Experiment 3							
Treated sheep	91	1192	65	94.5	88	13	85.2
Controls	18	683	772	0.0	15	17	0.0
Treated goats	47	494	6	98.8	41	2	95.1
Controls	19	647	639	1.2	19	18	5.3
Experiment 4							
Treated sheep	28	138	7	94.9	22	1	95.5
Controls	20	187	148	20.9	18	9	0.0
Experiment 5							
Treated sheep	38	250	1	99.6	37	1	97.3
Controls	30	552	672	0.0	30	25	16.7

94.5 and 98.8 per cent respectively. The numbers of the animals excreting worm eggs were reduced by 85.2 and 95.1 per cent respectively.

No reduction in the average faecal worm egg count or number of animals excreting worm eggs occurred amongst the untreated sheep, while only slight reductions occurred in the untreated goats.

Slaughter of some of the untreated goats revealed that they were infested with *H. contortus, T. colubriformis, S. papillosus, O. columbianum* and *Trichuris* spp.

Experiment 4

The average *Nematodirus* spp. faecal egg count in the treated group was reduced by 94.9 per cent and the number of animals excreting these eggs by 95.5 per cent. A decrease of 20.9 per cent occurred in the average *Nematodirus* spp. egg count of the control group, but the number of animals excreting eggs increased by one.

Experiment 5

In the treated sheep, the average faecal worm egg count was reduced by 99.6 per cent and the number of animals excreting eggs by 97.3 per cent. No decrease occurred in the average faecal egg count of the controls, but the number of animals excreting eggs decreased by 16.7 per cent.

Differential faecal cultures showed that the sheep were infested with *H. contortus, Trichostrongylus* spp. and *O. columbianum.*

Experiment 6

The average monthly faecal worm egg counts in the three flocks of sheep and the percentage positive of those sampled, are summarized in Table 4.

Table 4: Experiment 6: FAECAL WORM EGG COUNTS IN THE TREATED, UNTREATED AND NATURALLY INFESTED FLOCKS

Flock	Average epg and percentage of sheep positive during						
	May	June	July	August	September	October	November
Average faecal worm egg count							
Low level	0	1	0	1	0	0	0
Control	0	3	5	18	9	174	451
Infested	545	373	620	965	290	853	971
Percentage excreting worm eggs							
Low level	0	3	0	2	0	0	0
Control	0	5	7	23	15	64	100
Infested	100	100	95	100	100	100	100

In the flock given medicated feed daily, there were positive faecal worm egg counts in June and August due to one sheep in each case. Negative counts were recorded in the other months.

In the untreated control flock a steady but gradual rise in average faecal worm egg counts and the percentage of samples containing nematode eggs occurred. A slight decline in both occurred during September.

The naturally infested flock exhibited an erratic rise in worm egg counts to a peak of 965 epg in August followed by a sharp decline to 290 epg in September, rising rapidly to 971 epg in November. The number of positive samples varied between 95 and 100 per cent.

Differential faecal cultures in the untreated control flock yielded *H. contortus*, *Ostertagia* spp. and *Trichostrongylus* spp. larvae and similar cultures in the naturally infested flock contained *H. contortus*, *Trichostrongylus* spp. and *O. columbianum* larvae.

DISCUSSION

In South Africa, the minimum dosage level of thiabendazole for sheep is 44 mg/kg or 1.0 g per 22.7 kg liveweight. In these experiments the minimum level was increased to 55 mg/kg or 1.25 g per 22.7 kg liveweight. This was done to compensate for the possibility of low feed intake by shy feeders and the less aggressive animals in a flock.

Where feed pellets are used as supplementary feed, the recommended daily feeding rate is 115 g per lamb (22.7 kg) and 230 g per ewe (45.4 kg). Sufficient pellets to supply the daily requirements are usually put out at two or three day intervals; the two day supply is generally consumed within minutes. In accordance with this practice, thiabendazole was incorporated in the feed mash or pellets so that approximately 230 g or 460 g of feed contained sufficient anthelmintic for a single therapeutic dose (55 mg/kg) for lambs or ewes.

Experiment 1

The efficacy of the thiabendazole in the mash was similar to that recorded by numerous workers after the oral or intraruminal administration of thiabendazole to artifically infested sheep[4-7].

Of particular note is the fourth stage *O. columbianum* burden in the control group. To obtain burdens of fourth stage *O. columbianum* slightly larger in number to those found in the control sheep, it is necessary to infest worm-free sheep with 600 to 800 infective larvae[3, 8, 9]. The sheep in this experiment must have ingested considerably more larvae as some harboured relatively large adult *O. columbianum* burdens as well. The immature worms can be considered as the remainder of a considerable, prolonged infestation, retarded in the fourth stage. Further evidence of this retardation is that some of the control sheep had been removed from the source of infestation for 18 days at the time of slaughter and still harboured undiminished numbers of fourth stage worms.

Not only was *O. columbianum* retained in the fourth stage, but similar retardation occurred with *H. contortus* and *Trichostrongylus* spp., both worms with relatively short periods between the third and fourth moult in the normal life-cycle. It would appear as if 'self-cure' was taking place in the case of *H. contortus*, if the high adult burdens in the indicator controls are compared with the burdens in the other controls slaughtered a few days later. It is possible that the alteration in diet assisted this process.

Experiments 2 to 5

Anthelmintic efficacy, based on the percentage reduction in the average faecal worm egg count or the number of animals excreting eggs, was above 85 per cent in each experiment.

The reduction to zero of the egg counts in the treated sheep in Experiment 1, four days after treatment, accompanied by the reduction in worm burdens, indicates that the reduction in egg counts in the later experiments would also be due to the elimination of worms.

In experiment 2, the prolonged feeding of non-medicated feed pellets seemed to be responsible for the decrease in the faecal worm egg count and the number of animals excreting eggs in the untreated control group. For this reason, shorter periods of adaptation to feed pellets were employed in Experiments 3 and 5 and the controls in Experiments 4 and 5 received no pellets whatsoever. The sheep in Experiment 4 were already receiving 13 mm diameter lucerne cubes, but when presented with 5 mm diameter feed pellets they failed to consume the full amount. A more gradual

change-over to the smaller pellets would probably have eliminated this.

The comparable rates of efficacy obtained in Experiments 2 and 5 indicates that storage for 10 months did not affect the anthelmintic efficacy of the medicated pellets.

In experiments 3 and 4 the pellets were fed in troughs, while in the other two experiments they were fed on sacks laid out on the kraal floor. Sufficient space for all the animals to feed is of prime importance.

Experiment 6

This experiment was included as a demonstration of an alternative method of supplying low level anthelmintic medication to the better known method of medicated licks[10, 11].

Although the pastures were irrigated, the untreated control flock became only lightly infested during the winter months, despite the fact that the infested flock was contaminating the pasture continuously. A rapid increase in infestation occurred in the untreated flock in October and November, indicating that the warmer weather created suitable conditions for the free-living stages on the pasture.

In spite of this increase of infestation in the untreated flock, the flock on low-level medication had negative egg counts, except on two occasions, when one animal had a count of 33 epg. Snijders, Stapelberg & Muller[12] have shown that these negative or low egg counts are indeed a reflection of negative or very small worm burdens in sheep receiving daily low-level medication.

CONCLUSIONS

The administration of an anthelmintic by incorporation in feed pellets or mash is particularly applicable to penned sheep or sheep already receiving supplementary feeding. It can also be adapted to sheep that are housed at night by first accustoming them to non-medicated feed similar in shape and feed formulation to the medicated feed.

Sufficient feeding space must be provided and allowance must be made for shy feeders and timid animals by incorporating more anthelminitic in the feed than would normally be required for oral drenching.

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OBSERVATIONS ON RED LICE *(DAMALINIA OVIS)* INFESTATIONS IN SHEEP ON THE TRANSVAAL HIGHVELD

G. F. ZUMPT*

SUMMARY

Observations on large scale infestation by red lice *(Damalinia ovis)* confirm the epizootiological pattern of this disease, namely:

1. that it occurs during relatively mild winter conditions when the preceding summer infestation has reached a certain base level; and in sheep in poor condition, mainly as result of overall nutritional deficiency;

2. that spread is greatly accelerated by large scale direct contact such as occurs in auction yards, and to a lesser degree by indirect contact when uninfested sheep are immediately brought into premises previously vacated by infested sheep.

As the eggs are resistant to the usual insecticide dips, a second dipping 10-14 days after the first, is required. This procedure, coupled with restriction of movement of infested sheep, ensures adequate control.

INTRODUCTION

Infestation of sheep by the red louse is a well-known occurrence in South Africa and the parasite is familiar to most farmers. In the past only sporadic cases of severe infestations were observed and the lice never really created any great problem nor caused economic loss to the sheep industry. Nevertheless, during the winter months of 1969 (March to September) on the Transvaal highveld in the Ermelo and Carolina districts, the incidence of the lice on sheep increased dramatically in number and severity. It is well known, and confirmed by excellent laboratory work in Australia[1], that infestation increases during the winter months, especially if the population on the host is at least two lice per square inch at the end of summer. During the period under review, conditions for high louse infestations had been favoured by comparatively mild winter weather. The grazing on the highveld consists mainly of sour veld which becomes unpalatable in winter. The grazing on the whole at this time was poorer than usual, as little rain had fallen during the summer; the drought conditions had also prevented farmers from growing sufficient feed for their livestock; all these factors had resulted in a poor condition of the sheep. From the observations made, it was clear that animals in such a condition are more susceptible to heavy infestations by the red louse. Infestations of sheep flocks had increased and several severe outbreaks had been reported. This constituted such an economic threat to the farming community, that the condition had to be proclaimed a scheduled disease.

AETIOLOGY

The parasite responsible is referred to as the red louse or red-headed louse of sheep, *Damalinia ovis* (Shrank, 1781) (Phthiraptera: Ischnocera: Trichodectidae). The insect (Fig. 1) can easily be seen with the naked eye when the wool is parted. Studies in Australia[1,2] have elucidated the exact distribution of the parasites in the fleece according to temperature gradients. As winter temperatures prevailed during the present study, lice were mainly found about 1.3-2.5 cm from the skin surface, with very few specimens more than 3.5 cm from the skin.

* State Veterinarian, Ermelo, Transvaal.

As the common name implies, the louse is reddish to light brown in colour. It injures the skin of the host by biting; this results in the secretion of serum, which serves as a source of food for the parasite. Only small quantities of the serum released are actually utilized by the lice; the rest congeals in the fleece, giving rise to the typical matted wool which characterizes severe infestations. In such an infestation, the whole or major parts of the lower half of the body are affected, resulting in difficulties in movement and walking by the affected animal. Although the "scratch symptom", as observed, for example, in sheep scab caused by the mite *Psoroptes ovis,* is not usual, it may occur particularly in severe louse infestations. This results in the partial loss of fleece with a typical "pulled-out" appearance (Fig. 2). Care must be taken not to confuse this with sheep scab, but diagnosis by the recognition of the lice is simple.

EPIZOOTIOLOGY

The parasite normally completes its life cycle on one individual host and is very host-specific. The shortest life cycle recorded is 34 days, in Australia[1]. Oviposition is a slow process and each ovum matures before it is laid. The ova are tightly affixed to the wool fibres, and as a rule are highly resistant to adverse conditions, including dipping. They are quite sensitive, however, to prolonged high environmental temperatures.

Under ideal conditions, the lice can spread rapidly by direct contact between hosts. This is important, especially in kraals, shearing sheds and auction enclosures. It was evident when severely infected sheep came into contact with "clean" animals at the weekly auctions held in the area mentioned. Although indirect contact as a mode of spreading has not been stressed by other workers — the lice being very sensitive to sudden fluctations in temperature — it is believed that this does take place to a certain extent, but then, only if contact of clean sheep with an infected area is almost immediate. It was observed at auction kraals, where sheep were constantly moved into different enclosures as they were sold.

Heavily infested sheep can easily be detected (Fig. 2), but the so-called "carriers" create problems. These animals appear completely normal, but small numbers of lice would be seen if the sheep were to be examined carefully. As a rule, infestation increases during the winter months, but if sheep are healthy and in good condition, the infestations do not become severe. As soon as stresses occur, however, such as insufficient winter feeding, the resistance of the animals is lowered and the louse population increases at an alarming rate. This is what happened in the winter months of 1969 on the Transvaal highveld, when the condition spread rapidly.

CONTROL

Because louse and other ectoparasitic infestations normally do not constitute a major problem in this area, sheep are usually dipped only once a year, i.e. after shearing in mid-summer from November to January. Under normal conditions the single annual dipping is sufficient to keep the parasite population down to a low level (less than 2 lice per square inch).

For the efficient control of *D. ovis* the sheep had to be shorn and dipped almost immediately afterwards, without regard to the state of growth of the wool. Naturally this resulted in considerable economic loss to the farmer, as such fleeces would not realize good prices. Nevertheless, this procedure was followed in order to control the outbreaks under discussion and very good results were achieved. No resistance to BHC dips was observed[4], but many other effective preparations are available which could have been used if resistance to BHC or other insecticides[3] had been a complicating factor.

Re- infestation was observed to be particularly high after the first dipping; this was explained by the fact that the eggs appear to be resistant to the effects of BHC A second dipping, 10-14 days after the first, became standard practice, with highly satisfactory results.

CONCLUSIONS

Most farmers are aware of the dangers and economic loss that will befall them if they fail to control lice adequately in their stock, and they therefore co-operate with the Division of Veterinary Services to de-

crease the incidence of the disease. Problems are encountered with speculators, who tend to spread the parasites by allowing heavily infected sheep to mix with others at public auctions. Fortunately most speculators today realize the importance of control at such places and prevent the marketing of infested stock. I am, therefore confident that the problem will be successfully controlled in the future, and only the occasional sporadic cases will be encountered.

ACKNOWLEDGEMENT

I thank Mr J. A. Ledger, Department of Entomology, South African Institute for Medical Research, for assistance in preparing this paper.

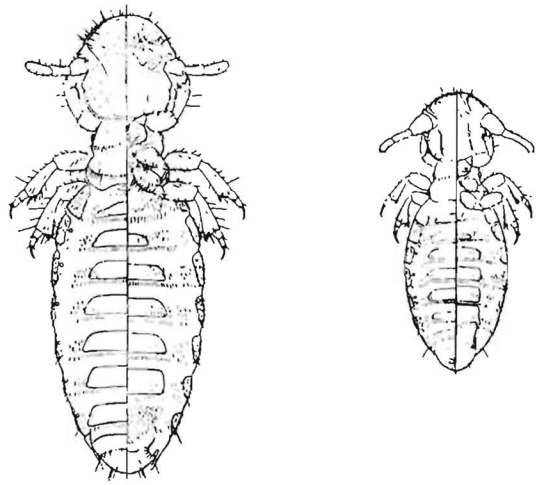

FIG. 1. Female (left) and male (right) of **Damalinia ovis** (after Werneck[5]).

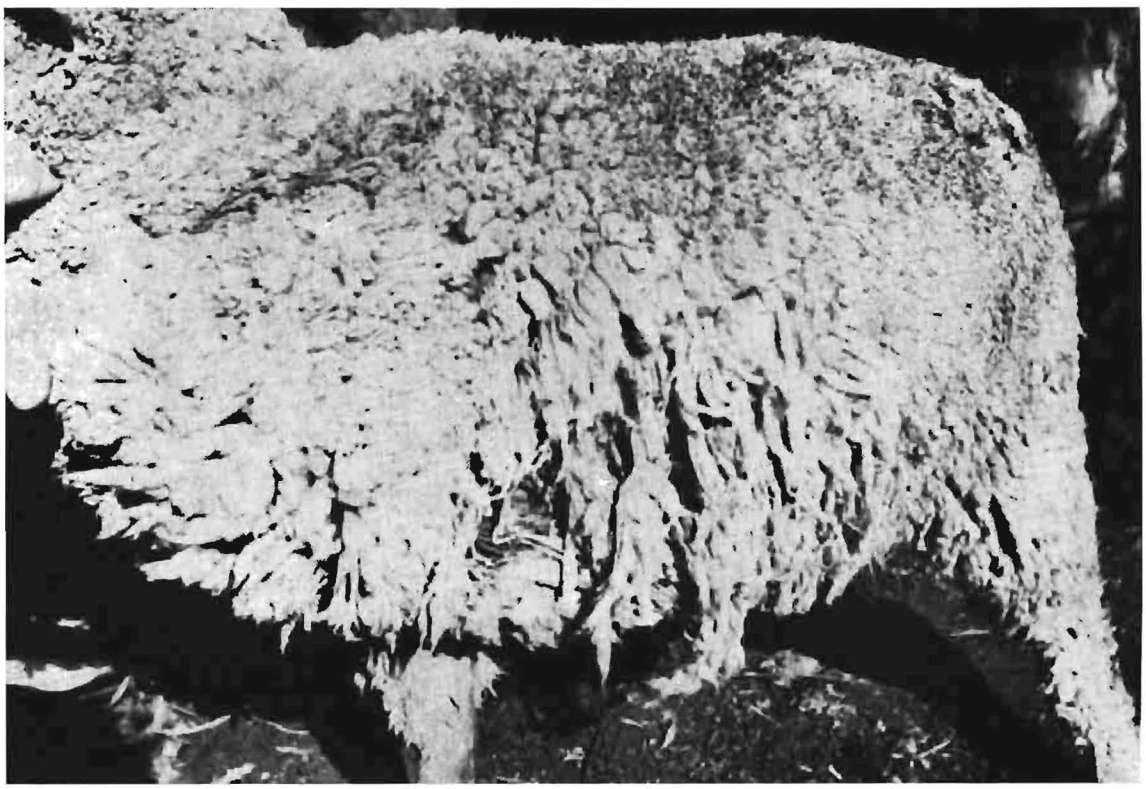

FIG. 2. Appearance of sheep heavily infested with **Damalinia ovis.**

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SOME ASPECTS OF THE MAINTENANCE OF COLONIES OF WILD ANIMALS FOR EXPERIMENTAL PURPOSES*

J. K. THOMSON**

SUMMARY

Colonies of the Cape chacma baboon *(Papio ursinus)*, the Cape hyrax (rock-rabbit) *(Procavia capensis)*, the black-backed jackal *(Canis mesomelas)*, and the caracal lynx *(Felix caracal)*, are maintained for experimental purposes at the Problem Animal Control Research Farm, Vrolijkheid near Robertson, Cape Province. The management of these species is described and discussed.

INTRODUCTION

The Department of Nature Conservation of the Cape Provincial Administration is responsible for the control of various species of problem animals in the rural areas for the Cape Province. Its services are based on the work done on a research farm "Vrolijkheid" situated 10 miles from Robertson, Cape Province, at which research is in progress on the ecology and control methods applicable to animals which cause appreciable damage to the local agricultural economy and at the same time play an important part in the general wildlife ecology. For the necessary research work small colonies of problem animals are maintained either in close captivity or comparative freedom. The species vary according to the current research programme but the Cape chacma baboon *(Papio ursinus)*, Cape hyrax or rock rabbit *(Procavia capensis)*, caracal lynx (*Felis caracal)* and black-backed jackal *(Canis mesomelas)* are of the greatest importance.

BABOONS

The baboons are mostly cage-trapped in the course of control operations on farms, although a few semi-tame animals are obtained through confiscation from owners who keep them in contravention of the legislation for the control of animals classified as vermin. Young baboons soon lose their fear of man and can be handled up to the age of nine months with a fair measure of success; the older ones become unpredictable and every precaution must be taken to ensure the safety of attendants and to prevent escape. This is especially necessary in the case of the larger males, which are extremely dangerous and capable of inflicting severe injury even after extraction of their incisor teeth. On arrival the baboons are generally put into cages of medium size and allowed to settle down for a period of two to three weeks; thereafter they are drafted into the colony, used for experimental work, or paired off for breeding.

Housing

The cages vary in size and shape according to the type of experiment but in the light of our experience, simple rectangular structures standing in the open, either partially roofed or under overall shelter give the best results. Originally, on grounds of economy, these were constructed from treated gum-tree poles and diamond mesh netting and have proved remarkably serviceable. Two guiding principles have proved their worth throughout the years which baboons have been kept at Vrolijkheid. First is the use of cages with a wire mesh floor about 30 cm from the ground surface. This permits frequent and efficient cleaning, easy removal of excreta and discarded fragments of food, resulting in a much higher standard

* Paper read at the symposium on the Production and Use of Laboratory Animals, Council for Scientific and Industrial Research, Pretoria, September, 1970.

** Veterinarian in Charge, Problem Animal Control Research Farm, Department of Nature Conservation, Cape Provincial Administration, Vrolykheid, Robertson, C.P.

of hygiene. Secondly, all doors through which baboons are handled or trapped must be of the vertical sliding type. Such doors must be the same size as the doors of the transporting crates, which must also be vertically sliding, so that the crates can be held in close proximity to the cage and thus prevent any possibility of escape or injury to the attendants. An additional improvement was devised in the form of a catching room inside the cages into which baboon could be enticed or driven with external and internal sliding doors, operated if necessary by remote control.

In recent years multi-purpose, perforated angle iron has become available as a proprietary product. It is, I believe, intended for shelving in big commercial stores and is issued with both bolts and nuts as in the case of a well-known constructional toy. Experiments have been carried out in cage construction and it has been found highly suitable: the size of the cages can be varied without expensive cutting tools. Large cages, 5m × 2.5m × 2m, have given excellent results with groups of six baboons over long periods.

Experiments have been tried with electric fencing. The results have been disappointing and baboons appear to be resistant to shocks which would deter cattle from touching the wire.

Baboons have kept in remarkably good health in these open air cages, although temperatures vary from 10°C to 38°C. They are protected from the prevailing wind by corrugated sheeting on two sides of the wall of the cage. Their good condition is attributed to the limitless supply of pure air, the easily controlled hygiene and, although this cannot be proved, the fact that they can look out over long distances. Baboons both in nature and in captivity spend long periods gazing over wide spaces and prolonged observation at Vrolijkheid has led to the conclusion that this is of psychological value to these animals. When they are kept under such conditions they appear to be much more contented than if kept in a carefully regulated environment surrounded by four walls. The psychological aspects of captivity play a very important part in the well-being of captive animals and should receive as much attention as diet and cage dimensions, if colonies are to be maintained under optimum conditions, especially if they are going to breed in confinement.

Handling

Small crush crates are essential. These permit easy administration of intra-muscular immobilizing drugs or tranquilizers such as phencyclidine. These crates are rectangular wth a moveable partition operated from the outside which holds the animal firmly against the end of the cage. Baboons are easily caught in game nets if the latter are allowed to hang loosely. Baboons climb over a tight net but apparently lose their heads and are completely baffled by a loose game net with a large mesh.*

Feeding

Feeding has never been a great problem: a diet of mealies and pumpkins varied with a proprietary balanced ration (dog food) has given excellent results if supplemented by a pint of milk per day. The average female receives about 450 g of dry food per day and large males up to 900g. The weight-range of adult females is from 10 to 13 kg and 20 to 30 kg in the case of males. Exceptionally large males weigh up to 35 kg. Baboons will eat meat and on occasion seem to prefer it. Numerous cases of lamb-killing by outcast males are reported to this Department. The baboons kill young lambs and goats, devouring the meat and internal organs with special preference for the curdled milk in the stomach. They also eat caterpillars with the greatest relish when plagues of these larvae infest the trees on the farm.

Breeding

It has been found that although sexual activity is not affected by capture and detention, conception does not readily occur until the animals settle down. Once this period has passed, breeding is fairly regular, although the conception rate is not as high as it appears to be in the natural state. The gestation period is generally about 217 days and lactation continues for about four months, although longer periods have been observed. It has been recorded that some baboons are indifferent mothers[1] but this has not been our experience at Vrolijkheid.

Disease

Baboons have remained remarkably free from disease at Vrolijkheid.

* Personal communication from Mr. John Clark, Natal Parks Board.

General behaviour

It is essential to group baboons according to age. If this is not done, the weaker baboons are dominated by their seniors. Both in captivity and in the natural environment, the male baboon is probably one of the most unchivalrous wild animals in existence, and woe betide any younger member of the troop which attempts to feed within the elder's reach before the leader's wants are satisfied. Females receive very harsh treatment in similar circumstances. This can only be accounted for by the suggestion that the safety and survival of the troop, especially when such predators as leopard are a threat, depend largely on the presence of a large male literally in fighting condition. These factors must all be taken into consideration when the movement of the baboons is limited in any way. It is a pity that electric fencing has not given better results, as with a greater area a more natural pattern of life with better breeding results would be possible.

CAPE HYRAX

Hyraces *(Procavia capensis)* have been kept in colonies for over 9 years. The Cape hyrax is a slow breeder, with a gestation period of 225 days; from the examination of a large number (281) of females in 1968, the average number of foetuses per female was found to be 2.3[2].

These animals are generally kept in artificial rock habitats built in small enclosures surrounded by wire netting. The hyrax is a very agile climber and it was amusing to discover that a small group climbed up a drain-pipe about six feet high during the evening and invaded a lucerne patch for some hours prior to a voluntary return to captivity. The poor temperature adaptation of this species, coupled with their fear of human beings, are factors which complicate their maintenance in captivity.

During experiments, they are usually kept in cages 50cm × 50cm × 50cm in experimental rooms and keep in good bodily condition much better than in the comparative freedom of artificial habitats. Their natural timidity and readiness to leave food for cover may be responsible for this*, as they take refuge in rocks at the slightest provocation. The hyrax camps are now on a new site. Formerly they were surrounded by walls; it has been suggested* that screens may give them a greater sense of security.

* R. P. Millar, personal communication.

Handling

The metal tube "dog catcher" with a loop of cord is invaluable, as these animals can inflict a vicious bite. Marking provided a problem until the numbered wing bands for young chickens were used as ear-tags.

Disease

Hyrax are susceptible to a contagious pneumonia and a haemolytic streptococcus has been isolated from lung lesions. They are also invariably heavily infested with ecto- and endo-parasites when captured and are obviously able to tolerate these stress factors to a marked degree without any noticeable deterioration in condition. Ecto-parasites should be controlled by dusting powders under artificial conditions.

Diet

Lucerne provides a satisfactory ration in quantities of 200 to 300 g per head per day.

JACKAL

The black-backed jackal *(Canis mesomelas)* is kept for control experiments.

Housing

In general, jackal are kept in large camps or enclosures of up to a hectare in extent, surrounded by netted wire fencing with a "verandah" i.e. a horizontal wire attached to the top, as jackals are adept at running up a fence and climbing over. Gates must be carefully netted. There is no foundation in the belief that a grid type of cattle gate will keep jackal in. Jackal will detect an escape avenue quicker than any other animal.

Handling

They are either cornered and caught in box traps or with a sack and the attendants must wear leather gloves. The "dog-catcher" can also be used. A noticeable feature of captivity it the fact that they soon lose their fear of hounds and come up to the wire to grin at the packs as they go out to train. I may mention that the captured jackal are seldom used in hound training, as not only do they lose their fear of hounds, but they also loose stamina and cunning and have very little chance of extending hounds. This has a deleterious effect on the latter.

Breeding

Jackal breed fairly well in captivity but they do not rear the pups well; the adults either

neglect or kill them for no apparent reason. This may be due to the presence and scent of humans. As in the case of the ferret and other species, the female will kill the litter if they are handled.

Disease

They are of course susceptible to rabies but no cases have occurred as it is not enzootic in the area. During one year, fatal distemper occurred in the jackal at Vrolijkheid. Apart from this, disease has been no problem.

Feeding

In nature the diet is highly variable, from the carnivorous to the insectivorous; they do eat grapes at the appropriate season, hence the biblical allusion. In actual practice 500 g of meat daily will keep a jackal in excellent condition. In nature they will require more, as they travel long distances in search of food. Offal, unwashed stomach and intestines are relished and are useful variants to the diet.

CARACAL LYNX

The caracal lynx *(F. caracal)* is kept for observation and control experiments. Slightly larger than the jackal, it is very vicious and dangerous to handle. It is fairly easy to catch in cage traps but must be handled with extreme care. It does well in camps but these must be roofed to prevent escape.

Feeding

When initially captured it may be necessary to give them live rabbits to kill, otherwise they may refuse food. Meat must be fresh — freshly killed carcasses with the skin on are preferred; 500 to 700 g will keep them in good health.

Breeding

These animals do not breed well in captivity and are prone to devour the kittens. Lynx kittens are very susceptible to enteritis but an effective solution to this problem was found[3, 4]. An ordinary farm cat was given lynx kittens to rear with her own kittens and made a superb success of this task, even teaching them to hunt rats and mice. It is advisable to take away two domestic kittens for each lynx, as the latter at a few weeks are nearly as big as the mother. This has been repeated with great success.

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RESEARCH NOTE

AN APPROACH FOR THE STUDY OF DRUG DISTRIBUTION ACROSS RUMINAL EPITHELIUM *IN VIVO**

W. L. JENKINS** AND L. E. DAVIS***

SUMMARY

An experimental model is described which proved to be useful for the study of drug distribution across ruminal epithelium *in vivo*. The approach is based on the occlusion of both potential outlets from the ruminoreticulum and the establishment of a closed and continuously mixed system.

INTRODUCTION

There exists a paucity of basic pharmacodynamic and pharmacokinetic studies in the ruminant animal[1]. The presence of a complex and voluminous hollow organ such as the ruminoreticulum as a component of the gastro-intestinal tract might well be expected to influence the absorption, distribution and excretion of any drug administered by practically any route. Furthermore, posology in domesticated ruminants presents a problem, as up to 20 per cent of the body weight may be attributable to the ruminal contents[2], while the forestomach may or may not constitute a distribution compartment for a particular drug.

In our investigations on the exchange of drugs across the ruminal epithelium it became essential to prevent any inflow or outflow from the rumen during a 6 to 10 hour experimental period. In addition, it was highly desirable to establish a permanent preparation which would allow the utilization of fully conscious, normal animals in repetitive experiments. The methods previously employed in the study of ruminal absorption[3] were unsatisfactory for our purposes and an apparently new approach was adopted.

METHOD

Double ruminal cannulae and an oesophageal cannula were introduced into goats by standard surgical procedures. The material employed to fashion these cannulae was unvulcanized silastic sheeting, .06″ thick and reinforced with dacron mesh[4]. These animals were fed a commercial pelleted feed and finely ground hay. The experimental model was prepared as follows. The ruminoreticulum was thorougly washed out with warm water (about 39°C) introduced through the ruminal cannulae and then completely emptied. A balloon attached to a polythylene tube in the form of a cuff, which resembled a thin modified Magill tube in design, was introduced into the reticulo-omasal opening by manipulations carried out through both ruminal cannulae and by using an elongated endoscope as a light source. Following inflation of this balloon, a second balloon was inserted into the rumen via the oesophageal cannula, and inflated. By careful withdrawal, the cardia and terminal part of the oesophagus were occluded. A suction catheter was also passed through the oesophagael cannula: the saliva which accumalated was removed in this manner. Prevention of the contamination by saliva of the fluid in the rumen obviated a possible source of error, as some drugs are known to be excreted in ruminant saliva[5]. Finally, between 5 and 7 litres of a buffered

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Krebs-Ringer phosphate solution, containing .001 per cent phenolsuphonphthalein as a volumetric indicator[6], were allowed to run into the empty ruminoreticulum and this fluid was then continuously circulated extracorporeally at a rate of one litre per minute. The final arrangement is diagramatically illustrated in Fig. 1. Any leakage past the balloon into the oesophagus could be observed and corrected immediately, while the intactness of the omasal balloon was periodically verified by aspiration at the end of the appropriate tube. At the conclusion of an experiment the physiological solution was pumped out of the rumen and replaced by 2 to 3 litres of a suspension of food in warm water and 1 to 2 litres of dilute ruminal liquor freshly collected from the rumen of a mono-fistulated goat which had been receiving the identical ration. Normal ruminal function appeared to be re-established within 24 hours in most cases.

USE

This fundamental experimental approach was used for bidirectional flux studies. Specific compounds were added to the buffered physiological solutions and their rate of disappearance from the rumen, recorded. Concentration and pH dependent effects were demonstrated and the concept of the nonionized fraction of a drug in solution traversing a membrane by simple diffusion seemed to be confirmed[7]. In the above case no application of the diffusion equation could be made, because the loss of absorbed compound by biotransformation, excretion, etc. precluded a steady state distribution. In the

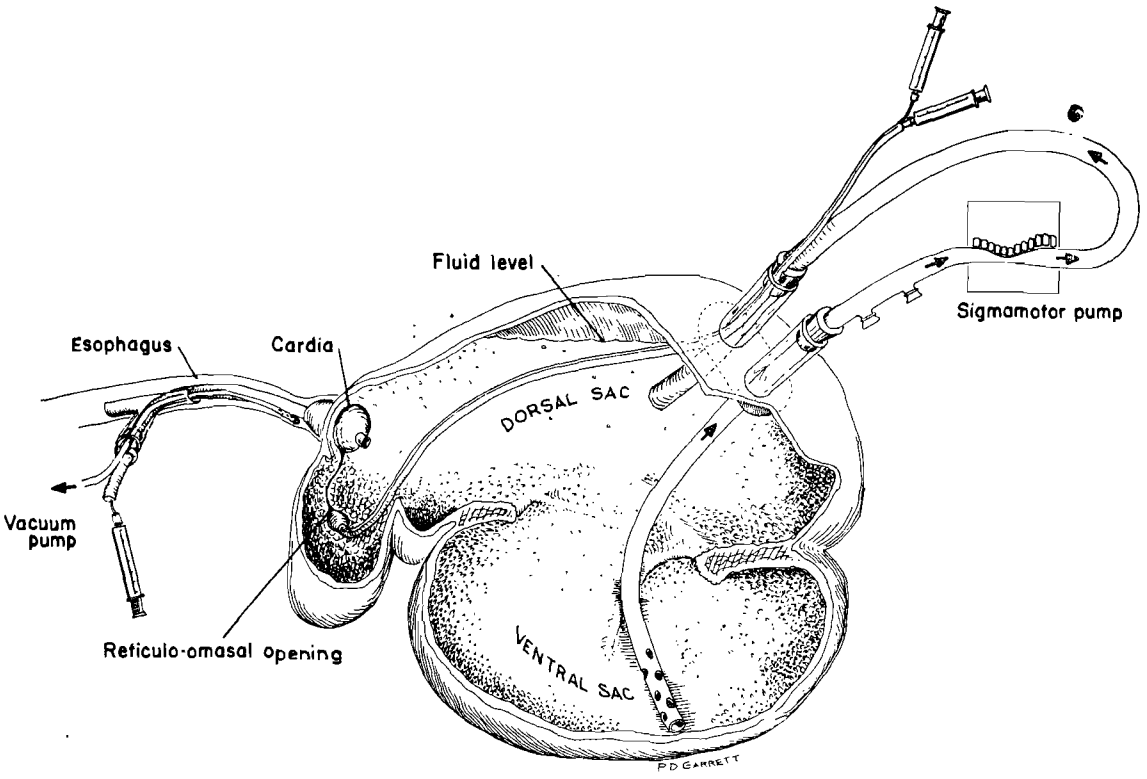

FIG. 1. Final experimental arrangement illustrating the occlusion of both potential outlets and the establishment of a closed but continuously mixed system.

investigations involving drug transfer into the rumen, however, it was possible to maintain constant plasma levels[8,9] of the compounds used for periods of 6 to 8 hours, and thus to establish an evenly balanced distribution (steady state) which permitted the calculation of the relative rates of drug transfer and the permeability coefficients[10] for the ruminal epithelium of goats.

CONCLUSION

We feel that this *in vivo* model could be employed with equal success in quantitative studies on the absorption of normal nutrients and end-products of microbial fermentation from the ruminant forestomach.

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BOOK REVIEW

TROPISCHE TIERSEUCHEN UND IHRE BEKÄMPFUNG

E. Mitscherlich and K. Wagener

Paul Parey, Berlin/Hamburg, 1970. Pp 383, 73 illustrations, 17 colour plates and 23 tables. Price: DM 86.

In the foreword the authors state that their aim has been to provide veterinarians, students of veterinary medicine and agriculturalists with a review of tropical diseases of animals. In this they have certainly succeeded. Although the specialist will not find everything he wishes to know in this book, he can also make good use of it.

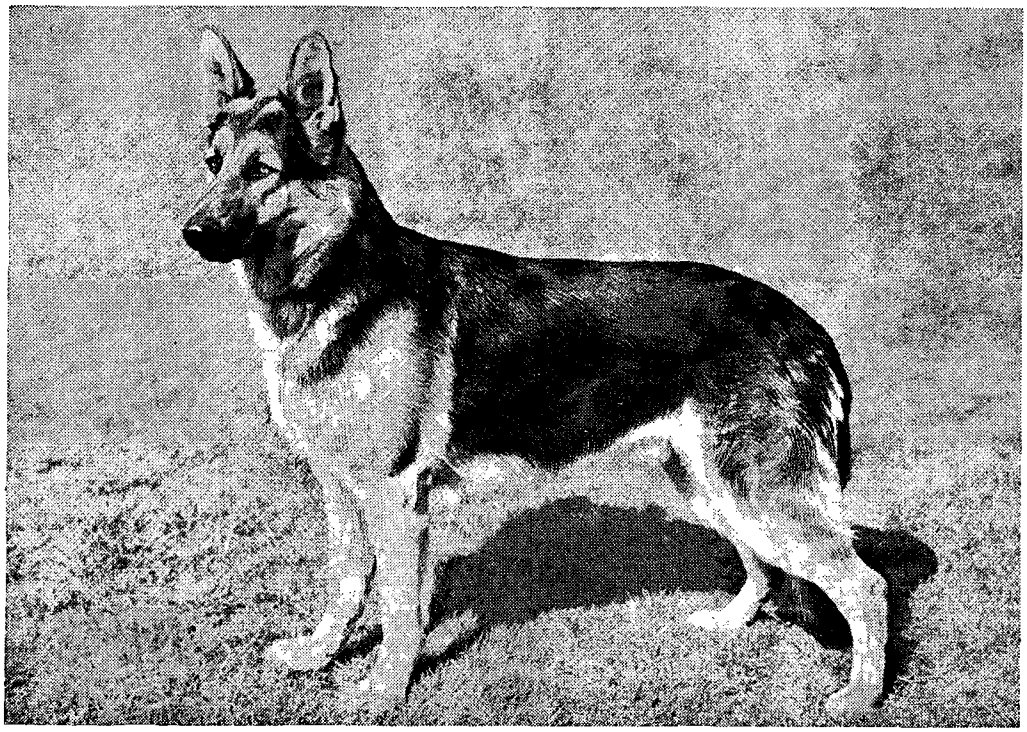
The writers have intentionally concerned themselves only with diseases confined to tropical and subtropical regions, and have, with few exceptions, excluded diseases that also occur in temperate zones, which has limited their scope somewhat. A disease like besnoitiosis might well have been included.

The book has a comprehensive index and the typography, illustrations and colour plates are generally of a high standard. The subject matter is divided into two major sections. In the first, the role played by wild animals, ticks and insect vectors in the epizootiology of tropical diseases is outlined. The second section deals specifically with the aetiology, epizootiology, symptomatology, necropsy findings, diagnosis, treatment and prophylaxis of tropical diseases of animals caused by protozoa, anaplasmas and related organisms, rickettsias, bacteria, fungi, mycoplasmas and viruses. Finally there is an appendix listing a few useful laboratory techniques.

With few exceptions, like the perpetuation of the antiquated description of the structure of the kinetoplast and the incorrect definition of crithidial forms of trypanosomes, the text is abreast with recent developments. Typographical errors are rare. The Afrikaans names of diseases are, however, consistently misspelled and bluetongue is incorrectly referred to as "Vuil bek". A comprehensive list of references appears after the discussion of each disease.

This book will undoubtedly be of great value to German veterinarians and scientists who have to deal with tropical diseases.

R. D. B.



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SERUM TRANSFERRIN TYPES IN HEALTHY MERINO RAMS AND IN THOSE AFFECTED WITH EPIDIDYMITIS

D. R. OSTERHOFF*, HELGA G. KASSIER* AND I. S. WARD-COX*

SUMMARY

An attempt was made to correlate the genetically variable ironbinding proteins, the transferrins, in Merino rams with the degree of resistance to infectious epididymitis caused by three types of organisms, *Brucella abortus, Brucella ovis* and *Actinobacillus seminis*. Transferrin types were determined in 2 230 serum samples of Merino rams; 512 of these samples were recorded as being either positive or suspicious to one or more of the serological tests for the three types of organisms. No correlation between specific transferrin phenotypes and positive or suspicious bacterial tests could be established.

INTRODUCTION

The importance of polymorphic variants in veterinary investigations has been realized during the past few years[1]. Genetically controlled polymorphism in ovine transferrin was first reported by Ashton[2, 3]. Since then the results of further investigations have been published by workers in different countries[4-12]. In 1966, the European Society for Animal Blood Group Research held an international sheep transferrin standardization trial (Istst)[13], the results of which indicate the presence of at least nine transferrin zone pairs in the electrophoretic pattern. On the basis of this trial a final nomenclature, mainly based on Ashton's original suggestions[2], was accepted; this is followed in the present paper. Recently, however, several new transferrin alleles have been found in Hungarian Karakul and fine-woolled Askanian Merinos[14].

In 1968, epididymitis in a Dorper ram was described with the subsequent isolation of *Actinobacillus seminis* from the ram's semen[15]. At the same time, *A. seminis* was isolated independently from semen of 21 rams on seven properties[16]. Nothing was known of the incidence or economic importance of this condition in South Africa and a survey was therefore performed to obtain a complete picture of the seriousness of the situation.

In the present paper an attempt was made to correlate specific transferrin phenotypes with the presence or absence of any of the organisms being responsible for infectious epididymitis.

MATERIALS AND METHODS

More than 12 000 serum samples were collected in different regions of the Karoo and the incidence of epididymitis and other abnormalities of the testes and accessory glands was correlated with the results of the serological tests. The tests for *Actinobacillus seminis* and for *Brucella abortus* and *Brucella ovis*, fully described elsewhere[15, 17], were performed in the section of Bacteriology of the Veterinary Research Institute. The titres for all three tests were evaluated as follows: 1/10 = negative; 1/20 = suspicious; 1/40 and 1/80 = positive. The complete results of the investigation, including the actual correlation between serological test results and the incidence of abnormalities of the sexual organs, will be published shortly by Worthington and van Tonder.

Of the serum samples mentioned above, 2 230 were available for the investigation presented here. These formed a representative random sample since they originated from different farms in the following magisterial districts: Graaf Reinett, Philipstown, de Aar, Colesberg, Victoria West, Hanover, Britstown, Richmond and Murraysburg.

Zone electrophoresis was carried out in the manner described by Kristjansson[18]. Samples of serum were adsorbed onto filter paper (Whatman 3MM) and were inserted in incisions 5 cm from the cathodic end of the gels. The inserts were removed after

* Dept. of Zootechnology, Faculty of Veterinary Science, University of Pretoria, P.O. Onderstepoort,

applying 165 *v* for 10 min. Electrophoresis was then continued at 300 *v*, until the borate boundary had migrated about 12 cm beyond the origin. The gels were sliced, stained with amido black and destained in methanol, water and acetic acid at 5:5:1 parts respectively. Reference samples from the ISTST were available and used in each electrophoretic run. It was found that the BD-control was the most useful one, because all other phenotypes could then easily be recognized. Difficulties were encountered in the electrophoresis of the last third of the serum samples because of the protein deterioration which had taken place during the storage of samples at room temperature over a period of four to five weeks. Under these conditions bacterial contamination may bring about a loss of sialic acid residues with a consequent decrease in electrophoretic mobility. To ensure correct identification many sera were retested by repeated electrophoresis with fresh sample controls migrating on either side of the samples in question.

RESULTS

The frequencies of the transferrin alleles, together with the number of rams tested, are given in Table 1 for the different areas respectively.

A few special transferrin types were also found; these could not be grouped according to the existing nomenclature and were called phenotypes E_2 and P_2 because the bands migrated somewhat slower than the known E and P bands. Examples of the less frequent types are given in Fig. 1.

These special types could be the result of mutations not seen before or the result of decreased electrophoretic mobility due to bacterial contamination. The latter was clearly shown in the samples tested towards the end of the testing period and examples are given in Fig. 2.

All samples giving doubtful results at initial electrophoresis were retested and required to migrate between the corresponding fresh controls to ensure the correct typing.

In the electrophoretograms of 7.6 per cent of all samples, abnormal migration rates of the slow alpha globulins were observed. In Fig. 1, the second sample from the left is called the FF-type (fast homozygous) and the sample on the extreme right is called the IN-type (intermediate-normal heterozygote). Of the abnormally migrating 169 samples, 31 per cent belonged to the FF-type, 57 per cent to the IJ-type and the remaining to the FI and IN types. It was interesting to note that this abnormal migration coincided mainly with the slow-migrating transferrin types; the abnormally slow alpha types were associated in the following way with the different transferrin alleles: Tf^A 9.2%, Tf^B 11.5%, Tf^C 12.4%, Tf^D 20.7%, Tf^E 24.9% and Tf^P 21.3%. Since the frequencies of the transferrin alleles differ widely—see Table 1—the association with these alleles became significantly clear: Only 2.6% of all Tf^A-alleles were conjugated to the abnormally slow alpha types, 9.3% of all Tf^B-, 4.4% of all Tf^C-, 4.2% of all Tf^D-alleles but 53.2% of all Tf^E- and 72.7% of all Tf^P-alleles. Further work, however, is necessary to prove

Table 1: FREQUENCIES OF THE DIFFERENT TRANSFERRIN ALLELES IN DIFFERENT MAGISTERIAL DISTRICTS IN THE KAROO

District	No. of rams tested	TRANSFERRIN ALLELES							
		Tf^A	Tf^B	Tf^C	Tf^D	Tf^E	Tf^P	Tf^M	Tf^G
Graaff-Reinet	438	.214	.073	.256	.347	.063	.039	.008	—
Philipstown	336	.313	.111	.164	.389	.021	.002	—	—
De Aar	189	.339	.140	.185	.307	.019	.010	—	—
Colesberg	223	.226	.102	.193	.352	.061	.054	—	.012
Victoria West	286	.219	.047	.267	.446	.012	.009	—	—
Hanover	152	.180	.105	.180	.431	.066	.038	—	—
Britstown	211	.313	.083	.185	.400	.014	.055	—	—
Richmond	111	.339	.114	.217	.330	—	—	—	—
Murraysburg	284	.288	.097	.193	.347	.044	.031	—	—
Total	2230	.262	.094	.212	.372	.035	.022	.002	.001

the genetic background of the association between these protein types.

Altogether 512 serum samples gave either positive or suspicious reactions to the three serological tests; the complete results are given in Table 2.

From the positive group nine rams and from the suspicious group 18 rams reacted

EXAMPLES OF SHEEP TRANSFERRINS — LESS FREQUENT PHENOTYPES

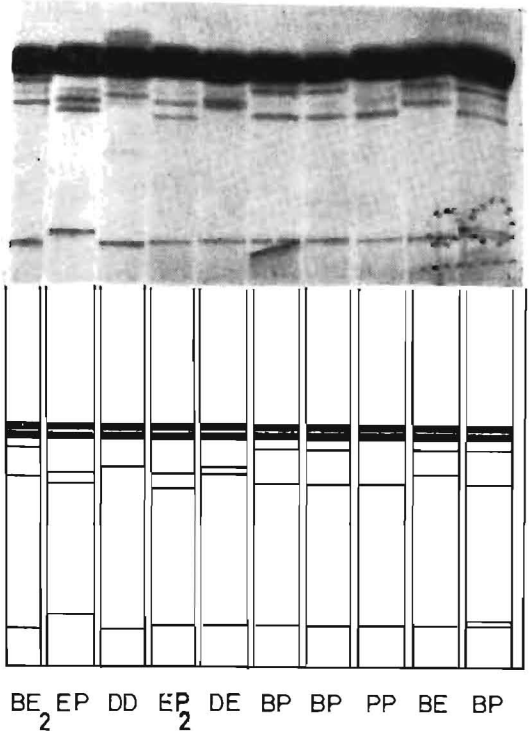

FIG. 1

in more than one test. These animals thus appear twice in the positive or suspicious group.

The groups were combined for calculation of gene frequencies so as to ensure sufficient numbers and because one may reason that the suspicious animals are, in fact, showing serological reactions. In Table 3, the transferrin gene frequencies of positive reacting rams are compared with negatively reacting samples.

COMPARISON OF TRANSFERRIN PHENOTYPES — FRESH AND PARTLY DENATURIZED SAMPLES

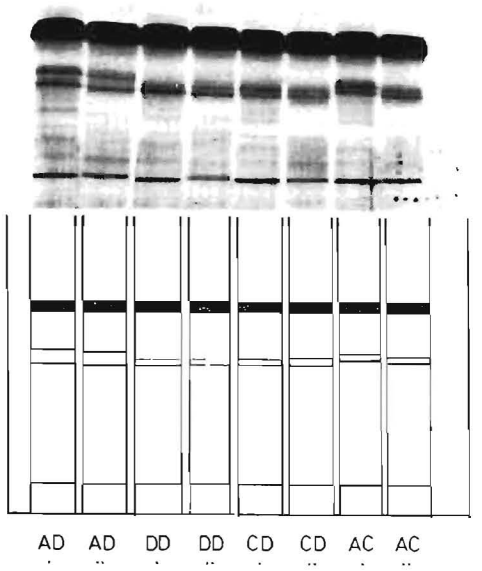

FIG. 2

Table 2: NUMBER OF RAMS REACTING IN THREE SEROLOGICAL TESTS

Infection	Positive	Suspicious
Br. abortus	87	81
Br. ovis	51	54
Act. seminis	80	159
Total	218	294

DISCUSSION AND CONCLUSIONS

From the results presented in Table 1 it appears that no clear differences exist in the transferrin gene frequencies of different magisterial districts. The material may be regarded as a random sample of the Karoo Merino population, but no environmental influence, which would in any event be slight, could be established. The gene

Table 3: TRANSFERRIN GENE FREQUENCIES OF SHEEP WITH POSITIVE/SUSPICIOUS (P/S) REACTIONS TO THREE DIFFERENT SEROLOGICAL TESTS

INFECTION	No. of reacting animals P/S	TRANSFERRIN ALLELES					
		Tf^A	Tf^B	Tf^C	Tf^D	Tf^E	Tf^P
Br. abortus	168	.245	.070	.195	.422	.042	.026
Br. ovis	105	.291	.120	.177	.366	.034	.012
Act. seminis	239	.269	.079	.211	.395	.032	.014
Negative samples	1745	.270	.096	.214	.362	.038	.022

frequencies obtained compare well with those obtained in Merino sheep by other workers[4, 9, 10].

In the Chi square analysis of the total material an excess of 159 animals possessing the genotypes Tf^A/Tf^A and Tf^C/Tf^C was found, whilst the expected heterozygotes Tf^A/Tf^C amounted to 83 animals fewer than than the numbers observed. Statistically significant differences between the expected and observed numbers, according to the Hardy-Weinberg equilibrium, were found in almost every district: this was mainly due to an excess of the homozygotes Tf^A/Tf^A and Tf^C/Tf^C and a deficiency of the heterozygotes Tf^A/Tf^C. Technical errors have to be excluded as a possible reason; further studies are indicated, since certain authors report on the heterozygote advantage in sheep and also in cattle[2, 8, 19, 20]. It is of interest to note, furthermore, that no deviations of the expected from the observed numbers were encountered in the other alleles.

The slow alpha globulin variation is most interesting and has not been reported before. Studies of the abnormal phenotypes should be performed in families to verify the mode of inheritance of these types.

The transferrin gene frequencies of the samples reacting positively and suspiciously to the serological tests for three types of organism did not differ from the transferrin gene frequencies of the normal controls. The gene frequencies were also calculated separately for the positive and suspicious samples, but the results do not add any new information and are therefore omitted. There is thus no difference in serum transferrin types in healthy Merino rams and in those affected with epididymitis, and predisposition of certain genotypes to bacterial infections could not be established. A discussion of the reliability of the serological tests for the detection of epidiymitis is beyond the scope of this paper. Furthermore. the geographical distribution of the positive rams will not be discussed since the results of the full investigation will be reported on in due course.

ACKNOWLEDGEMENTS

Mrs. M. S. G. Mulders and Dr. R. W. Worthington, Section of Bacteriology, Veterinary Research Institute, are thanked for making the sera and the results of their serological tests available to the authors for this investigation.

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BOOK REVIEW

BOVINE TUBERCULOSIS CONTROL IN MAN AND ANIMALS

J. ARTHUR MYERS AND JAMES H. STEELE

Warren H. Green, Inc., St. Louis, Missouri, U.S.A., 1969, p.p. 403. Price: $19.50

The volume consists of two individual contributions made by Dr. Myers, professor of Internal Medicine, Minnesota, and Dr. Steele, Chief of Veterinary Public Health in the U.S. Public Health Service. The first section by Myers is a shortened version of his book "Man's Greatest Victory Over Tuberculosis", (1940), and is devoted to the eradiction campaign of bovine tuberculosis in the U.S.A. from 1917-1940. It deals with the veterinary organization of the U.S. TB control programme, the epidemiological aspects, especially infection of man by *M. bovis,* the economics of the campaign, the aetiology, diagnosis and prevention of TB in cattle, and TB in animals other than cattle. It contains a strong plea to the medical profession to co-operate with the veterinarians.

The second section by Steele is a comprehensive description of the world situation as far as bovine TB is concerned, and the infection of man by *M. bovis.* The different chapters deal with more than 80 countries. How difficult this is in a continent like Africa, is shown in that only 8 of over 40 states are covered. The Americas, Europe and Australia are rather complete. The facts about South Africa are partly based on old information, but S.A. research work on the control of bovine TB by isoniazid chemotherapy is described in both sections of the book, and in the conclusion as part of the third report of the FAO/WHO Expert Committee on Zoonosis.

The book does not cover pathology, bacteriology or clinical symptoms, and gives little about the tuberculin test itself. Non-specific reactions are discussed in the chapters on Kenya and Australia.

There are the frequently-found mistakes in terminology, like mycobacterial dermatitis nodosa being called "skin TB" *M. intracellulare* is referred to as "Battey strains of *M. tuberculosis*", and *M. paratuberculosis* is called *"M. johnei"*.

The book is well printed, and contains many references and valuable tables.

The book makes available a large amount of information on developing countries so far never accumulated in one volume —and it can be highly recommended to the epidemiologist and as a reference work. It is amazing to see the differences between a country like the U.S.A. where 470 million tuberculin tests were done on cattle between 1917 and 1967, and other countries where the disease in cattle is still rampant and the transmission to man quite common but little or nothing is being done.

H. H. K.

UNIVERSITY OF EDINBURGH

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BOOK REVIEW

VETERINARY RADIOLOGICAL INTERPRETATION

S. W. Douglas and H. D. Williamson

London; William Heinemann Medical Books Ltd., 1970. Pp. viii & 303, Figs. 364.

Price approx. R9.

The authors' previous work "Principles of Veterinary Radiography" is well known and has proved itself an invaluable practical guide. The book under review complements their previous book in that it instructs the reader in the interpretation of radiographs.

The book is unique in veterinary radiological literature in so far as emphasis is placed on the general radiological recognition of pathological evidence.

A number of common pathological conditions are described and illustrated with reproductions of radiographs but the authors have purposely refrained from compiling a comprehensive atlas of radiological pathology of which several good ones already exist.

By way of introduction, the reader is briefly familiarized with radiographic factors which can influence interpretation of radiographs, and the correct procedure for examining radiographs is outlined.

The remainder of the book consists of two roughly equal parts, respectively dealing with the radiology of the skeletal and soft tissue systems.

After describing the detailed structure of normal growing and mature bone and tabulating the sequence of epiphyseal closure in the dog, the general radiological evidence of pathological changes applicable to bones and joints is described. Changes like osteoporosis, new bone formation and sclerosis are discussed. Specific pathological conditions e.g. neoplasia, osteomyelitis, dietetic deficiencies, osteo-arthritis and septic arthritis then receive attention.

The section on skeletal radiology also includes chapters on the normal anatomical features and common pathological conditions of the skull, vertebral column, ribs, limb bones and joints, and concludes by a short chapter on large animal radiology.

The section dealing with the radiology of the soft tissue systems includes chapters on the respiratory, cardio-vascular, alimentary, genital, urinary, muscular and central nervous systems.

The book is printed on good quality paper, is properly indexed, profusely illustrated with well-chosen radiographs and contains adequate references.

Criticism of the factual content of the book can be limited to a few minor points. The radiograph chosen to illustrate normal hip joints (Fig. 6.21) is not convincing, especially when examined in the light of the very next illustration, which depicts the parallel lines formed by the femoral head and the acetabulum of a normal hip joint. Should Norberg's Scale not be used to measure the depth of the acetabulum — with the limbs in the flexed position—instead of using it to demonstrate displacement of the femoral head in the extended position (Fig. 6.34)? Codman's triangle cannot be considered as typical of malignant bone neoplasia only according to texts on human radiology. A notable omission from the chapter dealing with cardiovascular radiology is the work of Wyburn and Lawson on measurement of cardiac enlargement from straight radiographs. Finally, the reviewer is of opinion that simpler terminology, like increased or decreased density, is preferable to terms like sclerosis, osteolysis and osteoporosis.

In a book covering such a wide field, the same high standard cannot be maintained throughout. When compared with the outstanding chapters on intra-thoracic structures, the superficiality of the discussion of basic bone pathology and its radiological interpretation is somewhat disappointing. Since the subject of bone pathology lends itself admirably to classification and systematic radiological examination, a more comprehensive, coherent and detailed discussion

appears desirable. Much more could have been said, for instance, about three of the basic radiological changes of bone, viz. increased and decreased density and new bone formation, their causes, underlying pathology and the diagnostic significance of the various known patterns shown by them.

Furthermore, an understanding of this section can be considerably enhanced by discussion of the pathogenesis of conditions like osteomyelitis and avascular necrosis of the femoral head, and correlating the different phases of the underlying pathology with the various radiological manifestations in these conditions. With the same end in view, explanations of relative and absolute increases or decreases in density, tumour- and reactive new bone, dystrophy and dysplasia, sequestrum and involucrum, etc., can help considerably. The difficult subject of bone dystrophies also calls for more extensive discussion.

This is an excellently produced and instructive book which can be recommended to students and practitioners.

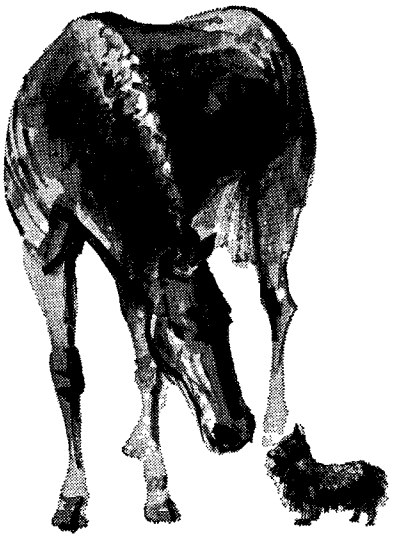
C. J. R.

STATISTICS ON HOUSEHOLD PETS

"A SURVEY OF EUROPE TODAY"

This massive document of 212 pages, gives 55,000 statistics on household possessions and other demographics, plus opinions and attitudes on many of today's trade-related questions, covering all 16 of the countries making up the European market of 320 million with an estimated spending power of R275,000 million a year. It includes statistics on household pets, their numbers, exports, pet food bought (both tinned and fresh) and the types of homes in which they are kept. Figures on pedigreed dogs are also given. With all the concern about human co-existence, it is interesting to note that of the 31 per cent of European households having a cat or a dog, seven per cent have **both** a cat and a dog.

The survey is a follow-up, on larger scale, of a similar one also sponsored by the Reader's Digest in 1963. It is available, bound in cloth, at R45.00 per copy from The Reader's Digest Association Limited, 1125 Parkade, Strand Street, Cape Town.



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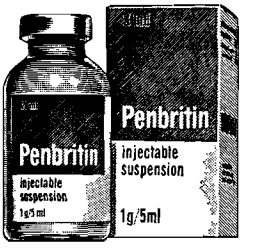

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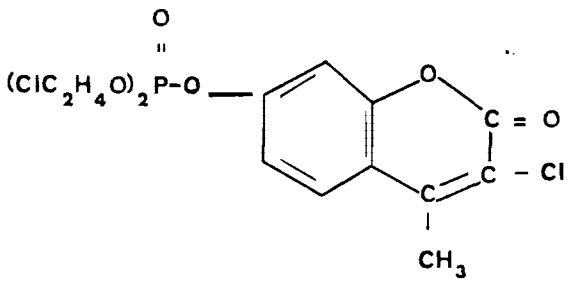

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J. 4790

Obituary - Ter Nagedagtenis

JOHN BAGOT QUINLAN

The death of John Quinlan in Durban on the 12th August, 1970, removed one of the oldest, most successful and most respected members of the veterinary profession.

Born on the 12th July, 1887, of farming stock, who bred Thoroughbred horses, cattle and sheep at Fethard, Country of Tipperary, Eire, he truly was gifted with an inborn knowledge and love of farm animals. These qualities, together with his academic training, undoubtedly made him one of the most outstanding veterinary surgeons and animal husbandrymen of his time.

He obtained M.R.C.V.S. with honours in Dublin in 1912, Dr. Med. Vet. with honours in Hannover in 1922, F.R.C.V.S. London, in 1928 and D.V.Sc., University of South Africa, in 1929. He twice undertook post-graduate work overseas, doing surgery, gynaecology, obstetrics and medicine at Hannover and Berne in 1922, and further courses in surgery, medicine and radiology in Hannover and Leipzig in 1928.

Dr. Quinlan began his career as Lecturer in Veterinary Science at Potchefstroom College of Agriculture in 1912. During the 1914-1918 war, he served as Captain in the S.A. V.C. and subsequently left Potchefstroom to take up a post at Allerton Laboratory, Pietermaritzburg, in 1920.

On the establishment of the Veterinary Faculty at Onderstepoort, he was appointed the first Professor of Veterinary Surgery, Gynaecology and Obstetrics in 1922. In the Division of Veterinary Services, he was promoted to the post of Sub-Director in 1931 and Assistant Director in 1937.

After his retirement from these posts in July 1947, he went into private practice, first in Cape Town, thereafter in Mooi River, and finally in Durban.

He became a member of the S.A.V.M.A. in 1913, served as Treasurer for several years and was later elected an Honorary Life Vice-President of the Association. In 1937 he was appointed a member of the

Advisory Council on Animal Nutrition of the Department of Agriculture.

He was a member of the Thoroughbred Breeders Association since 1923. Although specially interested in the Thoroughbred, he was also a very sound judge of other breeds of horses and of cattle, and frequently judged at various agricultural shows throughout the country. His practical knowledge of animal husbandry as well as of veterinary science gained him the highest respect of the farming community.

It was, however, his ability and achievements as a research worker and teacher that made John Quinlan world famous. He was the undisputed pioneer and leader of researches into fertility, infertility and sex physiology of farm animals in this country, and was the author of numerous articles published in various scientific journals.

"J.B." was not only an indefatigible worker himself but also had the happy gift of inspiring and encouraging all those who worked with him. He had the rare ability of being a stern and demanding disciplinarian while at the same time showing a sympathetic, guiding and helpful interest in the activities and problems of his co-workers. Consequently he sponsored numerous researchers in their endeavours for higher academic honours.

As a teacher, his guiding principle was constantly the upholding of a high standard of veterinary education and practice in this country, and to ensure that none but the competent be admitted to the ranks of the profession. His apparently grave external appearance masked a kind, sympathetic and kind-hearted nature, and his sound and friendly advice was at all times sought by and readily available to even the lowliest.

He married Miss Erna Kluge in February 1916, and is survived by her, their one daughter, two sons, all married, and ten grandchildren. To them the profession extends its profound sympathy in their great loss.

BOOK REVIEW

DESINFEKTION

Edited by W. STELLMACHER, K. SCHOLZ AND K. PREISSLER

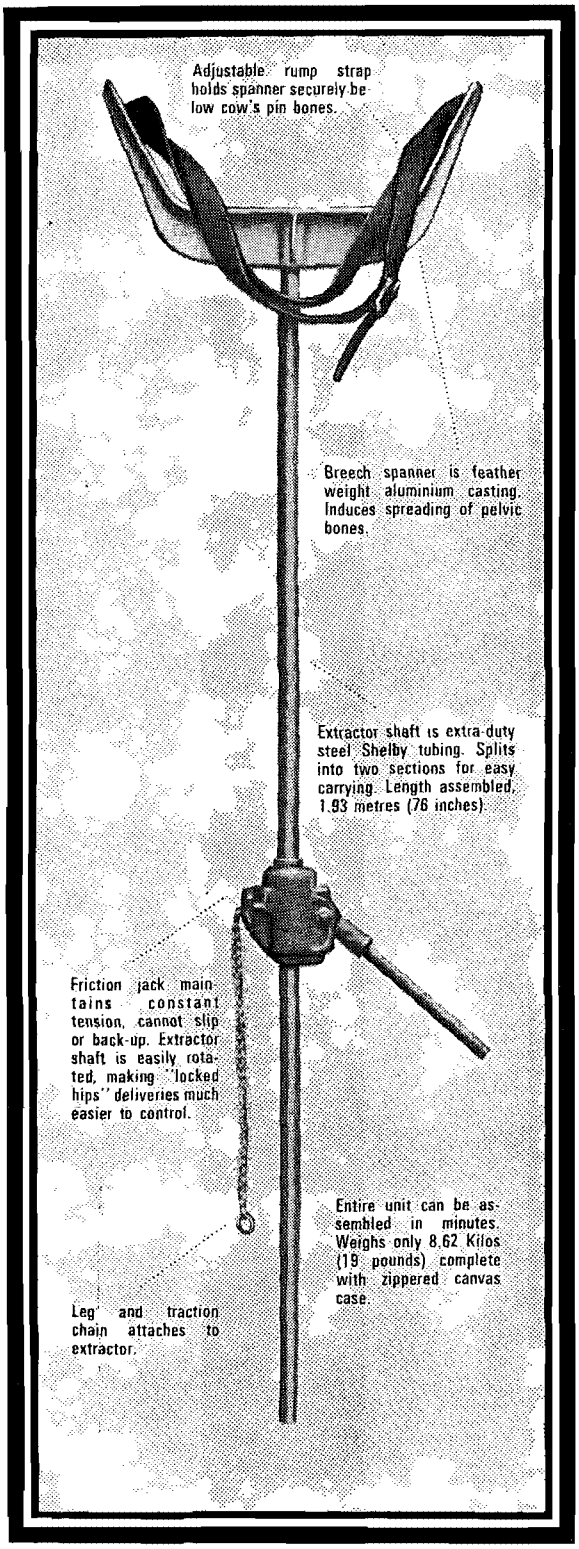
G. Fischer, Jena, 1970 pp. 255. Price: 21.10 mark.

This is a pocket book intended to assist veterinarians and others in the agricultural field in choosing and applying suitable and practical methods of disinfection under a wide range of circumstances. It is written in German and obviously intended for use in East Germany (D.D.R.) The index is clear and detailed. It raises expectations which are, however, not always realized. Some definitions are not clear and precise, disinfectants are discussed rather superficially but not always concisely, whilst cleaning agents are dealt with so briefly that the two chapters involved could well have been eliminated in this present form.

The special chapters on disinfection, on the other hand, provide considerable valuable information on a wide range of practical applications of disinfection, particularly to someone resident in East Germany (D.D.R.). The "foreign" reader, however, has to work his way through sections dealing in some detail with East German (D.D.R.) regulations and conditions in order to find reference to more generally applicable aspects of disinfection.

It is unfortunate that the book also attempts to deal with special matters such as disposal of carcasses, sterilisation, the control of rabies, insects and rodents. This has been done at the expense of disinfection—the true subject of the book. Greater detail and clarity concerning disinfectants and factors influencing their efficiency such as the relative sensitivity of micro-organisms, and condition of surfaces would have made the book of greater value.

W. H. G.



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The Faculty of Veterinary Science expresses its sincerest appreciation and thanks to the donors of the above-mentioned medals and prizes.

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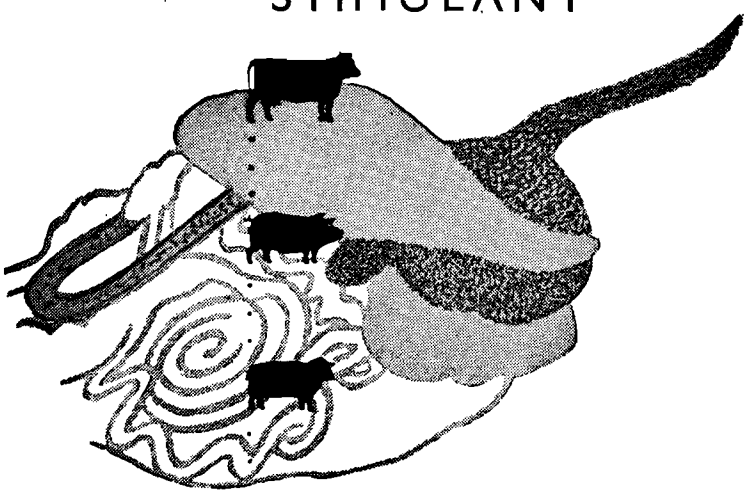

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No./Nr. 1 March/Maart ..... 1— 76
No./Nr. 2 June/Junie ..... 77—134
No./Nr. 3 September ..... 135—254
No./Nr. 4 December/Desember ..... 255—352

SUBJECT INDEX / INHOUDSOPGAWE

Scientific Articles **Wetenskaplike Stukke**

VOLUME 41 JAARGANG

Author Index — Naamlys van Outeurs

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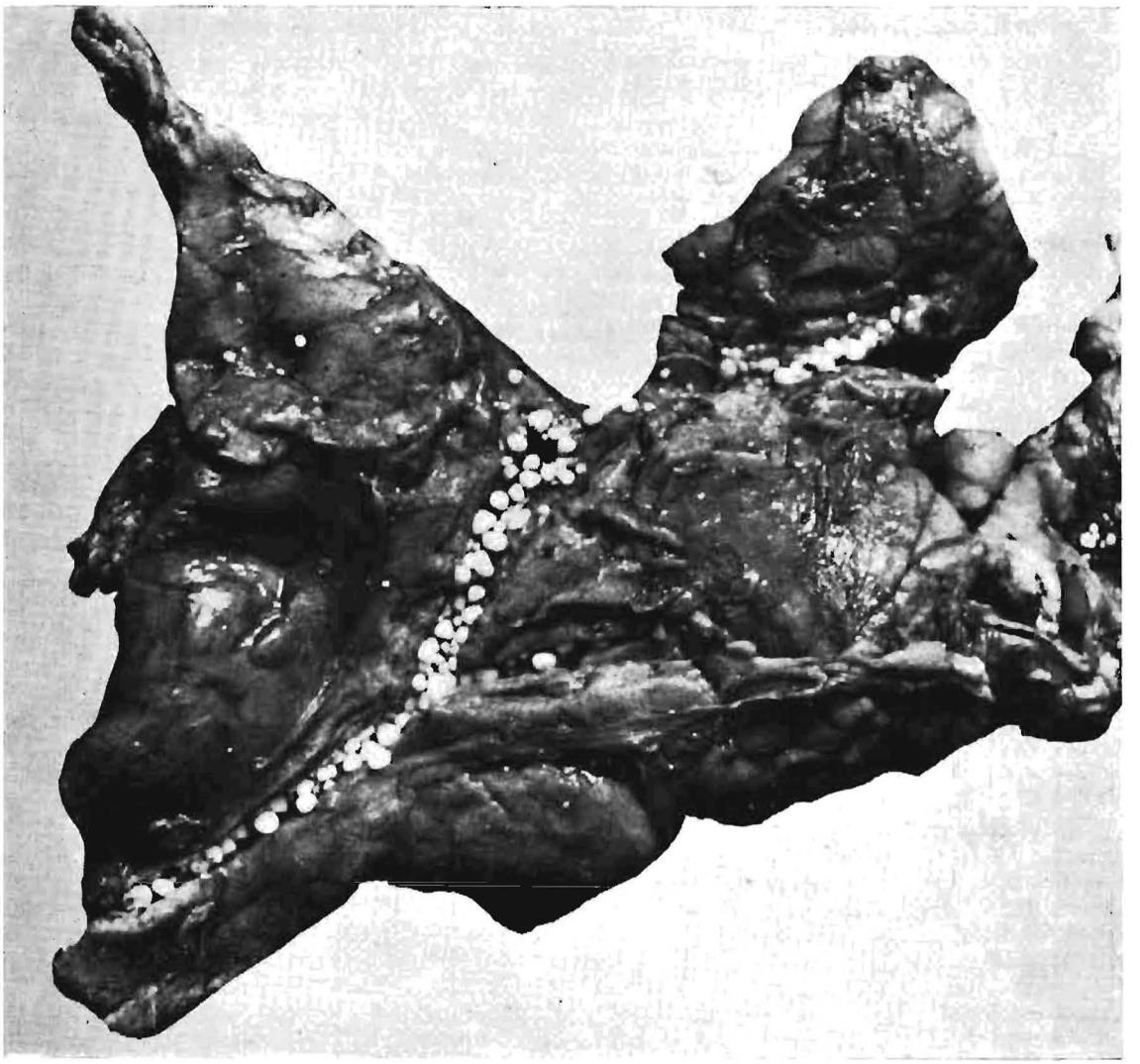

PANCREATIC CACULI IN A COW

The accompanying photograph is of the pancreas f a two year old South Devon cow which had died f postoperative complications. All the major pan-reatic ducts were filled with variable sized hard white alculi composed of 93.6% calcium carbonate and a mall amount of organic material, probably of protein-ceous nature. Other than mild inflammation and hickening of the affected ducts, this rare condition vas not associated with any significant pathologic hange of the pancreas. The cause of the stones is not nderstood although in man they are associated with lcoholism and diabetes mellitus.

PANKREATIESE STENE IN 'N KOEI

Die bygaande foto is van die pankreas van 'n twee-jaar oud South Devon-koei wat aan postoperatiewe komplikasies dood is. Al die hoofbuise van die pankreas was gevul met harde wit stene van verskillende groottes. Die stene het bestaan uit 93.6% kalsiumkarbonaat en 'n klein hoeveelheid organiese materiaal, heelwaarskynlik eiwit. Behalwe 'n ligte ontsteking en verdikking van die aangetaste buise was hierdie seldsame toestand nie geassosieer met enige betekenisvolle patologiese verandering in die pankreas nie.

Die oorsaak van die stene kon nie vasgestel word nie, maar dit is bekend dat hulle in die mens met alkoholisme en diabetes mellitus geassosieer word.

ubmitted by/Ingestuur deur: E. E. McConnell, Major, USAF, VC. Staff Member of Geographic Pathology Division, Armed Forces Insti-ute of Pathology, Washington, D.C., Temporary Assignment, Depa rtment of Pathology, Veterinary Research Institute, Onderstepoort, nd/en R. C. Tustin, Department of Pathology, Veterinary Research Institute, Onderstepoort.

Photography/Fotografie: A. M. du Bruyn.