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DIE SUID-AFRIKAANSE VETERINÊR-MEDIESE VERENIGING

EDITORIAL

VETERINARY FOOD HYGIENISTS IN SOUTH AFRICA

South Africa has a total human population of about 17½ million people living in an area of 471,000 square miles. Fifty four per cent of the population are rural; of the urban population 5 million are resident in the major cities or towns. The farm-animal population comprises *inter alia* about 40 million sheep and goats, 12 million cattle and 2 million pigs. Climatologically the conditions vary from subtropical coastal areas to temperate grass-lands on the central plateau. Rainfall varies from below 5 to over a 100 inches per year. Most farming operations are, therefore, on the extensive scale. The subcontinent has a large number of infectious diseases which are basically enzootic in character and which have seriously hampered agricultural development, and economic growth, until their eradication or efficient control. The veterinarian's primary task to date has been the eradication of diseases such as Rinderpest, Bovine Pleuropneumonia, East Coast Fever, Trypanosomiasis, and Foot and Mouth Disease, and the effective control of babesiosis, African horsesickness, anaplasmosis, blue tongue, anthrax, clostridial infections etc. The result has been that vast areas in South Africa are now available for stock farming and food production. During this phase of veterinary activity, particularly in the last half century, veterinary food hygiene received relatively little attention except from the small number of veterinarians who were employed by the larger cities.

The veterinary complement of South Africa is only 410 strong, giving less than one veterinarian per 100,000 farm animals. Of these, 18 or 4.4 per cent are engaged in full-time public health work, which is mainly concerned with control of hygiene of primary production and processing of meat and milk. Veterinary activity in the field of control of fish, eggs, meat and milk products, retail distribution of foods of animal origin, secondary processing and laboratory control of such foods of animal origin is virtually non-existent. Central control over food in general is the responsibility of the State Department of Health, which delegates its powers to responsible local authorities. Employment of medical officers and sanitary inspectors by these bodies is obligatory, but veterinarians may be engaged at the discretion of the local authority. Exported agricultural products are, however, controlled by authorised municipal veterinarians or state veterinarians with respect to premises, ante- and post mortem insepction of food animals and certification of consignments.

A pattern of food hygiene control has, therefore, of necessity evolved wherein the veterinarian has limited scope. However, a recent Commission of Inquiry into abattoir matters has recommended that central control of meat hygiene be transferred to the Veterinary Division of the Department of agriculture, and it is envisaged that this will bring meat inspection and hygiene of primary processing into the undoubted sphere of responsibility of the veterinary profession, so that closer integration between meat inspection and promotion of farm animal health may result.

Regarding statutory provisions for the control of foods of animal origin, the State Department of Health has always utilised the advisory services of the Veterinary Research and Veterinary Faculty personnel; and close collaboration is maintained.

To summarise, the veterinarians engaged in public health work are employed almost exclusively by larger local authorities to control primary production and processing of meat and milk and to direct municipal abattoirs. Veterinary control over eggs and fish, or proccessed meat and milk products, or fresh meat and milk offered for sale by wholesale and retail traders, scarcely exists. Medical officers, health (sanitary) inspectors and microbiologists are largely responsible for these aspects. Veterinary supervision and certification of meat and meat products destined for export is, however, undertaken by the State Veterinary Services.

Only one faculty of veterinary science exists in South Africa. This is subsidised by the State, attached to the University of Pretoria, and situated adjacent to the National Veterinary Research Institute at Onderstepoort. Fortyfive students are admitted annually to the *B.V.Sc.* course which extends over 5 years. This degree allows registration with the Veterinary Board. Food hygiene is taught to undergraduate students in the last two years of the course, and includes lectures, demonstrations and practical training in meat and milk hygiene, eggs and fish, the zoonoses, and diseases of the mammary gland. The time allotted to this subject in relation to other subjects is illustrated in the histogram below (entire B.V.Sc. study period).

PERCENTAGE OF CURRICULUM TIME ALLOCATED TO VARIOUS SUBJECTS IN THE B.V.SC. COURSE, OF THE UNIVERSITY OF PRETORIA.

	Percentage Time
Anatomy-Embryol.	16.0
Physiol.-Biochem.	9.8
Zootechnology	12.2
Infect. Dis. & Microbiol.	8.7
State Vet. Medicine	1.1
Medicine	6.0
Pharmacology	2.1
Toxicology	1.8
Dis. of Poultry	3.0
Surgery	7.9
Gynecology	3.1
Path. & P.M.	7.8
Parasitology	4.5
Food Hyg. & Publ. Health	2.7
Clinics (various)	9.0

This course, to a very large extent, complies with the requirements of about 120 hours of instruction as recommended by the F.A.O./W.H.O. panel on Veterinary Education, and follows, in the main the outline proposed by Prof. Jepsen in his working paper No. 10 presented to the panel in 1963. It is considered more than adequate for the undergraduate.

Since 1963, two post-graduate courses are offered by the Faculty of Veterinary Science, viz.

(*a*) *For a Diploma in Veterinary Public Health*, awarded to successful graduates after completion of a two-year part-time course almost identical to that given to medical graduates for the Diploma in Public Health. This degree fully qualifies the veterinarian to become a member of the public health team, as the curriculum covers a wide range of public health subjects such as legislation and administration, water and sewage purification, physiology of nutrition, elementary geology and meteorology, medical sociology, environmental sanitation, epidemiology, genetics, statistics, radiation, microbiology, parasitology, infectious diseases, town-planning, maps and plans, building construction, etc. Veterinary candidates devote additional time to the Zoonoses and foods of animal origin.

(*b*) *For a Masters Degree in Vet. Medicine* (Food Hygiene), with Food Hygiene and Public Health as major and Parasitology, Infectious Diseases and Pathology as ancillary subjects. Two years of study are required, and the candidates must prepare and submit seminars apart from doing formal examinations. A treatise on original work is also required.

Veterinary activities in the field of food hygiene in South Africa are not yet as broad or well developed as in older countries who have had less recent problems with enzootic infectious diseases which limit agriculture and primary food production. Nevertheless 4.4 per cent of the veterinary profession is engaged in the public health control of primary production and processing of meat and milk. Statutory obligation of this is, however, confined to meat and meat products for export. Both undergraduate and post-graduate training in food hygiene is, however, of a standard which may be regarded as acceptable to international agencies and is ahead of the actual practical requirements of the country. This enables veterinarians to render the necessary services where expansion and development of veterinary food hygiene takes place.

L. W. v.d. H.

J. S. Afr. vet. med. Ass. 36 (2) 1965

CAPE TOWN CONGRESS PAPER 1964

CLINICO-PATHOLOGICAL STUDIES OF *BABESIA CANIS* INFECTION IN DOGS II. THE INFLUENCE OF THE INFECTION ON PLASMA TRANSAMINASE ACTIVITY

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SUMMARY

Hepatocellular damage in *Babesia canis* infection was studied by means of serum glutamic pyruvic transaminase determination.

It was found that even in early cases manifesting only moderate anaemia, there was already evidence of damage of liver cells. This suggested that anoxia resulting from impaired sinusoidal blood flow, at least in some cases, was damaging liver cells before anaemic anoxia could have become an important factor. It appeared unlikely that the level of SGP-T activity could be used to predict any subsequent development of icterus.

INTRODUCTION

The usefulness of determining the transaminase activity in the plasma of animals as a parameter in the assessment of damage of the polygonal cells of the liver has been discussed elsewhere[2,3].

To recapitulate some salient and relevant facts about these enzymes briefly: they are essentially intracellular in greatest concentration and the levels normally found in the plasma are considered to result from the normal wear and tear of tissue cells. The actual site in the cells where they are found and presumably synthesized is in the hyaloplasmic fraction of the cells and not in the mitochondria, as are some other enzymes[4].

In man glutamic oxalacetic transaminase is found[5,6,7] in the greatest concentration in heart muscle, skeletal muscle, brain, liver and kidney, in decreasing order. In general, a similar pattern has been found[2] in the domestic animals. Necrosis or degenerative processes affecting the myocardium, skeletal muscles and liver particularly are, not surprisingly, reflected in elevated SGO-T levels.

Glutamic pyruvic transaminase (GP-T) in high concentration has been found only in the hepatic cells in man, cats and dogs[2] and it is in fact found that significantly increased plasma levels of this enzyme are encountered only in liver cell damage in these species. In the dog then, as with man and cats, such increased levels provide a practical and specific indication of liver cell necrosis or degeneration[3,4], regardless of the degree of functional impairment of the organ.

The investigation of hepatocellular involvement in *B. canis* infection has only once previously received passing mention[3], using transaminase activity as a parameter. In the present study the results of this particular investigation are recorded as a further contribution to the charaterization of the clinico-pathological changes brought about by the infection.

MATERIALS AND METHODS

The source of animals used for this study was the same as that outlined in the previous paper[1]. Clinical cases were again divided into three more or less arbitrary categories, briefly:

Category I. Early acute cases with pale pink mucous membranes.

Category II. Cases showing great mucosal pallor, but no icterus.

Category III. Cases showing any degree at all of icterus.

The majority of samples was taken upon establishment of the diagnosis and before specific treatment. Potassium oxalate was used as the anticoagulant in the early part of the study but later heparin was given preference.

Transaminase activity was determined by the colorimetric method of King[8] which yields values roughly three times as high as the Sigma-Frankel units[9] commonly used in the United States. Another study[3] has shown normal values for dogs to lie in the range of 30-100 King units for SGP-T.

As a routine, SGO-T was also determined, but as it was manifestly less sensitive and specific it has not been included in this paper. Moreover, red cells are rich in this enzyme so that high values were usually encountered when massive intravascular haemolysis was a feature of the disease.

Results

SGP-T values found in the three clinical categories are presented in the Figure.

Of the 35 dogs placed in Category I at least five gave values above the normal range, and the mean was 68 King units. Forty dogs in Category II showed considerably more scatter, with more than half within the normal range, the mean being 139, decidedly higher than the upper normal level. In Category III however, the criterion was icterus and the SGP-T was almost invariably considerably raised above the accepted normal figure. The mean for the fourteen cases was found to be 287 King units.

Discussion

The figures obtained in the first two categories illustrate the fact that there is no clearcut division between the two and that there is a considerable overlapping zone with no evidence of liver cell damage. Misleading histories (not necessarily deliberate) could have influenced the placing in one or other category to some extent. Generally however the red cell count was the guide to placing the cases but as there is in this disease quite often a precipitate haemolytic crisis the choice of category would in some instances be due to an accident of timing. Occasional cases of concurrent and unrelated hepatic pathology could also affect a few of the figures.

In Category III, however, the criterion was the presence of icterus and the SGP-T values were all, with the exception of one border line case, elevated considerably above the normal range.

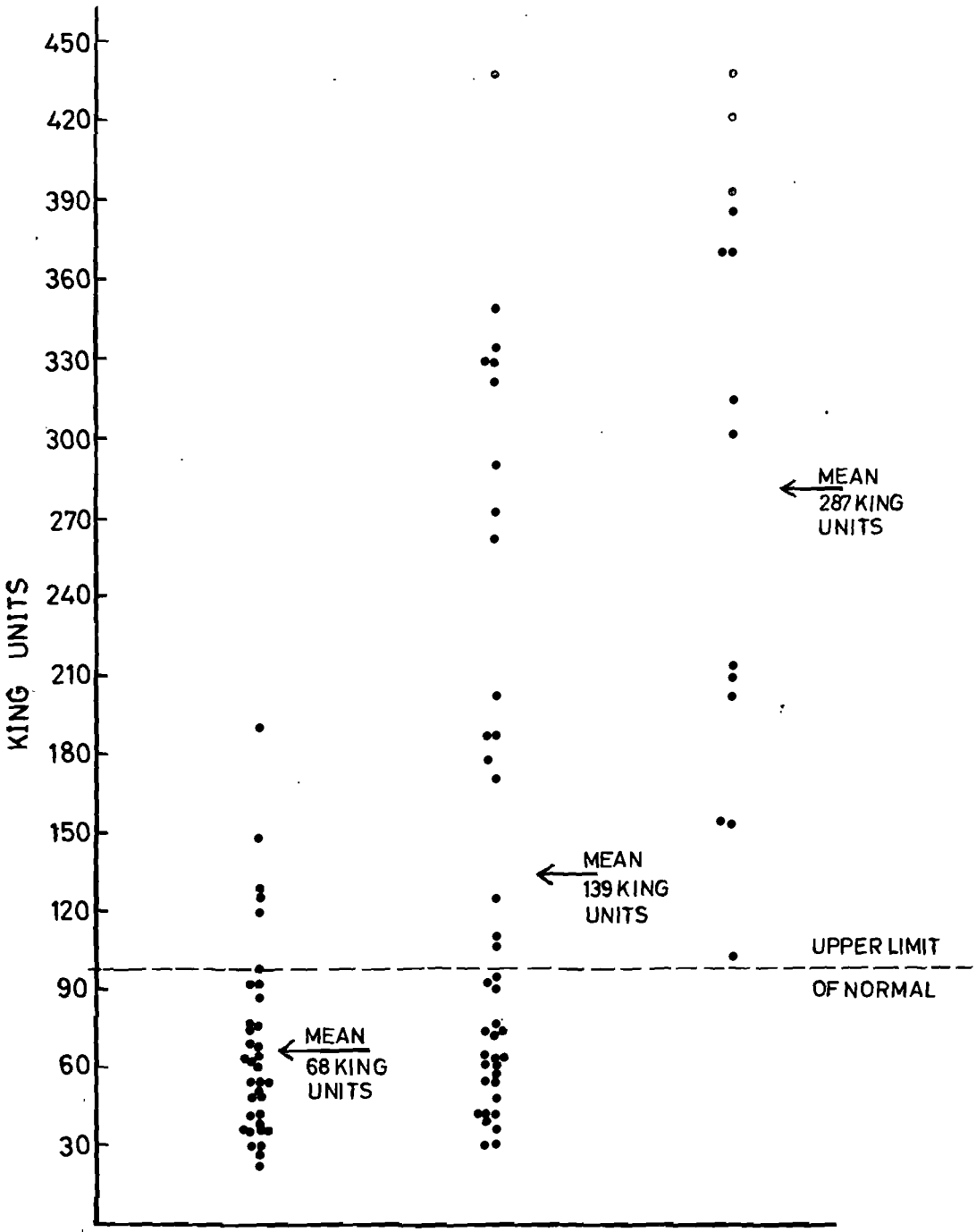
The results obtained suggest that liver pathology commences at a fairly early stage of the disease, as evidenced by SGP-T determination and that it is progressive during the course of untreated infection.

It is a frequent experience that dogs showing great pallor only on the day of treatment are found the next day to be markedly jaundiced, although parasites have disappeared from peripheral blood smears, indicating effective specific treatment. It has however not been found possible to correlate the height of the SGP-T figure with the likelihood of such an occurrence.

A few case histories may illustrate this irregularity.

Case 112/59 was a Category I animal clinically with a red cell count of 3.21 million per cu. mm. Its plasma was reddish brown and total bilirubin was 2.70 mg per 100 ml, more of it unconjugated than conjugated. SGP-T was 190 King units. It was treated immediately but was icteric on the following day.

Case 62/59 (Category II) was admitted very pale with a history of 24 hours inanition and malaise. BSP retention was 20.8 per cent, total bilirubin 0.65 mg per 100 ml. with 0.45 unconjugated and SGP-T 260. Three days after admission and specific therapy it was still not eating but SGP-T had dropped to 176 and the blood picture had deteriorated. No icterus developed. Blood urea nitrogen remained normal throughout indicating satisfactory kidney function. This animal made a slow but complete recovery.



FREQUENCY OF SGP-T VALUES

IN CANINE BABESIOSIS

A Category I case, 114/59, on presentation had a red cell count of 3.01 million, BSP retention of 38.4 per cent and SGP-T of only 54. The plasma showed evidence of marked haemolysis. Despite the low SGP-T, this animal was severely icteric the next day. Kidney function was disturbed as shown by a blood urea nitrogen of 27.6 mg per 100 ml on admission.

It would thus appear that SGP-T, while a good indicator of active degeneration and necrosis of liver cells, does not enable the clinician to predict the occurrence of subsequent icterus.

In summary, transaminase was found to be a useful indicator of the earliest stages of liver cell damage. Anoxia due to anaemia was obviously in the early stages not the major reason for such damage, and it could well be argued that anoxic damage resulted from defective sinusoidal blood flow[10] as discussed[1] in the earlier paper of this series.

ACKNOWLEDGEMENT

Permission by the Chief, Veterinary Research Institute, to publish this paper is acknowledged with thanks.

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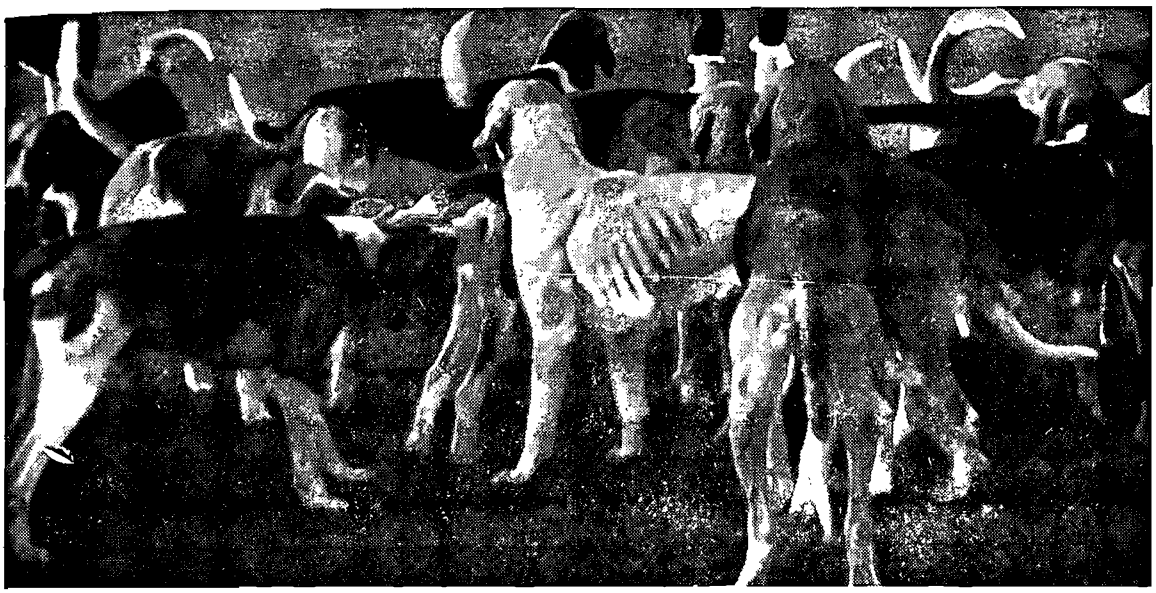

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CLINICO-PATHOLOGICAL STUDIES OF *BABESIA CANIS* INFECTION IN DOGS III. THE INFLUENCE OF THE INFECTION ON PLASMA ALKALINE PHOSPHATASE ACTIVITY

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SUMMARY

Serum alkaline phosphatase was the parameter used in this study of liver damage in dogs presented with natural infection with *Babesia canis*.

Since elevation of this enzyme is not specific for liver damage and values could be affected by conditions involving increased osteoblastic activity due to bone growth and bone disease, it was not possible to assess with certainty in early cases whether liver damage was responsible for rises or not.

As the disease advanced however, alkaline phosphatase activity was raised progressively, some times to levels as high as those found in obstructive types of jaundice. Impressive rises were already found in cases that had not progressed to the stage of clinical jaundice.

INTRODUCTION

The measurement of serum alkaline phosphatase activity was brought into clinical use in the early 1930's when it had been found[3,4] to be increased in certain types of bone disease. During that decade it received considerable attention[5,6,7,8] in diseases of the liver and has subsequently become firmly established[1] in the clinical chemical diagnostic evaluation of liver function.

Alkaline phosphatase is one of a group of phosphatases present in the blood and tissues and which hydrolyse organic phosphoric acid esters, primarily hexose monophosphate and glycerophosphate, liberating the phosphate ion. The different phosphatases are differentiated by the pH range in which each is most active. "Alkaline phosphatase" is most active at a pH of 9.3 but is also quite active at the pH of blood, about 7.4. It is present in blood, bones, ossifying cartilage, intestinal mucosa, kidney, liver and other tissues[22] and is believed[14] to be derived from the osteoblasts for the greater part. The route of excretion from the body is almost entirely via hepatic cells into the bile in man and dog. In cats, however, the kidney[2] also participates in excretion.

The amount of alkaline phosphatase activity has been expressed in a number of systems[9] of units per 100 ml, depending on the substrates used and the conditions and time of hydrolysis. Two of them extensively used today are Bondansky[12] units (United States) and the King-Armstrong[10] units (in Britain and in this country). Normal figures for man and most of the domestic animals usually fall within the range of 1–4 Bodansky[8] or 3–13 K.A. units per 100 ml[9].

Figures above these levels are obtained as the result of overproduction, as in young growing animals, rickets, osteomalacia, osteogenic sarcoma and in secondary hyperparathyroidism, or as the result of interference to the escape from the circulation via bile as in cholestatic conditions and in cases of hepatitis. The question of this interference as the sole mechanism has been described as controversial, and Popper & Schaffner[13] and Gutman[22] have discussed the evidence concerning increased formation in liver cells.

It follows that in any evaluation of increased levels of activity the clinical circumstances should be given due weight and that this deter-

mination should be combined with others in the assessment of hepatic functional impairment.

Alkaline phosphatase activity follows more or less the following pattern in the differential diagnosis of jaundice. It is regarded as less sensitive than bromsulphalein retention for early disturbance of function. Transaminases are much more sensitive to liver cell damage than phosphatase, and, between hepatocellular damage and cholestatic lesions, tend to react quantitatively in opposite ways. Transaminase rises moderately in obstruction and very much more in liver cell necrosis or degeneration while alkaline phosphatase shows considerably higher values in obstruction than it does in hepatitis and other forms of cellular damage. Latner and Smith[15] have in fact suggested the use of a transaminase/phosphatase ratio as being useful in the differential diagnosis of jaundice.

Working with dogs Freeman *et al*[16] found for instance values of the order of 100 K.A. units in experimental obstruction compared with 20 K.A. units in leptospirosis. Bloom[17] has observed values of between 6 and 10 Bodansky units in dogs with hepatocellular damage while in obstruction they were usually much in excess of 10 Bodansky units. The peak value found by Malherbe[18] in the course of a study of intrahepatic cholestasis (an obstructive lesion) was 499 K.A. units.

This difference is however, not always so clear cut since an obstructive element (presumably enzymatic in nature) may supervene in severe hepatitis and the resulting levels may be as high[19,22] as in obstruction.

An assessment of the significance of alkaline phosphatase levels must thus take into account all the factors listed above.

This paper studies the effect of *Babesia canis* infection on alkaline phosphatase levels in the plasma.

Materials and Methods

As in the first two studies[20,21] of this series, clinical cases of *Babesia canis* infection presented at the Small Animal Clinic at Onderstepoort were divided into three more or less arbitrary categories:

Category I: Early acute cases with pale pink mucous membranes.

Category II: Cases showing great mucosal pallor, but *no icterus*.

Category III: Cases showing any degree at all of clinical icterus.

Sampling was carried out as described before and alkaline phosphatase activity in the plasma determined according to the method described by King and Armstrong[10], as modified[11]. This procedure estimates the activity from the amount of phenol liberated from a substrate of di-sodium phenylphosphate when hydrolysed by the enzyme and measured with the phenol reagent of Folin and Ciocalteu.

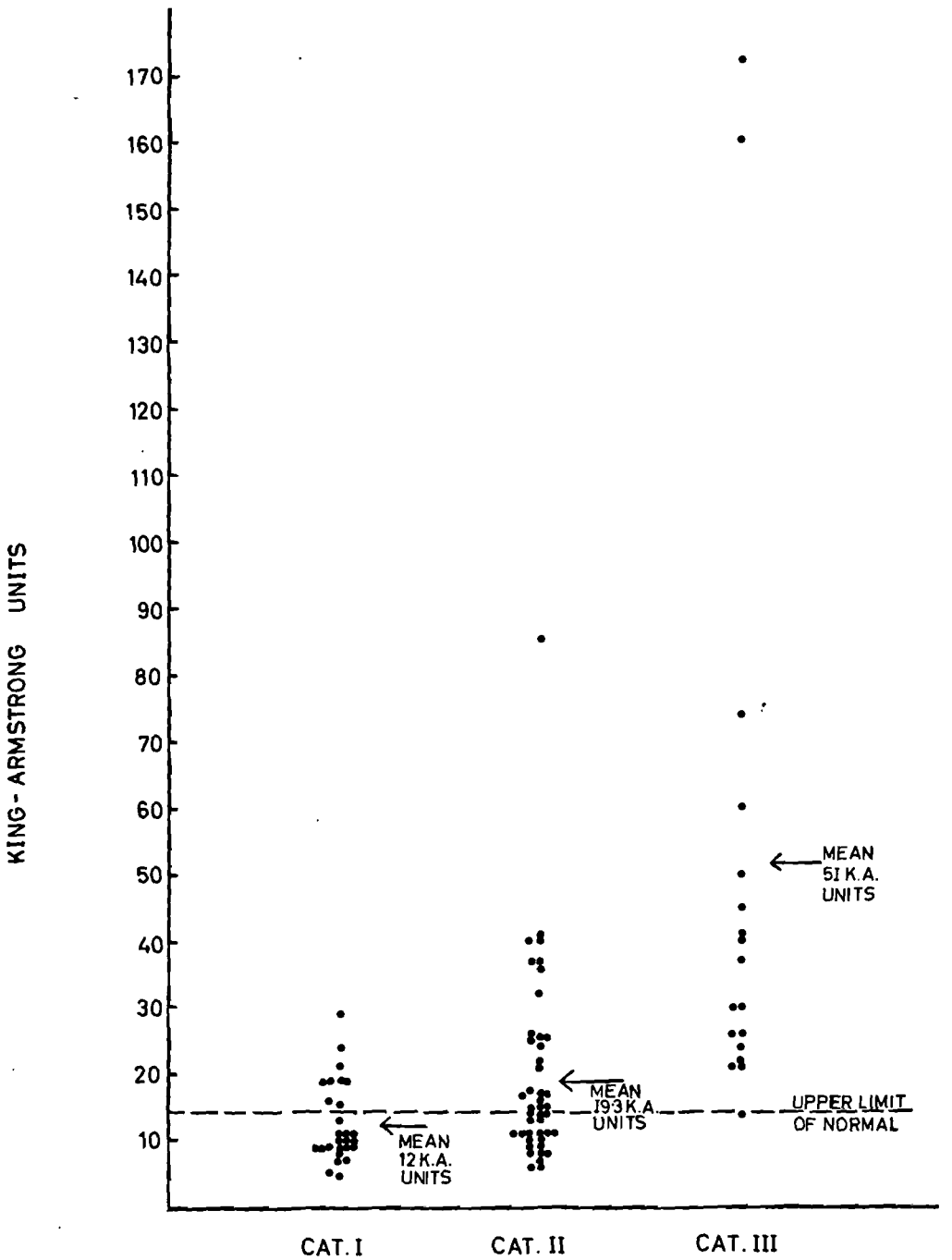
Results

The figure shows the scatter of plasma alkaline phosphatase values obtained in the three clinical categories.

Of the twenty seven dogs in Category I about one third gave values above the accepted normal of 3–13 King-Armstrong units. Since many of the dogs presented were young and some showed signs of rickets, this proportion explained on a basis of heightened osteoblastic activity, would not be unreasonable. The mean of 12 was within normal limits.

In the second category about one third again were distinctly elevated in a sample of 44 dogs and one value was as high as 85. If one takes the incidence of higher values due to bone growth and disease and consider the generally higher abnormal values there is certainly evidence of rises due to liver pathology. The mean at 19.3 K.A. units is above the normal range.

In Category III, all the values in a group of 18 icteric animals with the exception of one, were elevated, two to very high levels of 160 and 172 units. The mean was 51.



FREQUENCY OF ALKALINE PHOSPHATASE VALUES

IN CANINE BABESIOSIS

Discussion

The exact incidence of liver damage and early disturbance of function in the first category is somewhat problematical since most dogs presented with babesiosis are young. In the second category however the probability favours the increasing incidence of liver function disturbance, while in the icteric dogs the value is almost invariable quite considerably increased to levels found in hepatitis and liver cell necrosis. The two very high levels recorded were considered to be of the order of those found in severe cholestasis. As these were proven cases of babesiosis it might reasonably be assumed that the anoxic damage was severe enough to inactivate the transfer system between the cells and the bile caniliculi, thus giving rise to figures more usually found in obstructive lesions.

ACKNOWLEDGEMENT

Permission by the Chief, Veterinary Research Institute, to publish this paper is acknowledged with thanks.

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C. M. CAMERON AND C. F. SWART,

Department of Bacteriology, Veterinary Research Institute, Onderstepoort

Received for publication, April, 1965

SUMMARY

Nutrient broth containing 0.5 per cent yeast extract (Difco) and 1.0 per cent lactalbumin hydrolysate (N.B.C.) yielded, when inoculated with *C. pseudotuberculosis* and incubated at 37°C for 72 hours, an average packed cell volume of 1.84 per cent which corresponds to 7.36 g dry cells per litre.

Cells obtained from this medium contained endotoxin and, when killed by formalin, produced sterile abscesses in guinea-pigs. Although the best biological activity was shown by cells obtained from 48-hour cultures, the yield was somewhat lower.

Neither blood nor agar was necessary for producing cells containing endotoxin.

INTRODUCTION

A fluid medium suitable for the cultivation of *C. pseudotuberculosis* has been described in a previous publication[1]. It contains 2.0 per cent Tryptone (Oxoid) and 0.5 per cent yeast extract (Difco) in nutrient broth. However, cells grown in it contained little endotoxin, did not, after being killed with formalin, produce abscesses in guinea-pigs and had only poor immunizing properties. Cells which were grown on blood tryptose agar, however, possessed endotoxin, could, after being formalinized, produce abscesses in guinea-pigs and were immunogenic.

As only small quantities of cells containing endotoxin could be obtained on blood tryptose agar, experiment were carried out to find the factors responsible for conferring the desired properties on the cells.

Because lactalbumin hydrolysate in fluid media stimulated the growth of pleuropneumonia-like organisms, it was tested for its ability to support the growth of *C. pseudotuberculosis* in the hope that it would yield cells possessing the same characteristics as those grown on blood tryptose agar. The value of dipthheria medium[2] and different methods of aeration were also examined.

The possibility of replacing commercial yeast extract (Didco) with a watery extract of brewer's yeast was investigated.

MATERIALS AND METHODS

The methods of preparing media, inoculating the flasks, preparing the testing endoplasma for toxigenicity and for determining the pyogenic effect of formalinized cells were the same as those previously described[1]. *C. pseudotuberculosis*, strain 137B, was used in all the experiments.

The watery extract of brewer's yeast was prepared in the following way. Ninety grams of

dry brewer's yeast was boiled in 2 l of distilled water for 15 minutes, allowed to stand at room temperature overnight and the supernatant fluid filtered through muslin, paper pulp and Ford F.C.B. pads. Of this solution 1,500 ml were added to 3 l broth to give a final concentration equivalent to 1.5 per cent dry yeast. When a concentration of 3 per cent yeast was required the original extract was prepared by boiling 180 g of yeast in 2 l distilled water.

The diphtheria medium was a modification of that formulated by Pope and Linggood[3].

The yield of bacteria obtained after incubation was found by measuring the packed cell volume of each of the eight flasks used in every experiment. This was done by centrifuging a measured volume of culture in a Hopkins tube at 3,000 g for 30 minutes.

Results

The results of an experiment designed to find if lactalbumin hydrolysate could replace either yeast extract or tryptone in the original fluid medium are shown in table 1. It is evident that although tryptone is replaceable and almost certainly inhibitory, yeast extract is essential for pellicle formation.

Table 1

Effect of different concentrations and combinations fo tryptone, yeast extract and lactalbumin hydrolysate on yield of microbes.

Broth plus			% packed cell volume after 72 hours' incubation
Tryptone %	Yeast extract %	Lactal-bumin hydroly-sate %	
2.0	0	0	Neg.
2.0	0.5	0	0.925 (0.8—1.0)
2.0	0.5	1.0	1.7 (1.6—1.8)
2.0	0	1.0	Neg.
0	0.5	1.0	3.0 (2.0—4.5)
0	0	2.0	Neg.
0	0.5	0	Neg.

Neg. = growth too poor to measure.

The results in tables 2 and 3 show that maximal yield was obtained when the broth contained 1.0 per cent lactalbumin hydrolysate and 0.5 per cent yeast extract.

Table 2

Effect of lactalbumin hydrolysate on yield.

Broth plus		% pooled packed cell volume after 72 hours' incubation
Yeast extract %	Lactalbumin hydrolysate %	
0.5	2.0	1.75
0.5	1.5	1.75
0.5	1.0	2.0
0.5	0.75	1.325
0.5	0.5	1.4
0.5	0.3	1.3

Table 3

Effect of various concentrations of yeast extract on yield

Broth with 1.0% lactalbumin hydrolysate plus		% pooled packed cell volume after 72 hours' incubation
Yeast extract (Difco) %	Watery extract of brewer's yeast %	
0.5	0	1.4
0.3	0	1.3
0	1.5	1.0
0	3.0	1.0

Table 4

Packed cell volume in nutrient broth containing 0.5 per cent yeast extract and 1.0 per cent lactalbumin hydrolysate with inocula from 2 different media.

	Inoculum grown on			
	Blood tryptose agar		Nutrient agar with 0.5% yeast extract and 1.0% lactalbumin hydrolysate	
	1st Experiment	2nd Experiment	1st Experiment	2nd Experiment
	0.8	1.6	1.8	3.4
	0.9	1.7	1.6	2.8
	1.1	2.3	1.7	2.4
	1.1	2.2	2.0	3.2
	1.2	2.2	1.9	3.6
	0.9	1.8	2.1	2.8
	0.8	1.8	1.7	2.6
	1.2	2.4	1.6	3.1
Average	1.0	2.0	1.8	3.0

Table 5 demonstrates the presence of endotoxin in cells grown in different media and their ability to produce abscesses after having been killed by formalin.

TABLE 5.—Presence of endotoxin in cells, and pyogenic effects of formalin-killed cells cultivated on various media for 48 hours at 37°C.

Medium					Inflammatory effect of endotoxin in rabbits			Pyogenic effect of cells in guinea pigs after treatment with 0.4% formalin for 48 hours at 37°C			
					Dose 0.5ml intradermally			0.15g cells/100 ml.			
					Dilutions			Volume injected s.c. in ml.			
Base	Blood (Bovine)	Tryptone (Oxoid)	Lactalbumin hydrolysate (N.B.C.)	Yeast extract (Difco)	1:2	1:5	1:10	1.0	1.0	0.5	0.5
	%	%	%	%	increase in skin thickness in mm						
Tryptose agar (Difco).....	10	0	0	0	5.0	3.0	2.0	++	+	+	+
Nutrient agar.....	0	2.0	0	0.5	1.0*	1.0*	0.0	—	—	—	—
Nutrient broth.....	10	2.0	0	0.5	0.0	0.0	0.0	—	—	—	—
Nutrient broth.....	0	2.0	0	0.5	2.0	1.0*	0.0	—	—	—	—
Nutrient broth.....	0	0	1.0	0.5	6.0	4.0	3 0	++	++	+	+

* = No erythema s.c. = subcutaneously; + and ++ = abscesses present
Footnote.—Each cell suspension was tested repeatedly in 1.0 ml and 0.5 ml doses for pyogenicity in guinea pigs.

The effect of vortex aeration and of shaking on the yield was investigated. For comparative purposes the broth used for the production of *C. diphtheriae* toxin was also examined[2]. The yields obtained after 48 hours' incubation at 37°C and the biological characteristics of the cells are shown in table 6. In vortex and shake cultures both media supported good growth. The yield was, however, not as high as that regularly obtained with 72-hour static cultures when a pellicle is formed but was similar to the average yield obtained for 48-hour static cultures.

Examination of the biological properties showed that cells from 48-hour static cultures were considerably more active than those from 72-hour cultures. The activity of cells from vortex cultures, using the same medium, was as pronounced but the cells from diphtheria medium vortex cultures were less active. Very little growth was obtained, and no pellicle developed, in static cultures in diphtheria medium.

TABLE 6.—Yield and biological characteristics of cells grown under various conditions.

Medium	Cultivation method	Incubation time in hours	Average % Yield	Inflammatory effect of endotoxin in rabbits				Pyogenic effect of cells in guinea pigs after treatment with 0.5% formalin for 48 hours at 37°C			
				Dose 0.5 ml intradermally				0.15 g cells per 100 ml.			
				Dilutions				Volume injected s.c. in ml.			
				1:2	1:5	1:10	Aggregate	1.0	1.0	0.5	0.5
				Increase in skin thickness in mm							
Nutrient broth with 0.5% yeast extract and 1.0% lactalbumin hydrolysate pH 7.8	Vortex aeration	48	1.3	8.3	7.0	5.7	21.0	++	++	+	+
	Roux shake flasks	48	1.25	7.2	5.8	5.0	18.0	++	+	+	+
	Static Roux flasks	48	1.35	9.0	7.4	4.7	21.10	++	++	+	+
	Static Roux flasks	72	1.84	5.5	4.0	2.5	12.0	+	+	+	+
Diphtheria broth.....	Vortex aeration	48	1.3	7.1	5.0	3.9	16.0	+	+	+	—
	Static Roux flasks	72	*	—	—	—	—	—	—	—	—

* no pellicle or measureable growth; + and ++ = abscesses present: s c. = subcutaneously.
Footnote.—Each cell suspension was tested repeatedly in 1.0 ml and 0.5 ml doses for pyogenicity in guinea pigs.

CONCLUSIONS

From the results obtained it is evident that yeast extract is essential for pellicle formation and that it cannot be replaced by lactalbumin hydrolysate. Further, it is clear that lactalbumin hydrolysate is much superior to tryptone as a growth-promoting substance because it gives three times the yield at half the concentration. Further, it is at least twelve times cheaper than

tryptone. The maximum yield was obtained with a combination of 1.0 per cent lactalbumin hydrolysate and 0.5 per cent yeast extract. The commercial yeast extract can be replaced by a 1.5 per cent watery extract of brewer's yeast but the yield is somewhat lower, and the time and labour employed in preparing it do not warrant its use.

It was observed that higher yields were obtained when the flasks containing the fluid medium were inoculated with agar cultures grown on the same medium than when blood tryptose agar cultures were used.

As shown in table 5 the cells obtained from the new medium not only contained large amounts of endotoxin but were able, when killed by formalin, to produce sterile abscesses. Cells grown on the old medium containing agar or 10 per cent haemolysed bovine blood did not have these properties.

Aeration of the medium by shaking or vortex did not improve the yield. In 'diphtheria medium' a pellicle was not formed and, although the yield in vortex cultures was good, this medium had no exceptional growth-promoting properties.

The biological activity of the cells derived from the two media and cultivated by various methods and for 48 and 72 hours, showed considerable differences. It appears that cells from 48-hour cultures are much more active than those from 72-hour cultures and that aeration does not improve the activity.

Cells from 'diphtheria medium' were biologically more active than cells from 72-hour cultures of the new medium but less active than cells grown for 48 hours.

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PRACTICAL PROBLEMS IN TUBERCULIN TESTING IN CATTLE

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SUMMARY

The reason for the occurrence of non-specific reactions to the tuberculin test is briefly discussed. Methods by which non specific reactions can be distinguished from specific reactions are considered. The importance of using the test as a herd test wherever possible is stressed. A study has been made of the frequency distribution of tuberculin reactions in tuberculous animals, non-specifically sensitized animals, and reactors from herds where both types of sensitization occur. The importance of retesting and the interpretation standards which should be used are discussed.

INTRODUCTION

The success of an anti-tuberculosis campaign will depend essentially on the accuracy of the diagnostic methods used. For both the success of a campaign and in the interests of preserving the best possible relationship between the farming population and the veterinary profession it will be necessary to maintain the highest possible diagnostic standards.

The tuberculin test is a very reliable method of diagnosing bovine tuberculosis. There are, however, a number of problems which may be encountered in using the test in the field. The operator should therefore be fully acquainted with these difficulties so that unnecessary mistakes in the interpretation of tuberculin reactions can as far as possible be avoided.

It is the aim of this paper to discuss the interpretation of the tuberculin test with special reference to the non-specific reactor problem. The standard test used in South Africa is the single intradermal test using 7,000 T.U. of bovine tuberculin injected in the neck. Investigations to find the most suitable second tuberculin for comparative testing under South African conditions are not yet complete. Remarks will therefore mainly be confined to the standard bovine test as it is hoped to publish work on comparative testing at a later stage.

THE CAUSE OF NON-SPECIFIC REACTORS:

The literature on the causes of non specific reactions in cattle is voluminous, but it is not the intention to summarize all the available information in this paper.

When an animal becomes infected with *Mycobacterium tuberculoses* the body becomes sensitized by certain fractions of the organism—possibly the lipid or phosphatide fractions[1]. The animal is then in an allergic state and an acute defensive reaction (Koch's reaction) will occur at the infection site if the animal is reinfected with *M. bovis*. Reactions will also occur to the extracellular protein fraction of cultures of mammalian tubercle bacilli. It is therefore possible to produce a diagnostic agent—tuberculin—which will cause reactions in sensitized animals but will not sensitize a non-infected animal to further injections of tuberculin.

The genus Mycobacterium contains a large number of species including pathogens, free living saphrophytes and potential pathogens. A great number of these organisms contain sensitizing fractions similar to those contained by *M. bovis*, which can sensitize animals to

mammalian tuberculin. These reactions are always smaller than the reaction to a similar dose of their own homologous tuberculin would be[2,3,4,5], but they can cause problems of interpretation when testing is carried out in the field using a single tuberculin. Although these organisms cause tuberculin sensitivity they usually fail to produce any lesions in the affected animal and thus we have the position of the no-visible lesion (N.V.L.) reactor. The literature on non-specific reactions in cattle has been adequately reviewed by Karlson[6] and by Paterson[7]. Organisms which have been incriminated include *M. tuberculosis* (human type), *M. avium*, *M. johnei* and *M.sp* which can be demonstrated in "skin lesions". There are almost certainly many other undescribed Mycobacteria which are capable of causing tuberculin sensitivity. Many organisms from outside the genus *Mycabacterium* have also been described as being able to sensitize animals or as possible sensitizers e.g. organisms of the genera *Norcardia*[8], *Aspergillus* and *Trichopyton*[9], *Brucella*[10,11] and Actinomyces[12]. Liver fluke[13] hormonal influences[14] and non specific infections, such as peritonitis and pleuritis, have also been suggested as possible causes. The most convincing evidence is however confined to the *Mycobacteria.*

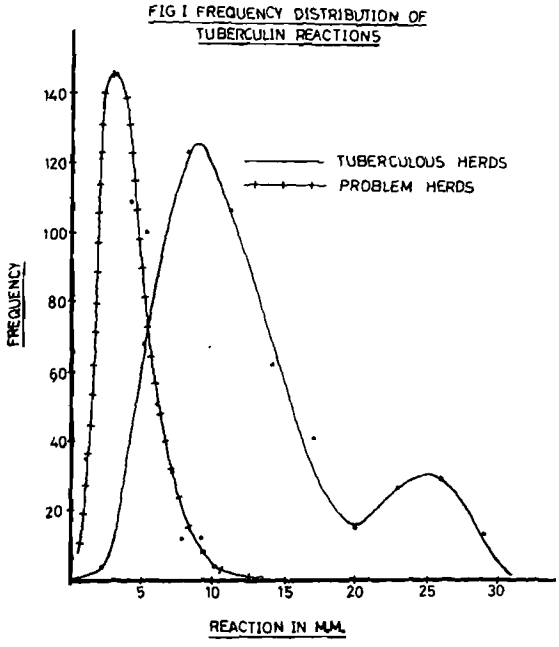
Recognition of Non-specific reactions in the field:

In South Africa we are in the fortunate position of having many large herds, in which the reading of tests can be facilitated by making interpretations on a herd basis. Before branding any animal, it is the duty of the veterinarian to satisfy himself that there is bovine tuberculosis in the herd. Judgment should not be made on each animal individually but only after the results of the whole herd have been considered. A few reactions in heifers can hardly be considered positive when there are no reactors amongst the older cows. Similarly, if only a few reactions occur in a large herd, especially in a herd which has been closed for some time, they should be considered with extreme caution. When any reactors are found unexpectedly in previously clean herds a careful check should be made, to find out whether there have been any new introductions or whether the cattle have been in contact with other cattle. While testing, a careful watch should be kept for the occurrence of "skin lesions" in the herd. It has been stated that non specific reactions are more common in heifers and oxen than in other cattle[14].

The nature of the reaction as well as its size should be considered and recorded. The typical specific reaction is hot, painful and diffusely oedematous, whereas non specific reactions are hard and circumscribed. It is unwise however to attach excessive value to the nature of the swelling as hard, circumscribed reactions are sometimes found in tuberculous animals. If there is reason to suspect *M. tuberculosis* (human type) sensitization then all farm labourers and people having contact with the animals should be screened for active lung tuberculosis.

A most useful guide for the demonstration of specific sensitivity is to study the frequency distribution of the reactions in the herd. To demonstrate the point, data was collected from 10 herds in which tuberculosis was present in a high percentage of the cattle. The animals in these herds, which were under Isoniazid treatment, have been retested a number of times, but only the original reactions at the first test have been used. The subsequent tests have however been carefully considered and any reactions which were thought to be possibly due to non-specific sensitization were excluded from the data. The frequency distribution for the skinfold increases in the 500 reactors tested with 7,000 units of standard bovine P.P.D. is shown in figure I. The tuberculous cattle fell into two groups. In the largest group comprising about 85 per cent of the cattle the reactions were approixmately normally distributed having a mean skinfold increase of 10.4 mm and a standard deviation of 4.1. The second group comprising about 15 per cent of the cattle in this series was highly sensitive to tuberculin. This group had a mean skinfold increase of 24.5 and a standard deviation of 2.9. The highly sensitive cattle may represent a more sensitive portion of the cattle population or they may be the recently infected animals. The distribution shown for this group may not however be strictly accurate as many reactions in this range were merely recorded as 30 or 30-plus, this being the limit of the calliper in general use.

A frequency curve has similarly been constructed from 616 reactions which were recorded from non-specifically reacting cattle in eight different problem herds, see Fig. 1. In this

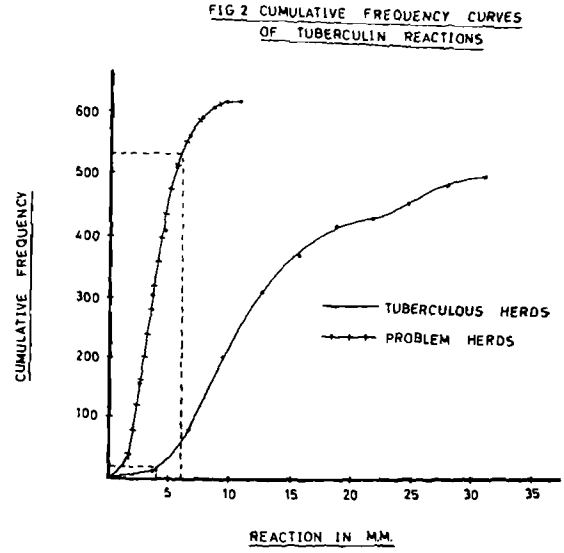


case the reactions are distributed about a mean of 3.9 with a standard deviation of 1.9. The curve shows considerable positive skewness which can be attributed to the fact that a number of small reactions of 1—2mm are not measured or recorded by many operators, and furthermore a number of reactions of less than nil would have to occur to make the curve completely symmetrical. In this case it is also possible that the data was not drawn from an entirely representative population, as the causes of sensitization in each herd are unknown and may be different in each. It should be noted that high reactions of over 10mm do occasionally occur in non specifically sensitized animals.

In practice we cannot construct frequency polygons for each herd as the numbers are usually too few to be significant and this would in any case be impractical. However, we can reasonably expect that the mean reaction should be around 10 mm in tuberculous herds, whereas in non-specifically sensitized herds the mean reaction will be considerably lower. It must, however, be stressed that if distributions of reactions are to be studied it is necessary to measure and record all reactions even although they may be low and in the negative range. The finding of a percentage of highly sensitive animals in a herd is useful additional evidence of the presence of specific sensitization. A history of animals having been condemned at abattoirs, tuberculosis having been found in the milk or positive findings at test slaughter would of course provide conclusive evidence of the presence of tuberculosis. Problem herds are however comparatively rare and where we find a typical picture, post mortem corroboration is not always necessary. In all cases of doubt animals should be regarded as suspicious and retested.

Interpretation Standards

Where there is tuberculosis in a herd, interpretation should be strict, while in herds in which non specific sensitization is present, interpretation should be more lenient. It is therefore necessary to consider what reactions should be regarded as positive in each case. It is not possible to calculate expected frequencies from probability tables for our curves in Fig. 1, as in the case of the tuberculous animals we have two peaks of distribution and in the case of the non-specifically sensitized animals we have an obvious positive skewness. Less than cumulative frequency curves were therefore constructed for the two distributions, (Fig 2). Considering



the standards suggested by Kleeberg[14], it will be seen that there are about 15 animals out of the 500 with increases of 4mm or less, so that this limit should select 97 per cent of the tuberculous cattle at a single test. On retesting after 2–3 months we should be able to find virtually all tuberculous animals. If a small percentage of non specific reactors are included at this stage in a tuberculous herd it is of minor importance.

Kleeberg[14] also suggested that in herds where tuberculosis has not yet been established that a skinfold increase of 6mm should be regarded as positive and an increase of 3–6mm as suspicious. While this formula is perhaps suitable for herds in which the type of sensitization has not yet been established it can be seen from Fig. II that 14 per cent of the non-specific reactions recorded in our series were over 6mm. In herds where non-specific infection has been demonstrated this formula may therefore be too strict. In such herds it is perhaps best not to lay down a definite formula for interpretation, but rather to resort to retesting and comparative testing until the picture becomes clear.

Retesting

Retesting is essential in both tuberculous herds and in problem herds. Tuberculous herds should be retested after 2–3 months as the animals which were in the pre-allergic state at the first test will have converted to positive by this stage. Tuberculous animals having become positive will retain their sensitivity indefinitely, the only exception being the animal which has become de-sensitized when the disease has reached an advanced state. It is however believed that the completely anergic tuberculous animal is extremely rare under practical conditions.

In the case of non-specifically sensitized animals it has been said that they should be tested until they become negative. This statement, although somewhat cynical, contains a great deal of truth. At a retest of a non-specifically sensitized herd we generally find that some of the reactors have become negative, some have decreased reactions, some have similar or increased reactions and a number of new reactors may be found. The problem may continue in the herd for a long time, or the reactions may suddenly and inexplicably vanish.

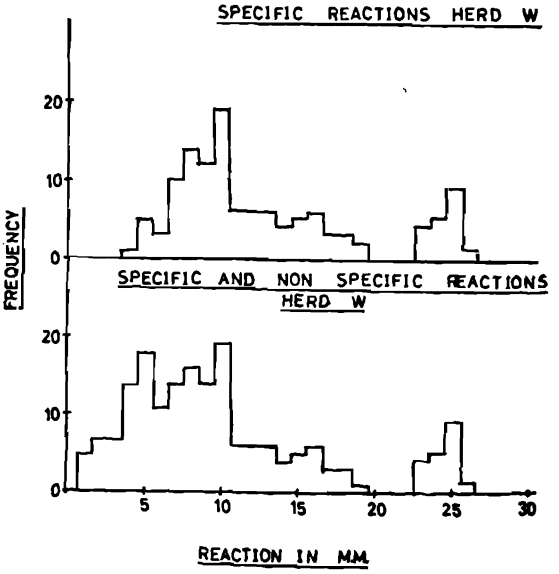
In problem herds special records should be kept of the skinfold increases of all reactor cows over a number of tests. By studying these records the non specific sensitivity pattern can be more easily regognized.

In our opinion the interpretation in problem herds will be greatly facilitated by using the comparative bovine-avian tuberculin test. This test will however be more fully discussed in a later publication.

Mixed Sensitization

The most difficult problems are likely to arise when there are both tuberculous animals and non-specifically sensitized animals in the same herd. Where such a problem is suspected the comparative test should be extensively used. In some cases a study of the frequency distributions of the reactions can give a clue that such a problem exists in a herd. As an example a study was made of the reactions occurring in a herd of 470 cattle of which 181 showed some reaction to tuberculin at the first test. The herd has since been under isoniazid treatment and regularly tested. A histogram was constructed of the 181 reactions occurring at the first test, see Fig. 3. It can be seen that there were three peaks

FIG 3



of distribution representing the usual two peaks seen in tuberculous herds, and one peak representing the non-specifically sensitized animals. On following the reactions of each animal over a number of subsequent tests, we were able to decide which animals were, in our opinion, non-specifically sensitized. A second histogram was then constructed from the reactions of those animals which were considered definitely tuberculous, see Fig. 3. In this case the distribution was found to approximate the typical type of distribution seen in tuberculous herds.

The Accredited Herd:

It is common experience that there is little difficulty in the interpretation of reactions in heavily infected herds. Difficulties are far more frequently encountered in accredited herds. The tuberculin test is frequently criticised on this account. The occurrence of these reactions should however be seen in the right perspective. Various authors have found the test to be between 96 per cent and 98 per cent accurate when used in cattle populations in which a fair number are infected with tuberculosis[14,15,16,17]. However, as a country becomes progressively more free from tuberculosis, the number of reactors to the tuberculin test in which no gross lesions can be demonstrated at post mortem, increases. Thus in 1961 after the incidence of tuberculosis had been reduced from 5.0 per cent to 0.15 per cent in the United Stated it was found that 73 per cent of reacting cattle did not have gross lesions of tuberculosis at post mortem[18]. It must however be remembered that the total number of reactors had been greatly reduced. Paterson[7] states that "the danger of exaggerating the importance of N.V.L. cases is well illustrated by figures given by Gow[19]; of 4,796 cattle shipped from Colorado to California in 1945 only 21 reactors were disclosed, with no visible lesions in 19, on post mortem examination. Here the incidence of N.V.L. animals is 95 per cent of the reactors but only about 0.004 per cent of the total number tested."

A similar position can be expected in testing accredited herds and it is therefore necessary to exercise considerable caution in interpreting reactions in these herds.

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OBSERVATIONS ON THE EPIZOOTIOLOGY OF FOOT-AND-MOUTH DISEASE IN SOUTH AFRICA WITH SOME REMARKS ON THE USE OF ATTENUATED SAT 1 AND SAT 2 LIVE VIRUS VACCINES IN FIELD TRIALS

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SUMMARY

In briefly describing the occurrence and epizootiology of Foot-and-Mouth Disease in the Republic of South Africa, the Author stresses the rôle of wild free-living cloven-hoofed animals. Some explanations are offered to support circumstantial evidence pointing to possible persistence of the disease in certain localities where large numbers of game of different species abound. Mention is made of the possibility of asymptomatic infection in game as well as the unknown pathogenicity of the virus for species hitherto unsuspected.

A number of examples are given to illustrate the variable nature and "plasticity" of the virus under field conditions in South Africa.

The evolution of control measures developed and successfully applied in the Republic are indicated as a background to co-operative field trials with SAT 1 and SAT 2 live attenuated virus vaccines produced by Pirbright Institute. A brief summary is given of some already published data in this respect, which shows that the vaccines are completely safe but the efficacy depends largely on the level of antigenic similarity between the vaccine and current field viruses. He concludes that at the present stage the use of such vaccines in the Republic of South Africa can only be considered as an adjunct to proven physical control measures during active outbreaks and not for precautionary vaccinations in the absence of a direct threat of infection.

South Africa is at present free from Foot-and-Mouth Disease since August 1961. The disease does not occur enzootically in any of the farming areas, but the country has been subject to repeated incursions across its land borders since 1933, outbreaks sometimes occurring at intervals of several years. With a susceptible animal propulation of over 50 million it is of the utmost importance that outbreaks be combated in the most safe and efficient manner.

The approach to control in South Africa has been based on the epizootiological manifestations of Foot-and-Mouth Disease in this region. As previously reported the disease was present in all provinces up to the time of the Rinderpest panzootic at the end of the previous century. The first record of the disease in the country dates from 1850 (Gordon Cumming) but it is not known when it was first introduced. It may have been present in wild animals for centuries and its disappearance at the time of the Rinderpest panzootic in 1896 may have been the result of disappearance of susceptible animals.

The re-occurrence of Foot-and-Mouth Disease in Southern Rhodesia in 1931 was associated with the development of cattle farming in an area of high game concentration. It therefore, appears that the disease could have persisted in wild cloven-hoofed animals. Such a contention is supported by subsequent observations that all initial outbreaks in South Africa were of "game origin".

Lesions of Foot-and-Mouth Disease in game animals are often so small that transmission to domestic stock possibly occurs more readily in the presence of large numbers of wild cloven-hoofed animals. Once domestic stock become infected the disease is usually transmitted readily to susceptible wild animals living in association

with them. Furthermore it has been observed that although game dispersed amongst domestic stock may become infected during an outbreak, the disease again disappears after a period of time, which may be somewhat longer than that required in domestic animals. This is, however, not the case in purely game areas where large numbers of game of different species abound. Experience has shown that wider contact of stock with wild cloven-hoofed animals from such areas often results in outbreaks of the disease.

The ecology of Foot-and-Mouth Disease in wild free-living animals is not yet fully understood. As already mentioned circumstantial evidence indicates that the disease persisted in these wild hosts during the period of approximat ly 1900 to 1931, without any manifestsation in domestic stock. It is probable that infection was limited to certain regions up to a certain stage.

The epizootiology of the disease in free-living wild animals may be interpreted in various ways: the bionomics and habits of the different species in relation to reproduction and relevant seasons, feeding, habitat, migrations, etc., may serve to supply a continuous chain of susceptible populations and successive contacts. On the other hand little is known about the symptomatology of the disease in various species. There is at least a suspicion of asymptomatic infection, which has never yet been established in domestic stock in these regions. The extent of the pathogenicity of the disease for species which have so far not been suspected as susceptible is also unknown.

It is believed that the elucidation of this complex problem will lead to the development of ways and means of finally eradicating Foot-and-Mouth Disease in this part of the world. In the interim it is important to avoid contact between domestic stock and game areas with high game populations.

The "plasticity" and variable nature of the virus was again recently referred to in an article by Dr. René Willems. Evidence of these aspects have been observed during various outbreaks of the disease and in the course of the experimental application of vaccines. The following instances may be mentioned:

In 1954 an outbreak of SAT 1 virus spread from game to cattle in the Eastern Transvaal. Some two months elapsed from discovery of the infection before cordon fences and other preparations for aphthasisation were completed. At that stage it was found that natural recovery of infected animals had progressed to the extent that it was extremely difficult to obtain virus material for aphthasisation purposes.

When the cattle were finally aphthasised some 40 per cent of animals in certain infected herds, in which no active infection existed any more, proved fully susceptible. This was probably an instance of low invasiveness of the virus.

The same virus type caused an outbreak on a farm in Barberton district, bordering on the Kruger National Park, in May 1958. The infection appeared self-limiting and disappeared in a short period of time after strict isolation of the property. Towards the end of that year an outbreak involving SAT 3 virus spread from game in the Park to cattle in the same area in the Barberton district. The virus this time was highly invasive and spread rapidly to a number of properties. The outbreak was eradicated in June, 1959, but during October 1959, the same and other areas became infected with SAT 2 virus, again originating from game in the Kruger National Park. Instances were observed where fresh foot lesions occurred simultaneously with remains of old lesions of the previous infection in the same animals. The virus, later typed as S.A. 106/59, was highly virulent with an incubation period a few days shorter than experienced during the earlier SAT 3 outbreak.

At the time of the SAT 3 outbreak four head of cattle known to have passed through an SAT 1 infection in May 1958, were bled and serum submitted to the Pirbright Instituted for determination of antibodies. The animals were thereafter transported to an SAT 3 infected property and aphthasised with SAT 3 field virus.

Two of the four animals proved immune, which fact corresponded with the results of their serum examinations at Pirbright. It was established that all four animals were immune to SAT 1 virus whilst two also had a sufficient level of immunity to SAT 3 virus to protect them. The animals had not previously been infected with SAT 3.

The three outbreaks just referred to were an instance where all three types, SAT 1, 2 and 3, occurred in the same area within a periofd of seventeen months. In every case the infection originated from game. In the latter two instances infection was actually diagnosed in game before the cattle became infected. Infection in game was fairly widespread.

In July 1961, an outbreak of SAT 3 virus occurred in the South-Eastern corner of the Letaba district, adjacent to the Kruger National Park. The game-proof fence around the Park was still in the process of erection. In the course of investigations to determine the origin of the infection, shooting of game and collection of serum samples were undertaken in the whole area around the outbreak, including the relevant area of the Park. The serum of one Impala (*Aepyceros melampus*—Lichtenstein) shot in the process, indicated that the animal had recently recovered from an SAT 3 infection.

No signs of Foot-and-Mouth Disease lesions were however, observed in any of the game animals so destroyed. Domestic animals, cattle, sheep and goats, concerned in this outbreak were isolated within cordons and aphthasised. At the stage when all animals had recovered from the reaction resulting from the artificial infection, one bovine on each of two properties was found to have lesions resembling Foot-and-Mouth Disease, during the course of detailed routine inspections. Material taken from their lesions was forwarded to the Pirbright Institute and typed as SAT 1. Subsequent extended quarantine and thorough short-interval inspections did not reveal any spread of infection whatsoever.

Towards the middle of 1960 an outbreak due to SAT 3 was diagnosed in Bechuanaland Protectorate. One cattle ranch adjoining the Republican border was involved. Precautionary quarantine and other measures were forthwith instituted on the South African side of the border. During the course of inspection and "mouthing" of cattle on the farm Witkopje, situated on the border, tongue lesions resembling Foot-and-Mouth Disease, were observed in one animal. Four weeks later there were 60 animals involved in the infection, which spread to two adjoining farms. The lesions were not typical and could be best described as necrotic papillitis. One of a few specimens of epithelial material submitted to Pirbright from the original farm was typed as SAT 1. A few months later cattle on the farm became infected with SAT 1 virus which had spread across the border from the major outbreak in Bechuanaland Protectorate.

The cattle proved to be 100 per cent susceptible to this type of infection.

During 1960 an outbreak caused by SAT 1 virus was also diagnosed in Bechuanaland. As the result approximately one million head of cattle were aphthasised to control the outbreak, as previously reported by Galloway. The infection ultimately spread into the Republic of South Africa during February 1961, and it could reasonably be duducted that this outbreak was also the source of infection which found its way to South West Africa in July, 1961. Infective material from the three respective countries were typed as SAT 1 at Pirbright. At the same time they were established to be distinct antigenic sub-types. It therefore, appears that the virus responsible for the original outbreak in Bechuanaland Protectorate became differentiated into at least two new antigenic sub-types within the course of approximately one year. In the face of this kind of evolution of the virus control by means of expecially modified live virus vaccines becomes problematical. Experience in South West Africa has, however, shown, that a large measure of success can be achieved by the use of an attenuated live virus vaccine in spite of differences between the vaccine- and field viruses. The circumstances of the relevant campaign are fully reported in a paper to be presented to this Conference (J.H.B. Viljoen).

The control of Foot-and-Mouth Disease in South West Africa has been developed against this background.

Most of the outbreaks were experienced in the Eastern Transvaal in areas adjoining the Kruger National Park. Initially the slaghter policy was applied up to approximately 1940 when it was found impractical for epizootiological reasons as sketched above. Extended isolation and quarantine of infected and in-contact herds were then practised during the next decade, sometimes for periods up to 12

months to allow the disease to disappear completely. This approach could also not be sustained due to economic hardships imposed on stock owners as well as the extended periods of persistence of infection and concomitant danger of further dissemination.

It was then decided to embark on a system of strict isolation of infected herds with aphthasisation to ensure that an immune population was created in the shortest possible time. This system allows the quarantine slaughter of stock three months after aphthasisation and the raising of strict quarantine two months later. It has, however, many disadvanteges in that a massive body of infection is created during the reacting phase with possible danger of spread, and will infect game if game is present.

Aphthasisation has been practised since 1951 and 419,750 head of cattle have been so treated in 23 districts. Not in a single instance has a "carrier state" resulted in consequence of this approach.

In an effort to find a safer method of dealing with outbreaks live attenuated vaccines of the virus types SAT 2 and 1, respectively, were experimentally applied in co-operation with the Pirbright Institute which had developed the vaccines. Favourable circumstances for such field trials occurred during 1959–1961, when outbreaks were encountered in the Eastern and Western Transvaal border regions, respectively. Details have been previously reported by Martin, Davies and Smith (1962) and Galloway (1962), whilst a further paper by Martin and Edwards has been prepared for publication.

SAT 2 (Rho 1) vaccine was used in five localities in four districts in the Eastern Transvaal involving 81,690 head of cattle. It was established that

— vaccine reactions were negligible, ranging from less than half to approximately 1 per cent;

— the vaccine virus was not transmitted to susceptible cattle in contact with vaccinates. This finding has endured since the field trial was carried out four years ago;

— at least 70 per cent of animals were protected under severe challenge conditions;

— different dilutions of the vaccine gave equally good results;

— strategic vaccination of cattle around a large active focus of infection had a dramatic effect on the spread of the disease. Rapid dissemination was halted in a matter of days, even in areas that were already infected at the time of vaccination. Cattle in the central area were aphthasised three weeks after vaccinations in the peripheral area. At least 60 per cent of these unvaccinated cattle reacted to artificial infection.

Some 3 to 5 weeks later cases of Foot-and-Mouth Disease were progressively found in some localities in the peripheral vaccinated area. The highest percentage was 4 per cent in one locality which remained active up to 12 weeks after vaccination. Cattle in these localities were revaccinated, followed by disappearance of infection approximately two weeks later. Circumstances were not favourable for observations to determine whether infection would have died out in any event after the first vaccination. The findings do, however, demonstrate the importance of comprehensive physical control;

— 70 per cent protection in South Africa compared with 91.5 per cent at Pirbright, when the homologous virus was used as the challenge virus. It was concluded that this was due to an antigenic sub-type difference between the vaccine virus and the current field strain in South Africa.

The circumstances in which the same type of vaccine was used in other localities did not allow of the proper evaluation of its protective qualities.

SAT 1 (R.V. 11) was used along an approximately 200 mile front on the international border with Bechuanaland Protectorate during the ealy part of 1961. The circumstances were that SAT 1 Foot-and-Mouth Disease infection had broken out in the protectorate during 1960. Large numbers of cattle were aphthasised as already mentioned. Towards the end of 1960 the disease reached the Tuli Block and in spite of stringent precautions the disease spread across the border in six places. At that stage the decision was made to conduct a field trial with SAT 1 live attenuated vaccine in an effort to create an immune barrier. A stretch of country along the exposed section of the border and a few farms deep was contained within a

cordon. Both cattle and small stock were double vaccinated at an interval of approximately three weeks. In one herd of 400 head of cattle, 200 were vaccinated to determine whether the vaccine virus was transmissible to susceptible in-contact animals. Just over 50,000 head of cattle and some 4,000 head of small stock were vaccinated and all animals on infected properties were finally aphthasised.

The vaccine was employed under rather difficult conditions: a severe drought prevailed and fair numbers of game were present in most of the area. Several farms were infected and aphthasisation of cattle on them was carried out progressively to speed up eradication. Other farms were infected at the time of the first vaccination or became infected between the first and second vaccinations. All these factors must have placed considerable stress on the vaccine immunity. The most important observations may, however, be summarised as follows:

(*a*) Vaccine reactions were again negligible—less than 2 per cent—and lesions were generally mild. In one instance a few newborn lambs showed symptoms of paresis, paralysis and subsequently died, following vaccination. The incident was not considered very significant at the time.

(*b*) The immune response was very variable. The following instances may be quoted:

(i) Vaccinated cattle on six farms were challenged I/M with virulent field virus after 23, 24, 41, 60, 60 and 60 days after vaccination and 67 per cent did not show reactions.

(ii) 3,516 head of cattle on four farms became naturally infected after 27, 29, 29 and 51 days, respectively, after the second vaccination. On subsequent aphthasisation an average of 41.75 per cent of the animals reacted.

(iii) Cattle on another 16 farms became infected 6 to 116 days after vaccination (Five properties after less than 21 days) and on subsequent aphthasisation 50 per cent proved to be immune.

Other variations were also encountered and the officer immediately in control of the campaign estimated that the average immune response varied between 40 and 50 per cent.

(*c*) There was no evidence of transmission of the vaccine virus to susceptible in-contact animals at the time or during the succeeding three years.

(*d*) An antigenic sub-type difference between the vaccine and field viruses has also been established in this instance at the Pirbright Institute. It may therefore be concluded that attenuated live vaccine viruses of the types used in field trials in South Affica are completely safe, as an additional measure to physical control of outbreaks and will not set up infection in susceptible animals in contact with vaccinates. It would appear that the efficacy of the vaccine depends on the level of antigenic similarity between the vaccine and current field viruses. In view of the known "plasticity" of Foot-and-Mouth Disease virus this latter phenomenon represents probably the biggest obstacle to the effective application of vaccines of this kind.

In view of our experience in the control of Foot-and-Mouth Disease in the Republic of South Africa it is considered that the use of vaccine, in our particular circumstances, should only be contemplated as an additional measure of control during active outbreaks of the disease in contradistinction to preventive vaccination.

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THE TOPOGRAPHICAL DISTRIBUTION OF NEGRI BODIES IN THE BRAIN

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SUMMARY

1. The number and character of Negri bodies were recorded in sections prepared from five different parts of the brain of 90 rabid dogs, bovines, cats and meercats.

2. In dogs, the cerebellum, and in cattle, the medulla oblongata proved to be the sites where Negri bodies could be demonstrated most readily in cases where they were absent in the hippocampus.

3. In dogs the hippocampus and cerebellum, and in cattle the medulla oblongata showed the greatest numbers of Negri bodies.

4. The hippocampus in cats and meercats proved to be the brain region where Negri bodies were superior in number, size and inner structure.

5. To ensure the greatest possible accuracy in the histological diagnosis of rabies, the hippocampus and cerebellum should be examined in the dog, the hippocampus and medulla oblongata in cattle and the hippocampus alone in cats and meercats. As a second choice the medulla oblongata is recommended in each of these species, except for the cerebellum in bovines.

INTRODUCTION

The fluorescent antibody technique is fast gaining prominence as a dependable method for the routine laboratory diagnosis of rabies. During a recent study of this technique in rabies, Schneider enumerated the most important factors responsible for non-specific fluorescence and stated that influences by such factors can now be eliminated and controlled with such a degree of success, that an accurate diagnosis of rabies can be made in the majority of cases. The histological diagnosis of this disease, based on the demonstration of Negri bodies (NB) in brain preparations, in conjunction with the biological test, however are still the sole diagnostic methods employed in many countries.

Tustin and Smit[2] found the overall efficiency of the histological test over a ten year period ending in March 1962 to be 66 per cent. In this analysis 71 and 46 per cent respectively of biologically positive cases were found to be histologically positive where the hippocampus alone and other parts of the brain were examined.

In 595 biologically positive cases submitted to the Veterinary Research Laboratory, Onderstepoort since the above date, a negative histological diagnosis was made in 88 cases or 15 per cent. In 60 of these latter cases the hippocampus had been available for histological examination, indicating that the histological diagnosis was negative in ten per cent of all biologically positive cases where the hippocampus alone was examined. It must be emphasized that 25 cases where a suspicious histological diagnosis had been made, were excluded from the total of 88 but were included with the histologically positive cases. These figures, illustrated in table 1, are representative for the four species dealt with in this study.

TABLE 1.—NUMBERS AND PERCENTAGES OF HISTOLOGICALLY NEGATIVE CASES OF FOUR SPECIES WHICH WERE BIOLOGICALLY POSITIVE FOR RABIES DURING THE PERIOD 15.1.62 TO 1.7.64%

Species	Number of biologically positive cases	Histologically negative	%Histologically negative	Histologically negative where hippocampus was available	% Histologically negative where hippocampus was available
Canine.....	201	47	24%	37	19%
Bovine.....	150	23	15%	15	10%
Meercat.....	207	14	7%	6	3%
Feline.....	37	4	11%	2	5%
Total.....	595	88	14.8%	60	10%

From the above table it can be seen that the highest percentages of histologically negative cases were associated with the canine and bovine species, whereas the corresponding figures for meercats and cats were considerably lower.

With the object of improving the efficiency of the histological examination it was deciced to determine the incidence and distribution of NB in five different parts of the brain of four species of animals. These findings would then enable the selection of a suitable part or parts of the brain in each species that would, in combination with the hippocampus, ensure the demonstration of NB in the highest possible percentage of cases. It is evident that these sites would automatically serve as the regions of choice either in combination with or in the absence of the hippocampus.

The species selected for this study were the canine, bovine and feline and the meercat species of the family *Viverridae*, because they are the species in which the highest incidence of rabies occurs in this country and which would consequently ensure sufficient material for the investigation.

The parts of the brain selected for this comparative study were the hippocampus, the mesencephalon in the region of the nucleus oculomotorius, the cerebellum, the medulla oblongata and the cerebral cortex. The hippocampus has been considered the site of choice for the detection of NB since their discovery in 1903 by Negri[3]. This worker, after whom the specific intracytoplasmic inclusion bodies are named, found the hippocampus to be the most suitable area for demonstrating his "protozoon parasites", which he held responsible for the disease. The mesencephalon was included because Thomas and Jackson[4] stressed the importance of examining the nucleus oculomotorius in rabid rabbits. They were able to demonstrate NB in this in almost every case in which they were present in the hippocampus and also in a considerable number of cases in which they were absent in the latter. In experimentally infected mice, Muratowa[5] found that the first appearance of NB was in the midbrain around the central canal and not in Ammon's horn. He also found that in advanced rabies of mice NB may be absent in the latter and the cerebellum while demonstrable in other parts of the brain.

Not only the number of NB but also their size and inner structure are important features in their identification; these characteristics were therefore also taken into consideration in this study.

MATERIAL AND METHOD

Formalin preserved specimens of biologically positive cases only were examined. From these only those cases in which the five specific brain areas could be identified, were selected. A block of tissue for embedding in paraffin wax was excised from each of these zones. A special attempt was made to include as many cases as possible of each species in which an original negative histological diagnosis based on examination of the hippocampus only, had been made but which subsequently proved to be biologically positive. Such highly selective material was employed because the chief object of this work was to detect the possible occurrence of NB in regions of the brain other than the hippocampus in cases where NB were absent in the latter. Sections were cut at five microns and stained by the Acid Fuchsin-Methylene Blue method described by van der Merwe[6].

The total number of NB in 50 neurones from each of the five parts was counted with the help of

a counter operated manually. In the cerebrum 50 consecutive neurones were examined. In the hippocampus groups of neurones throughout the entire length of the pyramidal layer, in the cerebellum the Purkinje cells and in the medulla oblongata neurones in various nuclei in close proximity to the central canal were examined. In the mesencephalon neurones of the nucleus oculomotorius were given preference and, in its absence, neighbouring nuclei close to the central canal were examined.

The size and definition of inner structure of the NB in each site were recorded. Sizes of NB were estimated arbitrarily and recorded as large, medium or small. NB were recorded to possess an inner structure if spherical basophilic bodies of. Varying size could be distinguished within the outlines of the N.B.

Results

The results obtained are shown in tables 2 and 3.

Table 2.—Tabulation of the number of cases and the regional indicence of NB in the four species.

	Canine	Bovine	Feline	Meercat
Total number of cases examined	42	18	13	17
No. of cases where NB absent in each of 5 parts	10	1	0	0
No. of cases where NB present in each of 5 parts	6	6	2	7
No. of cases where NB in hippocampus only	4	0	2	0
No. of cases where NB in medulla oblongata only	1	2	0	0
No. of cases where NB in cerebellum only	3	0	0	0
No. of cases where NB absent in hippocampus but present in more than one of other parts	4	1	0	0
No. of cases where NB in hippocampus and one or more of other parts but not in all 5	14	8	9	10

It is evident from table 2 that in eight dogs (20 per cent) NB could be demonstrated in parts other than the hippocampus, while being absent in the latter. The same was true of three bovine cases, whereas in cats and meercats not one such case could be demonstrated.

In the dog NB were present in the cerebellum only in three cases and in the medulla oblongata only, in one case. In an additional two cases NB were in evidence in the mesencephalon, cerebellum and medulla oblongata only and in another two cases in the cerebellum and medulla oblongata only.

In cattle the medulla oblongata proved to be the single site where NB could be demonstrated in two cases, while in one case only both the medulla oblongata and cerebellum gave such evidence. A significant feature of the NB in the medulla oblongata of these three cases was their small size and large numbers. The cytoplasm of a large number of neurones in various medullar nuclei was packed with numerous small NB. There was no indication of inner structure.

Table 3.—Table indicating the incidence of superior numbers of NB of medium to large size and with distinct inner structure.

	No. of cases per brain region where NB were superior in number.					No. of cases where large to medium sized NB with clearly distinguishable inner structure were predominant in area indicated.			No. of cases where NB of superior size and inner structure were demonstrable in largest numbers in one particular site.	
	Hippo campus	Cerebrum	Mesencephalon	Cerebellum	Medulla oblongata	Hippocampus	Cerebellum	Medulla oblongata	Hippocampus	Cerebellum
Canine	8	0	5	8	3	5	7	0	5	5
Bovine	3	0	4	3	6	5	2	0	3	1
Feline	5	1	4	0	1	4	1	0	1	0
Meercat	12	0	2	0	3	10	0	1	11	0

In the above table estimation of size and inner structure of NB was based on the overall impression of the majority of NB in a particular area of the brain. These characteristics were considered together because it was generally found that large NB evidenced a definite and clearly visible inner structure more frequently than did small and medium sized NB. Furthermore, only cases in which the characteristics mentioned, singly or combined, were superior in one particular site, are indicated in table 3. Those in which largest numbers of NB of superior size and inner structure were demonstrable in more than one brain region, were therefore excluded.

As shown in table 3, the hippocampus and cerebellum were the sites where the largest number of NB per fifty nerve cells could be demonstrated in an equal number of dogs (eight or 19 per cent). Although this was the case with five and three dogs respectively where the mesencephalon and medulla oblongata were examined, the NB in these areas were of a medium to small size with indefinite or no inner structure, so that not one case could be demonstrated where from size and inner structure point of view, these areas were superior.

In cattle the largest numbers of NB in the highest percentage of cases (35 per cent) were in evidence in the medulla oblongata, whereas the hippocampus, mesencephalon and cerebellum jointly follow on the former. The hippicampus again emergesas the site where NB show superior development in size and inner structure.

Both the feline and meercat species were characterised by a high incidence of great numbers of large-sized NB with well-developed inner structure in the hippocampus. Similarity in these two species was also evidenced by the fact that with the exception of one case in a cat, the cerebellum never was the site where NB were superior in number, size or inner structure.

The region of the oculomotor nucleus in the mesencephalon was the site where the greatest number of NB could be demonstrated in a considerable number of cases in each of the four species, but in no instance was this the exclusive site. The NB were usually small with poorly defined inner structure. In no single case in any of the species were NB of superior size and inner structure in this site (Table 3). In a total number of 43 cases of all four species the oculomotor nucleus could be located with certainty. No significant difference in number and character of NB existed between this nucleus and neighbouring nuclei of this region.

The number of cases in which the three characteristics of superior numbers, size and inner structure were combined in one particular region of the brain is indicated in the last column of table 3. Such a combination could be demonstrated in the hippocampus and cerebellum only in an equal number of cases (12 per cent) in the canine species. In the other species the hippocampus was the only site where such a specific combination occurred, in cattle in 18 per cent and in meercats in 65 per cent of cases.

Conclusions and Recommendations for the Collection of Specimens for Histological Examination.

From the above findings the following conclusions may be drawn, and these in turn permit certain recommendations regarding the most suitable parts of the brain of each species that should be submitted and examined for the histological demonstration of NB. In addition the second and third most suitable parts in each species, that should be examined in the absence of the first choice, may be deducted. These recommendations are indicated in table 4.

TABLE 4.—THE MOST SUITABLE OF THE FIVE SITES, IN ORDER OF PREFERENCE, FOR THE HISTOLOGICAL DEMONSTRATION OF NB IN SECTIONS.

	First choice	Second choice	Third choice
Canine.....	Hippocampus and cerebellum	Medulla oblongata	Mesencephalon
Bovine.....	Hippocampus and medulla oblongata	Cerebellum	Mesencephalon
Feline.....	Hippocampus	Medulla oblongata	Mesencephalon
Meercat.....	Hippocampus	Medulla oblongata	Mesencephalon

In the canine species examination of the hippocampus and cerebellum should facilitate the demonstration of N.B. in the highest possible percentage of cases. Inclusion of the cerebellum is justified by the eight cases indicated in table 2 in which NB were absent in the hippocampus and by the large number of dogs in which the cerebellum proved superior in numbers, size and inner structure. In the absence of the hippocampus and/or the cerebellum, the sites recommended are, in the order of preference, the medulla oblongata and mesencephalon.

In cattle the diagnostic significance of the medulla oblongata is supported by the 35 per cent of cases in which NB were most numerous in this area, by the two cases in which NB were present in the medulla oblongata only and the one instance where this was the case in the medulla oblongata and cerebellum. The former is therefore the area of choice as a supplement to the hippocampus. If during the microscopical examination the hippocampus should prove negative, particular attention should be paid to the neurones in the nuclei of the medulla oblongata, for the presence of numerous, small NB, particularly in animals that have been destroyed early in the course of the disease. In spite of their small size and absence of inner structure these NB have considerable value in the diagnosis of rabies. Although it must be admitted that these particular NB in the bovine medulla oblongata could be found in only three cases, it would appear that NB make their first appearance in this part of the central nervous system.

In meercats and cats the hippocampus alone is considered adequate firstly because not one case out of 30 could be demonstrated where NB were present in any one of the five sites while being absent in the hippocampus and secondly because of the high incidence of large numbers of NB of fair size with distinct inner structure. Should the hippocampus not be available, the medulla oblongata and mesencephalon have equal value as substitutes.

There were no cases in any of the four species in which NB could be demonstrated in either the mesencephalon or cerebral cortex while being absent in all other parts. Although fair-sized NB with distinct inner structure could be demonstrated in the cerebral cortex, only one case in a cat is shown in table 3 where the greatest number of NB were recorded in this site. This part of the brain would therefore only be chosen for histological examination in the absence of all other parts.

During this investigation evidence was sought on the question of whether the number and character of NB from animals that have been destroyed differ from those from animals that have been allowed to die after the full course of the disease. Of the ten cases in dogs in which N.B. were absent in everyone of the five parts examined, six had been destroyed, two of which after an illness of three days. No history of the immediate cause of death was available for the remaining four dogs. In another 52 cases where the mode of death was known, it was found that 32 cases which had been destroyed evidenced NB of small size and in small numbers, whereas in 11 animals that were left to die, moderately large to large NB in large numbers could be demonstrated in the majority of sites examined. Ther e-maining nine cases that had been destroyed showed fair-sized NB in large numbers. These findings and particularly the total absence of NB in the case of six dogs that had been destroyed, support the view that destruction of rabid animals early in the course of the disease lessens the chances of a dependable histological diagnosis.

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VIRUSES AS ETIOLOGICAL AGENTS IN EQUINE RESPIRATORY DISEASE*

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SUMMARY

During the last decade a number of different viruses have been isolated from cases of respiratory disease among horses. In this paper the available information is collated and briefly reviewed and a short description of the symptom complexes produced by these agtents is given.

The differential diagnosis and economic importance of these diseases are discussed.

INTRODUCTION

Respiratory infections and the symptom complexes associated therewith are commonly found in horses and have been recognised for centuries. However the etiological agents were, until recently, practically unknown and the diseases were therefore named after certain prominent clinical features. The terminology was highly confusing and included diseases such as "equine influenza", "infectious bronchitis", "catarrhal fever", "epizootic cough" and "contagious pleuropneumonia". The etiological relationship of these conditions was completely unknown.

Although viruses were previously suspected as causal agents of equine respiratory disease it was only during the last ten to fifteen years that definite attempts were made to isolate and identify the viruses concerned. During this period a number of distinct viral entities have been identified in the former "equine influenza complex".

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It is the object of this paper to give a brief description of these viruses and the disease syndromes they produce.

EQUINE RHINOPNEUMONITIS VIRUS

Jones and his co-workers[1] succeeded in isolating a virus, which they called "equine influenza virus", from horses with an upper respiratory infection. A similarity between the "equine influenza virus" and the "equine abortion virus" isolated by Dimock and his associates[2,3], was first suggested by Manninger[4] who regarded abortion as a sequel of equine influenza virus infection. Doll *et al*[5]. subsequently proved that these two viruses were identical and proposed the name equine rhinopneumonitis virus.

Detailed reviews of the disease caused by this virus have appeared recently [6,7]. Rhinopneumonitis virus has a world-wide distribution, including South Africa where its presence has recently been established[7]. The respiratory manifestations of the disease are mainly observed in weanlings and yearlings whereas older horses usually only suffer from a very mild or inapparent infection. The incidence of infection is highest during autumn and winter.

The incubation period varies from 2–10 days[1]. Animals suffering from an uncomplicated viral infection show mild fever (102–105°F), congestion of the nasal mucosa serus rhinitis, mild conjunctivitis and slight enlargement of the mandibular lymph nodes. The febrile reaction is generally accompanied by a marked leucopenia[5]. Complete recovery usually occurs within one week, but in cases compli-

cated by secondary bacterial invasion recovery may be protracted.

Pregnant mares which become infected during the latter semester of pregnancy may abort from three weeks to three months after exposure. It is important to note that the mare shows no premonitory signs of impending abortion and is not visibly ill at the time. During the course of the transient viraemia in the dam, the foetus is infected *in utero* (foetal rhinopneumonitis) with subsequent death and expulsion. The afterbirth comes off readily and as a rule the future breeding efficiency of the mare is not impaired[8].

Mares infected late during the gestation period may give birth to live foals, but these foals are usually weak and very prone to secondary infection[3,6], with bacteria such as *Actinobacillus equuli* and haemolytic *Streptococci*[3]. In such cases the underlying viral condition is frequently not recognized and its importance overlooked.

Infection with rhinopneumonitis virus is followed by antibody formation and a temporary refractivity to reinfection. Although virus-neutralizing antibodies may persist the respiratory tract may be re-infected within 3 to 6 months. Immunity against abortion is more durable but still incomplete and variable[6].

Equine Herpes Virus 2

During the course of investigation at the Wellcome Research Laboratories in England, Plummer and Waterson[9] isolated a virus from a horse with respiratory catarrh and coughing. This virus was found to be similar to equine rhinopneumonitis virus in various respects such as size, morphology and the characteristic cytopathic effects in tissue cultures with the formation of intranuclear inclusion bodies. Complement fixation tests also revealed some antigenic overlap with equine rhinopneumonitis virus but no cross-neutralization could be demonstrated[10].

In view of the apparent relationship between the two viruses Plummer and Waterson[9] suggested that both viruses should be considered as members of the herpes virus group and further proposed that equine rhinopneumonitis virus be known as equine herpes virus-1 and the newly isolated virus as equine herpes virus 2.

The clinical symptoms and the pathological lesions produced by this virus have not as yet been described, but its isolation from a horse showing nasal catarrh and coughing, and its close relationship to equine rhinopneumonitis virus suggests a clinical syndrome very similar to that produced by the latter virus.

Equine influenza viruses

An unidentified equine respiratory disease occurred in Sweden during 1955 and 1956[11] from where it presumably spread extensively over Eastern Europe. A severe epizootic occurred in Czechoslovakia from which Sovinova *et al.*[12] isolated a type A-influenza virus, classified as Myxovirus influenzae A/equi-1/Prague/56[13].

In 1957 the World Health Organization sponsored a serological survey in some twenty different countries in order to gain information about the incidence of influenza-A virus infections in horses and swine. Particular emphasis was placed on the type A_2 (Asian) influenza virus which had been active in pandemic form among humans at the time.

The results of this survey indicated that the Asian (A_2) strain of influenza virus could cause natural infection in horses and swine, and further demonstrated the presence of antibodies against the A/equi-1 strain in many countries from which the virus infection had not been previously reported[14].

Gaidamaka *et al.*[15] investigated an outbreak of upper respiratory disease among horses at a race-course in Kharkov, U.S.S.R. The equine disease occurred shortly after an epidemic of Asian influenza among the race-course staff. Although virus could not be isolated from the sick horses, serological evidence indicated that the animals apparently had suffered from an influenza-A_2 virus infection, which suggested a relationship between the human and equine diseases[15].

During an influenza epidemic in Moscow in 1959, a local outbreak of acute respiratory dis-

ease among horses coincided with the occurence of influenza among the attending staff[16]. Strains of influenza virus isolated from the horses and from the attendants were closely related to each other and to other strains of influenza-A_2 virus isolated from humans in different parts of the city. These findings were subsequently confirmed by serological tests on equine and human sera. Domracheva[16] therefore concluded that the human influenza-A_2 virus was responsible for the equine respiratory disease.

Antibodies against influenza-A virus in equines have been reported from the United States of America prior to 1963[14,17,18,19]. Early during 1963 an outbreak of respiratory disease occurred among horses in Florida, from where it extended to almost the entire North-American continent[20]. The etiological agent proved to be an influenza virus classified as Myxovirus influenzae A/equi-2/Miami/63[21,22].

Marois *et al.*[23] reported the isolation of type A_2 influenza virus from horses in Canada. Ditchfield and his co-workers[24], who thoroughly investigated the viral etiology of equine respiratory disease in the Toronto area, isolated influenza viruses similar to the A/equi-1/Prague/56 and the A/equi-2/Miami/63 viruses.

The first evidence of equine influenza in the British Isles was obtained by the demonstration in 1962 of antibodies against the Prague virus in a small percentage of horses in Ireland[25]. During the autumn of 1963, however, a widespread epizootic of equine influenza occurred in England which was shown to be due to a virus classified as A/equi-1/Cambridge/63[26], The latter authors also showed that a virus identical or closely related to the A/equi-1 virus had occurred in England at least as far back as 1948. It is interesting to note that antibodies to A/equi-1 virus were also found in zebra kept in captivity[26].

The various reports[11-26] concerning infection of horses with influenza A viruses fully justify the proposal that the term "equine influenza" be restricted to infection of horses with true influenza viruses[12,19].

Equine influenza is characterized by its extreme contagiousness and the explosive nature of the outbreaks[20]. Horses of all ages are infected and the morbidity is usually very high (50–90%). The clinical symptoms[12,15,19,20,21,23,26,27] seem to vary in intensity with different virus strains and may be severe, mild or frequently inapparent. The onset of the disease, after an incubation period of 2 to 4 days, is sudden and is usually associated with a rise in temperature (102–106°F) The febrile reaction may last from 1 to 5 days or longer and is frequently accompanied by serous rhinitis, excessive lachrymation, anorexia, fatigue, muscular pains and swelling of the limbs. The most constant symptom, however, is a severe dry cough, particularly in animals that are raced or kept in training. The course of the disease may vary considerably, but in the absence of complications recovery takes place within two weeks. No mortality as a result of an uncomplicated viral infection has been reported.

PARA-INFLUENZA VIRUSES

Para-influenza viruses constitute a wellknown cause of human respiratory infection, especially in young children[28]. This group of viruses is however, by no means confined to man since Myxovirus para-influenzae 3 was isolated from calves suffering from "shipping fever"[29] and an equine strain of Myxovirus para-influenzae 3 (RE 55) was recently recovered from horses in Canada by Ditchfield *et al*[30].

Serological investigations conducted in Canada further indicated a very high prevalence of antibodies against all three para-influenza virus types, particularly in older horses[24].

Horses suffering from natural infection with para-influenza 3 virus do not present pathognomomic clinical features. The symptoms are those of acute respiratory tract infection, accompanied by a mild febrile reaction, marked sero-purulent nasal exudate, conjunctivitis, anorexia and dyspnoea. Adenitis of the submaxillary lymph nodes appear to be a constant finding. Spontaneous recovery usually occurs within about a week but bronchitis and purulent rhinitis may develop and can persist for several weeks.

Equine Rhinoviruses

Various viruses have been shown to contribute to the "common cold" syndrome in man, of which the rhinoviruses[31] may be regarded as the most important single group. The rhinoviruses have certain properties in common with the true enteroviruses (e.g. small size, cubic symmetry, absence of essential lipids), but differ in being acid labile and incompletely stabilized by magnesium ions[32]. At least 53 distinct serotypes have been isolated from man[33] and one from a calf[34].

The first equine rhinovirus was isolated by Plummer from horse faeces[35]. A virus subsequently isolated from horse serum was shown to be similar to Plummer's rhinovirus[36]. Ditchfield *et al.*[24] recently reported the isolation of two distinct equine rhinovirus types.

The incubation period after natural or experimental infection of horses is 3 to 8 days. The duration of viraemia is 4 to 5 days and is often accompanied by pyrexia, a mucoid or mucopurulent pharyngitis, swelling of the pharyngeal lymph nodes and a nasal exudate of varying severity. There is no evidence that the virus multiplies in the tissues of the gut and the small quantities of virus found in the faeces probably originate from the pharyngeal region[35,37].

The uncomplicated viral disease is usually mild but secondary bacterial invasion readily occurs[37].

Serological surveys have indicated a very high incidence of infection among selected equine populations[37]. Antibodies to this virus were also demonstrated in some of the stable workers which indicated that the human being is susceptible[35]. A human volunteer, infected intranasally with the equine rhinovirus developed severe pharyngitis, swelling of the pharyngeal lymph nodes, fever, and a viraemia which lasted for 4 days[38]. Plummer also recorded the susceptibility of monkeys and rabbits to this virus[35,36].

Epizootic cough ("Hoppegartener Husten")

In 1934 Waldman, Köbe and Pape [39]described a lower respiratory tract infection of equines in Germany. These authors succeeded in producing the typical disease in horses by means of bacteria-free filtrates of lung and bronchial tissues. Young pigs were shown to be susceptible to experimental infection and cattle contracted the disease by natural contact with diseased horses as well as by artificial intranasal inoculation of infective tissue suspensions[39].

It was not until 1963 however, that Böhm and Straub[40] succeeded in isolating a viral agent from the nasal passage of an infected horse. Cytopathic effects were only apparent after five serial passages in calf kidney cells and a sixth passage in a culture of foetal calf lung. They subsequently failed to re-isolate virus from the original material.

The virus which they isolated was shown to consist of two different particle sizes, both of which grew readily in various tissue culture cell types and in embryonated eggs. Only one of these agents proved to be infectious for calves and pigs[40] and produced symptoms indistinguishable from those described by Waldmann *et al.*[39]. Böhm and Straub[40] therefore considered this virus to be the causal agent of epizootic cough in spite of the fact that no indication of infection was obtained in two horses infected intranasally with tissue culture material.

The clinical symptoms in horses as described by Waldmann *et al.*[39] and Röhrer[41] followed after an incubation period of 2–6 days and generally consisted of a transient febrile reaction, serous rhinitis, epiphora, swelling of the pharyngeal lymph glands and a dry painful cough.

The most prominent lesions found in animals killed during the reaction period were catarrhal bronchitis and peribronchitis with oesinophilic infiltration[39].

Equine arteritis virus

Doll and his co-workers[5] proposed the name equine arteritis for a virus which they isolated from an aborted horse foetus in 1953. Confirmed outbreaks of the disease have been confined to a few isolated foci in the United States of America. Consequently most of the information concerning this disease was derived from experimental work[19,42]. The disease produced by equine arteritis virus resembled some

of the classical descriptions of conditions such as "equine influenza", "pink eye" and epizootic cellulitis[42].

Although equine viral arteritis is not a true respiratory disease in the strict sense of the word, the respiratory system of the horse is nevertheless severely affected and mild cases of viral arteritis cannot readily be differentiated from the other viral diseases discussed in this paper. It was therefore thought advisable to include a brief description of this disease.

The morbidity may be very high, especially under conditions favouring close contact of horses. The incubation period is usually very short but may be as long as 10 days. The first clinical symptom is a rise in temperature (104–106°F) followed by a clear serous nasal exudate. In addition, affected horses may show conjunctivitis, lachrymation, palpebral oedema, congestion of the nasal mucous membranes, dyspnoea, colic, diarrhoea, dehydration and reduced sensation[5,19,27,42,43]. Symptoms less frequently encountered are keratitis, photophobia, icterus and oedema of the legs, abdomen, mammary gland, scrotum and sheath[27]. The febrile reaction is almost invariably accompanied by a marked panleucopenia, the lymphocytes being affected more severely than the neutrophils[44]. Virus is present in the blood, nasal secretion and in other body fluids.

Recovery from viral arteritis depends upon the severity of the condition but is usually complete within 14 days. Mortality is very variable, but seldom exceeds 30 per cent.

The pathognomonic lesion is found in the vascular system and is characterised by degeneration and necrosis of the media of small muscular arteries in all parts of the body[45]. Other lesions such as haemorrhage, thrombosis, infarction and oedema are probably the result of the arterial lesions.

Field and Laboratory diagnosis of respiratory viral diseases

The clinical and epidemiological features of the respiratory diseases produced by the variety of viral agents described in this paper are remarkably similar and it is frequently impossible to differentiate between these conditions by field investigations only. The recognition of certain outstanding clinical symptoms may however aid the laboratory isolation of the causal agent. For instance, a severe dry cough may suggest influenza virus as a cause of the condition whereas an acute pharyngitis is suggestive of rhinovirus infection. Respiratory symptoms occurring in association with abortion, subcutaneous oedema and wide-spread haemorrhages point to arteritis virus as the causal agent. Furthermore, the appearance of equine disease concurrently with a respiratory epidemic among stable personnel may suggest either influenza or rhinovirus as a cause in view of the possible relationship between human and equine infections with these viruses.

A definite diagnosis can only be made by laboratory isolation and identification of the virus concerned or by means of serological tests on acute and convalescent phase serum samples from selected cases.

Most respiratory viruses can be isolated from nasal swabs or nasal washings. These should be collected during the early febrile phase of the disease and submitted to the laboratory in a frozen state. If para-influenza or rhinovirus infection is suspected, swabs of the laryngopharyngeal region should preferably be taken. In addition, blood specimens collected during the acute stages of the disease, are suitable for the isolation of equine influenza, equine arteritis and equine rhinoviruses.

Most of the viruses concerned can be isolated in tissue culture and some indication as to the identity of the agent can be derived from the cytopathic effects which occur. The herpes viruses grow readily in horse and rabbit kidney cells and produce typical ballooning of the cells with large eosinophilic type A intranucleur inclusion bodies. Para-influenza viruses multiply in monkey kidney cells and exhibit haemadsorption with guinea-pig erythrocytes. Rhinoviruses are propagated in monkey kidney cells and produce cytopathic effects typical of the Picorna virus group[31]. For the isolation of influenza viruses, 10:day old embryonated hen's eggs are inoculated into the amniotic sac, and the amniotic fluid is tested for haemagglutinin to human O, chicken or guinea-pig erythrocytes 3 to 5 days after infection.

Since virus isolation is a tedious and costly procedure, serological tests on paired serum samples are valuable as alternative methods of diagnosis, particularly when large numbers of animals are involved and serves as a method of screening before virus isolation is attempted.

General Discussion

Equine respiratory disease, with its complex viral etiology, may be responsible for considerable economic losses particularly in racing establishments. The diseases may adversely affect the racing ability of horses and interfere with their training programme. These indirect losses are not always appreciated.

Although the primary viral infections are usually mild, secondary bacterial invasion invariably follows especially in animals which are kept under unhygienic conditions or subjected to work or other stress factors during the acute phase of the disease. The secondary complications, commonly due to haemolytic *Streptococci*, *Staphylococci*, *Neisseria catarrhalis* and *Pseudomonas* spp.[6,37], aggravate the symptoms and prolong the recovery period. These infections are characterised by a mucopurulent rhinitis and pharyngitis, suppuration of the pharyngeal lymph nodes, a persistent cough and less frequently by bronchopneumonia, pleuritis, enteritis and arthritis. As a rule these conditions do not respond well to systemic treatment with antibiotics. Although favourable results are sometimes obtained by topical treatment of the affected mucous membranes and careful nursing symptoms may linger on for several months. Apart from the cost of treatment, these conditions may permanently affect the racing ability of certain animals.

Outbreaks of equine rhinopneumonitis and arteritis may also be responsible for considerable financial losses in breeding establishments due to abortions in pregnant mares.

The presence of rhinopneumonitis virus infection in horses has definitely been established in South Africa and the disease appears to be widespread. In addition, a large number of other viral agents have also been isolated from horses in this country, but they have not yet been sufficiently studied to allow their identification[46]. Some of these agents appear to belong to the herpes virus group, but on cross-neutralization tests with the Kentucky-F strain of equine rhinopneumonitis virus no serological relationship could be demonstrated. These strains may prove to be similar to equine virus 2 or may be unrelated viruses of the herpes group. Infections with equine influenza and rhinoviruses have not yet been recognised in South Africa but it is quite possible that these viruses are responsible for some of the unidentified respiratory diseases among horses in this country.

As in other countries, attempts should be made to isolate, characterize and identify the complete range of etiological agents responsible for equine respiratory diseases in South Africa. Veterinarians in equine practice are therefore urged to co-operate fully with diagnostic centres and the Research Institute in order to achieve this aim. Until this knowledge becomes available it will not be possible to control equine respiratory diseases in South Africa on a sound scientific basis.

Since the preparation of this manuscrip a few relevant papers have appeared to which reference should be made in this article.

An outbreak of equine influenza due to a virus identical to or closely related to the A/equi-2/Miami/63 influenza virus was recently reported from Britain[47]. Further comments and observations on equine influenza were given by Beveridge[48] and Miller[49]. The latter author places particular emphasis on the occurrence of influenza in new-born foals. Foals born during an epizootic are highly susceptible and contract the disease in a far more severe form. Such animals usually show pyrexia, anorexia and severe respiratory distress. The prognosis is usually poor but encouraging results have been obtained by hyperimmune serum therapy, particularly when used as a prophylactic measure within three days of birth. It is also advisable to institute heavy post-natal antibiotic therapy for foals exposed to viral infection since they are very prone to secondary bacterial infections[49].

A good account of an epizootic of equine influenza A which occurred in Sweden in 1960 was

given by Espmark and Salenstedt[50]. The etiological agent was not isolated but serological investigations suggested it to be an A/equi—1 virus.

Kasel and his co-workers[51] published a most interesting report on the experimental infection of human volunteers with equine influenza virus. Five individuals were infected with Myxovirus influenzae A/equi—2/Miami/63. One of these volunteers developed illness which consisted of fever, mild sore throat, nasal congestion and obstruction and myalgia. Virus was reisolated from throat washings of all five volunteers but homotypic neutralizing antobodies only developed in four.

A detailed description of the properties of two equine rhinoviruses was given by Ditchfield and Macpherson[52]. These authors proposed the name "equine rhinovirus type 1" to include Plummer's respiratory enterovirus"[35] and their E 14 virus. The name "equine rhinovirus type 2" was suggested for their E 26 virus isolate.

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INVESTIGATION INTO A NATURAL OUTBREAK OF INFECTIOUS PUSTULAR VULVO-VAGINITIS (IPV) IN CATTLE IN SOUTH AFRICA

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SUMMARY

An outbreak of IPV amongst a group of cows and bulls in the Republic of South Africa has been described. The disease was characterised by the presence of a pustular vulvovaginitis and a pustular balanoposthitis in the cows and bulls respectively. The diagnosis was confirmed by the isolation of IPV virus from the naturally infected animals and by the reproduction of the disease in experimental animals with the virus isolated from the naturally infected cases. The marked rise in the level of antibodies to IPV virus in the convalescent phase sera of affected animals was added confirmation of the diagnosis.

INTRODUCTION

The disease, infectious pustular vulvovaginitis (IPV), named as such by Kendrick *et al.*[16], has been described in different countries under the names of vesicular venereal disease (Gibbons[9],), coital vesicular exanthema (Barker, 1958[1]), vesicular exanthema (Law, 1912[17]), and Bläschenausschlag (Kampmann, 1887[15]).

The clinical disease and its infectious nature have been recognised since the latter half of the previous century. The condition has been reported on numerous occasions from Western Europe and Germany (Kampmann, 1887[15]) and less frequently from the United States of America, (Steddon, 1895[25]). More recently it has been reported in New Zealand (Fastier and Smith[6]), and Australia (Parsonson, 1963[22]).

Reisenger and Reimann (1928[24]) were the first to transmit the disease with bacteria-free filtrates. Kendrick *et al.*[16] in the United States of America, succeeded in isolating the virus from vaginal exudate of typical cases and demonstrated that it was the cause of the disease.

In South Africa, Maré and van Rensburg[20] isolated a number of viruses from the genital tract of cattle. Certain of the virus strains isolated showed complete reciprocal cross neutralisation with IPV virus. The clinical syndrome associated with natural and experimental infection with these virus strains differed markedly from the classical IPV syndrome described by Kendrick *et al*[16]. Up to the present the latter syndrome has not been described in South Africa.

This article describes one of two outbreaks of IPV occurring in the Pretoria district during 1964 and the artificial transmission of the disease to bulls and heifers.

It occurred among a small group of experimental cows and bulls at the Onderstepoort Veterinary Research Institute. The presence of the animals at the Institute created an ideal situation for the early diagnosis of the disease, daily observation of the symptoms as the outbreak progressed and the collection of specimens for laboratory examination.

MATERIALS AND METHODS

Experimental animals:

In the natural outbreak of the disease six mature Swiss cows and four 2-year old bulls of various breeds became infected. For the trans-

mission experiments nine yearling Jersey and Jersey cross heifers, and 1 yearling Jersey cross bull were used.

Restraint, examination and infection of experimental animals-

Prior to examination of the female animals, the external genital orifice was thoroughly cleansed with G11 soap. The vulva was examined by manual separation of the labia permitting direct inspection of the vulva. The vagina was inspected with a vaginal speculum lubricated with liquid paraffin and a torch as a source of artificial light.

To prevent cross-infection from the examination of the genitalia, a separate, sterile speculum was used for the examination of each animal and the operator's hands were thoroughly washed with G11 soap after examining each animal.

The bulls were tranquilised prior to examination of the penis to avoid mechanical damage to the organ. Acepromazine* was found to be the most satisfactory ataractic. The drug gave adequate tranquilisation and relaxation of the penis when injected intramuscularly at a dosage of 10.0 mg per 100 kg body weight. To avoid cross-infection the precautions drescribed above were observed.

Artificial transmission of the disease was accomplished in the following manner: Eight susceptible heifers were infected intravaginally by inserting a gauze tampon that had been previously impregnated with 1 ml of 3rd tissue culture passage material containing $10^{5 \cdot 7}$ $TCID_{50}$ of virus per ml. The tampon was removed from the vagina after one hour.

One susceptible heifer was infected intravaginally with a tampon collected from the vagina of a naturally infected cows.

The bull was infected by instilling 2 ml of the 3rd tissue culture passage of the virus intrapreputially.

* Manufactured by Boots Pure Drug Company, Ltd., Nottingham, England.

Collection and preparation of specimens for virus isolation:

Vaginal exudate and semen were collected from the naturally infected cows and bulls. Nasal, conjunctival and rectal swabs were collected from all the animals.

Where possible vaginal exudate was aspirated with a sterile pipette and placed in a sterile container. When this was impossible, specimens were collected with sterile cotton wool swabs on wire holders. Before and after sampling the swabs were kept in sterile test tubes.

From the heifers used for experimental transmission of the virus, nasal, conjunctival, rectal and vaginal swabs were collected prior to, and on the 2nd, 4th, 7th, 10th, 15th and 20th day after infection. From the bull, nasal, conjunctival and rectal swabs in addition to semen specimens were collected.

As soon as possible after collection the specimens were treated for at least 1 hour at 4°C with a suitable volume of Hank's buffered salt solution (BSS) containing 2000 units penicillin, 2 mg streptomycin, 1.1 mg neomycin and 0.02 mg Fungizone* per ml. The fluid from all the treated specimens was then decanted and centrifuged at 2,000 rev./min. for 10 minutes. The supernatant fluid was either seeded direct onto roller tube cultures or stored in a dry ice cabinet at –70°C for subsequent inoculation.

Specimens for bacteriological examination:

Vaginal swabs were collected from the animals as described above, extreme care being taken to avoid skin and faecal contaminants. The material obtained from the swabs was cultured on blood tryptose agar.

Preparation and infection of tissue cultures:

Roller tube cultures of calf kidney cells were prepared according to Bodian's[3] modification of the method described by Youngner[28]. A modified Hanks BSS (Weiss and Geyer[27]) con-

* Manufactured by E. R. Squibb & Sons, New York.

taining 0.5 per cent lactalbumin hydrolysate and 10 per cent bovine serum was used as growth and maintenance medium.

Prior to seeding the tubes, the maintenance medium was decanted and the monolayer washed with phosphate buffered saline (PBS). Each of three roller tube cultures were seeded with 0.2 ml of specimen material. After an absorbtion period of 1 hour at 37°C, the monolayers were again washed with PBS and 1.0 ml of maintenance medium without serum was replaced. The cultures were placed in a roller-apparatus in an incubator at 37°C and examined daily for 14 days for cytopathic effects.

Virus strains:

The 8th tissue culture passage of the FH335 strain of the virus, which was shown to be antigenically identical to IPV virus by Maré and van Rensburg[20], was used as antigen in the serum virus neutralisation tests.

The strain of IPV virus isolated from the outbreak was used at the 4th tissue culture passage level for parallel serum virus neutralisation tests.

Type-specific antiserum:

Type-specific antiserum to IPV virus was prepared by inoculating a susceptible steer intramuscularly with the FH335 strain of the virus. The steer was bled 4 weeks after inoculation and the serum was separated as soon as possible. After Seitz filtration, 2.5 ml amounts were placed in ampoules, freeze-dried and stored at –20°C. The pre-inoculation serum sample obtained from the same steer was used as normal control serum.

Serum virus neutralisation tests:

The tests were employed to compare the level of circulating antibodies in the acute and convalescent phase sera of the naturally and experimentally infected animals and as a means of determining the identity of the various viruses isolated from these animals.

The procedure was as follows: All the sera were inactivated at 56°C for 30 minutes prior to use. Serial decimal dilutions of the stock virus were prepared. Equal volumes of undiluted serum and dilutions of the virus were mixed and incubated for 1 hour at 37°C. Roller tube cultures were seeded with 0.2 ml. aliquots of each mixture. The tubes were incubated at 37°C for 1 hour to allow adsorbtion of the virus before the maintenance medium was added. The cultures were then incubated at 37°C and examined daily for 8 days for cytopathic effects.

The end points were calculated according to the method of Reed and Muench[23] and expressed as the neutralisation index of the serum.

Staining of tissue culture monolayers:

Monolayers were prepared and stained according to the method described by De Lange[5].

Observations and results

Clinical observations on the naturally infected animals:

The naturally infected animals were first examined when two of the cows showed a discharge from the external genitalia. Examination of the two cows revealed the presence of an acute vaginitis, characterised by a severe hyperaemia of the mucosa, the presence of numerous pustules and 20—30 ml of a seropurulent exudate in the vagina. A thorough examination of the genitalia of the other animals in the herd revealed no clinical abnormalities.

Four days later the disease had spread to all the animals in the herd. The cows showed an acute pustular vulvovaginitis, accompanied by oedema of the vulva and a copious seropurulent discharge. Within 72 hours of first being observed, the pustules in some areas appeared to coalesce, resulting in an acute pseudomembranous vaginitis which was obviously painful. Small shallow erosions also occurred in certain areas. Figure I shows the vulva and posterior vagina of one of the cows with typical lesions.

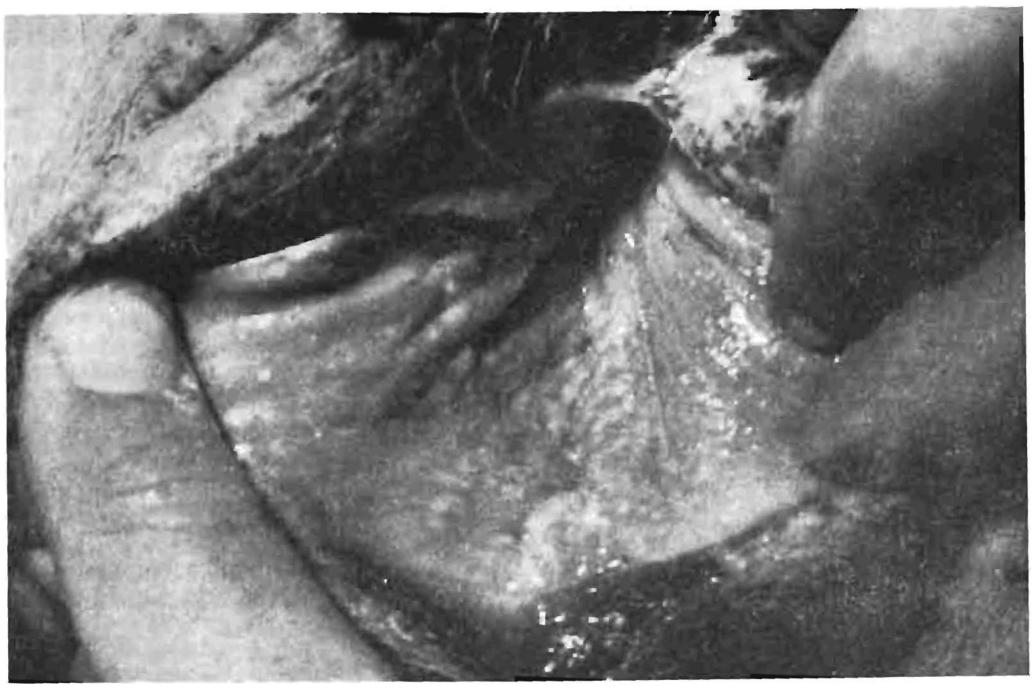

Figure I.
Posterior vagina of naturally infected cow showing typical pustules.

Ten days after first being observed, the symptoms had abated noticeably and only a mild hyperaemia and a slight purulent discharge was present. Complete resolution of the lesions occurred within 2–3 weeks of the outbreak being observed, except in one case where the vaginal discharge persisted for over 12 weeks. It is worth noting that all the cows, except the last-mentioned, conceived within 6 weeks of having had the disease.

In the bulls the severity of the lesions varied markedly. One bull showed only a transient hyperaemia of the preputial mucosa. Another had an acute, uncomplicated pustular balanoposthitis.

The first symptom observed was a slight prolapse of the prepuce in one of the bulls. Closer examination of the penis revealed the presence of an acute pustular balanoposhtitis accompanied by oedema, petechiae, echimoses, small shallow erosions and a slight purulent discharge. In two cases the balanoposthitis became pseudomembranous. In one case the oedema of the prepuce became so severe that a paraphymosis, persisting for 5 days, ensued. (See Fig. 2.).

There was no significant deterioration in the semen quality of the bulls during or after the outbreak. It is interesting to note that they showed no reluctance to serve a cow on heat during the acute stages of the disease.

The mild cases recovered within a few days. In the severe cases the symptoms only started regressing after 8 days. Healing was then remarkably rapid and complete resolution of the lesions occurred within 14 days of their appearance.

Laboratory investigations of the naturally infected animals:

The results of the laboratory examinations are given in Table I.

IPV type virus was isolated from the vaginal exudate of all six cows involved in the outbreak and from semen samples of three of the four

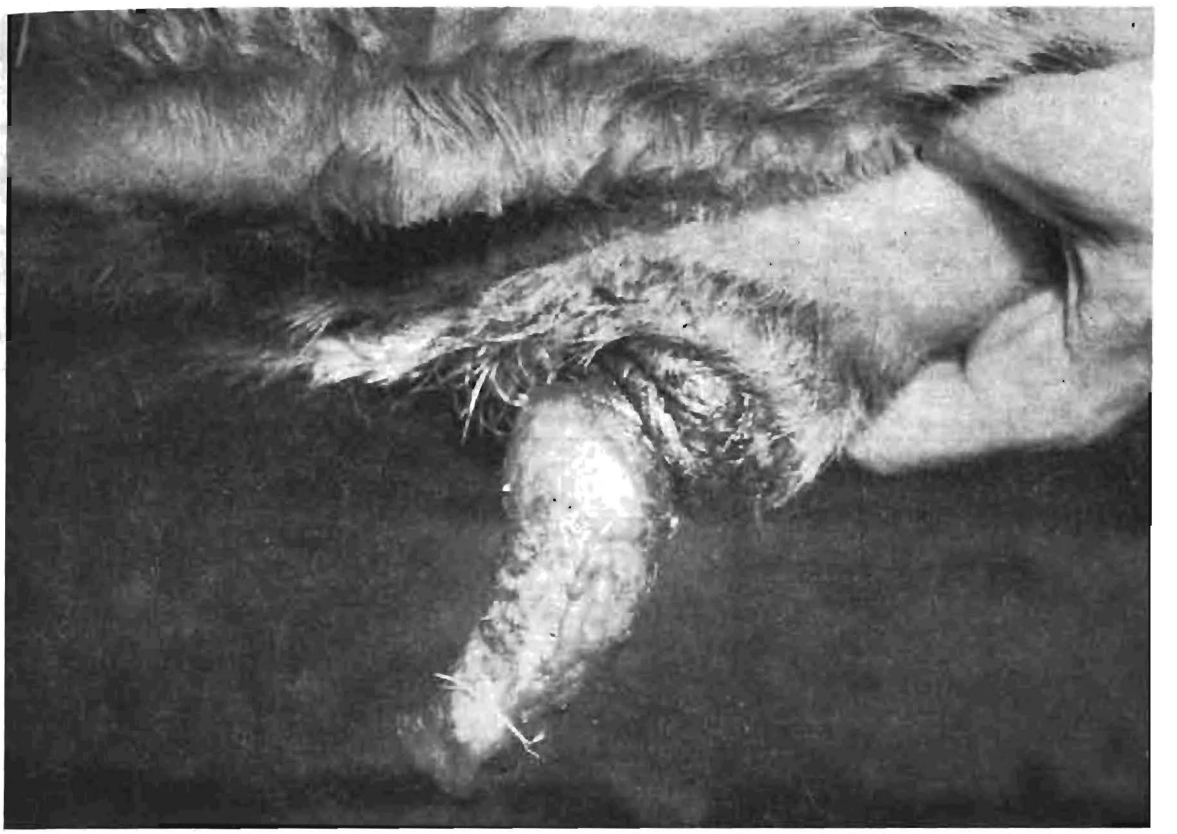

Figure II.
Penis of a naturally infected bull showing paraphymosis and a pseudomembranous balanoposthitis.

bulls. No viruses were isolated from the nasal, conjunctival and rectal specimens collected from the animals. The viruses isolated from the naturally infected animals were all neutralised by FH335 antiserum. Stained preparations of infected monolayers of each virus isolate were examined and showed cytopathic changes and intranuclear inclusion bodies (See Fig. 3), similar to those described for IPV virus by Kendrick et al.[18] and Maré and Van Rensburg[20].

Serum samples collected from the animals during the acute stages of the disease failed to neutralise FH335 virus or those viruses subsequently isolated from the infected animals. All the convalescent phase sera showed a significant rise in antobodies against FH335 virus and the homologous virus isolates.

Bacteriological examination of vaginal and preputial exudate collected from the animals on the 3rd day after the disease was first noticed, revealed the presence of a variety of bacteria. There was, however, no single predominant species of micro-organism which could be incriminated as a primary cause of the condition.

On the 7th day after the disease was first ob-observed vaginal biopsies were taken from each of the six cows.

Histopathological examination revealed the presence of an acute vaginitis accompanied by hyperaemia, haemorrhage and oedema. A generalised leucocytic infiltration of the mucosa and submucosa was present in some cases. In others, however, the infiltration of leucocytes was focal in nature. Although polymorphonuclear cells predominated lymphocytes were more prominent in a few cases. The epithelial cells showed hydropsic degeneration and focal necrosis. In some cases there were foci in which

TABLE I.—RESULTS OF LABORATORY EXAMINATION OF SPECIMENS FROM NATURALLY INFECTED ANIMALS.

Identification of animals	Specimens for virus isolation	Isolation of virus	Neutralisation of the homologous virus isolate by FH335 type antiserum	Neutralisation index of acute phase serum		Neutralisation index of convalescent phase serum		Bacteria isolated from vaginal and preputial exudate
				Homologous virus isolate	FH335 virus	Homologous sviru isolate	FH335 virus	
Cow 2549.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	10	<10	100,000	10,000	Negative
Cow 2550.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	10	10	100,000	100,000	Pseudomonas spp.
Cow 2551.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	10	<10	10,000	10,000	Streptococcus dysgalacteae
Cow 2863.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	10	<10	100,000	100,000	Corynebacterium pyogenes
Cow 2864.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	10,000	10	<10	100,000	100,000	Staphylococcus epidermides (non pathogenic)
Cow 2865.....	Vaginal exudate Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	10	10	100,000	100,000	Corynebacterium pyogenes
Bull 1903.....	Semen Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	10,000	10	<10	10,000	100,000	Mixed culture
Bull 2913.....	Semen Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100,000	..10	<10	100,000	100,000	Staphylococcus epidermides (nonfi pathogenic)
Bull 2997.....	Semen Nasal swab Conjunctival swab Rectal swab	Negative Negative Negative Negative	—	10	<10	100,000	100,000	Negative
Bull 2999.....	Semen Nasal swab Conjunctival swab Rectal swab	Positive Negative Negative Negative	100.000	10	<10	100,000	100,000	Streptococcus uberis and Alcaligens spp.

TABLE II.—CLINICAL OBSERVATIONS ON EXPERIMENTALLY INFECTED ANIMALS.

Identification No. of animal	Sex	Incubation period	Maximum rectal temperature during the 7 days prior to infection	Macimum temperature during the 14 days following infection	Duration of acute pustular vulvovaginitis/balanoposthitis	Duration of vaginal discharge
2914.....	Female	72 hours	101.6°F.	101.8°F.	4 days	14 days
2915.....	Female	72 days	101.6°F.	101.4°F.	4 days	9 days
2926.....	Female	72 hours	102.0°F.	102.8°F.	4 days	55 days
2928.....	Female	72 hours	101.8°F.	102.4°F.	4 days	21 days
*2917.....	Female	72 hours	101.2°F.	105.0°F.	4 days	—
3473.....	Female	48 hours	101.2°F.	101.8°F.	8 days	21 days
3474.....	Female	48 hours	101.8°F.	102.4°F.	8 days	8 days
3475.....	Female	48 hours	101.2°F.	102.0°F.	6 days	14 days
3476.....	Female	48 hours	101.8°F.	102.0°F.	4 days	14 days.
2966.....	Male	96 hours	101.4°F.	102.4°F.	5 days	—

*Slaughtered on the 7th day post-inoculation.

Figure III.
Infected monolayer of bovine kidney cells with typical intranuclear inclusions.

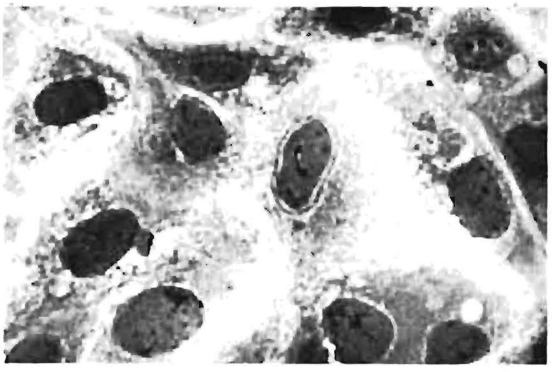

Figure IV.
Section of vaginal mucosa showing lymphocytic and neutrophilic infiltration.

complete desquamation of the epithelium had occurred. Intranuclear inclusions were not observed, probably due to the late stage at which the biopsies were taken.(See Fig. 4).

Transmission of the disease to experimental animals:

A summary of some of the observations on the artificially infected animals is given in Table II.

Symptoms indistinguishable from those observed in the naturally infected animals were seen in the experimental cattle. The incubation period after artificial infection varied from 48–96 hours. Only one of the heifers showed a febrile response which lasted for 3 days and reached a maximum of 105°F. The duration of the acute phase of the disease varied from 4–8 days. Most animals recovered within 8–21 days with the exception of one heifer in which the vaginal discharge persisted for 55 days. In the bull an acute pustular balanoposthitis lasting for 5 days was observed. The results of the laboratory investigations on the artificially infected animals are given in Table III.

Virus was reisolated from vaginal exudate of the heifers from the 2nd to the 10th day and from the semen of the bull from the 2nd to the 7th day after infection. The isolates were all neutralised by FH335 antiserum. The rectal, nasal and conjunctival swabs collected from the animals for a period of 20 days were all negative. Stained preparations of infected monolayers showed cytopathic changes and intranuclear inclusions similar to those produced by the viruses isolated from the naturally infected animals.

The pre-inoculation serum samples of the animals showed no detectable antibodies to

TABLE III.—ISOLATION OF I.P.V. VIRUS FROM VAGINAL AND SEMEN SPECIMENS AND NEUTRALIZING INDICES OF PRE- AND POST INOCULATION SERA FOR F.H. 335 VIRUS.

Identification No. of bovine	Specimen	Prior to In-infection	2 days post infection	4 days post infection	7 days post infection	10 days post infection	15 days post infection	20 days post nfection	Neutralisation iindex of pre-noculation serum for FH335 virus	Neutralisation index of post-inoculation (26 days) serum for FH335 virus
Heifer 2914...	Vaginal exudate	Negative	Positive	Positive	Positive	Negative	Negative	Negative	10	10,000
,, 2915...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	100,000
,, 2926...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	10,000
,, 2928...	,,	Negative	Positive	Positive	Positive	Positive	Negative	Negative	< 10	100,000
,, 2917*..	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	—
,, 3473...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	10	1,000
,, 3474...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	10	100,000
,, 3475...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	100,000
,, 3476...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	10,000
Bull 2966...	,,	Negative	Positive	Positive	Positive	Negative	Negative	Negative	< 10	100,000

*Slaughtered on the 7th day post infection.

FH335 virus, while convalescent phase sera obtained 26 days after infection showed a high titre of circulating antibodies to FH335 virus as well as to the particular virus isolate under investigation.

The results of the bacteriological and histopathological examination of specimens from the experimental animals were comparable to those obtained with material from the naturally infected cases.

Discussion and Conclusions

It has been shown conclusively that the viruses of infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV) are antigenically indistinguishable. McKercher *et al.*,[18,19]; and Gillespie *et al*[10]. These viruses have been incriminated as the cause of vaginitis, balanoposthitis, abortion, infection of the upper respiratory tract, keratitis, conjunctivitis, encephalitis and gastritis in cattle. (Kendrick *et al.*[16]; Studdardt *et al.*[26]; Chow *et al.*[4]; McKercher *et al.*[18]; Hughes and Olander[13]; French[7,8]. Cattle of all ages have been involved in one or more of the syndromes described above.

In South Africa antibodies to IPV virus are widespread in the cattle population (Hellig[11],) and a number of viruses antigenically indistinguishable from IPV virus have been isolated from the genital tract of cattle (Maré and Van Rensburg[20]). These viruses caused an acute anterior and posterior vaginitis in cows, and a seminal vesiculitis, transient orchitis and a balanoposthitis in bulls. At no stage were either pustules or vesicles observed in animals infected by these virus strains. The strain of IPV virus isolated during the outbreak described in this article, however, produced all the classical symptoms of IPV as observed by Kendrick *et al*[16]. Both naturally and experimentally infected cows showed a pustular vulvovaginitis and apustular balanoposthitis was observed in the bulls.

Infection of the respiratory tract of cattle, with seven of the strains of IPV virus isolated by Maré and Van Rensburg[20], elicited no symptoms apart from a mild febrile response (Maré[21]). Pathogenecity trials in cattle conducted with the strain of IPV virus isolated during the present outbreak showed that the virus could spread by contact and that, in addition to a vaginitis, it could cause a pustular rhinitis, an encephalitis and a pustular kerato-conjunctivitis. In some cases the keratitis was complicated by corneal opacity and ulceration (Hellig[12]).

Some of the earlier European literature reported that sheep, goats and horses are susceptible to the virus of coital exanthema (Hutyra and Mareck[14]). These observations have been confirmed in sheep and goats with the virus strain described in this paper as well as the FH335 strain of IPV virus. While there appears to be a marked difference in the pathogenicity of the two strains, both strains produced a vaginitis, rhinitis and conjunctivitis in sheep and goats.

Evidence has been produced that, apart from the strains of virus isolated by Maré and Van Rensburg[20], there is at least one strain of IPV virus present in the country capable of producing the classical symptoms of IPV. In addition preliminary unpublished observations have incicated that this strain also causes disease of the upper respiratory tract and a kerato-conjunctivitis. In the light of these observations it is highly probable that the IBR syndrome is also present in South Africa.

To date, a large number of strains of IPV/IBR virus have been isolated throughout the world. The various strains are indistinguishable from each other except in their ability to produce lesions in the different tissues of the body. There is, in fact, a considerable overlapping of this pathogenicity which varies from strain to strain. It would probably be more correct to regard all these isolates as different strains of the same virus.

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THE TUMBU FLY AS A PARASITE OF THE CHINCHILLA

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SUMMARY

The African tumbu fly (*Cordylobia anthropophaga* Grünberg), which causes cutaneous myisais of a furuncular type in dogs, rabbits, rats and also man, was found to attack chinchillas (*Chinchilla brevicaudata* Waterh.) in their breeding sheds. The boxes with fine river sand provided for their daily sand bath seemed to be very attractive to the gravid female fly as the chinchillas tend to soil the sand with their urine. Since the animals like to sit on their hind legs, most maggots are found on the underside of the hind legs, around the anus and on the root of the tail. Animals of all ages can be attacked but mainly those over three months old are the main sufferers. Losses amongst young and adult chinchillas have been experienced. Suitable methods of control is slight dusting of the fur with 0.5 per cent Lindane or 2½ per cent DDT powder or the addition of these insecticide to the sand for the bath. Keeping the flies out of the sheds by means of gauze wire is recommended.

BIOLOGY

The tumbu fly (*Cordylobia anthropophaga* Grünberg) also called "skin maggot fly" is a compactly built insect approximately one centimeter long. It has clear wings and the basic colour of the body is yellow brown with diffuse bluish-grey markings on the thorax. The posterior half of the abdomen is also grey.

The larvae of the tumbu fly are parasitic on mammals including man and produce a cutaneous myiasis which is furuncular in nature. It is the odour of the living animal and of their sleeping places that attracts the gravid females, and the eggs are deposited in the sleeping places and lairs on straw, sacking and also directly on the ground. In the case of human attack they are laid on washing and clothing laid out in the sun. One female produces some 500 eggs which are laid singly and scattered on the lairs of a large number of suitable hosts. For this reason, animals living in easily accessible burrows like rats or having a permanent lair like dogs are the main sufferers from these parasites.

The young larvae hatch after 2 to 4 days and the infestation of the host results from direct penetration of the healthy skin by the freshly hatched maggot. The penetration may take place immediately after hatching and can be achieved in less than ten minutes. Especially those parts of the body that are in close contact with the ground when the animal is resting, become infested by the maggots.

The maggots live singly in the subcutaneous tissue. Each is situated during the later stage of their development in a furuncle-like swelling approximately one centimeter in diameter, which is rather painful. There is a small central opening on top of the swelling for the larvae to obtain the necessary supply of air. The maggots obviously live mainly on lymphatic fluid secreted by the host which also oozes out continuously through the opening. The mature larvae are 10 to 12 mm. long and 4 to 5 mm. in diameter. Their body is entirely covered with numerous minute spines. The maggots reach their full length in 12 to 15 days after entering the host. They then leave the host through the opening and pupate in the soil or on the ground between the material covering the lair. They form a

typical Calliphorid-pupa dark brown in colour. The pupal period is said to last three to four weeks during the warmer months of the year.

Attack and Damage

Of the domestic animals, especially dogs and rabbits are known to be the main victims. During recent years the tumbu fly, however, is also attacking chinchillas (*Chinchilla brevicaudata* Waterh.) which are bred for their valuable pelt and which obviously are very suitable hosts on account of their habits of living.

The tumbu fly is a native species of Africa. The first attacks of caged chinchilla colonies were observed in the northern regions of South West Africa in 1960. Specimens of larvae off chinchillas and adult flies caught in the breeding sheds were identified as *Cordylobia anthropophagà* Grünberg. The commonest type of housing for chinchillas is a semi-open shed built on concrete with burrows into the cement for each female. Wire cages are erected on top of the burrows. Each cage is also fitted with a box of fine river sand, which these animal need for their sand baths. Fresh sand has to be provided once a week as the chinchillas are inclined to soil the sand with their urine.

The adult tumbu flies readily enter the breeding sheds and make straight for the sand boxes, occasionally they can also go into the concrete burrows. They are obviously attracted by the odour of the urine. The eggs apparently are deposited in most cases on the soiled sand of these boxes, as it was observed by several breeders that the animals always become parasitized by the skin maggots when the boxes has been visited by a tumbu fly previously.

The flies occur in the summer and mainly during the rainy season from November to January. They are particularly active on close days when a thunderstorm is threatening.

Animals of all ages can be attacked. Very young animals become seldom infested but those that are three months and over suffer most. Furthermore, animals in poor condition seem to be more prone to infestation according to the breeders. Since the chinchillas like to sit on their hind legs like squirrels, most maggots are found in the skin of the underside of the hind legs, around the anus and on the root of the tail. Only the odd larva will be seen in the front paws. The first sign of maggot infestation is a small reddish dot on the described parts of the body which normally are also less hairy. These spots swell up rapidly and develop into a boil-like eruption with the typical hole in the centre. Two to three days thereafter the maggots in the swellings are already 5 mm. long. The hair around the boils then starts falling out. This, however, is fortunately not of severe consequence for the value of the pelt as regrowth of normal hair is taking place as soon as the boil has healed and no damage will be seen by the time of slaughter six to eight months later during July and August.

Two to three maggots are found per infested animal as an average but up to 15 boils have been counted to appear simultaneously on a single animal. The rate of growth and development is impeded already by a very light infestation. Young animals will die when infested by several maggots and if the infestation is not discovered and treated at an early stage. Losses of adult chinchillas from this type of myiasis have also been experienced and eight to ten boils seem to be fatal even to adults if left unattended. The larvae can be easily pressed out and a disinfectant applied.

With regard to the type of damage the African tumbu fly can be regarded as the parasitical counterpart to the Fox Maggot fly (*Wohlfahrtia opaca* Coq.) which produces a similar type of myiasis and cause heavy losses among young foxes and mink in the U.S.A. and Canada. Regular treatment of the pups with DDT dusting powder has proved to eliminate strikes entirely (Gassner & James).

Control

In the case of the tumbu fly it was found that an adequate method of control was the addition of 0.5 per cent Lindane dust or 2½ per cent DDT dust to the sand bath which is used daily by all animals. Other animals were lightly dusted with these insecticides without any disadvantageous

effect. New outbreaks of myiasis were prevented by both types of treatment. Besides the use of insecticides, cleanliness and regular disinfection of the sleeping places is important. The addition of the abovementioned insecticidal dusts to the sand bath has the advantage that the individual animal need not be handled. Organo-phosphorus insecticides of low mammalian toxicity have not been tested on these valuable animals for fear that their breeding activity might be influenced by the normally strong odour of these compounds. The safest method to eliminate the tumbu fly problem is by keeping the flies out with gauze wire. When the shed is closed with a fly screen, care must be taken however, that the circulation of fresh air is not curtailed and the burrows become too warm for the well-being of the chinchillas.

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THE DEVELOPMENT OF NEW PROTECTING AGENTS AGAINST BLOWFLY STRIKE OF SHEEP*

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In practically all countries where the wool producing industry has developed, different species of sheep bloflies have become a serious menace and the modern type of Merino sheep itself is largely responsible for this plight. Due to continual selection within a comparatively short period the woolled sheep has undergone marked changes with regard to its fleece. The wool has become finer and denser and there is hardly a portion of the body that is not covered by wool. These desirable characteristics, on the other hand, are most favourable for the development of cutaneous myiasis.

As blowflies are not dependant on the sheep for their existence and possess prodigious powers of reproduction, their eradication is practically impossible and their control also most difficult. The only alternative as regards control is to break the link between the sheep and its aggressor.

The elimination of conditions like soiling and wetting of the wool and fleece rot, which act as attractants for the gravid blowfly females would offer a solution to the problem. This may be achieved by breeding a plain-bodied sheep or by the so-called Mule's operation, whereby skin folds at both sides of the breech and a portion of wool-bearing skin on the tail stump are cut away. Breeding, however, is a slow process and offers no immediate solution in a critical emergency, whereas the Mule's operation gives a satisfactory protection in normal years when the fly population is high[1].

For this reason, a substance is needed that can either repel blowflies or, alternatively, is able to destroy the young maggots before they commence feeding. There is, however, no effective repellent known up to the present but the discovery of the modern synthetic insecticides has opened up a new field of investigation into the blowfly problem. As far back as 1946, tests were conducted with the different formulations of these new compounds at the Veterinary Research Institute of Onderstepoort, and the very first substances to be tested (DDT and DHC) yielded a promising degree of protection[2,3]. It became clear that these insecticides would provide a satisfactory and immediate solution to the pressing blowfly problem.

During these initial insecticidal evaluation tests a very interesting phenomenon was discovered. Far better results than had been expected were obtained with BHC which provided sheep with an immunity for considerably longer periods than DDT used at five or ten times the concentration, in spite of the fact that DDT was generally known to provide a longer residual effect than the more volatile BHC when applied to exposed surfaces.[4] The differences in the behaviour of the two compounds on the living sheep suggested that some factor in the wool played an important part by either assisting the action of BHC or retarding that of DDT.

It was therefore obvious that an as yet unexploited field for research into the protective properties of the rapidly enlarging list of these new substances against strike, existed. It was clear, furthermore, that the guiding principle

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would be to retain a high concentration of the larvicidal agent in the wool for as long as possible especially in those parts normally attacked by the flies.

Since the serous fluid exuding from the superficial wounds caused by the larvae of the primary flies constitutes their principal food, and since these maggots tend to crawl about in the wool wetted by the exudate[5], it is obvious that the newly hatched maggots must remain in contact with the insecticide to achieve complete control. For this reason the larvicidal agent must be evenly distributed in the wool to provide, in addition, a lethal concentration at skin level.

To study the fate of the protective agent in the wool of living sheep the minimum concentration of all insecticides capable of destroying first instar larvae when the compound is added to the nutritional medium, had first to be determined. The tests conducted revealed clearly, that DDT and BHC fall into the same class, as both are capable of killing all young larvae at a concentration as low as 4 ppm. As a next step, a new bio-assay method had to be evolved to determine the quantity of insecticide remaining in the different zones of the wool staple after treatment[4,6]. The method indicated clearly that treated animals are no longer protected against strike when the insecticidal concentration in the proximal portion of the new growth wool falls below the level capable of killing the first stage larvae. As long as the half-inch of wool nearest to the skin contains sufficient insecticide to impede the normal growth of the young maggots, no strike will develop on the sheep.

The bio-essay method disclosed at the same time profound differences in the behaviour of the various insecticides in the fleece of living sheep and revealed furthermore the method by which this action was achieved. It was discovered that the fine layer of wool grease covering each wool fibre plays an important role and that the solubility of a compound in the grease of the wool also determines its period of protection.

DDT and related compounds like TDE and Methoxychor, which do not dissolve readily, remain confined to the zone originally treated and show no tendency to diffuse within the wool grease into the new growth of wool underneath. The result is that strikes can develop as soon as the new growth of wool is about half-inch in length. Compounds such as BHC, Aldrin and Dieldrin, on the other hand, possess the then newly discovered ability of diffusing along the wool fibres into the new growth.

The actual period of protection afforded by any agent, therefore, is dependant upon two important properties, namely, its larvicidal value and its diffusion power. Furthermore, a third factor influencing its action was discovered, namely its alkaline stability, which is the property possessed by a compound not to break down rapidly in the presence of an alkaline and watery medium. The more pronounced these three factors are, the longer is the resulting period of protection.

In the field trials which followed a number of additional factors were observed as influencing the duration of protection to a greater or lesser degree under field conditions. Such factors are introduced either by the protecting agent, by the sheep, or even by the blowfly[1].

Dust and wettable powders give longer protection than emulsions in clean and dry wool[7], whereas emulsions give better results on soiled sheep. The protecting agent must be evenly distributed throughout the vulnerable area of the fleece, right down to skin level, to ensure the maximum degree of protection. The reason for this is the fact that some of the compounds are even absorbed into the sebaceous glands and excreted again with the new wool grease[8].

Factors introduced by the sheep are the following: Soiled crutches disadvantageously influence the uniformity of the application and, consequently, shorten the duration of protection. Insecticides give better protection when applied to long wool than to short wool, the reason being that more insecticide can be retained by long than by short wool. The crutching of soiled breeches is therefore not advocated.

With regard to factors introduced by the blowfly itself it could be shown in the field trials that the density of the fly population had a profound bearing on the length of protection afforded by any of the insecticides. This factor, which is described as the "fly population pressure", was found to exert so marked an influence

that it overshadows all other factors. For this reason any figures expressing the duration of protection should always be accompanied by a statement on the fly population or otherwise it is not possible to assign to them either scientific or practical significance.

In the last few years resistant blowfly species have been encountered in most sheep-breeding countries. The maggots of these species can no longer be killed by the concentrations of chlorinated hydro-carbon compounds such as DDT, BHC, ALDRIN and DIELDRIN previously recommended. The phosphoric ester DIAZINON alone remained effective against these resistant strains[10].

Shortly thereafter, two further phosphorus compounds were introduced as effective protecting agents, namely, DIOXATHION (=Delnav, Hercules Powder Comp.) and COUMAPHOS (=Asuntol, Bayer). Both compounds, which are very lethal to ticks and flies, proved to be adequate protecting agents but did not actually represent a true step forward in the development of better blowfly remedies.

Further extensive search for new substances was undertaken with the particular aim of finding agents with even better properties. Two new compounds were found recently which possess definite advantages over the protecting agents so far in use. These are the phosphoric ester Diethyl-methylmercapto-methylphenyl-thiophosphate (= Lujet, or Lucijet, Bayer) and BROMOPHOS (by Cela, Ingelheim-Rhein), a Dimethyl-dichloro-bromophenyl-thionophosphate.

As Lujet is one of the most stable phosphorus compounds and possesses a higher degree of larvicidal action than Diazinon as well as very good diffusibility in wool grease, the prognosis that this compound will also produce a very long period of protection in practice was justified[9,10]. This was confirmed subsequently and spectacular results were obtained even under very high fly pressure and in cases where the wool was very dirty at the time of treatment.

Bromophos, on the other hand, possesses a very important character besides all the properties that make it a very efficient protecting agent, namely, its low mammalian toxicity. The acute oral LD50 for rats is between 3750 and 5050mg. per kg. body weight, which means that this new compound is far less toxic than pyrethrum[11,12]. The extremely low toxicity makes its use on slaughter stock possible, and application by hand does not entail any hazard to the health of the operator.

Research has progressed a long way from the first synthetic insecticides DDT and BHC that under severe conditions protected Merino sheep against strike for several weeks only, to the most modern compounds that exert protection for several months. The hope is entertained that agents capable of rendering sheep immune to strike for the entire fly season by means of a single annual treatment, will be discovered.

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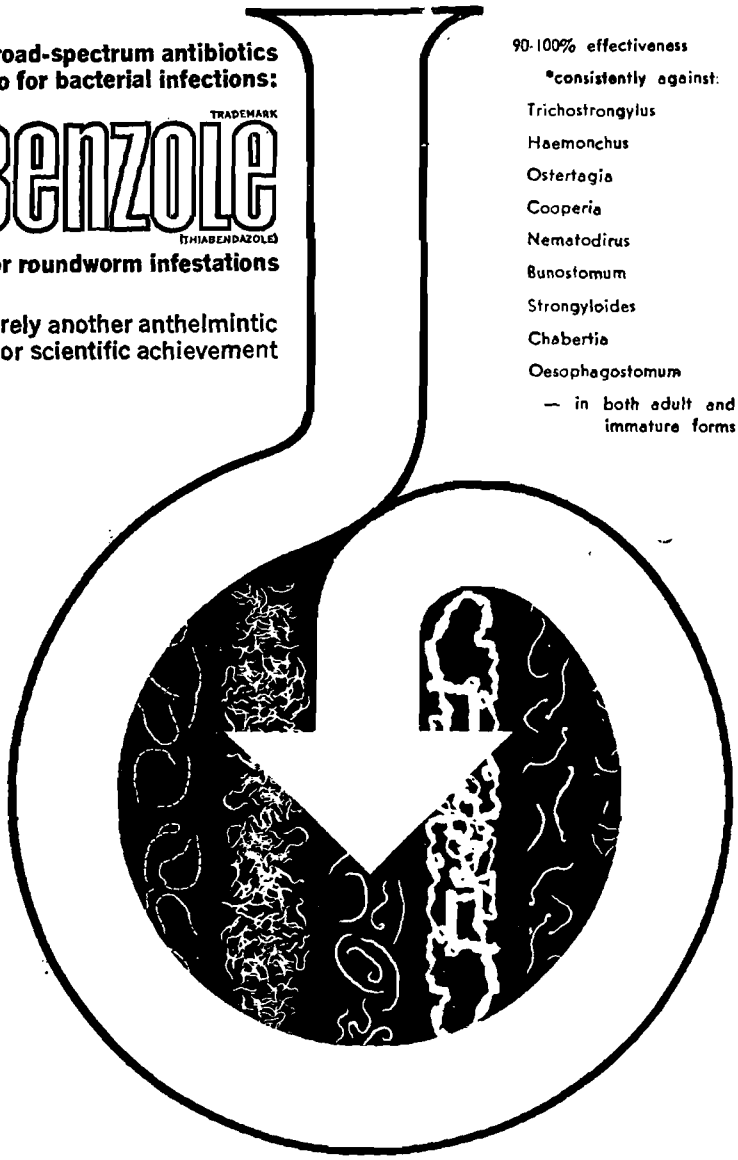

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A LITERATURE REVIEW WITH SOME COMMENTS ON THE SHEEP ITCH MITE (*PSORERGATES OVIS* WOMERSLEY)

W. M. McHardy

Cooper & Nephews, S.Af. (Pty.) Ltd., Johannesburg

Summary

This review of the more important publications on the sheep itch mite, with references and some comments, is presented under the following headings:— description of adult mites, life cycle, transmission, diagnosis, seasonal incidence, economic importance, control methods, *in vitro* tests and criteria for testing of insecticides.

Introduction

For some 40 years before Carter[1] (1941) first reported *Psorergates ovis*, a mite had been suspected as the cause of dermatitis of sheep. In the same year, Womersley[2] described the mite—the fourth species of the Genus *Psorergates* Tyrrell[3] (1883) described *P. simplex* from the mouse, Zumpt and Till[4] (1955) *P. cercopitheci* from the monkey and Till[5] (1960) *P. oettlei* from the multimammate rate. The C.S.I.R.O. (Australia)[6] mentioned that *P. ovis* had been found in at least two flocks in New South Wales in 1941, and the next year in South Australia and Tasmania[7]. Graham[8] found mites in specimens from South West Queensland, Northern and Southern Tablelands, the Riverina of North South Wales, Western Victoria and Tasmania. In 1948, it was reported from Western Australia[9], by Bell *et al*[10] in the United States of America, by Fiedler and du Toit[11] (1954) Republic of South Africa, in New Zealand[12] (1965) and in 1960 Argentina (Ault cited by Downing and Mort[13]).

Seddon[14] recorded these mites in Merinos and Corriedales and Armstrong[15] (1961) added half breeds. He also believed the itch mite was assuming greater incidence and importance because of the failure of certain modern dipping chemicals to control it. Circumstantial evidence has shown that sheep dipping in arsenical washes prevented infestations becoming serious but never achieved total eradictaions[15].

Description of Adult Mites

Murray[16] described the adult mites as having four parts of well developed legs in both sexes and gave the following details:—

Male, width 110–140μ, oval-shaped; the dorsal penis visible and a single pair of setae seen posteriorly from medial ventral tubercles.

Female, width 130–160μ, rounder than the male and with two pairs of setae posteriorly situated arising from ventral tubercles situated on either side of the medial line.

Lapage[17] described the sides of the adults as indented between the legs which had paired claws. Each femur carried relatively long setae and on the ventral side a large curved spine directed inwards; the chelicerae were very small stylets and the pedipalps short and conical. Downing and Mort [13] were unable to find the large curved spine mentioned by Lapage[17] which was first described by Baker *et al*[18]. They[13] said the spine was not illustrated in Womersley's[2] original description but Carter's[1] photographs showed it.

Life cycle

Womersley[2] described the life cycle consisting of egg, larvae, two nymphal stages and the adults. The number of nymphal stages and the

length of the life cycle, however, remained in doubt. According to Murray[19] there are three nymphal stages and the life cycle may be completed in 35 days. After placing 100–200 females, collected daily, on unaffected sheep, he found eggs after 8 days, larvae after 11 days, protonymphs after 20 days, deutonymphs after 20 days, tritonymphs after 27 days and females after 35 days. Downing and Mort 13 agreed with Murray's findings[16].

Murray[16] reported the egg as measuring 70–98μ X87–117μ, and Lapage[17] said the developing larvae could be seen within the egg shell, but that the eggs were spherical and measure 48μ in diameter. Murray[16] gives the width of the larvae as 70–90μ, having a protruding head, three pairs of rudimentary legs and a visible developing nymph inside during the larval moult.

The nymphs have four pairs of legs and pass through three stages. Firstly, the protonymphs, having a width of 80–130μ, are small and roundish, and the legs and head of the deutonymphs can be seen inside them. Secondly, the deutonymphs have a width of 120–170μ, are medium in size, roundish, and the legs and head of the tritonymphs can be seen inside them. Thirdly, the tritonymphs are 150–200μ in width, pear-shaped, and larger than the adults which develop inside them.

Transmission

Murray[16] found that only the adult stage was motile possessing well developed legs, and both he and Toop[9] agree with Graham[8] that infestation by direct contact occurred soon after shearing when the short wool favours the transmigration of the parasite, e.g. ewe to lamb, and that migration of mites from full fleece sheep is unlikely. New Zealand investigators[14,15] showed that apart from a short period immediately after shearing the transmission of mites from one woolled sheep to another was extremely slow, and that the infestation apparently spread very slowly so that three or four years could elapse before the condition became widespread in a flock.

Occurrence in South Africa

Sheep Itch mites have been found in the following areas:—

CAPE PROVINCE—
Graaff-Reinet, Middelburg, Cracdok, Paerston, Tarkastad, Maclear, Barkly East, Indwe, Sterkstroom, Queenstown, Cathcart, Fort Beaufort, King William's Town, Stutterheim, Kei Road, Komgha, Humansdorp, Mossel Bay, Swartberg (East Griqualand).

ORANGE FREE STATE—
Bethlehem, Harrismith, Vrede, Senekal.

TRANSVAAL—
Ermelo, Carolina, Bethal.

NATAL—Donnybrook.

Diagnosis

According to Toop[9] when, in the absence of lice, there is rubbing and biting with damage to the fleece, itch mite infestation should be suspected and microscopic examination of skin scrapings is essential for demonstrating the mite. A number of scrapings taken from different body sites may be necessary before obtaining a positive finding.

Graham[8] used a light mineral oil when scraping and subsequently Fiedler and du Toit[20] used white oil. The site selected for scrapings should be prepared by clipping the wool very short with scissors on an area approximately 4" square. A small quantity of liquid paraffin is rubbed into the clipped area and then scraped with a blunt, straight-edged knife, using a fair amount of pressure but not sufficient to break the skin. The oil and debris thus collected is transferred onto a glass slide and examined under the microscope. The mites, when alive, are readily detected by their leg movements.

Downing and Mort[13] illustrated the predilection sites and in a table indicated that they found most mites along the back, along both upper and lower sides and on the hips. Mites were also demonstrated from the poll, upper neck,

shoulders, loins, rump, both sides of the lower neck and dewlap, the belly, crutch and scapular regions, but in lesser numbers than on the predilection sites quoted. Downing and Mort[13] stressed the influence of temperature on the activity of mites in scrapings and, not having facilities for working under controlled temperatures, discarded the *in vitro* technique later described.

Downing and Mort[13] said "It is interesting to note that Mellanby[21] (1943) reported that in early infestations of human scabies, intense reaction and sensitivity occurred although only few mites were present, whereas as the disease progressed and become chronic, with increasing mite population, the reaction and sensitivity abated. We think that this may offer an explanation of the discrepancy between clinical reaction and mite population observed in mite infested sheep of different ages."

In their opinion, seventeen sites[13] per sheep should be scraped to obviate erroneous conclusions[22] and the same site should not again be scraped, for at least a month. By using a large number of sites both "insecticidal efficiency" and "distributional efficiency" could be determined. Where failure to eradicate[13] the mite populations on dipped sheep, the live mites were usually found along the back and upper surfaces when liquid or miscible dips were used, and along the belly or crutch when washes were derived from powder (particulate) dips. They found that in their single sheep trials the results obtained were often better than those obtained under field conditions.

Murray[16] found most mites on the well covered parts but he also found them on the legs, face, eyelids and commissures of the lips. All stages of mites were under the *stratum corneum* where they lay in small depressions of the *stratum lucidum*. All stages were found singly and not in clusters. Adults and tritonymphs were the only stages found frequently on the surface of the skin or in the wool within 5 mm. of the skin.

Downing and Mort[13] express some doubt as to the place on the skin on which eggs are laid but say it may be on the surface of the skin, possibly under the scurf as described by Carter[1], and agree that the larva may attack the epidermis[22].

Seasonal incidence of mites

All stages of mites can be found on the sheep throughout the year. Armstrong[15] in New Zealand, claims diagnosis is difficult during January, February and March (warm months). Downing and Mort[13] agree with Graham[8] that there is a marked reduction in the mite populations on recently shorn sheep. They[13] state this decrease is greater if the sheep are exposed to high temperatures and sunlight, and an increase in mite populations follows a period of cold weather. Skerman *et al*[23] mentioned that there was danger in assessing insecticidal action on a falling mite population in summer. It follows, therefore, that on recently shorn sheep the mites are affected, their numbers decrease and transfer most readily to other shorn sheep. Downing and Mort[13] agreed and furthermore stated that any results from assessing an insecticide on a "falling parasite" population might be completely reversed on a "rising active parasite" population as usually occurs following a cool spell. Although infestation was reduced by shearing and warm, dry weather, no evidence of self-cure was encountered by them[13].

Economic importance

Accurate information on the economic importance of itch mite is not readily available because of the insidious nature of the condition. It is known that the wool fibres are seriously affected, the weight of wool is decreased, the cross fibres, matting and cotting makes it difficult to shear, the irritation may affect the condition of the sheep and, in general, the value of the shorn wool is appreciably lowered[9,11,14,15,17].

Because the spread of this parasite is slow[1] and can be kept within bounds by annual dipping in certain washes, the disease is often over-looked and sometimes ascribed to lice infestations or malnutrition. Losses due to "plucked" wool and thinner wool fibres are greater than generally realised although actual figures are not available. However, Armstrong[1,5] believed the itch mite was assuming importance and becoming of greater incidence.

Control methods

A thorough wetting of the fleece to skin level is essential in any treatment. For this reason, irrespective of what suitable insecticide is chosen, short spray races or other apparatus which only wet the tips of the wool, are unlikely to control itch.

Whether by dipping or showering, the wool should not be so long as to make wetting to skin level difficult and should preferably be of about a month's growth. Arsenical preparations may be irritant, and foul washes may contaminate wounds, hence dipping in arsenicals should be practised as soon as shear wounds have healed and before much growth of wool has taken place. Each sheep should be kept at least 30 seconds in the dipwash or shower to ensure thorough saturation and, when dipping, the head must be submerged once or twice.

Because of the long life-cycle and the apparent slow multiplication rate of the mite, the period of observations must be prolonged, particularly if the insecticide being studied has an effect before one can claim a cure. Downing and Mort[22] felt that this period could well be up to two years.

1. Lime Sulphur. A number of Australian workers[7,8,14,24,25] reported that lime sulphur eradicated the mites at a wash concentration of 1 per cent polysulphides to which 0.03 per cent Agral 3 was added to improve wetting. Graham[8] found lime sulphur at 0.4 per cent wt./vol. polysulphides gave complete kill, but in order to cover exhaustion of the wash during dipping, a 1 per cent polysulphide wash was recommended. Fiedler and du Toit[12] claimed that in a single dipping 1.23 per cent lime sulphur by no means cured advanced cases and even a second dipping several weeks later did not always succeed. Baker[26] obtained good results with 0.6 per cent polysulphides, recovering only one mite from one of nine sheep a year after treatment. Downing and Mort[22] in their 1944–45 trials, using 1.0 per cent polysulphides plus Agral 3, did not achieve eradication and although no live mites were found in their 1958–9 trials, the latter result must be accepted with some reserve as it was carried out during a time of "falling mite populations".

2. Arsenic. Sodium arsenite (0.2% As_2O_3) controlled clinical infection by markedly reducing mite infestations[14,27]. The latter report also mentioned that a single sheep, tip-sprayed with 1 per cent arsenic and examined two months after treatment, had no mites. No subsequent confirmation of this has been found. Downing and Mort[22], from their "single sheep" dipping, did not expect that a high degree of control would be obtained in the field under ordinary dipping practices.

3. Derris Root—Rotenone. Washes of 0.004 per cent or 0.005 per cent rotenone[8,14] reduced itch mite populations but were considered less efficient than arsenic (0.20% As_2O_3).

Downing and Mort[22], after numerous trials using various concentrations, found a 0.01 per cent rotenone gave excellent results and that the cube extract miscible oil was superior to the suspension. On five sheep dipped, taking 293 scrapings, 3,388 dead mites were counted and only 1 live mite found on the head of a large sheep with big horns.

Downing[28], found rotenone (0.005%) most efficient and subsequently used it with piperonyl butoxide and considered it conferred a synergistic factor of X4 in itch mite control and suggested the maximum efficiency to lie within the rotenone/piperonyl butoxide ratio of 1:0.4 and 1:1.2.

4. Arsenic—Sulphur—Rotenone. Fiedler and du Toit[12] mentioned that "Field experiments with the so-called 'quick acting dips' in the Karroo area have shown them to be ineffective against *Psorergates* mite infestation" but gave no details or data. Baker[26] obtained excellent results finding no mites on 18 experimental sheep a year after treatment and complete control on six sheep after 11 months, but on another occasion found a few mites on one of nine sheep eleven months after treatment. In these trials the possibility of reinfection from contact sources was not entirely ruled out. Downing and Mort[22] stated that within the limits of their trial, the arsenic (0.10%)—sulphur (0.30%)—rotenone (0.0012%) wash (dip B), although it did not eradicate the infestation, nevertheless proved equal to lime sulphur dips. No mites were, however found during 100 days after "single sheep" dippings

in 0.19 per sent arsenic—0.56 per cent sulphur—0.0012 per cent rotenone. They also stated that this dip gave a high degree of control over many years in the field, although it did not eradicate the mite.

5. GAMMA ISOMER OF BHC. Scott[29] showed that even at 8 times the concentration used to control lice, gamma BHC was ineffective. Seddon[14] also mentions that BHC suspensions, with and without wetting agents, or as phenolic emulsions, or as oil emulsions at concentrations from 0.007 per cent to 0.056 per cent gamma isomer, did not eradicate *Psorergates ovis*. Fiedler and du Toit[20] found that neither technical BHC at gamma wash concentrations from 0.0043 per cent to 0.34 per cent, nor Lindane at 0.05 per cent, gave any worthwhile control of the itch mite.

6. DELTA ISOMER OF BHC. Washes containing 0.1 per cent delta isomer of BHC were claimed by Fiedler and du Toit[20] to cure mite infestations in a single dipping and they stated "The discovery of the potent acaricidal property of the delta isomer of BHC against *Psorergates ovis* is of the greatest significance". Du Toit and Fiedler[30] drew attention to an antagonistic effect between the delta and gamma isomers and considered a ratio of 4 delta to 1 gamma to be desirable in the simultaneous eradication of infectious itch and other parasites at a single dipping, provided the wool was $\frac{1}{2}$" long or less and the concentration of delta isomer at least 0.06 per cent. Downing and Mort [22] with "single sheep" dippings failed to confirm these findings. Murray[24] and the C.S.I.R.O.[27] (Australia) were also unsuccessful.

7. MERCAPTOTHION. Fiedler and du Toit[20] claimed, and Baker confirmed, that 0.2 per cent Mercaptothion washes cured *Psorergates ovis* in a single dipping. Murray[24], using the "Patch Test" Technique, found treatment with 0.2 per cent, 0.4 per cent and 0.8 per cent washes efficient. The Australian Veterinary Association[25] stated that further "patch" and field tests in Australia were not so promising.

8. D.D.T. Itch mites increased after DDT. dip was used in the annual dipping procedure[20].

9. ALDRIN. DIELDRIN. These have proved quite ineffective[31].

10. DIAZINON. This was promising in "Patch" tests[24] but further tests and field trials gave poor results[19,31].

REGISTERED REMEDIES IN SOUTH AFRICA

At present, in the Republic of South Africa, Lime Sulphur (1.0% poly-sulphides), Arsenic—Sulphur—Rotenone (Quick Acting) (0.16% As_2O_3), 0.00155% rotenone), Mercaptothion (0.2% A.I.) and delta isomer (0.06%) of BHC. washes, are approved by the Registering Officer of Act No. 36/1947 for the control of sheep itch mite.

IN VITRO TESTS

Du Toit and Fiedler[30] developed an *in vitro* test by incorporating candidates insecticides at 0.05 per cent active ingredient in liquid paraffin, and observing motility of the mites for up to six hours later. Candidate insecticides were considered potentially active if all mites were immobilised within four hours.

The adoption of this *in vitro* method has proved of value in selecting the more promising candidate insecticides (acaricides) for subsequent sheep dipping trials. In South Africa, it has been found that insecticides rejected in the *in vitro* tests constantly failed in field dipping trials, and even some potentially active compounds, as adjudged by the *in vitro* method, have failed when field tested.

Sheep dipping trials, where the same insecticide and dipping techniques have been employed, have given different results on farms geographically removed from one another. This again stresses the value of repeated field trials to confirm seemingly promising results.

CRITERIA FOR TESTING OF INSECTICIDES

Some standard or uniform approach to the assessment of the efficacy of insecticides against this mite is most desirable. In addition, the season when tests are conducted is of paramount

importance as mites, in common with ticks, are more difficult to control when infestation is waxing than when it is waning. It follows that itch mite assessments should be made during winter but not during summer.

The technical committee of the Australian Veterinary Association[25] found that the "patch test", as described by Graham[8] and later Murray[24] was suitable only for determining whether or not a compound was active against itch mite and, furthermore, that sheep were suitable for "patch" testing only if the mite population yielded 10–20 mites from each scraping taken just prior to treatment. Results must only be based on repeated examination of treated patches starting 3–4 weeks after treatment. "Patch tests" should be followed by treating the entire sheep. Compounds that appeared to eradicate or reduce mite populations after repeated examinations warranted field testing.

For field tests, only sheep on which mites have been demonstrated by skin scraping prior to treatment may be used. No reliance can be placed on clinical signs alone. The sheep should preferably be heavily infested and must be suitably identified. Tests should be commenced on sheep 2 weeks off shears and conclusions should be based on repeated examinations of the identified sheep at regular intervals and through at least one winter season. Assessments made in the summer months are useful but suspect because of summer population decline. Trials to confirm any good results from one farm must be repeated on other sheep farms in other geographical areas.

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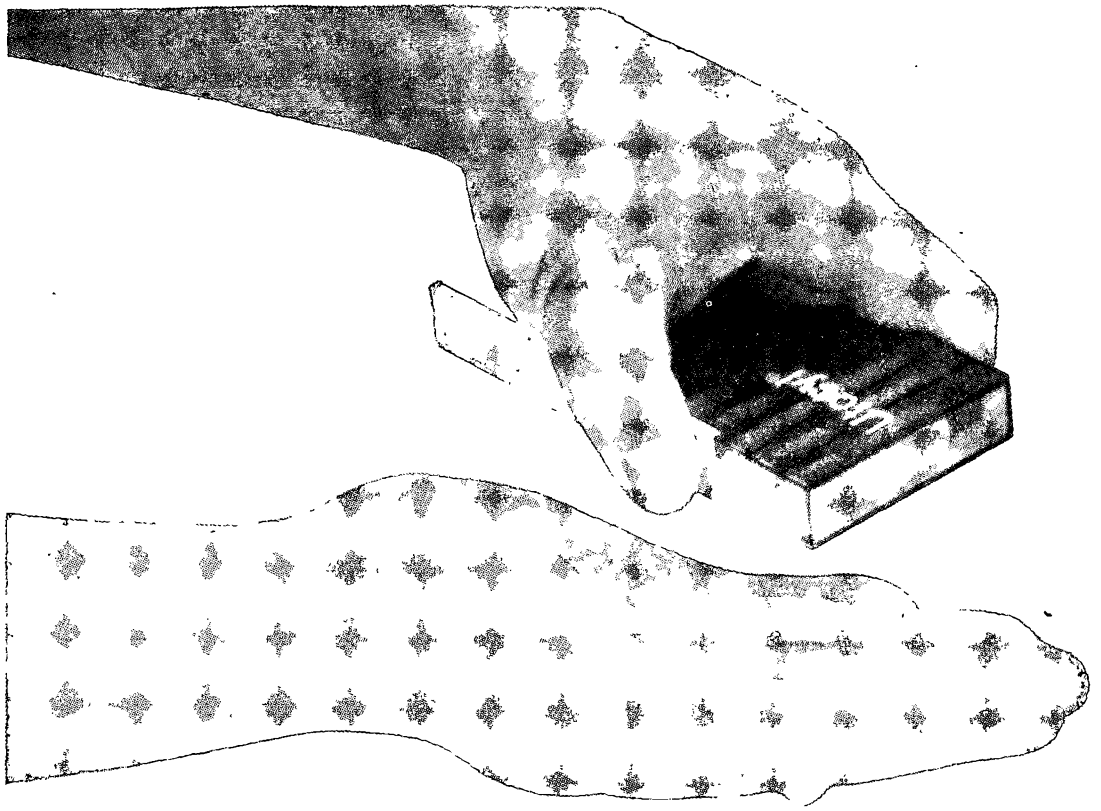

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BLOWFLY (*LUCILIA CUPRINA*) STRIKE IN SHEEP: A COMPARISON OF PROPHYLACTIC EFFICIENCIES OF CHLORFENVINPHOS* (AC 4072) AND VC-13**

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SUMMARY

Chlorfenvinphos at concentrations of 0.1 per cent, 0.5 per cent and 0.025 per cent, and VC-13 at 0.2 per cent were used on adult merino ewes carrying approximately eight months wool, and three to four month-old lambs at foot.

Insecticidal washes were applied to crutch areas only, by jetting at a pressure of 25 lbs. p.s.i.

Chlorfenvinphos at 0.1 per cent and 0.05 per cent and VC-13 at 0.2 per cent gave protection to the sheep for the twelve week duration of the trial.

The trial extended from late October to mid-January and was terminated when the sheep were shorn.

INTRODUCTION

The prevalence of blowfly strike on woolled sheep in many areas of South Africa has made it essential to protect these animals against this scourge. The first effective prophylactic treatment of animals was by means of the chlorinated hydrocarbon insecticides, namely, BHC. dieldrin an aldrin. However, in several areas the blowfly has developed a high degree of resistance to these insecticides, and a search was instigated for replacements. Several of the organophosphorus group of insecticides were tested, and of these some have been used commercially with success. As yet no organophosphorus resistant strains of *Lucilia cuprina* have been reported in South Africa.

The search for new organophosphorus compounds with higher biological efficiencies and longer residual actions for use against these fly larvae continues. Chlorfenvinphos recommended itself as suitable for this use as early investigational work of Wrich *et al*[1,2] with this compound showed that it had a very high biological efficiency against the larvae of the screw worm fly (*Chrysomia bezziana*). Screening tests by the Cooper Technical Bureau revealed Chlorfenvinphos' activity against *Lucilia* larvae. VC-13 was used as a comparison as this is an organo-phosphorus compound which is already in use commercially for blowfly control. Scott and Forsythe[3] reported the efficiency of VC-13 against blowfly in Australia.

METHOD AND PROCEDURE

Two grazing flocks of merino ewes with three to four month-old lambs at foot were available. These consisted of 197 ewes and 85 lambs running on irrigated lucerne pastures and 90 ewes with 109 lambs running on vlei veld. All adult sheep carried eight months growth of wool.

Each flock was divided into approximately equal groups by random selection and suitably identified. Individual identity was established by means of ear tags.

* Supona (Shell) (G.C. 4072) (2-chloro-1-(2, 4-dichlorophenyl) vinyl diethyl phosphate).

** Nemacid (Virginia Carolina Chemical Corp.) (0-2, 4-dichlorophenyl 0, 0-diethyl phosphorothioate).

Application of insecticides was affected through an engine-driven vane pump operating at an average pressure of 25 lbs. p.s.i. Two hoses with lance and nozzle attachments were employed. Spraywashes were mixed in ten gallon quantities immediately prior to application. Approximately 100 sheep per hour were treated.

A platform constructed of corrugated iron sheets on a wooden frame measuring 10'x2'6" was placed on the ground. As many sheep as possible, usually four, were laid on their backs thereon to prevent soiling of the wool. The hind legs of each sheep were firmly held in an open position to expose the whole of the crutch and to prevent kicking. It was thus possible to wet the tail and surrounding wool area with a minimum effort. This factor was important when sheep having folds or pleats around the tail root were soaked.

During treatments the spray nozzles were kept firmly pressed into the fleece ensuring a thorough wetting of wool at skin level. Simultaneously, the wool was gently squeezed by the operator's free hand to distribute the wash evenly throughout the fleece. The woolled areas above and on each side of the tail, the tail itself and the whole of the crutch area extending to the udder or scrotum and down the inner surfaces of the hindlegs, were saturated before the sheep was released. No attempt was made to collect and re-use the run-off which resulted.

One treatment only was applied to each sheep and thereafter the flocks as described above were reconstituted and returned to pasture.

Inspections were carried out at seven day intervals depending on the availability of labour for sheep collection. Each sheep was examined in a standing position. Any sheep showing any suspicion of an abnormality was turned up onto its back and a detailed examination carried out and recorded.

All strikes which showed no signs of spreading into the surrounding woolled areas, all strikes containing affected or dead larvae at any stage of development, and all strikes which had numbers of dead larvae in the surrounding wool, were classified as 'abortive strikes'. Thus, only thriving strikes containing reasonably large numbers of active, healthy larvae in all stages of development, and showing signs of rapid spread into the wooled areas, were classified as 'progressive strikes'. In general strikes under 2" in diameter were regarded as 'abortive strikes' pending further development. Instances occurred when no clearcut differentiation would be made under this arbitrary classification and such strikes were designated 'query abortive'. Such sheep were marked and at the next inspection were subjected to a particular examination and a definite classification made. The farmer co-operator agreed to this procedure with the reservation that he would treat such affected sheep if the suspect strikes spread rapidly between investigator visits. This occurred three times during the experiment.

Results

Neither VC-13 nor Chlorfenvinphos had any repellent effect on the adult blowfly. Numerous egg batches or 'blows' were found on sheep in all groups at the first post-treatment inspection. Evidence of good fly activity, such as the presence of numerous 'blows' on the sheep and calliphorine flies accompanying the flocks, was observed over the first five weeks of the trial. Thereafter, fly density was at a lower level but continued until the end of the experiment.

Tables I and II summarise the 'progressive' strikes recorded in the treatment groups in each flock. In Table III are recorded the number of 'abortive' strikes that occurred in all treatment groups plus the number of 'progressive' strikes recorded, giving an overall picture of blowfly activity.

Chlorfenvinphos at 0.1 per cent and at 0.05 per cent concentrations permitted progressive strikes to develop after nine and ten weeks, respectively. In the 0.05 per cent Chlorfenvinphos treatment group, however, only two cases of developing strikes were seen, an incidence of 5.4 per cent, whereas two of the 189 animals treated with 0.1 per cent Chlorfenvinphos or 1.1 per cent were found to be struck. Furthermore, the earlier progressive strike in the 0.1 per cent Chlorfenvinphos group occured in a young

lamb which was purging badly due to a heavy *Monezia expansa* infestation. The protection offered by 0.025 per cent Chlorfenvinphos is of doubtful practical value as two progresesive strikes occurred three weeks post-treatment. Altogether only three progressive strikes were recorded in the 0.025 per cent group over twelve weeks. Although this constitutes only 6.9 per cent of the group, nearly 5 per cent were protected for less than three weeks.

No progressive strikes were recorded amongst those sheep which were treated with 0.2 per cent VC-13.

In the control group a total of seven (17.9 per cent) of the 39 sheep suffered developing strikes. The first of these were discovered at the third inspection, and thereafter occurred regularly until the ninth week after treatment. In each case these animals were then treated with VC-13

TABLE I.—RESULTS FROM IRRIGATED LUCERNE PASTURED FLOCK

Treatment weeks	No. of Sheep	No. of sheep struck/weeks after treatment												% Struck During Trial
		1	2	3	4	5	6	7	8	9	10	11	12	
Controls.....	39—32	0	0	2	0	1	1	0	2	1	0	0	0	17.9%
VC-13 0.2%.....	125	0	0	0	0	0	0	0	0	0	0	0	0	0
Supona 0.1%.....	38	0	0	0	0	0	0	0	0	0	0	1	0	2.6%
Supona 0.05%.....	37	0	0	0	0	0	0	0	0	0	1	1	0	5.4%
Supon 0.025%.....	43	0	0	2	0	0	0	0	0	1	0	0	0	6.9%

TABLE II.—RESULTS FROM SHEEP DEPASTURED ON VLEI VELD

Treatment	No. of sheep	No. of sheep struck/Weeks after treatment												% Struck during trial
		1	2	3	4	5	6	7	8	9	10	11	12	
VC-13 0.2%.....	48	0	0	0	0	0	0	0	0	0	0	0	0	0
Supon 0.1%.....	151	0	0	0	0	0	0	0	0	1	0	0	0	0.6%

TABLE III.—SUMMARY OF ABORTIVE STRIKES PLUS PROGRESSIVE STRIKES FOUND DURING TRIAL

Treatment weeks	No. of sheep	No. of abortive strikse/weeks after treatment												Totals	%	Progressive strikes recorded	Total progressive & abortive	%
		1	2	3	4	5	6	7	8	9	10	11	12					
Controls.....	39—32	6	2	2	4	2	2	2	0	0	1	1	1	23	59	7	30	76.9
VC-13 0.2%.....	173	5	6	5	7	3	1	5	2	1	2	2	1	40	23.1	0	40	23.1
Supona 0.1%.....	189	2	4	5	2	1	3	3	4	5	4	8	2	43	22.7	2	45	23.8
Supona 0.05%.....	37	0	0	1	1	0	0	0	1	3	3	0	0	9	24.3	2	11	29.7
Supona p.025%.....	43	0	1	1	2	0	1	2	1	3	3	2	0	16	37.2	3	19	44.1
Total of Abortive strikes/week		13	13	14	16	6	7	12	8	12	13	13	4					

The number of animals in the Control Group was, therefore, progressively reduced.

'Abortive' strikes occurred in al groups from the first post-treatment week onwards.

Discussion

Comparison of strike incidence in the control and Chlorfenvinphos treated groups reveals that 'progressive' strikes occurred in the control group and the 0.025 per cent Chlorfenvinphos treated group at the same time. It is probable, therefore, that the prophylaxis offered by 0.025 per cent Chlorfenvinphos is of dubious quality, as nearly 5 per cent of the sheep obtained less than three weeks of protection. However, the remaining Chlorfenvinphos treatments, 0.05 per cent and 0.1 per cent gave very satisfactory results which do not differ sugnificantly from the excellent record achieved by 0.2 per cent VC-13 under the conditions of this experiment. It was unfortunate that farm management necessitated the shearing of the sheep at this juncture, as prolongation of the trial may well have provided a better basis for comparing the two insecticides.

The continued occurrence of 'abortive' strikes throughout the trial was a useful indication of fly density. It is of interest to note that a high proportion of blowfly strikes on the control animals failed to develop into progressive or clinically visible strikes. The reasons for this are not demonstrated by this experiment but individual sheep susceptibility, and lack of follow-up strike are likely to be implicated. It is probable that the majority of the 'abortive' strikes recorded above would not be noticed by the average shepherd. 'Clinically abortive' is possibly a better term as many of the strikes so classified undoubtedly give rise to fully developed larvae which go on to pupate and complete a normal life cycle.

ACKNOWLEDGEMENTS

The writer thanks the Board of Directors of Cooper & Nephews S. Af. (Pty.) Ltd., for permission to publish this work, and Mr. H. G. Milne and Son of 'Bracklyn', Fishriver, who made their sheep available for the experiment.

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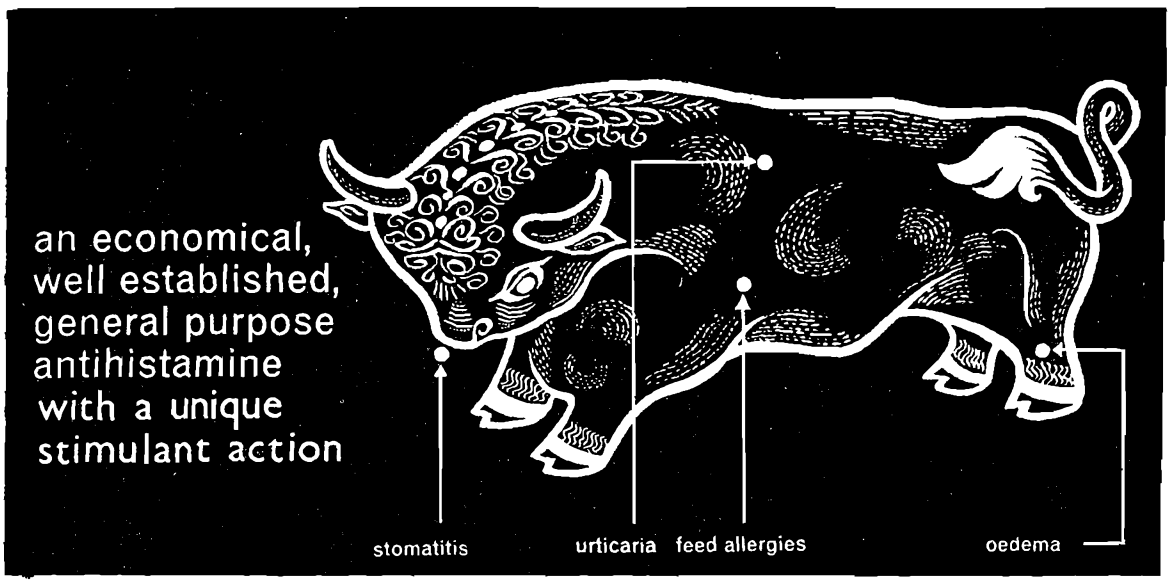

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ANTHELMINTIC TESTS WITH HALOXON* IN CATTLE

C. J. BOSMAN

Cooper and Nephews S.Af. (Pty.) Ltd., East London.

Received for publication January 1965

SUMMARY

Haloxon was dosed orally at 38.48 mg/kg to bovines naturally infested with *Haemonchus* spp, *Ostertagia* spp, *Trichostrongylus* spp, *Cooperia* spp, *B. phlebotomum*, *N. spathiger*, *N.* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp., *B. phlebotomum*, *N. spathiger*, *N. vitulorum*, *O. radiatum*, *Ch. ovina* and *Trichuris* spp.

It was highly effective against all adult worms with the exception of *B. phlebotomum* where efficiency was erratic. Some of the fourth and early fifth stages of *Ostertagia* spp. were not always removed.

Toxicity was tested in over 3,000 cattle, not only in different areas, but also under different stress conditions, e.g. nutrition—including feedlot cattle; dry and lactating cows; various animal husbandry methods, dipping procedures etc. Even overdosages at 80 100 mg/kg failed to cause any toxic effects. Haloxon dosed animals did not show any cholinesterase inhibition even although they had been regularly dipped in a Dioxathion** wash (0.065%) concentration.

The milk of dairy cows dosed with this product was not tainted and the yield not depressed. The seasonal decline in milk production was halted.

INTRODUCTION

In Southern Africa internal parasites combined with such stress factors as variable climatic conditions leading to summer abundance and winter famine; mineral deficiences; sub-clinical chronic plant poisoning etc. are annually responsible for great losses in beef and milk production. A safe anthelmintic under these various conditions is essential.

The low toxicity of Haloxon even when given at doses above the therapeutic level was reported by Malone[3] (1964). He dosed sheep subjected to a variety of environmental and stress factors: e.g. pregnancy, copper deficiency, treatment with hexachlorophene and carbon tetrachloride, dipping in Dioxathion washes, without any increase in toxic hazards. Sheep dosed with ten times the therapeutic dose, demonstrated only a slight to moderate depression of red cell cholinesterase activity.

Hart[2] (1964), working with Nigerian zebu cattle, showed that Haloxon at 30.50 mg/kg was highly effective against adult *Haemonchus*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum*. Efficiency against the immature stages was variable, but high against *Haemonchus* spp., *Cooperia* spp.

Armour[1] (1964), in controlled critical tests with calves experimentally infested with *Ostertagia* spp., found that at 40 mg/kg Haloxon was highly effective against fully mature adults but only moderately efficient against young adults. He demonstrated that Haloxon had little effect against the histotropic larvae even at 100 mg/kg, but no toxicity was evinced.

ANTHELMINTIC AND DOSAGE RATE

The Haloxon drenches were prepared by adding 1-lb. 77 per cent Haloxon wettable powder to 90 fl. oz. of water, giving 100 fl. oz. of

*"Haloxon" = O,O di (2-chlorethyl) 0-(3-chloro-4-methyl coumarin-7yl) phosphate = 'loxon' R.T.M. Cooper, McDougall and Robertson Ltd., Berkhamsted, U.K.

**"Dioxathion" = 2, 3-p-dioxane S, S-bis (0, 0-diethyl Phosphorodithioate) 'Delnav' R.T.M. Hercules Powder Co. Wilmington, U.S.A.

suspension; each ounce contained 3.50 gm active ingredient. The dosage rate was 34–48 mg/kg bodyweight estimated by weighband.

Faecal egg count test

Materials and methods

A modified McMaster's technique for counting eggs in the faeces was employed. Fifty-four infested calves were divided into two uneven groups and suitably earmarked. Forty were treated at 38.48 mg/kg and 14 left as untreated controls. Faeces samples were collected from each animal on the day of dosing and seven days later. Results following treatment with Haloxon showed that there was 100 per cent reduction in 'other' eggs, but a variable reduction of '*Bunostomum* eggs' ranging from nil to 100 per cent reduction (Table I).

Controlled anthelmintic test

Materials and methods

Calves from three different farms were purchased. Faeces passed after dosing were collected to note the numbers of *Bunostomum* spp. expelled. The animals were sacrificed 72 hours after dosing. The contents of the abomasum, small intestine and colon were collected and the linings of the organs thoroughly washed. A nylon scrubbing brush was used to remove any *Ostertagia* spp. still attached to the mucosal wall of the abomasum.

All the ingesta and washings were thoroughly mixed and stirred by bubbling air through the column of liquid. The total volume was measured and a 10 per cent aliquot was taken from the material from each animal. This was searched for worms which were collected, identified and counted, and the number found multiplied by ten.

Results: The high efficacy against all worms present, with the exception of *Bunostomum* sppc., is shown in Table II.

The worms encountered were not all identified but the following species were listed:—

Haemonchus contortus (although it is known that *H. placei* occur in the areas from which the calves for critical trials were selected).
Ostertagia ostertagia and *O. trifurcata*
Trichostrongylus axei and *T. colubriformis*
Cooperia mcmasteri, *C. punctata* and *C. pectinata*
Bunostomum phlebotomum
Nematodirus spathiger
Chabertia ovina and
Oesophagostomum radiatum

Critical Anthelmintic Test

Seven infested calves were housed individually in concrete floored pens and the amount of food restricted to a quarter of the normal intake, but water was freely available. After dosing, the faecal output of each calf was collected every 12 hours until no more worms were expelled. Each individual faecal sample was washed through a 100 mesh to the linear inch sieve. The faeces trapped on the sieve were then washed into a suitable container to form a thin suspension and a 1 per cent aliquot of the total volume of this suspension was examined microscopically.

Table I.—Differential e.p.g.* counts before and after dosing with haloxon.

Group and Number of Cattle	Examination of Faeces for:	Before Dosing 8.1.64		After Dosing 15.1.64	
		(Range)	Mean	(Range)	Mean
Control Untreated 14 animals	'*Bunostomum spp*'. Eggs	(0–600)	150	(0–700)	228
	'Other eggs'.....	(500–1000)	892	(400–1300)	813
Haloxon dosed 40 animals.....	'*Bunostomum*' eggs.....	(0–1000)	130	(0–500)	32
	'Other eggs'.....	(400–2100)	1002	0	0

*e.p.g.—eggs per gram of faeces.

The aliquots were stained with iodine, worms collected, identified and counted. The total of each species of the different aliquots from each calf was multiplied by 100 to give the total number expelled.

The balance of the faecal suspension, on each occasion, was passed through a 40 mesh sieve and the larger species, i.e. *B. phlebotomum*, *O. radiatum* and *Ch. ovina* collected, identified and counted.

Calves were slaughtered 72 hours after dosing, and the same methods of examination *post mortem* followed as described in the previous experiment.

Results

The data presented in Table III show the treatment was highly effective against the majority of species present; moderately effective against *N. spathiger* and poor against *B. phlebotomum* (Table III).

Anthelmintic test with *Neoascaris vitulorum*

Materials and Methods

Two calves known to be infested with *N. vitulorum* were dosed with Haloxon and housed in concrete floored pens, faeces collected, worms counted until no further ascarids were expelled. They were then dosed with 220 mg/kg Piperazine adipate and the expelled ascarids collected.

Table II—Controlled autopsy trials

Calf Identification	Weight kg.	Dose mg/kg.	*Haemonchus* spp.	*Ostertagia* spp.	*Trichostrongylus axei*	*Bunostomum* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*Oesophagostomum* spp.	*Trichuris* spp.
			NUMBERS OF WORMS FOUND AT AUTOPSY							
Trial I										
No. 216	114	Control	2,800	2,260	1,920	132	6,700	4,250	20	—
No. 250	114	48	0	200	0	49	0	0	0	—
No. 92	109	40	0	145	0	23	0	0	0	—
Mean of Treated Animals			0	172	0	36	0	0	0	—
Efficiency.....			100%	92%	100%	71%	100%	100%	100%	—
Trial II										
No. 20	114	Control	1,260	2,560	26,860	150	8,300	2,800	159	0
No. 15	159	Control	1,040	4,860	39,750	122	10,300	12,100	251	10
Mean of Controls.....			1,150	3,710	33,305	136	9,300	7,450	205	5
No. 11	135	48	0	140	0	58*	30	20	0	0
No. 10	116	42	0	66	33	0*	0	30	0	0
No. 9	163	39	0	300	0	15*	0	0	0	0
Mean of Treated Animals			0	169	11	24	10	17	0	0
Efficiency.....			100%	96%	99%	82%	99%	99%	100%	100%
Trial III										
No. 162	80	Control	274	5,280	8,940	74	15,500	2,360	196	89
Nol 161	115	Control	3,640	3,860	9,100	144	11,320	2,480	228	11
Mean of Controls.....			1,857	4,570	9,020	109	13,410	2,420	212	50
No. 163	95	37	0	50	0	14*	0	0	0	0
No. 18	68	50	0	10	0	40*	0	0	0	0
No. 165	120	46	0	30	0	141*	0	0	6	0
Mean of Treated Animals			0	30	0	65	0	0	2	0
Efficiency.....			100%	99%	100%	42%	100%	100%	99%	100%

* An average of 16 *Bunostomum* spp. were expelled from these animals, giving an average reduction of 26%.

Results

Forty worms were expelled after Haloxon dosing and only one on subsequent dosing with Piperazine (Table IV).

Table IV
Worms expelled after dosing calves with Haloxon and then with *Piperazine adipate* at 220 mg/kg.

	Haloxon			*Piperazine adipate*		
Number of calf...	1	2		1	2	
Weight kg.....	39	32		39	32	
Dose mg/kg.....	45	54		220	220	
	Nos. Expelled		*Average*	*Nos. Expelled*		*Average*
Neoascaris vitulorum.....	19	21	20	0	1	0.5

Attempts to produce toxicity

Over 3,000 cattle of different breeds and ages, in various parts of the Republic were successfully dosed with Haloxon at 34–48 mg/kg without any loss or evidence of clinical intoxication. One cow aborted 24 hours after dosing and blood collected from her ten days later revealed agglutinating antibodies of *Brucella abortus bovis*. Two other cows lost their calves. One was a fortnight overdue and her calf was delivered dead in attempting to alleviate dystokia, and the other calved 13 days after dosing, but its full-term calf was found dead in the morning. In none of the above cases was Haloxon responsible.

Table III—Critical Trials

Calf Identification	Weight Kg.	Dose mg/Kg.		*Haemonchus* spp.	*Ostertagia* spp.	*Bunostomum* spp.	*Cooperia* spp.	*Trichostrongylus* spp.	*Oesophagostomum* spp.	*Trichuris* spp.	*Chabertia* spp.	*Nematodirus*
Trial I												
No. 1	49	57	E	650	11,400	—	11,200*	8,800	2	117	—	—
		@	A	0	450x	—	0	30	0	0	—	—
No. 2	50	57	E	1,200	3,800	—	9,200*	5,500	11	2	—	—
		@	A	0	230x		0	20	0	0	—	—
No. 3	70	57	E	1,100	3,100	—	4,200*	9,020	10	0	—	—
		@	A	0	200x	—	60	30	0	0	—	—
Mean expelled.....				983	6,100	—	8,200*	7,773	8	39	—	—
Mean @ autopsy.....				0	293x	—	20	27	0	0	—	—
Efficiency.....				100%	95%	—	99%	99%	100%	100%	—	—
Trial II												
No. 5	115	48	E	348	2,400	23	3,100	13,600	224	—	10	100
		@	A	0	66x	148	0	0	0	—	0	30
No. 6	138	46	E	240	4,400	38	6,300	28,200	278	—	—	60
		@	A	0	200x	256	0	0	0	—	—	30
No. 7	136	47	E	—	4,000	28	5,100	10,900	218	—	—	300
		@	A	—	500x	181	0	200	0	—	—	100
No. 8	142	45	E	300	9,900	27	10,000	28,100	154	—	—	—
		@	A	0	1,800x	170	0	500	0	—	—	—
Mean Expelled.....				296	5,450	29	6,125	20,200	218	—	10	153
Mean @ autopsy.....				0	641	189	0	175	0	—	0	53
Efficiency.....				100%	89%	14%	100%	99%	100%	—	100%	74%

* *C. mcmasterei* and other species present.
x All the *Ostertagia* spp. found at autopsy were immature stages.

E = expelled
A = found at autopsy

Some calves in poor condition were given several times the therapeutic dose, and the only symptom displayed was a softening of the faeces which lasted two days.

There are certain parts of South Africa where it is accepted that anthelmintic dosing often has toxic effects. Stress factors, such as grazing where poisonous plants occur, mineral deficiencies, severe winter conditions associated with low nutrition etc, exert an influence. In such cases, at first 10–20 representative animals were dosed, and if no side effects were noted the whole herds were dosed 48 hours later. Cattle under feed-lot conditions destined for slaughter, were also safely and successfully dosed.

The results of these experiments are summarized in Table V.

Sixteen calves which had been dipped every week in 0.065 per cent Dioxathion wash for six months. were earmarked and divided into four equal groups. One group was undosed; the second group was dosed midweek between dippings; the third immediately prior to dipping, and the fourth half an hour after dipping. Whole blood cholinesterase determinations were taken from all animals 0, 6, 24 and 168 hours after dosing. No depression of whole blood cholinesterase was found, using the Lovibond method. A further 77 calves in the herd were successfully and safely dosed with Haloxon. Finally, a herd of 500 cattle of all ages and in varied condition, which had been regularly dipped in Dioxathion, were successfully and safely treated with Haloxon.

Two dairy herds were treated with Haloxon with no drop in milk yield attributable to the drenching. The milk was not tainted and was of normal colour. The seasonal drop in yield was also checked.

Discussion

The number of worm eggs in faeces before and after treatment is an unreliable method of checking its efficacy. This applies particularly to *B. phlebotomum*. A possible explanation of

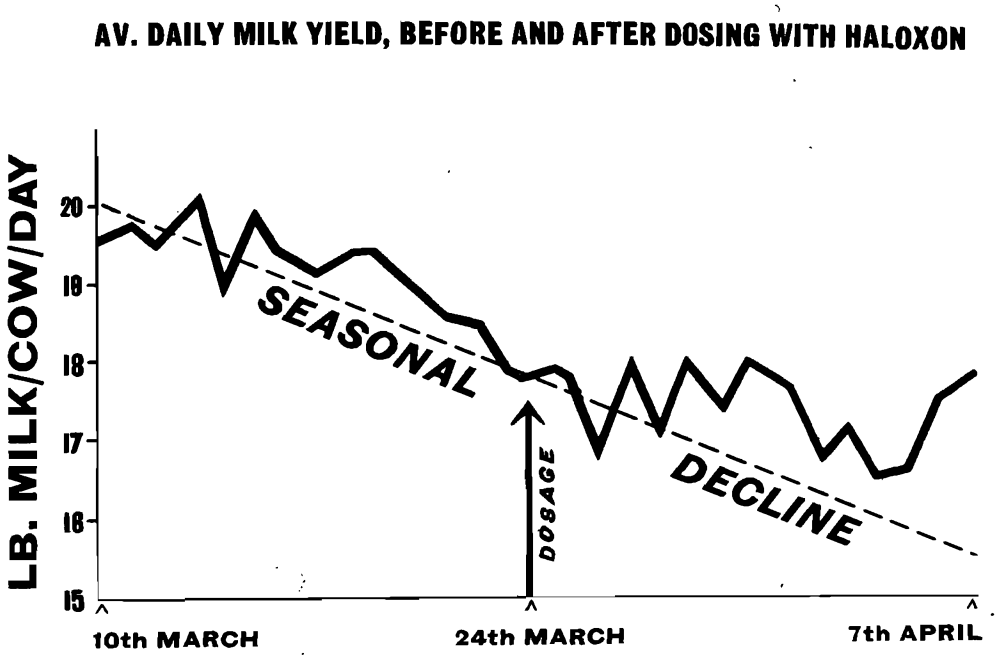
Table V
Safety trials: Cattle 'field' dosage.

Dosing Table used: 1-lb to 90fl ozs. water giving 100 fl. oz. drench.

70—100 lb.	½ fl. oz.
101—150 lb.	¾ fl. oz.
151—200 lb.	1 fl. oz.
201—250 lb.	1¼ fl. oz.
251—300 lb.	1½ fl. oz.
301—350 lb.	1¾ fl. oz.
351—400 lb.	2 fl. oz.
401—500 lb.	2½ fl. oz.
501—600 lb.	3 fl. oz.
601—700 lb.	3½ fl. oz.
701 lb. and over	4 fl. oz.

Areas		
Orange Free State	456	cattle of all ages.
Transvaal	574	cattle of all ages.
Eastern Cape Province	512	cattle of all ages (dipped in chlorinated camphene*** and Dioxathion combination wash.
	77	calves (dipped in Dioxation at 0.065%)
	136	all ages (dipped in Dioxathion at 0.065%)
Eastern Cape Province (*Senecio* veld)	769	cattle of all ages.
South Western Districts (Legume grazing)	546	cattle of all ages.
Age and weight groups		
Calves	476	70—400lb.
Young Stock	914	400—600 lb.
Adult animals	572	600—1,200 lb.
Cows and heifers in calf	377	500—1,000 lb.
Cows in milk and Suckling calves	731	600—1,000 lb.

*** Chlorinated camphene (av. 68% chlorine) 'Toxaphene' *R.T.M.* Hercules Powder Co., Wilmington, U.S.A.



the variable production of eggs by this species following dosing with Haloxon, may be that this coincided with the termination of an egg laying cycle. Sprehn[4] (1932) reported that the females lay eggs in short cycles and resume egg production after refertilisation. It is therefore, doubtful whether Haloxon is solely responsible for the variable reduction in egg production. Critical tests confirmed the low efficacy against *B. phlebotomum* in contrast to the good results with the faecal egg count method.

The controlled and critical tests demonstrated the high efficacy of Haloxon against most of the other gastrointestinal roundworms of cattle, with moderate efficiency against *N. spathiger* and the immature stages of *Ostertagia* spp.

Comparison of the three methods for assessing anthelmintic efficiency of a drug, confirms that critical tests are the most reliable under field conditions except possibly when *Haemonchus* spp. are involved. These worms are very fragile and are digested, decomposing on their route out of the animal. This drawback may be overcome by also counting the tails of the parasites expelled in the faeces. The summary of controlled and critical tests clearly demonstrates this fact (Table VI).

The action of Haloxon against immature stages of *Ostertagia* spp., i.e. late fourth and fifth stages, is variable, and if it were not for this it would be a near 100 per cent remedy against these species. This confirms Armour's (1964) findings. Hart[2] (1964), has reported that Haloxon was very effective against the fourth and fith stages of *Haemonchus contortus* at 30 mg/kg. He remarked, however, that the results were variable against fourth and fifth stages of *Cooperia* spp., *Trichostrongylus axei* and *Oesophagostomum radiatum*, but that it was excellent against the large roundworms (*N. vitulorum*) of calves at 45–55 mg/kg.

Under South African conditions, Haloxon as a 77 per cent wettable powder, proved a safe anthelmintic for cattle even in animals which were known to be suffering from a chronic liver damage from grazing on *Senecio* spp. veld. All that animals on *Senecio* veld showed, was a softening of the faeces for a few days after dosing. Some very emaciated animals were included in these trials without any ill effects from dosing. Of over 400 pregnant animals dosed, none showed any side effects attributable to the treatment.

TABLE VI.—SUMMARY OF CONTROLLED AND CRITICAL ANTHELMINTIC TEST.

	Controlled			*Critical*		
No. of animals	2	3	3	3	4	2
No. of controls	1	2	2	—	—	—
Haemonchus spp.	100%	100%	100%	100%	100%	—
Ostertagia spp.	92%	96%	99.3%	95%	89%	—
Trich. Axei	100%	99.9%	100%	—	—	—
Bunostomum spp. (Controlled)	71%	82%	42%	—	—	—
(Critical)	—	43%	17%	—	14%	—
Cooperia spp.	100%	99.8%	100%	99.7%	100%	—
Trich. spp.	100%	99.7%	100%	99.6%	99%	—
Oesophagostomum spp.	100%	100%	99.1%	100%	100%	—
Trichuris spp.	—	100%	100%	100%	—	—
Nematodirus spp.	—	—	—	—	74%	—
Chabertia spp.	—	—	—	—	100%	—
Ascaris spp.	—	—	—	—	—	97%

When the seasonal decline in milk production is studied in the graph, it is evident that the dosage of Haloxon checked the decline in milk yield. Because of the fact that Haloxon dosing does not interfere with milk production, the dosing of worm ridden cows-in-milk becomes feasible. The results clearly indicate the economic importance of worm control in dairy herds.

It is worthy of emphasis that the blood of Haloxon dosed animals, which had been regularly dipped in a wash containing an organophosphorous insecticide, did not subsequently show cholinesterase inhibition. This is an additional advantage as many animals which must be dosed are regularly immersed in organophosphorous washes.

ACKNOWLEDGEMENTS

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THE CONTROL OF PIGEON TAPEWORM INFESTATION WITH LINTEX(R)

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SUMMARY

(*a*) The common tapeworm species of pigeons in South Africa, according to this trial are: *Raillietina Raillietina columbiella* and *Raillietina Fuhrmanetta crassula*[12].

(*b*) The incidence of tapeworm infestation was found to be 35 per cent in an untreated flock of pigeons.

(*c*) Lintex at a dosage rate of approx. 200 mg/kg. was found to be highly effective against these tapeworms of pigeons. A 100 per cent result was obtained on all birds slaughtered five hours and more after treatment.

(*d*) The extreme safety of Lintex was demonstrated by dosing at a rate ranging from 356 to 1045 mg/kg. without any deleterious effects.

(*e*) Only 6 of the 21 pigeons harboured roundworms, which is an infestation rate of approximately 28 per cent. Of these six birds, three harboured one round worm each; two harboured two roundworms each and one harboured four roundworms. The species encountered were *Ascaridia columbae* and *Ornithostrongylus quadriradiatus*.

(*f*) Lintex is a specific cestocide and is known to have no effect on roundworms.

INTRODUCTION

It is estimated that approximately one million pigeons are owned by about 25,000 people in South Africa. There are 550 registered clubs which, in turn, are affiliated to 43 federations, combines or unions. These figures were obtained from the S.A. Racing Pigeon Association, who advised that this is only an estimate as exact figures are not avaialble. It must also be borne in mind that large additional numbers of racing and fancy type pigeons are kept by owners who do not belong to any clubs and it is, therefore, perhaps not unrealistic to estimate the number of pigeons in the Republic of South Africa at a figure of $1\frac{1}{2}$ millions. It costs about R2,000,000 per annum to feed these birds.

During the racing season (which opens in June) pigeons compete weekly for prize money of approximately R7,500. Pigeon racing, therefore, has become quite lucrative. It is essential that racing birds be kept in the best of condition. The aim of this trial was to establish to what extent pigeons are normally infested with tapeworms and how this can be controlled with a modern tapeworm remedy.

MATERIALS AND METHODS

(*a*) Lintex, which contains N-(2' chlor 4 nitrophenyl)-5-Chlorsalicyl-amid, is known to be of high efficacy against tapeworms in cattle, sheep, goats, humans, dogs, cats and poultry.

(*b*) A flock of 60 mature pigeons of varying ages was acquired from an owner in the Pretoria area. No anthelmintic treatment against roundworms or tapeworms had been carried out for a period of about 20 months.

(R) Lintex is the registered trade mark of Farbenfabriken Bayer A.G., Laverkusen Western Germany.

(*c*) For this trial all pigeons were individually caged in steel drums with a diameter of 12 inches and about 24 inches deep. Water and feed receptacles were fitted to a wire-mesh door and the drums kept flat. Faceal collections were made three to four days after caging the pigeons. The faeces were washed on 100 mesh to the linear inch sieves and thereafter microscopically examined for the presence of tapeworm segments. Twenty-one of the sixty pigeons were found to harbour tapeworm segments, which is a 35 per cent infestation rate.

Lintex Efficacy Trial

The 21 tapeworm-infested pigeons were again individually caged, numbered, weighed and dosed with Lintex capsules at various intervals.* Details of the numbers and types of tapeworm are given in Table I.

Lintex Tolerance Trial

Eleven pigeons were overdosed with Lintex to determine to what extent this material was tolerated. Details of dosing rates, etc. are given in Table II.

Table I—Efficacy Of Lintex Against Pigeon Tapeworms : Dose - 1 Capsule Each

NO	WEIGHT GRAMS	SLAUGHTER TIME AFTER TREATMENT—HOURS	TAPEWORMS EXCRETED AFTER TREATMENT	TAPEWORMS FOUND POST MORTEM	ROUNDWORMS FOUND POST MORTEM	DOSAGE MG/KG.
1	422	24	6 R.F.C.* spp., numerous segments and Strobila	Nil	1 *Ornithostrongylus quadriradiatus*	204
2	400	24	3 R.F.C. spp. -ditto-	,,		215
3	385	24	1 ,, ,, ,,	,,		224
4	500	14	6 ,, ,, ,,	,,	1 *Ascaridia columbae*	172
5	415	14	3 ,, ,, ,,	,,	1 ,, ,,	207
6	442	14	2 ,, ,, ,,			195
7	382	14	2 ,, ,,	,,		225
8	415	14	2 ,, ,, ,,	,,		207
9	523	14	4 ,, ,, ,,	,,		164
10	455	14	3 ,, ,, ,,	,,	1 *As. columbae and* 1 *O. quadriradiatus*	190
11	410	14	3 ,, ,, ,,	,,	2 *As. columbae*	210
12	400	5	3 ,, ,, ,,	,,	4 *O. quadriradiatus*	215
13	406	5	1 ,, ,, ,,	,,		212
14	495	2½	2 ,, ,, ,,	2 RFC. spp.		174
15	545	2½	1 ,, ,, ,,	Nil		158
16	355	6	5 R.R.C.** ,, ,,	,,		242
17	449	6	2 RRC & RFC ,,	,,		192
18	437	6	3 R.F.C. ,, ,,	,,		197
19	360	6	4 ,, ,, ,,	,,		240
20	462	6	2 ,, ,, ,,	,,		187
21	522	6	6 ,, ,, ,,	,,		164

* R.F.C. = *Raillietina Fuhrmanetta crassula*
** R.R.C. = *Raillietina Raillietina columbiella*

Table II.—Lintex: Tolerance to pigeons

NO.	WEIGHT	DOSAGE: CAPSULES	DOSAGE MG/KG.	RESULTS AND REMARKS
22	382	2	447	Nothing unusual
23	480	2	356	Nothing unusual
24	445	2	275	Nothing unusual
25	452	2	378	Nothing unusual.
26	447	3	580	Nothing unusual.
27	388	3	670	Moping after 2 hours. After 6 hours nothing unusual.
28	444	3	584	Nothing unusual.
29	431	3	600	Nothing unusual.
30	464	4	745	Moping after 2 hours. After 6 hours nothing unusual.
31	479	4	720	Nothing unusual
32	330	4	1045	Moping after 2 hours. After 6 hours nothing unusual.

Remarks and discussion of anthelmintic trial

The amount of Lintex dosed to every pigeon was contained in a sealed gelatine capsule, which holds 115mg. of the 75 per cent w.p. or 86.25 mg. of the active ingredient. Pigeon weights varied between 355 grams and 545 grams. The therapeutic dosage level, therefore, ranged between about 158 mg/kg. and 243 mg/kg. At no stage of the trial were any adverse reactions displayed by any of the dosed pigeons.

The first three pigeons were dosed and then left for a period of 24 hours in clean steel drums, which were standing upright with the wire-mesh door on top. Only water was offered at this stage. These three birds were slaughtered 24 hours after treatment. Faeces and excreted worms were again washed on 100 mesh to the linear inch sieves. The intestinal tract from behind the gizzard to the cloaca was opened, thoroughly washed and the mucosa stripped and washed on a sieve as above. Faecal and ingesta material were kept separately and examined under the stereo-microscope for tapeworm scoleces, strobila and segments. The faecal material obtained from these three birds indicated that the pigeons should be perched, after treatment, in the cages in order to avoid laceration and trampling of the tapeworms. All pigeons from Nos. 4 to 21 were, therefore, kept on 2-inch wiremesh about 3 inches above the cage floor.

The number and types of tapeworms excreted by the pigeons are given in Table I. Roundworms were also recorded and it seems from the above that only a very low rate of roundworm infestation is normally present in pigeons in the Pretoria area. The good hygienic measures which normally apply in the average pigeon loft must undoutedly account for the low incidence of roundworms. The very high incidence of tapeworm infestation on the other hand seems to point to an abundance of tapeworm intermediate hosts (various beetles and ants) in the Pretoria area.

As can be seen from Table I, the pigeons were dosed and slaughtered at various intervals after dosing. The only pigeon which still harboured a tapeworm was No. 14, which was sacrificed 2⅛ hours after tretament. Pigeons were closely observed after dosing, and it was noticed that tapeworms were excreted as early as 50 minutes after treatment. For the complete excretion of the entire tapeworm burden a period of approximately 5 to 6 hours should be allowed. Tapeworms collected 5 and 6 hours after dosing were recovered undamaged with the scolex intact.

Tolerance trial:

Eleven pigeons were exposed to double and higher over-dosages with Lintex to determine to what extent this material is tolerated. All birds were weighed individually, and 4 were given 2 capsules each; another 4, 3 capsules each and a further 3, 4 capsules each. Only 3 pigeons behaved in a slightly abnormal way by moping about 2 to 2½ hours after dosing. These slight side reactions were of a transitory nature only and passed off after approximately 6 hours. Thereafter all birds were normal when the loft was opened all birds flew out in normal fashion. For detailed information regarding this trial please refer to Table II.

ACKNOWLEDGEMENTS

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TRIALS WITH THIABENDAZOLE IN HORSES

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SUMMARY

Anthemintic trials with thiabendazole in horses based on faecal egg counts and critical slaughter trials are described. Thiabendazole was highly effective against small strongyle species, *Parascaris equorum*, *Oxyuris equi* and *Probstmayria vivipara.*

During toxicity studies no significant hamatological or clinical disturbances were demonstrable at dosage rates up to 940 mg/Kg.

INTRODUCTION

The anthelmintic efficacy of thiabendazole (2-(4'-thiazolyl)- benzimidazole) in horses has been described by Drudge, Szanto, Wyant and Elam[2,5]; Drudge, Szanto and Wyant[4]; Egerton, Cuckler, Ames, Bramel, Brightenback and Washko[6]; Turk, Ueckert and Bell[15]; McDonald[9]; Enigk and Stoye[7] and Skerman, Shahlapoor and Eshim[13]; effective removal of mature large and small strongyles and *Oxyuris equi* were obtained at dosage rates of 25mg/Kg and higher. Drudge *et al*[2], *inter alia* report that highly effective removal of *Parascaris equorum* was found at 100 mg/Kg. Some of these workers conducted toxicity trials and established a very high therapeutic ratio. Thus Drudge[3] and Drudge *et al*[4] demonstrated slight haemoconcentration at 600 mg/Kg, which increased at 1200 mg/Kg with an accompanying leucocytosis.

Since Reinecke and Rossiter[12] had reported high efficacy against *Parascaris equorum* at 50 mg/Kg which appeared at variance with some of the above authors, it was decided to conduct critical slaughter trials after initial trials based on faecal egg counts with this and other nematodes.

It was reported by Azzie[1] that Thiabendazole caused a change in the haematocrit readings at approximately 50 mg/Kg. Accordingly haematological determinations were carried out after treatment of 50 mg/Kg as well as after doses up to 942 mg/Kg.

FAECAL EGG COUNTS

MATERIALS AND METHODS

Faeces were obtained from the recta and faecal egg counts carried out according to the modified McMaster egg counting technique as described by Reinecke[10].

Trial 1.

Twenty-four thoroughbred brood mares were treated with twentyfive grams of commercial phenothiazine on Day 1. On Day 3, they were divided into two equal groups. The one group received thirty grams of a 68 per cent water dispersible formulation of thiabendazole* each i.e. 20.4 grams of active drug, in the feed. The other group was not treated with Thiabendazole.

Seven yearlings out of a group of fifteen which had received 15 grams of Phenothiazine were each treated three days later with 13.6 grams thiabendazole (20 grams of 68 per cent formulation) in the feed. Faecal samples were collected from all the horses on Day 3 and 27.

* c/o MSD (PTY) LTD.—P.O. Box 7748—JOHANNESBURG.

** Platkoppies, HEIDELBERG.

Trial 2.

Eighteen polo ponies, including thoroughbreds were divided at random into three groups. Group 1 received 20 grams thiabendazole each in the form of a 68 per cent formulation mixed with wheaten bran. Group 2 similarly received 20 grams thiabendazole in a 33⅓% formulation** in order to compare efficacies of various formulations. Group 3 served as untreated controls.

Faecal samples were collected on days 0, 5, 12, 26, 44, 62, 75 and 90.

Critical anthelmintic tests

It appears from these results that the 33⅓% formulation was more effective. It must, however, be noted that these are average egg counts of the different groups and only one of six horses was infested in group 1, 12 and 26 days after treatment, respectively.

(*a*) *Donkeys*: Rectal faecal samples from two naturally infested donkeys were collected on the day of treatment and strongyle eggs counted according to the modified McMaster technique[10]. Both donkeys were weighed and treated by stomach tube. Donkey 1 was treated at 88 mg/Kg liveweight with a water dispersible formulation of thiabendazole. Donkey 2 received 88 mg/Kg of a formulation containing 50 per cent coarse (44–400μ) particles.

The donkeys were stabled separately on concrete floors. All faeces were collected twice daily for four days after treatment when both were slaughtered for *post mortem* examination.

Total faeces or aliquots by weight were washed through a 44 mesh to the linear inch sieve and the sieve contents examined microscopically. Worms were collected for microscopic identification.

At autopsy ligatures were applied at the pyloric sphincter, ileo-caecal valve and anus.

Results

Table 1.—Results of faecal worm egg counts in trial 1.

Animals	Treatment		Average e.p.g.†	
	Day 0	Day 3	Day 3	Day 27
12 Mares	25 gm PTZ	20 gm TBZ	0*	0
12 Mares	25 gm PTZ	—	0	346
7 Yearlings	1 gm PTZ	13.6 gm. TBZ	0	0
8 Yearlings	15 gm. PTZ	—	0	729

† e.p.g. = eggs per gram.
* = 0 or = 50 e.p.g.
PTZ = Phenothiazine
TBZ = Thiabendazole
* Trademark THIBENZOLE, MSD (PTY) LTD.

Table II.—Results of faecal worm egg counts in Trial II.

Day	Average strongyle eggs per gram.							
	0	5	12	26	44	62	75	90
Group 1 (68% formulation)	1,958	0*	17	17	100	100	666	1,283
Group 2 (33⅓% formulation)	3,108	0	0	0	0	117	133	200
Group 3 (Untreated controls)	810	575	666	633	1,083	1,566	1,600	1,133

0* = 0 or <50 e.p.g.

The small intestine and large bowls were opened, washed thoroughly and the washing sieved through a 100 mesh to the linear inch sieve. Volumetric aliquots were prepared by the method described by Reinecke[11]. Microscopical examination was carried out on 1/10 aliquots from the small intestine and 5x1/50 aliquots from the large intestine. Worms were identified microscopically using the check list of Theiler[14].

To facilitate counting, preparations were stained with strong iodine prior to macro—or microscopical examination.

(*b*) *Horses*: Four yearling fillies were selected on the presence of ascarid and strongyle eggs in the faeces. These foals were then kept in separate loose boxes on concrete floors prior to treatment.

Fillies 1 and 2 were treated by stomach tube with thiabendazole at 88 mg/Kg liveweight. Fillies 3 and 4 were treated at 44 mg/Kg liveweight with thiabendazole containing 50 per cent coarse particles. Total daily faecal output was collected in the mornings and refrigerated in polythene bags if not examined immediately. Faeces were communited through ½" wire netting, coarse vegetable matter removed and *Parascaris equorum* and *Oxyuris equi* were collected. After comminution sievings were weighed and well mixed manually. Aliquots by weight were collected as follows:

Three x 1/100 for microscopical and two x 1/20 for macroscopical examination. The aliquots for microscopical examinations were washed through a 100 mesh sieve. Aliquots were stained with a strong solution of iodine prior to examination.

Fillies 1 and 2 were slaughtered four days and fillies 3 and 4, six days after treatment. *Post mortem*—the same techniques were used as for donkeys. In addition the lungs, liver and abdominal vascular systems was examined carefully.

Aliquots by weight were prepared and sieved through 100 mesh sieves. The small intestines' contents were examined macroscopically for *Parascaris equorum*, 2 x 1/10th aliquots were collected from the large bowel for macroscopical and several 1/100 aliquots for microscopical examination.

Results

Cross checking of weighed aliquots revealed accuracy within 10 per cent of the mean.

No significant difference was obtained at 88 mg/Kg between standard and coarse particles.

Highly effective removal of mature and immature small strongyles and *Probstmayria vivipara* were recorded both at 88 and 44 mg/Kg thiabendazole and with standard and 50 per cent coarse particles. In the second trial highly effective removal of *Oxyuris equi* at both dosage rates and particle sizes were obtained. Thiabendazole was highly effective against *Parascaris equorum* at 88 mg/Kg standard particles. No signs of aneurysms, few liver and no lung lesions were encountered at autopsy.

Toxicity and Haematology

1. Jugular blood samples were obtained in heparin from treated and control polo ponies (Trial 2) on Days 0, 5, 12 and 75. Haematocrit, sedimentation rate and bilirubin determination were carried out.

Table III.—Critical tests on Donkeys.

Donkey No.	Worms* Recovered						
	(*a*) In faeces after dosing on day					(*b*) Post Mortem	% Efficacy
	1	2	3	4	Total		
1	158*	16,524	3,750	280	20,712	450	97.9
2	452	818	21,794	5,380	28.444	500	98.3

* Small strongyles including the genera *Cylicocercus, Poteriostomum, Craterostonum* and *Triodontophorus.*

TABLE IV.—CRITICAL TESTS ON HORSES.

Filly 1—88 *mg/Kg on* 11.6.64.

Date	Ascaris	Oxyuris	Strongyles (small)	*P. vivi-para**	Immatures**
12/6	0	0	66	9,118	582
13/6	0	4	2,670	166,176	6,924
14/6	2	0	270	14,014	1,386
15/6	0	0	110	376	24
Total excr.	2	4	3,116	189,684	8,916
Post mortem 15/6	0	0	10	0	0
%Efficacy	100%	100%	99.68%	100%	100%

Filly 2—88 *mg/Kg on* 11.6.64.

12/6	0	37	1,010	10,430	4,470
13/6	8	6	3,200	77,865	11,635
14/6	11	14	700	7,254	2,046
15/6	3	8	125	2,340	660
Total excr.	22	65	5,035	97,889	18,811
Post mortem 15/6	2	0	80	0	300
%Efficacy	93.7%	100%	98.5%	100%	98.4%

Filly 3—44 *mg/Kg* (50% *coarse*) *on* 22.6.64.

23/6	0	1	100	0	0
24/6	0	0	1,550	72	28
25/6	5	0	2,930	644	256
26/6	8	0	600	144	56
27/6	0	0	80	0	0
28/6	0	0	10	0	0
Total excr.	13	1	5,270	860	340
Post Mortem 28/6	4	0	130	0	0
%Efficacy	76.5%	100%	97.6%	100%	100%

Filly 4—44 *mg/Kg* (50% *coarse*) *on* 22.6.64.

23/6	0	3	1,160	6,240	260
24/6	0	6	1,860	15,921	2,379
25/6	2	0	460	1,840	160
26/6	0	0	110	0	0
27/6	0	0	20	0	0
28/6	0	0	0	0	0
Total excr.	2	9	3,610	24,001	2,799
Post Mortem 28/6	5	0	65	0	150
%Efficacy	28.6%	100%	98.3%	100%	94.9%

* P.v. = *Probstmayria vivipara*

** Immatures = Not differentiated, small strongyles only. Small strongyles included the genera: *Craterostomum, Triodontophorus, Cylicocercus, Cylicocyclus, Poteriostomum* and *Trichonema.* Predominant genera were *Trichonema* and *Poteriostomum.*

2. Four horses (pony type) were purchased and stabled on cement floors separately for 13 days prior to treatment. Blood samples were obtained by jugular venipuncture in 1 per cent heparin for haematology and in Disodium ethylenediaminetetraacetate for blood chemistry. Blood samples were collected twice at 2-day intervals prior to treatment with thiabendazole, on day of treatment, day 2, 4 and 7. Erythrocyte and leucocyte counts, haemoglobin determination, blood urea nitrogen, total plasma protein, albumin, erythrocyte sedimental tion rate and bilirubin determinations were carried out at various times. Standard techniques were used. Horses were weighed at treatment and ten days later. Thiabendazole was administered by stomach tube at individual dosages of 117 mg/Kg, 225 mg/Kg, 471 mg/Kg and 942 mg/Kg liveweight respectively.

Results

1. Neither formulation at dosages in excess of 44 mg/Kg liveweight caused noteworthy deviations from the untreated controls as adjudged by heamatocrit, sedimentation rate and bilirubin. Horses from all three groups were ridden to play polo 48 and 72 hours after treatment without any obvious discomfort.
2. Variations in body weight, haematocrit, erythrocyte count, leucocyte count, haemoglobin content, blood urea nitrogen, total plasma protein, albumin, erythrocyte sedimentation rate, and bilirubin (direct and indirect Van den Bergh) could not be correlated with the dosage administered. No significant deviations from the normal were encountered at the dosage rates used.

Discussion

Equine anthelmintics have been reviewed by Gibson[8] *inter alia*. The anthelmintics reviewed had limited spectra of efficiency and varying degrees of toxicity.

Various authors have reported that the antelmintic thiabendazole exhibited a high degree of efficacy against all mature strongyles, *Oxyuris equi*, *Strongyloides esteri* and *Parascaris equorum*[2,3,4,5,6,7,12,13,15]. The effect against small strongyles, and *Oxyuris equi* at 44 mg/Kg have been confirmed. Highly effective removal of *Parascaris equorum* was obtained at 88 mg/Kg liveweight.

Anthelmintic Efficacy

Large strongyles (*S. vulgaris*, *S. edentatus* and *S. equinus*) were not encountered in these trials but highly effective removal was obtained by Drudge *et al*[4,5] at 50 mg/Kg; Enigk *et al*[7] at both 25 and 50 mg/Kg and Skerman *et al*[13] at 40 mg/Kg. Drudge *et al*[4] state that thiabendazole at 25, 50 and 100 mg/Kg was highly effective against 4th stage small strongyles. Drudge *et al*[4] demonstrated activity against the migrating larvae of *S. vulgaris* at varying dosage rates; 44 mg/Kg daily for 10 days starting on the day of infection, 440 mg/Kg on day 2 and 3 after infection and 440 mg/Kg on day 7 and 8.

Based on post treatment egg counts Egerton *et al*[6] concluded that thiabendazole killed immature strongyles. In our trials highly effective removal of 4th stage small strongyles in the intestinal lumen was obtained at 44 mg/Kg (50 per cent particles 40–400μ) and 88 mg/Kg standard particles. The examinations did not include pepsin digestion of the intestinal walls and no conclusions can be drawn regarding histiotrophic phases.

Haematology

Azzie[1] encountered fluctuations in the haematocrit in horses in training after the administration of thiabendazole at 20 grams per 1000 lbs liveweight (44 mg/Kg.).

Drudge *et al*[4,5] administered 200, 300, 600 and 1200 mg/Kg and noted that no untoward clinical effects were encountered at doses of less than 600 mg/Kg. At 600 mg/Kg slight haemoconcentration—31.0 per cent and 30.4 per cent

to 35.2 per cent and 35.0 per cent respectively—occurred. At 1,200 mg/Kg abdominal distress, haemoconcentration (31.6 per cent to 41.2 per cent) and leucocytosis was observed.

In the experiments reported above, changes in haemoconcentration at dosage rates of 20 grams/1000 lb, did not differ from those observed in the controls. During toxicity trials no significant haemoconcentration occurred at 117 to 942 mg./Kg.; nor could significant changes consistent with the dosage rate be demonstrated in total plasma protein, albumin, sedimentation rate, bilirubin (direct and indirect), erythrocyte and leucocyte counts, haemoglobin and blood urea nitrogen.

General

During the course of this work faecal samples from more than fifty donkeys of unknown origin and ranging from suckling to year old were examined. No ascarid eggs were encountered, few large strongyles but generally high counts of small strongyles. In a municipal stable, horses of six years and over had varying ascarid counts.

An interesting relation between ascarid egg counts and *Parascaris equorum* recoverd was encountered in filly No. 1. The egg count was 300 prior to treatment, but only two females were recovered.

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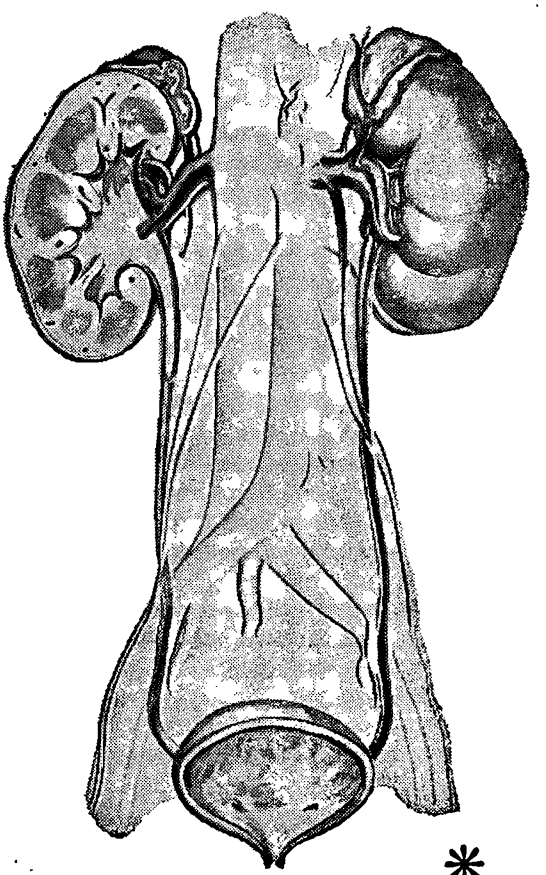
SPECIFIC FORMULA FOR URINARY TRACT INFECTIONS



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TDAP 3421

THE EFFECT OF FEEDING THE SODIUM SALTS OF THE FATTY ACIDS DERIVED FROM THE FISHER — TROPSCH PROCESS TO SHEEP AND CATTLE

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Introduction

The degradation of carbohydrates as cellulose, hemicellulose and simple sugars by the rumen micro organisms yield the steam volatile fatty acids as end products. The principle acids produced are acetic, propionic and butyric. In general, the molar proportions in which propionic and butyric acids occur remain relatively constant at 3:2 while the proportion of acetic acid to the sum of the other two acids varies according to the nature of the diet.

The volatile fatty acids are the major contributors of the free energy required for doing the work in maintaining the body of ruminants and in the synthesis of body tissues and secretions. Blaxter[1] has dealt in detail with the role of the steam volatile fatty acids in ruminant metabolism.

One of the end products of the synthesis of petrol and other hydrocarbons by SASOL, the South African oil from coal project, is an aqueous solution of fatty acids. These fatty acids occur in the product water of the American designed synthesis plant utilizing the Fisher—Tropsch process. The stream contains up to 1 per cent of acids (Rousseau[2]). The fatty acids are converted to the sodium salts by the addition of sodium carbonate, concentrated to a 50 per cent slurry by evaporation, and incinerated.

The resemblance of the proportions in which the acids occur to those found in the rumen under certain diets, led us to believe that the examination of this material could lead to its use as a source of supply of energy to ruminants.

The first essential was to establish the palatability of the material to sheep and cattle and observe gross effects.

Materials and methods

(1) Approximately 40 gallons of the 50 per cent slurry of sodium salts was provided by SASOL.. The pH was found to be 9.6 and this was adjusted to pH 7 by the addition of hydrochloric acid. A high pH may have a detrimental effect on the rumen where the pH is usually in the region of 7.0.

The slurry was dried by evaporation and approximately 350 lbs. of solids recovered. The salts were analysed and found to contain:

Sodium acetate	43.5%
Sodium propionate	11.3%
Sodium iso-butyrate	1.8%
Sodium n-butyrate	4.9%
Sodium valerate	1.2%
Sodium caproate	1.7%
Sodium chloride	4.2%
Sodium carbonate	1.9%
Water (loss on drying)	29.5%

(2) Two pens each containing 5 German Merino ewes were used. The sheep were penned throughout the trial and fed only on a dairy meal having a crude protein content of 18 per cent. No roughage was fed, Prior to the commencement of the trial these sheep had served as controls to a feeding trial and

were in a very poor condition. During this period they had been fed only on poor quality veld hay and salt *ad lib.*

Daily feed and water consumption was recorded. The fatty acid salts were included as part of the daily feed presented.

(3) Four cows were used. Nos. 2, 4 & 5 were Jerseys and No. 3 was a Friesland. The cows were penned throughout the trial and fed individually on a meal comprising:

Lucerne Meal	50%
Yellow Maize Meal	40%
Molasses	5%
Gluten Meal	3%
Salt	1%
Di calcium Phosphate	1%

No roughage was fed and the cows were adapted to the meal for four weeks before any salts were added to the feed.

The feed consumed within 60 minutes of presentation was recorded daily. The salts were added to a fixed quantity of feed offered to each cow.

Results

In figure 1 the average liveweight of the treated group (pen 13) and control group (pen 14) are presented together with the average daily quantities eaten per sheep. The marked difference in the liveweight gains between the two groups following the period of adaptation is striking. The increase in liveweights following withdrawal of the salts from the feed was immediate and marked.

In Table 1 are presented the daily quantities of food, water and salts eaten by each group for the periods depicted in Figure 1.

During the first 26 days of the trial the average daily quantity of feed eaten by the control group was 2.502 lbs. and for the treated group it was 2.317. The difference was not statistically significant.

The pH of the ruminal fluid of 3 of the sheep during the feeding of the salts was 5.7, 6.65, and 6.3. Sodium and chlorine determinations were undertaken on qualitative samples of urine collected from four of the sheep receiving the salts. Sodium values were 214, 268, 300 & 292 and chlorine values were 100, 106, 120 & 129m. equiv.

Blood samples collected from 3 sheep showed no difference in pH, sodium or potassium levels from those of untreated sheep and Rotheras test for ketones was negative.

The effect of the salts on feed intakes was virtually identical for all cows and only those of 2 are depicted in Figure II.

Discussion

The inclusion of the sodium salts derived from the Fisher-Tropsch process in operation at the South African oil from coal project in the feed of sheep had a severe depressive effect upon liveweight gains.

The reason for the depressive effect exercised by the salts was not fully established but it is thought to be due to the body having to excrete

Table 1.—Average quantity of feed, fatty acid salts and water consumed per day per pen of 5 sheep for the periods depicetd in figure 1.

	Average quantity of feed eaten in lbs.		Average quantity of fatty acid salts eaten in lbs		Average quantity of water consumed in lbs.	
	Pen 13	Pen 14	Pen 13	Pen 14	Pen 13	Pen 14
Period A. (days 0–12)	10.8	10.4	Nil	Nil	31.1	33.2
Period B. (days 13–20)	11.1	13.0	1.56	Nil	40.7	47.6
Period C. (days 21–30)	14.63	14.5	2.19	Nil	43.3	53.4
Period D. (days 31–49)	11.48		2.07		34.6	
Period E. (days 50–63)	9.19		1.37		37.4	
Period F. (days 64–75)	12.8		Nil		35.9	

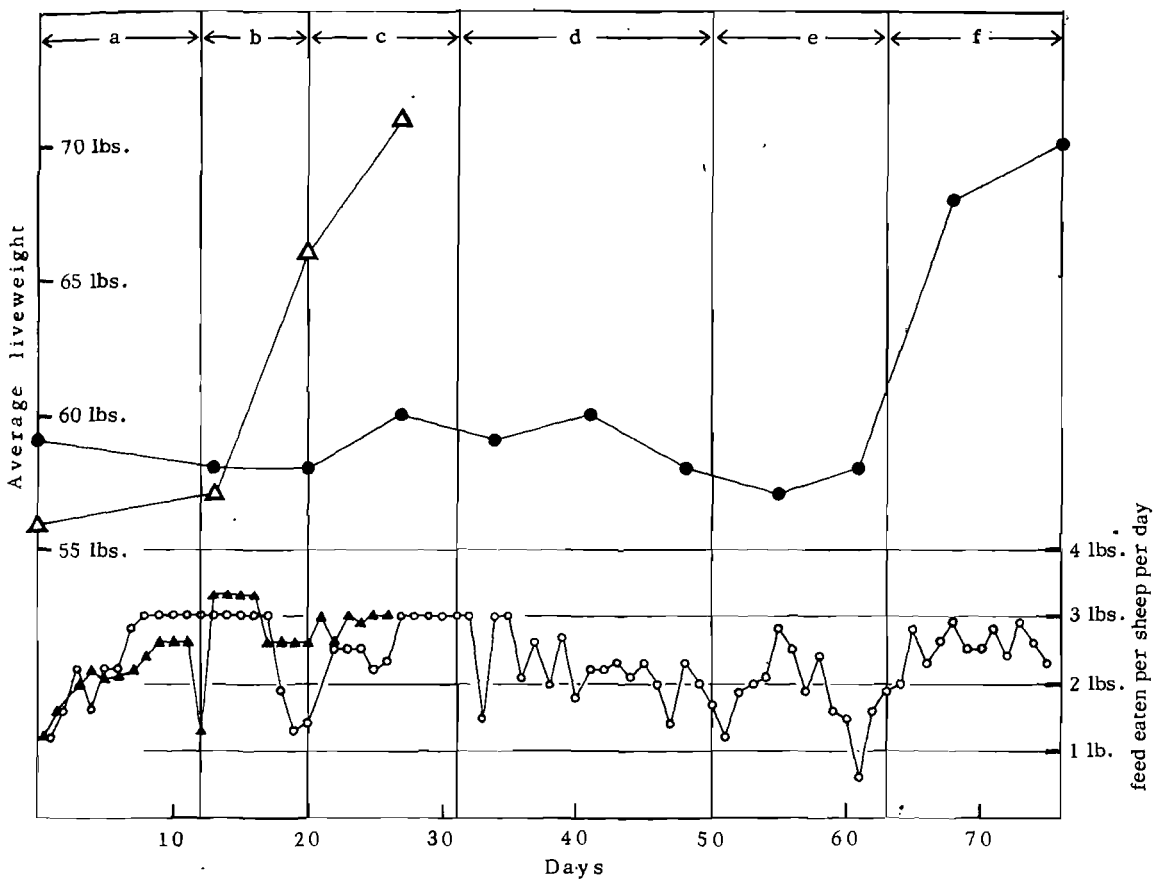

FIGURE 1

THE EFFECT OF FEEDING THE SODIUM SALTS OF FATTY ACIDS ON LIVEWEIGHT GAINS OF SHEEP.

Legend for table 1

●—●	Average liveweights of sheep fed sodium salts of the fatty acids (Pen 13)
△—△	Average liveweight of sheep not receiving fatty acid salts (Pen 14)
o—o	Average daily feed eaten by sheep receiving fatty acid salts (Pen 13)
▲—▲	Average daily feed eaten by sheep not receiving fatty acid salts (Pen 14)
(a)	Day 1 - 12 no salts in feed of treated group
(b)	Day 13 - 20 0.44 to 2.44 lbs. of salts in 13 lbs. feed presented
(c)	Day 21 - 30 2.2 lbs. salts in 15 lbs. feed presented
(d)	Day 31 - 49 2.6 lbs. salts in 15 lbs. feed presented
(e)	Day 50 - 63 2.2 lbs. salts in 15 lbs. feed presented
(f)	Day 64 - 75 No salts in 15 lbs. feed presented

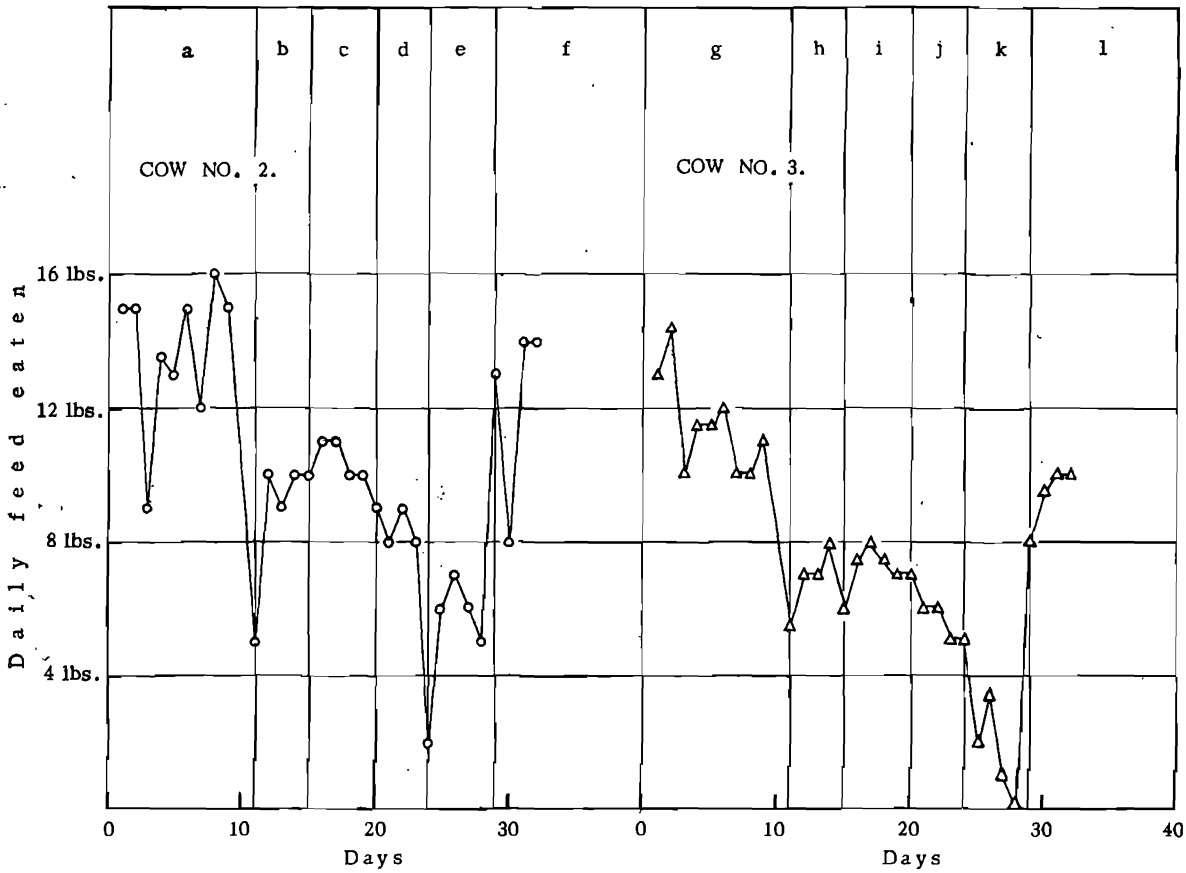

FIGURE 2

THE EFFECT OF SODIUM SALTS OF THE FATTY ACIDS ON FEED INTAKES OF 2 COWS

Legend for figure 2

o——o Δ——Δ	= Daily feed eaten	e.	= 14 lbs. feed plus 1.1 lbs. salts
a, f, g, l,	= Feed only	h.	= 10 lbs. feed plus 0.44 lbs. salts
b.	= 14 lbs. feed plus 0.44 lbs. salts	i.	= 10 lbs. feed plus 0.67 lbs. salts
c.	= 14 lbs. feed plus 0.67 lbs. salts	j.	= 10 lbs. feed plus 0.89 lbs. salts
d.	= 14 lbs. feed plus 0.89 lbs. salts	k.	= 10 lbs. feed plus 1.1 lbs. salts

large quantities of sodium ions released when the organic acid fractions entered directly into the metabolic cycle. There were no differences in the blood pH values and blood sodium and potassium levels between treated and control sheep. An examination of qualitative samples of urine collected drom treated sheep showed the presence of quantities of sodium in excess of what could be accounted for by the chlorine present. It is probable that this sodium was excreted in the form of the bicarbonate salt.

The depressive action upon the liveweight gains was not due to any detrimental effect upon the rumen microflora as withdrawal of the salts from the feed resulted in immediate weight gains as compared to the period of adaptation required at the start of the experiment.

It is of interest to observe that, despite the high nutrient value of the feed, the sheep at the start of the experiment made no gains in liveweight until after a period of adaptation.

The smaller quantity of feed eaten by the treated group cannot account for the failure of liveweight gains, as, based upon the National Nutritional Council standards 2.3 lbs. per day of this material would be adequate to produce weight gains of 0.3 lbs. per day.

ACKNOWLEDGEMENT.

Our thanks are due to Mr. P. Bloom of S. K. & F. Laboratories who undertook the arduous task of drying the salts, Mr. A. Cross of S.K. & F. Laboratories, Great Britain for undertaking the analysis of the salts, and Dr. W. Malherbe of Onderstepoort who very kindly undertook the clinical pathological tests.

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WATER INTOXICATION IN CALVES

J. A. LAWRENCE

Veterinary Research Laboratory P.O. Box 8101, Causeway Salisbury.

Received for publication April 1965

SUMMARY

An outbreak of water intoxication in dairy calves is described.

INTRODUCTION

Although the condition known as water intoxication is well recognised and is adequately described by Blood and Henderson[1] its occurrence is of sufficient rarity for a description of the condition, as it occurred on a dairy farm in Rhodesia, to be worth recording.

CONDITIONS

The herd was composed of good quality Jersey cattle. Calves were hand-reared, receiving whole milk for the first month of life, and skim milk for the next three months. Meal, home-mixed, containing 2.2 per cent salt and 2.2 per cent bonemeal, was fed from two weeks of age and hay was avaialble *ad lib.* At the time of final termination of milk supplementation calves were receiving 2 galls of a mixture of skim milk and water per day, and 3–4 lbs meal.

Calves were housed in two separate houses. In the one they were confined individually in pens for the first month, and thereafter given free access to a communal run in which water was always available. Average water consumption was between 1 and 2 galls per calf per day. No cases of the condition occurred in this house. In the other house calves were penned individually for the whole four months—with no access to water. It was noticed that calves in this house did not thrive quite as well as those in the other house, and it was five calves from this house that succumbed to water intoxication when they were turned out for the first time.

Calves were turned out in groups of about four into other pens or a small paddock. In the first group affected, methyridine had been administered two days before turning out. Immediately after being turned out the calves proceeded to the water trough and drank copious quantities of water. All four became ill and two died the same day. The other two recovered. The next group turned out did not receive methyridine. An intense thirst was exhibited by two of the four calves, and large amounts of water were consumed. These two calves became ill and died within 2 hours. By this time the aetiology of the condition was suspected, and a routine of giving water after terminating the milk supplement and before turning out was instituted. The next group received 3–4 galls water each per day for several days before turning out and was not affected, but in the following group one death occurred on the day that the calves were first turned out into a paddock. In this case the calves were not seen to drink, but water was available. On investigation it was found that, due to a misunderstanding, the calves had received only one gallon water each per day since the skim milk/water feeding had been stopped 10 days previously.

No calves were seen while alive by a veterinary surgeon, nor was a post-mortem carried out except on the last case. The owner described no striking symptoms other than slight adbominal distension, dejection and collapse, but muscular tremors were seen in a few cases, and red discolouration of the urine was noticed in two cases. Treatment was empirical and ineffective.

Post-Mortem Findings

A post-mortem examination was carried out on the last calf to die. The most striking feature was a marked congestion of the mucousmembrane of the small intestine and caecum, which was not noticeable before the intestines were opened. The intestinal contents were in places scanty, yellow and mucoid, and in places watery.

Oedema was noticeable in the lungs, which were moderately congested, heavy and not collapsed; under the peritoneum in the grooves on the surface of the rumen; around the gall-bladder where it was associated with haermorrhage; and also around the major bile ducts within the liver. The liver was enlarged, and the mucosa of the gall-bladder was congested.

The brain was firm, white and swollen.

The urine in the bladder contained haemoglobin.

There were a few petechiae on the mucous membrane of the upper part of the trachea and under the epicardium. The blood had not clotted well.

Histological Findings

Liver, kidney, heart, lung, spleen, lymph nodes, adrenal, brain and small intestine were fixed in 10 per cent Formalin and examined histologically.

The intestinal changes showed marked congestion of the blood vessels in the mucosa and sub-mucosa. There was a marked dilation of mucosal and sub-mucosal lymphatics in the duodenum which was not noticed in more distal protions of the intestine.

Oedema was confirmed in the lungs, where it was both intra-alveolar and interstitial, in the portal tracts in the liver, around the arcuate vessels in the kidney, in the myocardium and in certain lymph nodes. Histological evidence of oedema in the brain was not striking, the only unequivocal changes being distension of the perivascular spaces round the arterioles and venules in the cerebral cortex.

In the kidney, the glomeruli showed leakage of eosinophilic material into Bowman's capsules.

Very small foci of necrosis with a moderate inflammatory cell infiltration were noted occasionally in the liver parenchyma and in the renal medulla. There was a slight interstitial infiltration of lymphocytes and macrophages in the heart and in the lungs, where a few eosinophils were also present. Lymph nodes also showed a mild lymphadenitis. It is not certain whether these changes are relevant to the condition or incidental.

Blood and spleen smears showed no abnormalities and no parasites, although the calf had been inoculated with a vaccinal strain of *Babesia bigemina* two weeks previously, to which it had shown no clinical reaction.

Chemical estimations revealed no arsenic in the liver, and 8 p.p.m. lead in the kidney. The sodium chloride level in the rumen contents was 0.03 per cent.

Discussion

The circumstances of the cases and the post mortem findings lead to a firm diagnosis of water intoxication, although there might be some argument that the condition should in fact be regarded as a chronic salt poisionng due to a high level salt intake associated with insufficient water intake. Intestinal congestion is not described in water intoxication but is described in acute salt poisoning in cattle.

Prevention of the condition is quite simple, provision of water *ad. lib.* to the calves. It is of interest that the surviving calves from the last batch, which were immediately returned to the house, were given water at controlled intervals over several days before being turned out again, and were found to drink up to 7 galls each per day.

ACKNOWLEDGEMENTS

I am grateful to Miss R. Baillie for the preparation of the histological sections, and to Mr. E. Adamson of the Chemistry Branch of the Ministry of Agriculture for the chemical estimations.

I thank the Director of Veterinary Services of Rhodesia for permission to publish this account.

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A CLINICAL NOTE:

AN OBSERVATION ON INTUSSUSCEPTION

R. B. TRENGOVE, State Veterinarian, Upington.

INTRODUCTION

It is seldom possible to observe an intussusception uncomplicated by secondary changes such as swelling of the intestinal wall and compaction of ingesta. On a recent occasion I was fortunate in being able to examine a case of short standing and my observations prompted the following suggestions on the pathogenesis of the condition.

SUBJECT

The subject was a six-month old Karakul ewe which had merely shown anorexia, listlessness and ruminal atony. Intussusception was suspected as the owner had recently lost another sheep from that condition. The animal was sacrificed for autopsy.

FINDINGS

An intussusception approximately 6cm in length was found immediately proximal to the ileo-caecal junction. Other findings were an empty and flatulant intestine, slight fatty degeneration of the liver and hypertrophy of the right adrenal gland.

The freshly exposed intestine showed active motility on the post mortem table. As a ring of peristaltic contraction moved distally down the intestine it was seen to force an accumulation of gas towards the caecum as illustrated in *Fig.* 1. Unfortunately, due to the existing involution, the final effect could not be observed. However, this very interesting phenomenon gave rise to the following speculation.

It is commonly accepted that peristaltic movement is the result of combined autonomous contraction of longitudenal and circular smooth muscle fibres passing aborally down the intestine proceeded by a wave of relaxation. Viewed at any given point the lumen increases and diminishes with every passing ring of peristalsis. It is reasonable to assume that this pumping action would not only force ingesta down the lumen mechanically but would also create areas of positive and negative pressure. If an accumulation of gas were trapped in the intestine, it should pass swiftly down the intestine due to positive pressure behind and the negative pressure in front. If, for some reason, the passage of gas were blocked, for instance by a foreign body or impaction of the caecum, dysfunction of the ileo-caecal valve or any other possible obstruction, pressure in the lumen would build up until (a) passage were forced past the obstruction, (b) the positive pressure from behind is overcome and the gas passes backwards to the negative pressure area behind the contraction or (c) tonus of the smooth muscle wall is overcome. In case of (a) normal function will continue, while in (b) the process would repeat itself. In the case of (c) the position is diagrammatically illustrated in *Fig.* 2.

A distension of the intestinal wall would occur which would act as a cavity into which the still active proximal part would automatically form a prolapse. Once the involution has formed, subsequent peristalsis would aggravate the position and there would be no tendency towards reduction even without the presence of contributing factors such as nodular worm lesions which tend to obstruct reduction by mechanical interferance.

When the gas had finally escaped or been absorbed a typical intussussusception would result. *Fig.* 3.

Fig. 1.

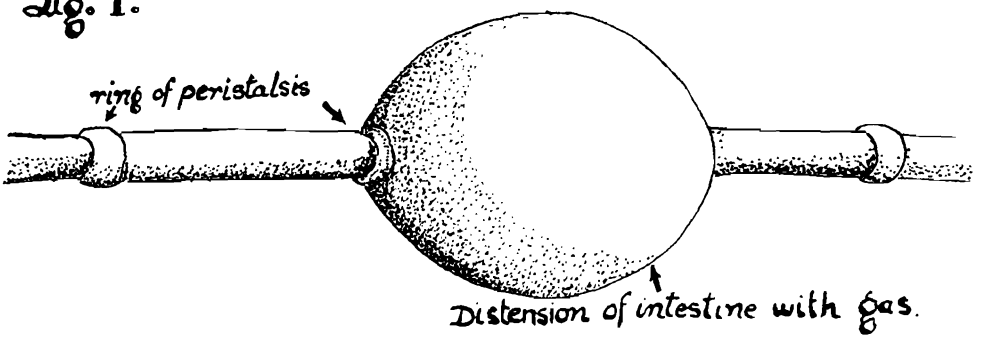


Fig. 2.

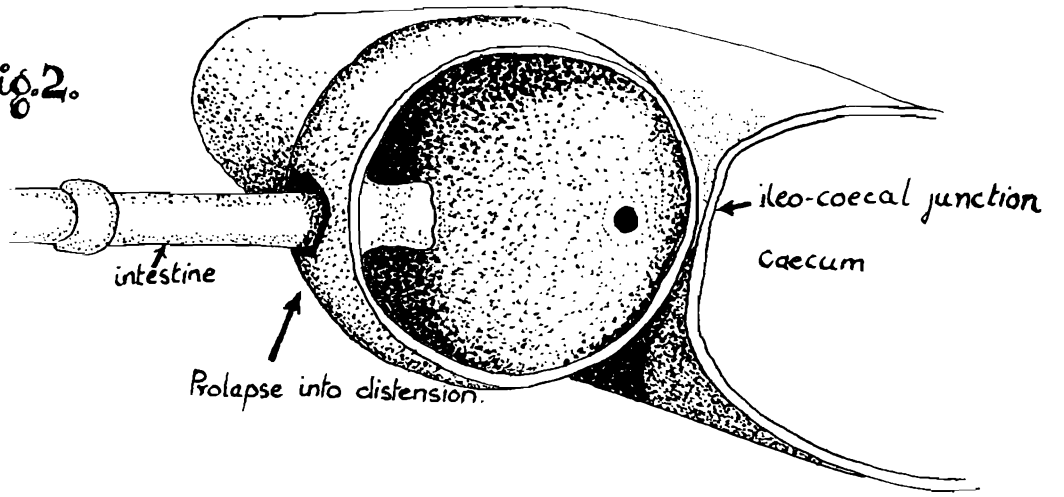
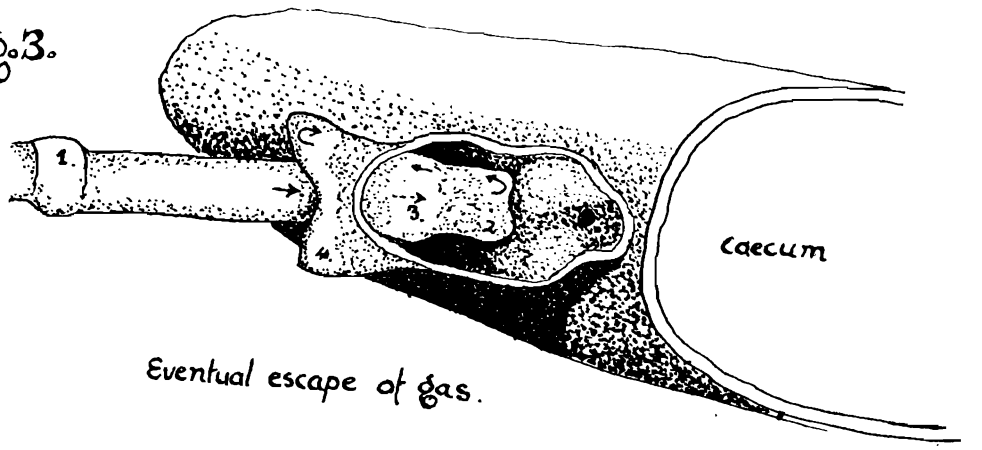


Fig. 3.



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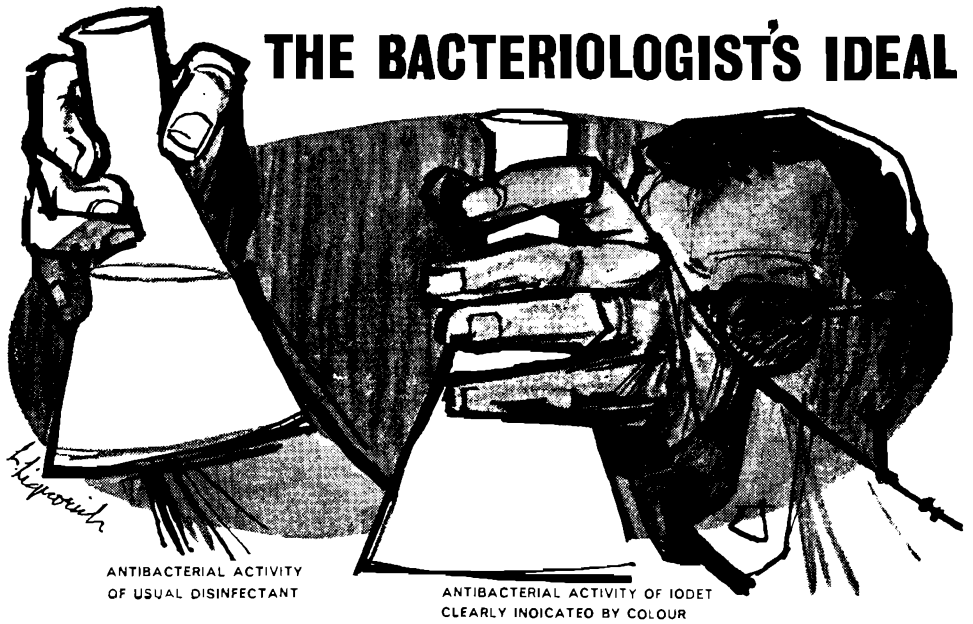

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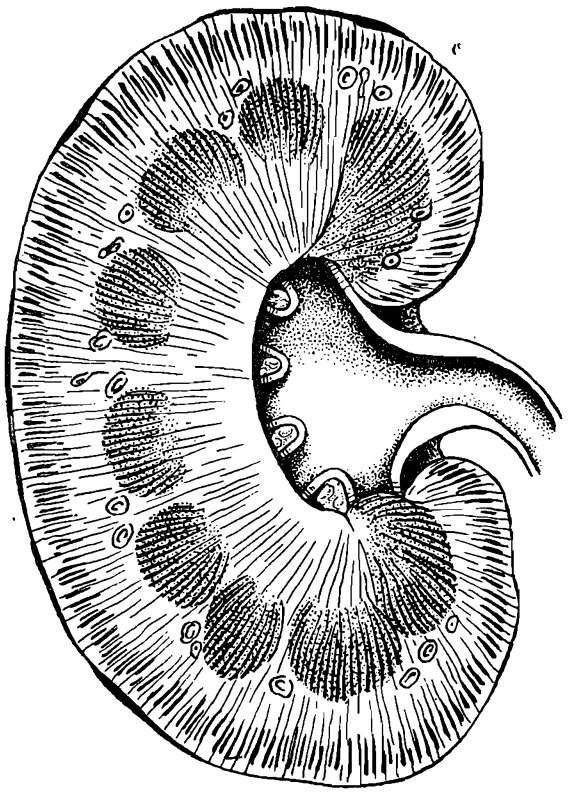

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A MEMOIR

WILLIAM HENRY CHASE, C.B.E. F.R.C.V.S.

It is with great regret that the death after a short illness, of William Henry Chase, F.R.C.V.S C.B.E., on 1st February 1965 in Pretoria is recorded.

William Chase, in latter years 'Pop' Chase to all his many friends and especially to his staff, was born on 10th November, 1880, at Tiverton

in Devon, England, where he attended school at Blundell's Boys' High School. He obtained his M.R.C.V.S. at the London Veterinary College on the 10th November, 1901, but being under age he continued his studies for admission as a Fellow.

'Pop' was a keen sportsman in his younger days, playing cricket, rugby and tennis. He was also interested in horse racing. In 1912 he imported two filly's from England and raced in the Durban 'July' of that year.

After serving in the Cape Administration he was transferred to the Bechuanaland Protectorate service on 1st March, 1905 when East Coast fever had rapidly spread westward through the Transvaal. His first duty was to enlist the co-operation of the African Chiefs, which he did so effectively that the disease was kept out of Bechuanaland.

He then found lungsickness to be widespread and despite the disruption of the first World War the country was freed of the disease by 1922. He made a worthy contribution to this task. Soon afterwards however (1933), foot and mouth disease control campaigns had to be undertaken. Over one million cattle were aphthasised by the blood-intranasal method in that campaign.

Up to 1908 he was the sole veterinary official in the Bechuanaland service. Then a stock inspector was appointed. In 1914 a second veterinary officer was added. When he retired in 1935 he had six veterinarians on his staff. He was decorated (C.B.E.) in 1932 in recognition of his services in the Bechuanaland Protectorate.

He was a life member of the Mafeking Club.

After his retirement he returned to England where be busied himself with part-time private practice. He also worked for the Ministry of Agriculture on foot and mouth disease outbreaks. In 1958 he returned to the Protectorate and settled in Mahalapye with his son Jack.

Only a few years ago he was again on duty in Bechuanaland helping with a foot and mouth disease outbreak.

He married in 1906 and is survived by a daughter Tommy and son Jack of this marriage. His first wife predeceased him soon after his return to South Africa. In 1959 he remarried, and is survived by his second wife. The sincere sympathy of the Profession is extended to his widow, son and daughter.

All who knew 'Pop' Chase had the greatest regard for his qualities. He had an exceptionally good memory. His passing is a great loss to the profession.

N. Boardman.

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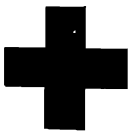

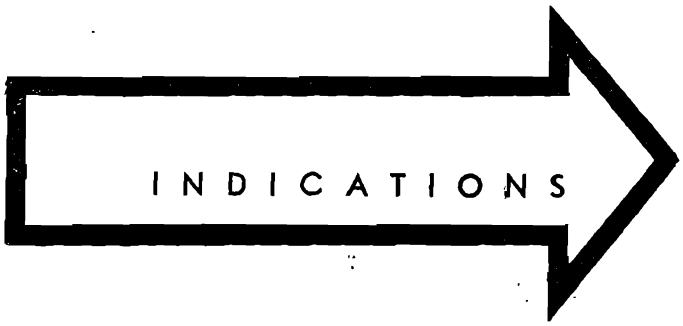

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BOOK REVIEWS

ELEMENTS OF HORSESHOEING

by

J. A. SPRINGHALL

University of Queensland Press, St. Lucia, Queensland.

Price 19s. 6d. *Published*: September, 1964.

The main essentials of the subject are covered in this 44 page book which includes chapters on the anatomy of the limb, the tools, making a shoe, the technique of shoeing, a description of some special shoes with their uses and shoeing of trotters, pacers and racehorses. There are a number of good illustrations giving a clear picture of what is being done.

The method given for picking up the fore leg where the operator stands facing towards the tail of the horse while effective, and in general use, is not favoured. It exposes the head and face of the operator to the danger of a cow kicker. This danger can be avoided by facing towards the head of the horse, placing the hand nearest its obdy over its scapula and running the other hand down the front of the fore limb to the pastern. Then lift the limb with this hand while simultaneously pushing the horse away with the other hand over the scapula. This latter method is not as neat or quick but far safer.

The use of the jaws of the pincers on the head of the nail below and clench above for bedding over the clenches tends to tear the wall of the hoof. It is preferable to do the whole process using the hammer and pincers, placing the pinchers with the jaws closed flat and sideways on the roof above the clench and hitting the head of the nail with the hammer until the clench is completely bent over ready for the final bedding.

There is a misprint on page 7 line (16). The words 'inside toe' should read 'outside toe'.

The recommendation for quiet handling in the forge is particularly useful advice which is frequently ignored. A shoeing crate or box has been found to facilitate the work of the farrier once he has become used to it; eliminating the repeated picking up of the limbs as well as promoting the safety of both the farrier and animal particularly with mules. A suitable design for one of these would be a useful addition to the book.

This book can be recommended. It fills a need for those who require a basic knowledge of horseshoeing without too much detail.

G.D.S.

VETERINARY MEDICINE AND HUMAN HEALTH

CALVIN W. SCHWABE. The William and Wilkins Co., Baltimore, U.S.A. 1964.

PR XVI, pp. 473 Fig. 93, Lab. 87 *Price*: R12.00 ($16.00).

This magnificent work is an unusually well documented exposition of the past contributions of, and future possibilities for veterinary science to the advancement, promotion, and maintenance of human health. It contains a wealth of information and succeeds most admirably in the author's expressed wish, i.e. to introduce to his readers a field of veterinary science in a manner which will somehow reflect his own enthusiasm. He has indicated how Virchow's contention that "Between human and animal medicine there is no dividing line—nor should there be" is true, how these two facets of medicine are in fact a single concept composed of a wide and singularly diversified number of subjects. The author presents us with a fascinating factual account of veterinary contributions to the entity of public health.

The book, a compliment to the printers, is divided into four sections: *Section I, The practice of population medicine*, deals with the implications of veterinary practice, the public's health, veterinary interests in public health, the

public health team and the place of the veterinarian on that team. *Section II*, Epidemiology, deals with *Homo Sapiens* as an animal species, the ecological study of disease, lower animals as hosts, agents and vectors, comparative approaches to chronic non-infectious disease, and the prevention, eradication and control of disease. *Section III, Food and Hygiene* covers veterinary mediciene and human intuition, rural health and environmental hygiene. *Section IV, Tools*, indicates how ecological methods, measures of health and disease, and the literature may be utilised in public health work.

The chapters dealing with statistical methods and evaluation of data, and with methods of study of literature, are outstanding and of immeasurable value to persons embarking on any aspect of biological work.

This book indicates just how much wider in fact is the field of veterinary medicine than the narrow concept which still prevails within and without the profession. Tjalma has said "The future of veterinary public health is limited only by the ability of the profession to recognise its opportunities and accept its responsibilities". By relating actual situations and numerous examples to certain concepts, the author has stressed how virtually all veterinary activity contributes to human health, and shown why the veterinarian is eminently equipped for the "herd" approach to human health.

Medical and veterinary administrators of public health will find a mint of information in the study of this essential work.

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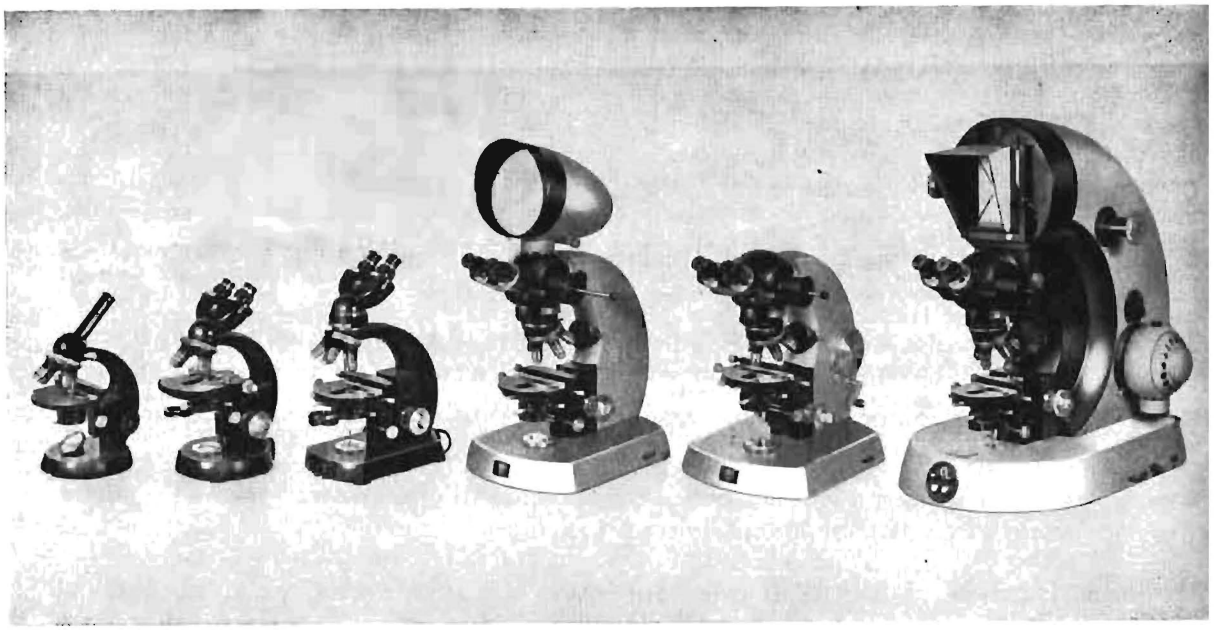

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PUBLIC RELATIONS SERVICE

On the 11th March, 1965, the Committee of the Natal Branch entertained at the Royal Hotel Durban, a very distinguished guest—Prof. E. Brayley Reynolds, O.B.E., M.R.C.V.S., of Fawsley, New Market, England. Present at the dinner were:—

Dr. J. L. Doré.
Dr. F. B. W. du Casse,
Dr. A. F. Tarr,
Dr. W. B. Hobbs.
Dr. J. M. O'Grady and
Dr. G. Cross.

Professor Reynolds was on the staff of the Royal Veterinary College, London, for a number of years and later settled at New Market where he developed a large practice. He is now 84 years, but still takes an interest in the care of a racing stud at New Market. With his wife he was visiting their son Mr. James Brayley Reynolds, a Judge of Thoroughbreds, and living at Durban.

Professor Reynolds in his day was one of the outstanding Veterinary Practitioners of the United Kingdom. He operated on his 80th birthday and claims that he will give a repeat performance on his 90th birthday.

Dr. 'Tom' Adelaar is on a visit to Europe and Britain and will be away for three months.

The first graduates to receive the specialist degree of M. Med. Vet. are:—

DR. J. F. W. GROSSKOPF—M.Med.Vet. (Phys.) who graduated with honours and DR. A. P. SCHUTTE—M.Med. Vet. (Gyn.).

The first Veterinarians to receive the Diploma of Veterinary Public Health (D.V.P.H.) are:—

DR. S. V. O'BRIEN
DR. P. L. UYS
DR. L. W. VAN DEN HEEVER
DR. J. P. VAN DER MERWE.

These colleagues were formally presented with their awards at the 27th Graduation Ceremony of the Pretoria University on 26th and 27th March, 1965.

Dr. R. Bigalke gained the Diploma in Applied Parasitology and Entomology (D.A.P. and E.) at the London School of Hygiene and Tropical Medicine while engaged in post-graduate study at that Institute.

Dr. B. A. Matson was admitted to the Degree of Doctor of Philosophy at a Congregation in the Senate House, Cambridge University, on 23rd January, 1965. His thesis was entitled 'Tick transmission in *Babesia divergens* and immunity in *Babesia rodhaini*'. (A non-pathogenie *Babesia* of Mice in the Congo).

After qualifying from the Veterinary Faculty at Onderstepoort in 1957 Matson spent a tour of service in the Colonial Veterinary Service in Nyasaland working mainly on Trypanosomiasis. The award of a Commonwealth Scholarship and an Hononrary Beit Fellowship in 1960 took Matson to the London School of Hygiene and Tropical Medicine where he read for the post-graduate Diploma in Applied Parasitology and Entomology. The award in 1962 of a Wellcome Veterinary Research Fellowship of the Animal Health Trust enabled Matson to proceed to Churchill College, Cambridge, where he read for the PhD. in the School of Veterinary Medicine. Matson has now returned to Southern Rhodesia in order to take up a research appointment as protozoologist in the Veterinary Research Laboratory, Salisbury. He is continuing with his research interests in the epizootiology of Tick and Tsetse transmitted diseases. While overseas Matson married Kathryn Gray and they now have a son and a daughter.

Dr. H. Graham Purchase has obtained the M.S. degree in Microbiology from the Michigan State University. He and his wife will be working at the Houghton Poultry Research Station on an Exchange Scheme for a year.

Dr. Ian Watt and Dr. Robinson from Ramathlabama, Bechuanaland Protectorate, are on a course of post-graduate study at the Veterinary Research Institute, Onderstepoort.

Dr. L. Abrams has terminated his service with the Veterinary Research Institute, Onderstepoort, and has joined the firm of Lion Bridge Feeds Ltd., Pretoria.

Dr. I. van Schalkwyk has left the service of Lion Bridge Feeds Ltd. to join the staff of Rainbow Poultry Farm (J. C. Methven) Hammarsdale, Natal.

Drs. W. A. de Klerck, S. K. Bakker and G. von Philipbourne (Miss) have terminated their service with the Veterinary Research Institute.

Drs. S. Lombard of Pretoria and Byron S. Johnson from the Field Division have joined the staff of the Veterinary Research Institute, Onderstepoort.

Dr. Susanne Solomon of White River has flown out to England and will marry Dr. A. M. Harthoorn and proceed with him to the Fort Collins Veterinary College Denver, Colorado, where Dr. Harthoorn, will pursue studies in Physiology, on a Fellowship granted to him.

Dr. E. Young has terminated his service with the National Zoological Gardens, Pretoria, to take up an appointment with the Division of Veterinary Field Serives and will be seconded to the staff of the Kruger National Park at Skukuza.

Dr. J. P. van der Merwe has left for Europe, the United Kingdom, America Australia and New Zealand where he will make a study of modern abattoir construction, meat processing plants, Meat Inspection standards, legislation, organization and control of meat and meat products. He will be away for about four months.

Dr. E. B. Kluge is visiting Britain and Europe officially on a survey concerned with the organization of the Division of Veterinary Field Services. He will be away for 80 days.

Dr. C. M. Cameron is due to depart on 5th October 1965 on a British Petroleum Post Graduate Agricultural Scholarship, which takes him to the United States of America for two years.

He will spend the first six months at the National Institute for Allergy and Infectious Diseases, Rocky Mountain Laboratory, Montana, studying antigenic fraction of bacteria.

For the subsequent 18 months he will work at the Michigan State University, East Lansing, Michigan, studying immunocytology and the mechanisms of immunity.

Before returning he will pay short visits to certain institutes in England. His wife and family will accompany him. We wish them happy days and much success.

Dr. L. W. van den Heever has been invited by the Free University of West Berlin to deliver a lecture on Zoonosis in South Africa with special reference to food-borne infections. The Faculty of Veterinary Medicine of this University holds an Annual Seminar on Tropical Veterinary Medicine, to which speakers from different parts of the world are invited. Professor B. C. Jansen and Dr. G. C. van Drimmelen are among the Veterinarians who have delivered addresses at this Seminar.

Dr. J. D. Poole of S.A. Cyanamid, P.O. Box g552, Johannesburg has been promoted to the post of Regional Technical Director, Africa Region, Cyanamid International.

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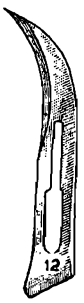

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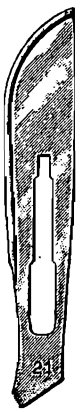

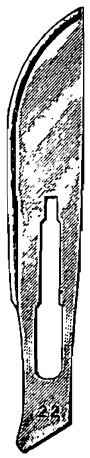

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VETERINARY NON-PROPRIETARY NAMES

List No. 3. March 1965

List No. 1. was published in the June 1964 issue and List No. 2 in the March 1965 issue of the Journal of S.A.V.M.A.

The British Veterinary Codex Revision Committee has adopted the following non-proprietary names for the veterinary substances indicated.

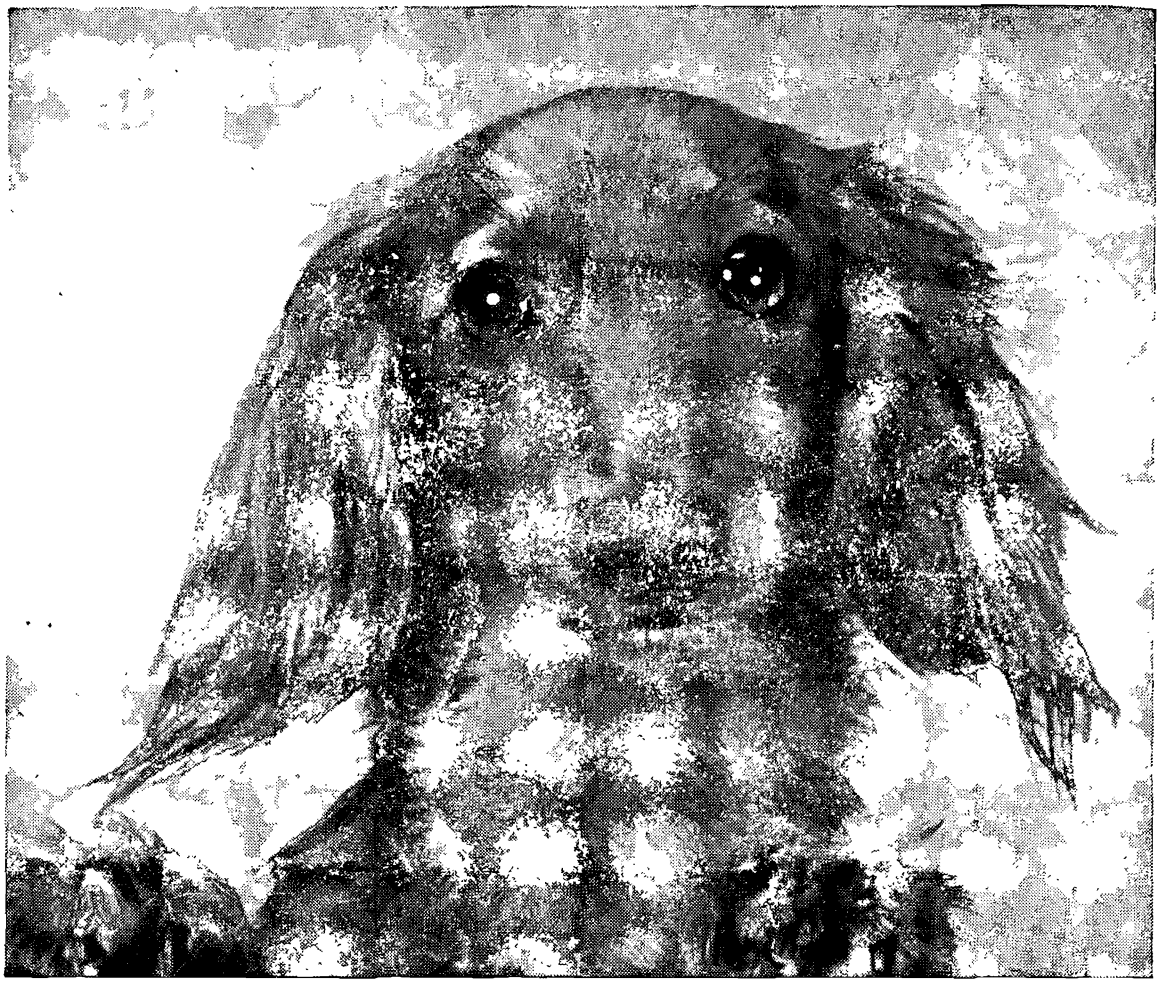
Non-proprietary name	*Other names*
BUNAMIDINE	*NN*-dibutyl-4-hexyloxy-1-naphthamidine; Scolaban is the hydrochloride.
CRUFOMATE	2-chloro-4-t-butylphenyl methyl *N*-methylphosphoramidate; Ruelene.
DIMPYLATE	diethyl 2-isopropyl-6-methylpyrimidin-4-yl phosphorothionate: *OO*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate; Diazinon. This is an ingredient of Basudin.
HALOXON	di (2-chloroethyl) 3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphate; *OO*-di (2-chloroethyl) *O*-(3-chloro-4-methylcoumarin-7-yl) phosphate; Loxon.
METHINDIZATE	2-(1-methyloctahydroindol-3-yl) ethyl benzilate. The hydrochloride is an ingredient of Isaverin.
PYRITHIDIUM BROMIDE	3-amino-8-(2-amino-6-methylpyrimidin-4-ylamino)-6-*p*-aminophenylphenathridine 5, 1-dimethobromide; Prothidium.

The non-proprietary names are reported to be free from conflict with trade marks registered in Great Britain and Northern Ireland, and these names, or names resembling these names, will not be registered as trade marks for pharmaceutical products or drugs in those countries. Some of the names, other than the chemical names, appearing in the second column above are registered trade marks.

The adoption of a non-proprietary name does not necessarily imply that the British Veterinary Codex Revision Committee recommends the use of the substance in veterinary medicine or that the substance will be included in the British Veterinary Codex, although if a substance is included, it is intended that the non-proprietary shall be the title of the monograph.

The British Veterinary Codex Revision Committee has undertaken, at the request of the Association of the British Pharmaceutical Industry, to provide non-proprietary names for veterinary products, and all requests from manufacturers and other interested persons for the provision of such names should be addressed to:—

The Secretary, British Veterinary Codex Revision Committee,
The Pharmaceutical Society of Great Britain,
17 Bloomsbury Square,
London, W.C. 1.



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Booster Dose When there is a possibility of waning immunity the Canilep booster dose ensures continuous protection. It is particularly useful for dogs in areas where there is a low incidence of disease and therefore less likelihood of natural infection reinforcing immunity; dogs at 'special risk', e.g. during kenneling, showing or when moving to another district. One vial of freeze-dried distemper, hepatitis and leptospirosis vaccine with 2 ml sterile water in snap-open ampoule.

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CONCLUSIONS OF THE Vth INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION AND ARTIFICIAL INSEMINATION

Contributed by Dr. S. W. J. van Rensburg, Veterinary Research Institute Onderstepoort

Emanating from the papers presented and the discussions at the above Congress held at Trento, Italy on September 6th to 13th last year and attended by over 1,100 delegates, the following conclusions and recommendations were approved at the closing session on the last day:

(1) Our knowledge of antigenicity of gametes and its importance in fertilization failure and embryo mortality must be extended. Foetal maternal incompatibility and their immune mechanisms are to be explored.

(2) Research is needed on the mechanism whereby changes in environmental temperature, light, feed supply and social environment affect gonadal development and function.

(3) Studies on the interaction of heredity and the many aspects of environment in determining reproductive performance are to be encouraged. They offer material highly suitable for analysis of mechanism of reproduction and their modes of action. They also offer approaches for the development of animal husbandry as a true biological science: the control of environment in relation to the control of heredity, in this instance to bring about the desired level of reproductive performance.

(4) To continue studies for prolonging the reproductive capacity and increasing the economic potential of domestic animals.

(5) To continue studies of the physical, chemical, genetical and immunological mechanisms acting on spermatozoa in the female genital tract.

(6) To further study the hormonal and genetic factors which control animal production and reproduction.

(7) To study the rôle of neuro-endocrine factors on reproduction.

(8) To continue studies on the detection and synchronization of oestrus to reduce operational costs and permit the use of Artificial Insemination in areas not adapted for year-round operation.

(9) It is important that research be continued on the factors affecting sex determination in offsprings because of its economic importance.

(10) Standardisation of the methods of calculating and expressing fertility results in artificial insemination is absolutely necessary to permit comparisons. It is recommended that all publications express these results in terms of 60 to 90 "non-returns", for cattle and comparable standardisation of other species.

(11) Intensified sanitary control of sires to prevent the spreading of diseases is recommended and exported frozen semen should come from tested disease-free sires.

(12) To develop acceptable health standards and uniform legislation for preventing the spread of pathological organisms from the wide distribution of frozen semen.

(13) To commend and approve the attempts of world organizations such as F.A.O. of the United Nations for legislation in the field of animal reproduction.

(14) To continue studies on the cause and control of embryonic mortality and early foetal death.

(15) To promote and encourage the exchange of information relevant to the field of reproduction among all countries.

Whereas there is a great possibility that research on animal reproduction could lead to the development of more effective means of human population control, and

Whereas each member of this Congress could call these facts to the attention of the responsible agencies and governmental authorities of his Country upon his return,

Therefore be resolved that this, the 5th International Congress on Animal Reproduction and Artificial Insemination go on record as urging every country of the world to give increased emphasis and financial support to research on animal reproduction in the hope not only of increasing food production, but also of increasing the chances of new discoveries leading to a better understanding of reproductive processes and thereby increasing the possibilities of developing more effective and more acceptable methods of human population control.

WORLD VETERINARY ASSOCIATION NEWS

1. PROF. WAYNE RIZER, President of the World Small Animal Veterinary Association will be in South Africa during September, 1965, and will address the Congress of S.A.V.M.A. on the diagnosis of Bone Diseases.
2. A joint Congress of the American Animal Hospital Association and the World Small Animal Association was held at the Sheraton Park Hotel, Washington D.C., between the 14th—19th March, 1965.

   Those interested in obtaining copies of papers could approach Dr. Frank Booth, Executive Secretary, American Animal Hospital Association 3920E Jackson Blvd. Elkhart, Indiana, U.S.A. or Mr. W. B. Singleton, Hon. Secretary, World Small Animal Veterinary Association.
3. The World Association of Veterinary Food–Hygienists, is organizing the presentation of its 4th Symposium at Lincoln Nebraska, U.S.A., from the 26th—30th July, 1965. Dr. J. P. van der Merwe of the Division of Veterinary Field Services will attend this Conference.
4. The XVIIIth World Veterinary Association Congress is to be held in Paris from the 17th—22nd July, 1967.
5. The preparation of a new film catalogue or an intimation of new films through its News Items, is being considered by the Permanent Committee of the W.V.A.
6. The W.V.A. from August, 1965, requires a membership fee of $1\frac{1}{2}$ shillings per Veterinarian. The S.A.V.M.A. is a member and will this year submit a membership fee of approximately 750 shillings.

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Attractive vacancies are available in the Department of Veterinary Services for candidates with recognised qualifications, under pensionable or contract conditions of service. The Rhodesia Veterinary Service offers excellent opportunities to Veterinarians to gain wide experience of animal diseases under sub tropical conditions.

GOVERNMENT VETERINARY OFFICERS are required in the Field Branch whose duties are the prevention and control of those diseases specified by regulations as being destructive diseases of livestock and the maintenance of a diagnostic and advisory extension service to the farming community.

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Vacancies exist in the Research Branch for officers who wish to specialise in *Laboratory Diagnosis*. These officers will be required to control and maintain the routine diagnostic service offered by the Laboratory. These posts offer opportunities for graduates or experienced research veterinarians with an interest in applied bacteriology, virology, protozoology and laboratory diagnostic procedures.

Credit will be given for previous laboratory experience, but the lack of research background will not preclude the consideration of an application.

A vacancy exists for a specialist in *PARASITOLOGY*. This officer is required to undertake surveys and perform experiment work on problems of applied parasitology relating to the livestock industry. A close liaison is expected with the Game Zoologist and other specialists.

The commencing salaries for the above posts will be assessed on the scale £1,650–£2,500 per annum.

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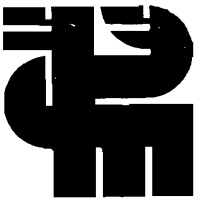

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CONGRESS BRITISH VETERINARY ASSOCIATION 1965

LIST OF SCIENTIFIC PAPERS TO BE PRESENTED AT THE EDINBURGH CONGRESS.

12th—18th September, 1965

TURKEY PRODUCTION AND DISEASE.
Author: R. H. Duff.

MODERN METHODS OF TURKEY PRODUCTION.
Author: Rupert Chalmers-Watson.

JAAGSIEKTE A HAZARD OF INTENSIFIED SHEEP HUSBANDRY.
Author: Dr. J. M. K. MacKay

SYMPOSIUM:

THE SMALLEST STOWAWAY"

African Swine Fever: *Author*: Dr. G. R. Scott.

AFRICAN HORSE SICKNESS AND BLUE TONGUE:
Author: Dr. D. A. Haig.

Rinderpest—*Author*: W. Plowright

PRINCIPLES AND APPLICATION OF INTERNATIONAL DISEASE CONTROL—*Author*: W. Ross Cockrill.

PLENARY:

HISTORY OF THE STATE VEVERINARY SERVICE IN BRITAIN
Author: Sir John Winnefrith.

RETINAL ATROPHY.
Author: Dr. K. C. Barnett

RECENT RESEARCH IN JOHNE'S DISEASE.
Author: Dr. N. J. L. Gilmour

CASTRATION IN FARM ANIMALS; ITS ADVANTAGES AND DISADVANTAGES.
Author: Dr. I. S. Robertson

THE RUMINANT LIVER
Author: E. J. H. Ford.

THE PATHOGENSIS OF FASCIOLIASIS.
Author: Dr M. H. Sewell—Co-Author: D. C. MacDonald

THE USE OF FLUID REPLACEMENT IN THE TREATMENT OF NEONATAL DISEASES IN CALVES.
Author: Dr. J. G. Watt

PREVENTIVE METHODS IN PIG FARMING.
Author: Dr. D. R. Melrose

PLENARY:

THE CHANGING ROLE OF ADVISORY SERVICES.
Author: W. Emrys Jones

SYMPOSIUM:

PRINCIPLES OF IMMUNISATION IN ANIMALS AND MAN.
The Veterary Aspect—Dr. D. G. Howell.
The Comparative Aspect—Dr. R. R. Gillies.

THE ROLE OF INFECTION IN INFERTILITY IN THE THOROUGHBRED MARE.
Author: A. M. Bain.

THE ROLE OF THE VETERINARIAN IN WILDLIFE CONSERVATION.
Author: M. Woodford.

UNIVERSITY APPOINTMENTS

THE UNIVERSITY OF MELBOURNE

Applications are invited for the following position:

LECTURESHIP OR SENIOR LECTURESHIP IN VETERINARY PATHOLOGY

in the

DEPARTMENT OF VETERINARY PARACLINICAL SCIENCES

QUALIFICATIONS: A degree in Veterinary Science registrable in the State of Victoria with post-graduate experience in teaching or research and investigation.

DUTIES. To conduct a laboratory of, and provide instruction to, senior students in Clinical Pathology, including Haematology, Clinical Chemistry and Clinical Microbiology. The appointee will be expected to atke up his duties on 1st January, 1966.

SALARY: -A2,300 — -A105 — -A3,150 for a Lecturer.
-A3,250 — -A110 — -A3,800 for a Sdnior Lecturer.

Initial salary within the range detailed will be determined according to qualifications and experience.

Further information including details of F.S.S.U. type superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Registrar, University of Melbourne, Parkville, N.2., Victoria, Australia, and applications should reach him by the 31st August, 1965.

13/5/65

F. H. JOHNSTON,
Registrar

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