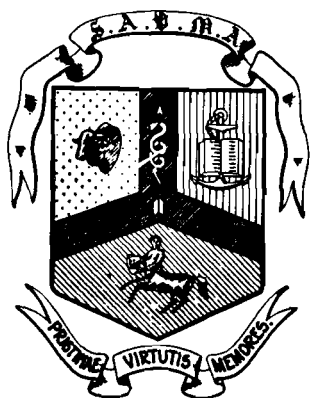


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## THE IMMUNIZATION OF RAMS AGAINST OVINE BRUCELLOSIS.

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(Received for publication March 1962)

### SUMMARY

1. Laboratory and field trials were made to determine the efficacy of strain 19 and Elberg Rev. 1 vaccines for brucellosis in rams.

2. Twenty five young rams not previously exposed to infection were inoculated with *Brucella* vaccine S.19. One contracted epididymitis and showed brucella in the semen before challenge. Five others became infected after exposure to infection. Three of these also showed epididymitis.

3. Twenty five young rams inoculated with Rev. 1 vaccine resisted two challenges and remained free from infection till the conclusion of the experiment 20 months after immunization. Eleven of the 21 controls became infected, 8 showing clinical epididymitis as well as *Br. ovis* in the semen.

4. In field trials 64 young rams were inoculated with Rev. 1 vaccine and were subsequently exposed by serving in infected flocks. All remained free from brucellosis and epididymitis up to 25 months after immunization. Out of 24 controls in the same flocks 21 became infected.

5. Fifty-one mature rams which had been serving in infected flocks but were not secreting *Br. ovis* in the semen, were inoculated with Rev. 1 vaccine. Thirty one became infected.

6. It is concluded that:

- (i) Strain 19 is not a completely effective vaccine against brucellosis in rams.
- (ii) In young rams at weaning age Elberg Rev. 1 vaccine confers a solid immunity lasting at least two years.
- (iii) The same success is not achieved in mature rams that had already been exposed to infection when inoculated.

### INTRODUCTION

*Brucella ovis* was first detected in sheep in South Africa during 1956 and reported by van Rensburg et al (1958)<sup>4</sup>. An extensive survey has since shown this infection to be so prevalent in flocks throughout the Republic that effective immunization would offer the only satisfactory method of control.

Although affected ewes evidence a depressed lambing percentage and serve to spread the disease, an infection by *Br. ovis* displays its main harmful effects on fertility in the ram. On the basis therefore, that a safe and efficient method of immunization of rams would furnish the best method of control, this work was undertaken. It aimed at determining the immunizing properties and safety of some of the vaccines used for the control of brucellosis.

## METHODS.

Preliminary trials had indicated that the two vaccines which may provide a solution to the problems were the live Strain 19 *Brucella abortus* and the live Strain Elberg Rev. 1 of *Brucella melitensis*. The latter was evolved by Elberg and associated in California and, according to Van Drimmelen (1960)<sup>3</sup>, it may be said to have higher virulence than S19 but not high enough to produce the clinical disease or the carrier state.

Both vaccines were administered by subcutaneous injection, the dose of S19 being  $20 \times 10^9$  organisms and  $5.0 \times 10^8$  organisms of Rev. 1. They were subjected to the following laboratory and field trials.

### *Laboratory tests:*

Seventy one young ram lambs of weaning age and not previously exposed to infection were divided at random into five groups and treated as indicated in table 1. Three groups were inoculated and two left untreated as controls.

All the five groups were challenged twice at intervals of 9 to 18 months after the three experimental groups had been inoculated. This was done by the application of a few drops of infected semen to the glans penis of the experimental rams, a method which was previously found highly effective for transmitting infection to susceptible rams.

During ten months following this exposure to infection all the animals were submitted to from 7 to 14 examinations at regular intervals. This consisted of palpation of the genitalia for evidence of epididymitis, and microscopic examination of smears from semen obtained by electric-ejaculation. The method of staining by the modified Ziehl-Neelsen technique described by Stamp et al (1950)<sup>1</sup> was used, but with a slight modification in that a 0.1% instead of a 0.30 solution of carbol fuchsin was used. It had previously been found that the higher concentration of carbol fuchsin ensured better differentiation of *Br. ovis*. Extensive tests convinced Van Drimmelen (1959)<sup>2</sup> that such direct microscopic examination of semen smears is the most reliable diagnostic method, and that serological tests are even less reliable in sheep than in human beings, pigs and goats.

### *Field Tests.*

The field observations were made on nine farms in the districts of Graaff Reinet, Murraysburg and Griquatown, on all of which *Br. ovis* infection had been established.

Since the laboratory trials had cast a doubt on the efficacy of Strain 19 for rams, only Rev. 1 vaccine was used in the field experiments. Here the available rams were divided into three groups as follows:

*Group A* consisted of a total of 64 young ram lambs of weaning age, all of which had not been exposed to infection.

*Group B* was composed of a total of 51 older rams that had already been exposed to infection, either by service or by contact with infected rams, but showing no evidence of epididymitis or infection at the time of inoculation.

*Group C* consisted of 24 young ram lambs of the same age and kept under identical conditions as those of Group A. They however were not inoculated and served as controls.

On the dates of inoculation the genitalia of all were palpated, semen was collected, and semen smears from each stained by the Ziehl-Neelsen

method, were examined. None of the animals revealed evidence of epididymitis or infection at this examination.

After inoculation all the rams in the three groups were exposed to natural infection by service in infected flocks, during the various periods specified for each farm in table 2. It will be observed that this challenge was made at intervals varying from one to 22 months after inoculation.

Finally all rams under observation on the different farms were again subjected to clinical examination of the genitalia, and microscopic examination of semen smears on the dates stated in table 2. This was from one to 16 months after exposure to natural infection, and from 9 to 25 months after immunization.

## RESULTS

The data in table 1 reveal that two of the five young rams inoculated with Strain 19 on 8 August 1958 started excreting *Brucella* in the semen 9 and 12 months after inoculation and 1 to 4 months after exposure to infection. Neither developed clinical epididymitis.

Of the 20 inoculated with Strain 19 two months later, four subsequently showed both a *Brucella* infection and epididymitis. One of these was actually found to be excreting *Brucella* and showing a well marked epididymitis on 24 September 1959. This was five days before he was challenged or exposed to infection. It is therefore possible that inoculation with Strain 19 might have caused this infection and the clinical manifestations.

Two of the four affected rams were excreting *Brucella* in the semen two months after the first challenge and one of them had also developed epididymitis by then, while the other showed it two months later, on 22 February 1960.

The semen of the fourth ram was found to be infected on 8 March 1960; and he had epididymitis on 1 August 1960, that is, 22 months after inoculation and 10 months after exposure to infection.

The remaining 19 rams that were inoculated with S19 remained free from infection and showed no clinical evidence of brucellosis up to their last examination on 24 November 1960, 25 months after inoculation and 14 months after the first challenge.

None of the 25 rams inoculated with Rev. 1 vaccine on 3 October 1958 showed any suspicion of *Br. ovis* in his semen or clinical evidence of epididymitis at any time up to the last examination on 6 October 1960, 20 months after immunization and 5 to 9 months after exposure to infection.

The 21 control rams were treated in exactly the same manner as the 50 experimental animals. Ten of them remained normal throughout the observation period, while 7 showed both infection of the semen with *Br. ovis* and epididymitis. Three became infected without developing lesions and one contracted epididymitis without revealing *Br. ovis* in the semen.

In the field experiments the data in table 2 reveal that all of the 64 (Group A) ram lambs inoculated with Elberg Rev. 1 vaccine at weaning age and subsequently exposed to infection by serving ewes in infected flocks failed to show evidence of *Br. ovis* infection in the semen or of clinical lesions of the genitalia up to 25 months after inoculation and 16 months after exposure to infection.

Of the 51 (Group B) mature rams that had previously been exposed to infection but were apparently free from brucellosis at the time of

inoculation, and were serving ewes in the same flocks as the above group, 20 were still free from *Br. ovis* and epididymitis when re-examined. Twenty others had contracted epididymitis as well as infection, and 11 had *Br. ovis* in the semen but showed no lesions.

The 24 controls (Group C) suffered even more severely than the mature group. When re-examined 2 to 16 months after serving infected ewes only 3 were normal. 14 showing both epididymitis and infection in the semen and 7 infection without lesions.

## DISCUSSION

The development of typical ovine brucellosis characterized by the presence of organisms in the semen and epididymitis in one of 24 rams after inoculation with Strain 19 vaccine and before exposure to infection, suggests that this vaccine on its own may produce lesions in a small percentage of animals.

Further, the appearance of organisms in the semen of 5 (3 of these developed clinical lesions) of the remaining 23 rams, within a short period after exposure to infection, proves that apart from its possible pathogenicity Strain 19 is not a completely effective immunizing agent for brucellosis in rams.

In this experiment inoculation with Strain 19 therefore resulted in failure in 24 per cent of cases. When however, this is compared with the number of cases in which the disease occurred during the same period and under similar conditions in the 21 controls, 11 of which (52.4%) became affected, it is evident that Strain 19 does confer some degree of immunity.

The results achieved in young rams with Elberg Rev. 1 vaccine can be regarded as spectacular. No breakdown occurred in any of the 25 in the laboratory experiment or in the 64 in the field observations. Present indications are that the immunity conferred lasts for at least 2 years and the results justify recommending Rev. 1 for young rams that have not previously been exposed to infection.

This confidence in the vaccine is further strengthened when one considers the high incidence of infection and epididymitis (21 out of 24 i.e. 87.5%) in the control Group (Table 2) which were kept under conditions identical to those of the inoculated rams.

The appearance of *Brucella* and epididymitis after vaccination in 31 of 51 (61%) older rams (Group B, Table 2) that were negative to the tests at the time of inoculation would, on cursory examination, appear to be inconsistent with the beneficial results obtained in young rams. This observation is of great practical importance as it may suggest that Rev. 1 vaccine is of limited value in adult rams. It therefore needs clarification.

The rams concerned had all been exposed to infection by natural service in infected flocks prior to inoculation and the majority of them were probably infected although they did not reveal this in the tests. Cognisance must however be taken of the fact that after natural infection adult animals may harbour the organisms for months without showing evidence thereof in the semen or blood serum.

In this investigation a large number of the rams serving in the nine flocks were found to be affected and were discarded, while those apparently normal were inoculated (Group B, Table 2). It can now be presumed that many of the latter were also infected despite the negative results to the tests. On that presumption the results establish that in rams that are already infected at the time of vaccination Rev. 1 vaccine has no inhibitory or other effect on the course of the disease.

The remaining 20 rams were probably free from infection when inoculated and remained so after subsequent exposure.

Certain aspects of immunization are still being investigated, but these results indicate that full protection against ovine brucellosis in rams can only be obtained if the animals are inoculated before exposure to infection. This fact constitutes the basis for the recommendation that ram lambs be inoculated with Rev. 1 vaccine at weaning age.

#### ACKNOWLEDGEMENT

This work was done while both authors were holding Senior Research Fellowships granted by the Stock Diseases Research Fund, and they are deeply indebted to the Executive of that Fund for enabling them to undertake these investigations.

The Chief, Veterinary Research Institute is thanked for granting all the necessary laboratory facilities and material, and Dr. G. C. van Drimmelen for making the vaccine available.

The valuable assistance given by Mr. W. H. de Vos in the routine examination of rams is gratefully acknowledged.

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TABLE I.

LABORATORY TRIALS WITH STRAIN 19 AND ELBERG REV. 1 VACCINES

Vaccine	Number of rams	Date of inoculation	Dates of challenge	Subsequent examinations from dates of challenge to 21/11/60				
				Number of examinations	Results			
					Normal	<i>Bru-cella</i> and epididymitis	<i>Bru-cella</i> only	Epididymitis only
Strain 19	5	8 Aug. '58	24 April '59 12 Jan. '60	12	3	—	2	—
Strain 19	20	3 Oct. '58	29 Sept. '59 12 Jan. '60	7	16	4	—	—
Rev. 1	25	3 Oct. '58	29 Sept. '59 12 Jan. '60	7	25	—	—	—
Controls	6	—	24 April '59 12 Jan. '60	14	3	2	1	—
Controls	15	—	12 Oct. '59 12 Jan. '60	9	7	5	2	1

TABLE II.

Farm Number	Date of inoculation.	Number of Rams.			Exposure to infection	Date.	Subsequent Examination							
		Inoculated.		Con- trols			Results.							
		Group A	Group B				Group C	Group A.	Group B.			Group C.		
									Nothing abnormal	Nothing abnormal	<i>Brucella</i> positive and Epi- didymitis	<i>Brucella</i> only	Nothing Ab- normal	<i>Brucella</i> positive and Epi- didymitis
1	(1)11/5/59	3	—	3	19/10/59 to 1/12/59..	4/2/60	3				2	—	1	
	(2) 7/3/60	20	16	—	April—November 1960	23/2/61	20	9	5	2	—	3	2	
2	26/8/59	3	2	5	March—April 1960..	16/6/60	3	—	2	—	—	3	—	
3	26/8/59	4	—	3	March—April 1960..	16/6/60	4				—	3	—	
4	26/8/59	5	—	4	15/4/60—30/5/60 ..	16/6/60	5				—	2	2	
5	13/5/59	5	12	4	August '60 to March '61	18/6/61	5	3	6	3	1	3	—	
6	10/2/60	6	—	2	May 1960—Feb. 1961	18/6/61	6				—	2	—	
7	23/3/60	5	8	—	Oct. '60 to April '61..	18/6/61	5	1	4	3				
8	11/5/59	9	—	3	Oct. '59—Feb. '60..	11/3/60	9				—	1	2	
9	March '60.	4	13	—	April—June 60. ....	22/8/61	4	7	3	3				
					March—June 61									



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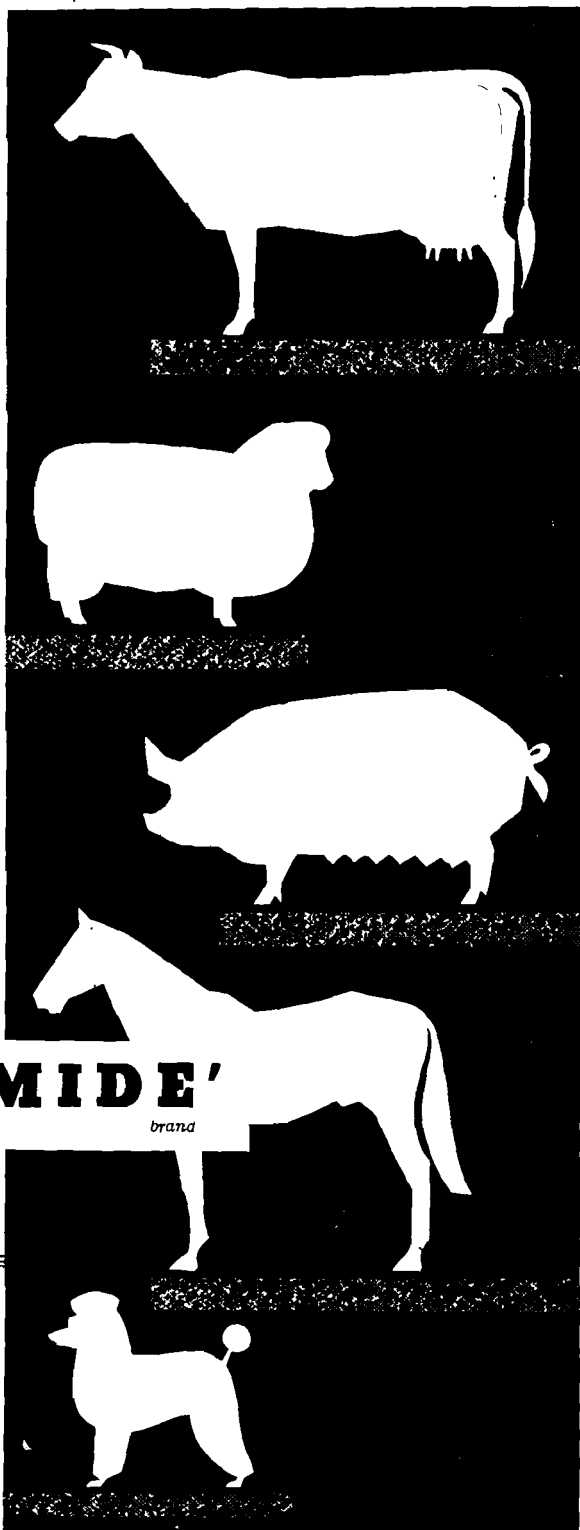
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## THE USE OF ADJUVANT WITH LIVE *BRUCELLA* VACCINE

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Received for publication March 1962.)

### SUMMARY

Live *Brucella* vaccine, used in the freeze-dried form for the immunization of cattle, sheep and goats, exerts its immunogenic effect largely by means of an invasion of the tissue cells during the transient infection and stimulates cellular resistance. Circulating antigen whether killed or living, produces humoral antibody which is more specific in its effect than the cellular immunity. Humoral antibody enhances the resistance of immune cells to invading organisms, and reduces their rate of multiplication within invaded cells.

Adjuvants promote humoral antibody production when they are used with killed vaccine. A substantial amount of nonviable *Brucella* antigen is present in freeze-dried vaccine. The addition of adjuvant increases the production of antibody, whereby the immunogenic effect is improved. The adjuvants have no deleterious influence on the viability of live organisms in the live vaccine.

### INTRODUCTION

Killed *Brucella* vaccines have been successfully applied to chronic cases of brucellosis in man. Clinical improvement after such antigenic stimulus has often been confirmed: yet killed *brucella* vaccine has usually been inferior to live vaccine in the production of immunity in man and animals<sup>1,2</sup>.

Russian and Californian workers have shown that immunity in brucellosis is principally due to the resistance of tissue cells against invasion by live organisms<sup>3,4</sup>. The highest degree of resistance is acquired after an infection of relatively long duration<sup>5</sup>. In the immune state the invading organisms are less numerous in the cells: the function and life of the invaded tissue cells are not adversely affected. The resistance to invasion is comparatively non-specific<sup>6</sup>.

Humoral antibody does not prevent infection and invasion of the tissues by virulent *Brucella* organisms. It has, however, been demonstrated<sup>7</sup>, that humoral antibody which is much more specific than cellular resistance to invasion, has a markedly favourable effect on immune cells. In the presence of humoral antibody, immune cells with intracellular parasites show improved survival value and phagocytic efficiency.

A long-standing chronic *Brucella* infection without circulating antigen often results in a measure of immunity showing little humoral antibody. Such cases may benefit from an antigen injected into, the blood stream by boosting the titre of the humoral antibody.

Adjuvant has been added to antigen to retard absorption and thereby prolong stimulation of antibody production. Renoux<sup>8,9</sup> has shown the remarkable increase in the degree of immunity in brucellosis obtained from killed vaccine by the use of adjuvants. The duration of such humoral

immunity is, nevertheless, probably shorter than that of the cellular immunity resulting from live vaccine prepared from strains of lesser virulence.<sup>10,11,12</sup>

The purpose of this article is to show that the freeze-dried live vaccines which contain a substantial amount of killed *Brucella* organisms, can be used to greater benefit if suitable adjuvant is added. This does not affect the viability of the live *Brucella* organisms in any way but utilises the killed cells to the best advantage.

Buddle<sup>11,12</sup> in New Zealand has advocated the simultaneous use of strain 19 (*Br. abortus*.) and killed *Br. ovis* vaccine with the addition of adjuvant. This vaccine has not been adopted in Australia, South Africa and California, where similar infections have been encountered.

Van Drimmelen<sup>13,14</sup> has obtained promising results with strain Elberg Rev. 1, *Br. melitensis* vaccine in sheep in South Africa.

## MATERIALS AND METHODS

### (1) THE VACCINES USED IN THESE STUDIES WERE:

- (a) Killed *Br. melitensis* and adjuvant. (Renoux)
- (b) Killed *Br. melitensis* and adjuvant. (Renoux)
- (c) Live freeze-dried *Br. melitensis* Strain Elberg Rev. 1. vaccine reconstituted in sterile water and mixed with equal quantities of Bayol F with 10% Arlacel A. (Onderstepoort)
- (d) Rev. 1. Live freeze-dried vaccine reconstituted as before without addition of adjuvant (Onderstepoort).

### (2) ADJUVANTS TESTED WERE:

- (a) Arlacel A.\* (emulsifier);
- (b) Bayol F.† (oily excipient);

They were tested by measuring the effect on growth of:

- (i) Fresh concentrated strain 19 and Rev. 1. vaccine organisms exposed to different dilutions of the substances and tested at different times after mixing.
- (ii) Rev. 1. *Brucella* organisms seeded on agar surface to produce a "mat" of culture exposed to blotting paper discs dipped in the substances.

### (3) THE FOLLOWING SHEEP WERE USED:

- (a) Six months old Merino ram lambs, grouped in 5 equal lots of six rams equal in weight, age and condition.
- (b) Six months old Merino ewe lambs, grouped in 5 lots of 3 to 5 ewes equal in weight, age and condition.

### (4) VACCINATION:

Four groups of six rams each and four groups of ewes, as summarised in Table 1, were inoculated subcutaneously behind the

\*Mannide monooleate Prep 9B CEL 69659. Atlas, Powder Co., Wilmington Delaware.

†Esso Petroleum Co., Ltd., 36 Queen Annes Gate, London SW 1.

shoulder with sterile (separate) needles: the skin being cleaned with 1% Cetrinide B. P. (Cetavlon)† solution. One group of each was kept unvaccinated as control.

TABLE I.

IDENTIFICATION NUMBERS OF THE SHEEP USED IN THE EXPERIMENTS ON  
*Brucella* VACCINES WITH ADJUVANT.

Group	1	2	3	4	5
Origin of vaccine	Renoux Tunis	Renoux Tunis	v. Drimmelen Ondersteprt.	v. Drimmelen Ondersteprt.	—
Organism...	<i>Br. melitensis</i>	<i>Br. melitensis</i>	<i>Br. melitensis</i>	<i>Br. melitensis</i>	—
Strain...	53—H—38	53—H—38	Elberg Rev. 1	Elberg Rev. 1	—
Batch of vaccine..	58	55	R.64	R.64	Unvaccinated
killed or- ganisms/ dose...	100000000000	100000000000	8 000 000 000	8 000 000 000	—
Live orga- nisms/ dose...	—	—	4 000 000 000	4 000 000 000	—
Adjuvant..	adjuvant	adjuvant	adjuvant	—	—
Volume of dose....	2 ml.	2 ml.	2 ml.	2 ml.	—
Animals ino- culated—					
(a) Rams...	7426	8747	7442	7427	7436
	8738	8749	8754	8762	8741
	8739	8750	8755	8763	8745
	8742	8751	8757	8764	8746
	8743	8752	8759	8765	8774
	8744	8753	8760	8766	8785
(c) Ewes...	7425	8791	7440	7431	7512
	7429	8792	7443	7433	8798
	8807	8793	8808	8794	8802
			8809	8799	8806
			8810	8803	

#### (5) SEROLOGICAL TESTS:

Serum was collected during 1 year at monthly intervals and tested by the:

- (1) 0.85% saline agglutination test.
- (2) 5.0% saline agglutination test.
- (3) Blocking antibody test.
- (4) Antiglobulin (Coombs) agglutination test.
- (5) Complement fixation test.

†Imperial Chemical (Pharmaceuticals) Ltd., Manchester, England.

## RESULTS

SENSITIVITY OF *BRUCELLA* TO ADDITIVES. Arlachel A and Bayol F were found completely harmless in vitro to the viability of *Brucella* organisms in reconstituted freeze-dried vaccine (see Table 2). As expected the addition of adjuvant slightly increased the size and persistence of the local reaction at the site of inoculation.

TABLE 2.  
VIABILITY OF *Brucella* IN THE PRESENCE OF ARLACHEL  
A AND BAYOL F.

Orga- nism	Strain	Batch No.	Recon- stituting fluid	Contact time (hrs.)	Diameter of zone of inhibition around discs soaked in			
					Arlacel A Bayol F	Arlacel A	Bayol F	Diluent
<i>Br. abortus</i>	S. 19	D 5388	0.85% NaCl in H <sub>2</sub> O	72	0	0	0	0
<i>Br. meli- tensis</i>	Rev. 1	R 109	H <sub>2</sub> O	72	0	0	0	0
		R 110	H <sub>2</sub> O	72	0	0	0	0
		R 111	H <sub>2</sub> O	72	0	0	0	0
					Surface growth on albimi agar after contact at 37°C			
					Arlacel A 10% Bayol F 40%	Arlacel A 10% + Diluent	Bayol F 40% + Diluent	Diluent
<i>Br. abortus</i>	S. 19	D 4893	0.85% NaCl	1	++++	++++		
				2	++++	++++		
				4	++++	++++		
				12	++++	++++		
<i>Br. meli- tensis</i>	Rev. 1	R 109		1	+++	+++	+++	++++
				4	+++	++++	++++	+++
				24	++	++	+++	++
		R 110		1	+++	+++	+++	++++
				4	+++	++++	++++	+++
				24	+	.	.	+
	R 111	1		+++	++++	+++	+++	
		4		++++	+++	++++	+++	
		24		++	+	+++	+	

+ = 100 × 10<sup>1</sup> viable organisms  
 ++ = 100 × 10<sup>2</sup> — 100 × 10<sup>4</sup> viable organisms  
 +++ = 100 × 10<sup>6</sup> — 100 × 10<sup>7</sup>  
 ++++ = 100 × 10<sup>7</sup> (confluent growth).

### SEROLOGY:

- (a) The serological evidence of antibody response in rams is illustrated in Table 3.

TABLE 3.

THE SEROLOGICAL EVIDENCE OF ANTIBODY RESPONSE AFTER VACCINATION OF MERINO RAMS WITH FOUR *Brucella* VACCINES.

(Average log 2 for all 12 of the monthly tests on all 6 rams in each group)

Group	Killed organisms plus adjuvant	Killed organisms plus adjuvant	Live vaccine plus adjuvant	Live vaccine	Controls
0.85% saline agglutination.....	7.94	7.57	5.65	1.29	0.39
5.0% saline agglutination.....	5.41	4.71	4.22	1.49	0.37
Blocking antibody.....	0.78	0.70	0.69	0.78	0.69
Antiglobulin agglutination.....	14.26	13.70	8.74	3.26	1.67
Complement fixation.....	4.41	4.21	2.29	4.17	2.29
Group averages.....	5.95	5.69	4.42	1.95	0.93

The differences are demonstrated by using numerals for the number of tubes showing positive reactions in each test of each serum specimen, and determining the average (log 2) for all tests on each group of rams. These figures show an overwhelming superiority in serum antibody response to adjuvant containing vaccine. (See Table 3).

- (b) The serological results in ewes are shown in Table 4. The differences are again demonstrated in a condensed form showing the average value (log 2) per test for each group.

TABLE 4.

THE SEROLOGICAL EVIDENCE OF ANTIBODY RESPONSE AFTER VACCINATION OF MERINO EWES WITH FOUR *Brucella* VACCINES.

(Average log 2 for all 12 of the monthly tests on all ewes within each group.)

Group	Killed organisms plus adjuvant	Killed organisms plus adjuvant	Live vaccine plus adjuvant	Live vaccine	Controls
0.85% saline agglutination.....	7.3	8.0	6.0	2.3	1.2
5.0% saline agglutination.....	5.3	5.7	5.0	2.6	1.3
Blocking antibody.....	0.5	0.3	0.3	0.3	0.2
Antiglobulin agglutination.....	9.6	10.5	8.5	4.9	3.5
Complement fixation.....	4.0	3.2	4.2	2.3	0
Group averages.....	5.34	5.54	4.80	2.48	1.24

The present results in rams (Table 3) show an antibody response with some outstanding features:

- (a) Figures representing antibody response to vaccination show a 2 to 3 times higher value for adjuvant containing vaccine than

for vaccine without adjuvant. The latter barely exceeds a level, double that of non-vaccinated controls.

- (b) Incomplete agglutination blocking antibody is not increased by vaccination; and complement fixing antibody in some groups, is only slightly increased by vaccination.
- (c) The 0.85% saline agglutination test shows a 20-fold increase for killed vaccine plus adjuvant, as against a 15-fold increase for live vaccine plus adjuvant, but only a threefold increase for live vaccine without adjuvant. (See Table 3).
- (d) The 5% saline agglutinins show a 13-fold increase for killed vaccine plus adjuvant, as against an 11-fold increase for live vaccine plus adjuvant but only a 4-fold increase for live vaccine without adjuvant. The antiglobulin agglutinins show a 5-fold increase for killed vaccine plus adjuvant, as against a 4-fold increase for live vaccine plus adjuvant, but only 2-fold increase for live vaccine without adjuvant. (See Table 3).
- (e) The highest response in terms of these values (average log 2), based on the average number of tubes agglutinated per test per  $10^8$  vaccine organisms, is shown by the live vaccine plus adjuvant namely 1.23 against 0.35 for live vaccine without adjuvant, and 0.12 for killed vaccine with adjuvant (See Table 5).

TABLE 5.

COMPARISON OF ANTIBODY RESPONSE TO DIFFERENT TYPES  
OF VACCINES

Type of vaccine used	Killed vaccine + adjuvant	Live vaccine + adjuvant	Live vaccine	Control
Amount antigen.....	2 ml.	2 ml.	2 ml.	—
0.85% saline agglutination (average tubes agglutinated per test).....	7.75	5.65	1.29	0.39
5.0% saline agglutination (average log 2 value per test).....	4.06	4.22	1.49	0.37
Number killed organisms inoculated.....	100 000 000 000	8 000 000 000	8 000 000 000	—
Agglutination per $10^8$ orga- nisms: (average log 2 value per test divided by number of bil- lions of organisms inocu- lated).....	0.12	1.23	0.35	

Similarly in ewes (Table 4), the response to live vaccine plus adjuvant is as good as that with the killed vaccines containing adjuvant, and considerably higher than the response to live vaccine without adjuvants.

## DISCUSSION

There is still a great deal to be discovered about immunity in brucellosis. Cotton, Buck and Smith, Mingle, Stableforth, Renoux, and Vereshilova have amply proved the sound foundation of immunization in practice. Elberg et al<sup>10</sup>, Vereshilova<sup>4</sup>, and others have created a better understanding of the immunising process. The present work may contribute to the knowledge of the different factors involved in vaccination.

The earlier virulent vaccines used in cattle were abandoned in favour of calfhood vaccination with Strain 19. Human vaccination with live non-H<sub>2</sub>S-producing variants of Strain 19 has been introduced in the U.S.S.R. Live Strain Elberg Rev. 1 and killed *Br. melitensis* culture plus adjuvant, are two vaccines fairly recently recommended.

The present results indicate a possible benefit to be derived from live vaccine applied with added adjuvant. The intracellular immunizing stimulus is derived from live cells which are unharmed by the adjuvant. The dead antigen, available as the result of manipulation of the culture during freeze drying and storage, is utilized to best advantage by the presence of adjuvant. By affecting the immune cells in a way which improves their resistance to invading bacteria and to the multiplication of the organisms that have invaded the cytoplasm, the humoral antibody supplies increased protection. The life of the cells is also prolonged by the humoral antibody. The method may enhance the immunity against genital infection of rams. This infection is believed to be confined to epithelial tissue in the prepuce and epididymides and it does not cause more than extremely weak antibody production.

## THE DURATION OF IMMUNOGENIC INFECTION

The artificial, active, anti-invasive immunity in brucellosis, as has been demonstrated by Elberg and his co-workers<sup>3,5,15</sup>, is primarily a consequence of intracellular contact with live antigen over a period of several months. The longer this contact is maintained the better the immunity. The degree this anti-invasive immunity finally reaches, would appear to depend on the kind of cells, and the number of cells parasitized without loss of function and without interference with cell division and growth. Therefore the most virulent strain of bacteria short of necrobiotic effect, would be expected to produce the maximum result in degree of cellular resistance. The highest degree of resistance probably also provides the greatest persistence of immunity. This is the reason for preference of the Elberg Rev. 1. strain to strain 19 for the vaccination of small stock.

## BETTER UTILIZATION OF KILLED CELLS

Some antigen in circulation results from the inoculation of a live vaccine, since all live cells are not immediately phagocytised and are therefore spread through the body via the blood stream. This provides the stimulus for production of humoral antibody with a bactericidal effect and, as mentioned earlier, a protecting effect on invaded immune cells. Live Rev. 1. vaccine is however given in doses that must be regarded as small, in terms of antigen available for circulation. This is where the use of adjuvant makes a difference. Adjuvant affects the rate of absorption of the antigen from killed cells causing a more persistent stimulation of the antibody forming apparatus over a lengthened period. The result is a higher titre from the amount of dead material injected.



The addition of adjuvant to freeze-dried *Brucella* vaccine which contains a proportion of the antigen in the form of killed cells, permits of better utilization of material.

It enhances the humoral antibody production and is likely to assist in the attainment of an immune state in which is combined:

- (a) Maximum cellular anti-invasion resistance of lifelong duration, obtained from long experience of intracellular parasitism.
- (b) High humoral bactericidal antibody content which also has a maximum protective effect on invaded immune cells.

#### THE PERSISTENCE OF AGGLUTININS

The results presented here justify the use of adjuvant with freeze-dried live vaccine. In circumstances where the persistence of agglutinating antibody is not of any consequence, further research is indicated. Calf-hood vaccination was introduced especially to avoid vaccinal agglutinin reactions in adult cattle.

Indeed the strong demand for non-agglutinin-producing vaccines, may have retarded the progress of work on immunogenesis in brucellosis in the past. Eradication is often preferred to immunization. Workers were perhaps over optimistic with regard to early eradication of *Brucella* Infection on a world-wide basis. Where nomadic farming is practised a different outlook is required.

The live vaccines, depending on their "invasive" properties, and the killed vaccines on their "absorptive" properties for immunogenesis, provide a field of study of great interest.

The immunogenic qualities of killed vaccine, as affected by absorptive properties, can be defined by a measure of contact of phagocytized antigen particles maintained in the living cell, as the main objective of the immunologist. By means of suitable adjuvants it may be possible to obtain the almost permanent positioning of antigens in the antibody-producing cells. In this event the immunologist's ideal would appear to be within reach. A vaccine entirely free from live antigen but capable of producing life-long protection by continued antigenic stimulation, may be possible.

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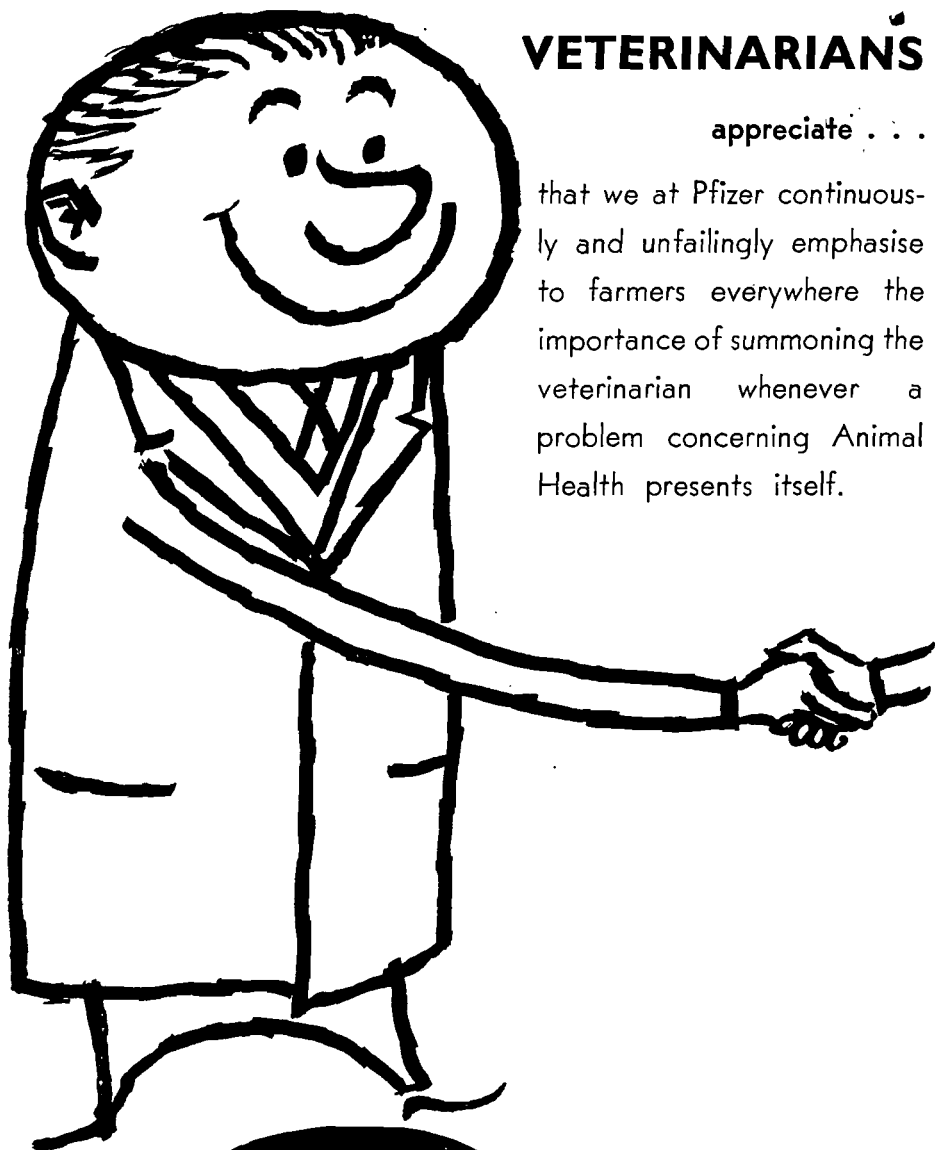
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## TICKS, THEIR HABITS AND BEHAVIOUR IN NATURE

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Louis Trichardt  
Transvaal.

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### SUMMARY

By means of daily counts of ticks taken off animals grazing on veld, some information has been gained on:—

- (a) the rate of tick build-up on an animal;
- (b) the preferred sites of attachment;
- (c) differences in cattle breed susceptibility to ticks;
- (d) some peculiarities of the bont and bontpoot ticks  
(*Amblyomma* and *Hyalomma* spp.)

### INTRODUCTION

A great deal of work has been done on ticks and their life history under laboratory conditions, especially in regard to transmission of disease. However, there are still many gaps in our knowledge of their habits under natural conditions.

The observations which follow represent the beginning of an attempt at determining the pattern of behaviour of some of the more common ticks in this area (Northern Transvaal).

### EXPERIMENTAL MATERIAL AND METHODS

Six yearling oxen born and reared at Mara Research Station were selected for reasonable docility and tractability by courtesy of the Officer in Charge. Three were Afrikaners with typical short, dense, smooth hairy coats. The other three were Hereford grades with average woolly coats.

As these animals had been exposed to ticks since birth, no serious complications due to tick bites were expected and none were experienced.

The animals were paired off into three groups A, B and C., each pair comprising one Afrikaner and one Hereford. At night all three groups were housed together in a large opensided shed, provided internally with a short race in which the animals could be confined and tied, while ticks were being counted.

During the day those animals going to graze were allowed free range in camps of about 20 morgen each. The grazing in these consisted of natural sour veld on the Louis Trichardt municipal land, where cattle had grazed for the past four years. The locality was thus known to be fairly heavily infested with ticks, typical to this area namely:—

*Rhipicephalus appendiculatus*

*Rhipicephalus evertsi*

*Amblyomma hebraeum*

*Hyalomma* spp

*Boophilus decoloratus*

Throughout these experiments no insecticides were used on the animals, nor were other cattle allowed to run in the camps. For convenience of counting and allocating ticks to the various parts, the body was arbitrarily divided into regions as shown in Table 1.

#### EXPERIMENT 1—

*Object:* To determine by actual count, the total number of ticks picked up by animals from day to day.

Four animals were used in this experiment. *Group A* (Nos. 361 and 548) and *Group B* (Nos. 415 and 545).

Prior to the beginning of the experiment these animals were kept in the shed for a few days to ensure that they were completely tick free. All ticks were removed by hand.

The different animals although free to range as they pleased, actually stayed close together and thus grazed the same places in a camp on a given day.

At intervals of about 14 days the animals were changed to different camps.

From 11.1.1961 and daily thereafter, both groups A and B went off together to graze in one camp and returned to the shed for the night. Each morning, except Sundays, the animals were seen at 8 a.m. when counts were made as follows:—

*Group A* (Nos. 361H and 548A). All ticks were counted as they were removed by hand, systematically from each body region. The ticks so removed were dropped at once in a receptacle containing paraffin (Kerosene) to ensure that none fell on the ground and so found their way on to the animals again.

*Group B* (Nos. 415H and 545A). All ticks were removed and counted every other day only. In other words group A went off to graze daily starting with zero ticks while group B carried a two day crop of ticks before being relieved of it and started with zero ticks only every 2nd day.

The number of ticks so removed, the species and in case of *Amblyomma* the sex, was recorded on a sheet for each animal as shown in Table 1.

The somewhat arbitrary anatomical division was influenced in the first place by the more obvious preferred sites of attachment of some ticks e.g. eye lids, ears, tail etc. It was realised later that there are other preferred sites of lesser importance such as front of hump, base of horns and base of dewclaws.

For the purpose of this preliminary experiment the tick count included only adults of readily identifiable species namely *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Amblyomma hebraeum*, (♂ and ♀) *Hyalomma*. Immatures were ignored, as also all stages of *Boophilus*. (It was found later that some of the ticks taken from the back of dewclaws and recorded as *Rhipicephalus appendiculatus*, were in fact *Rhipicephalus simus*. The proportion of this tick was small, however, and would not materially affect the conclusions arrived at).

Table 1 shows how the counts were recorded each day for each animal.

TABLE I.  
EXAMPLE OF DAILY RECORDING OF 4 SPP. OF TICKS ON ONE ANIMAL

Hereford 361		Date 13/4/61 ♂ ♀			14/4/61			15/4/61			17/4/61			18/4/61			19/4/61			20/4/61			21/4/61			22/4/61		
		Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H	Ra.	Ah.	Re. H
Eyelids . . . . .	L	1			1			2			1			3			2						4			3		
	R	2			2			1			5			1						1			7			1		
Ears . . . . .	L	66			59			59			136			47			22			21			80			42		
	R	45			56			53			95			43			35			23			79			39		
Head . . . . .	L	12			10			11			15			6			6			10			9			6		
	R	5			5			18			24			4			3			6			8			6		
Neck . . . . .	L	4			15			7			8			32			18			7			13			9	1/	
	R	5			14			14			15			24			11			5			11			8		
Axilla . . . . .	L	3			3	1/		5			2	3/		4	3/		1			4	1/		1	1/		5	1/	
	R	7			5	1/		9	4/		4	2/		1/			4			1/			6	1/		5		
Groin . . . . .	L	2	2/		3	1/		1			1	1/		5	4/											4	2/	
	R				1			5	1/1		1	2/2		4/2			6	1/		3			1	2/		1	1/	
Body . . . . .	L																5			1								
	R				3																							
Forelegs . . . . .	L				2		3					1		1			2		1	1				1		1		
	R							1			/1						1		1				1/					
Hindlegs . . . . .	L	3	/1	1			1	1		4	3	1/		1			1						2		4	2		
	R				1		2				1			1			1		1	1						1		
Scrotum . . . . .		2	2/									5													2			
Prepuce . . . . .	L	L/			1						5	2/1		3			4	1/1								1		
Perineum . . . . .				2			5				2	3	1			2				1			3		1			
Tail . . . . .		3	♂	2	3		1				33		2	2			1						1			3		
Totals . . . . .		161	5/1	2	3	184	3/	5	7	187	5/1	—4	321	11/4	8	4	177	12/2	2	2	123	2/1	—	—	84	2/	—	—

♀

L=Left.

R=Right

/ distinguishes ♂ of *Amblyomma hebraeum* only.



## ANALYSIS OF COUNTS

From counts so recorded, abstracts and tables were prepared to show the characteristics of each species of ticks separately or comparatively or in relation to other factors.  
e.g.—

- (a) The rate of tick build-up (total daily counts);
- (b) Effects of season and climate;
- (c) Preferred sites of attachment;
- (d) Relative susceptibility of cattle breeds, and
- (e) Other peculiarities.

### *Rate of Tick Build-up and Effects of Season and Climate*

The observations were carried out only for approximately six months from 11/1/1961 — 15/7/1961 and obviously cannot be regarded as complete or conclusive. For this reason only a portion of the data in regard to (a) and (b) above is presented in graphic form (Fig. I).

It should be pointed out at this stage, that if ticks were not removed every day or every other day from an animal, their distribution on the animal could conceivably be affected, for instance by overcrowding or even by injury (bleeding or softening) of skin.

In Fig. I only the daily count of *Rhipicephalus appendiculatus* and *Amblyomma* are given over the period 11.1.1961.–28.1.61 together with relative humidity, temperature and rainfall. It will be noted that the two Herefords collected 5624 *Rhipicephalus appendiculatus* and 302 *Amblyomma hebraeum* as against 2561 and 131 for the two Afrikaners respectively. The figures for *Rhipicephalus evertsi* and *Hyalomma* spp. (not shown on graph) are Herefords 197; Afrikaners 180; Herefords 12; Afrikaners 24, respectively.

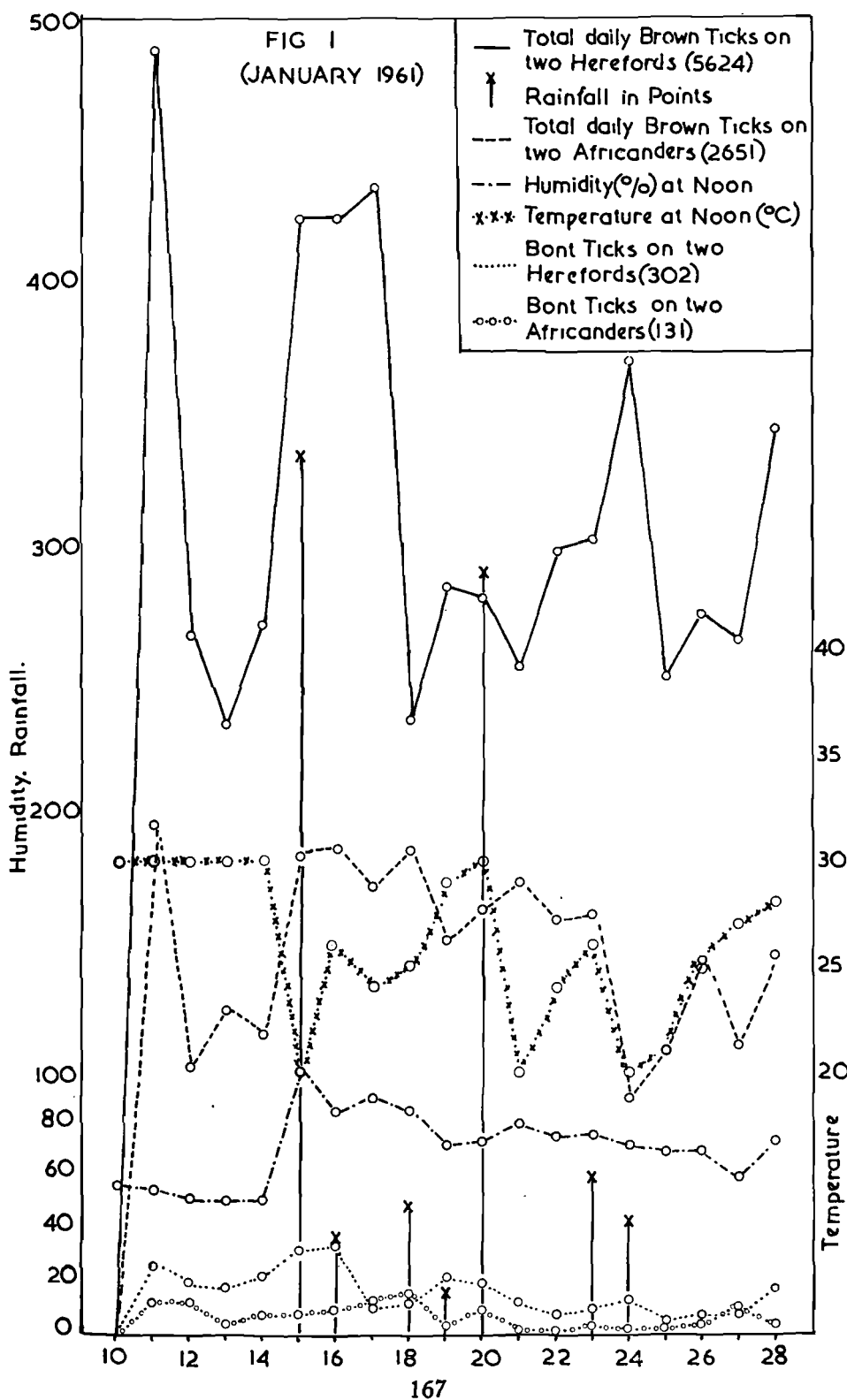
In the course of six months, ticks collected by groups totalled:—

	HEREFORD	AFRIKANER
<i>R. appendiculatus</i> .....	34,889	19,806
<i>Amblyomma</i> .....	1,415	649
<i>R. evertsi</i> .....	655	527
<i>Hyalomma</i> .....	460	214

(See Fig. II)

### *Preferred sites of Attachment*

In regard to the preferred sites of attachment tables II, III, IV and V give the total number of ticks collected from the different body regions of Afrikaners as against Herefords.



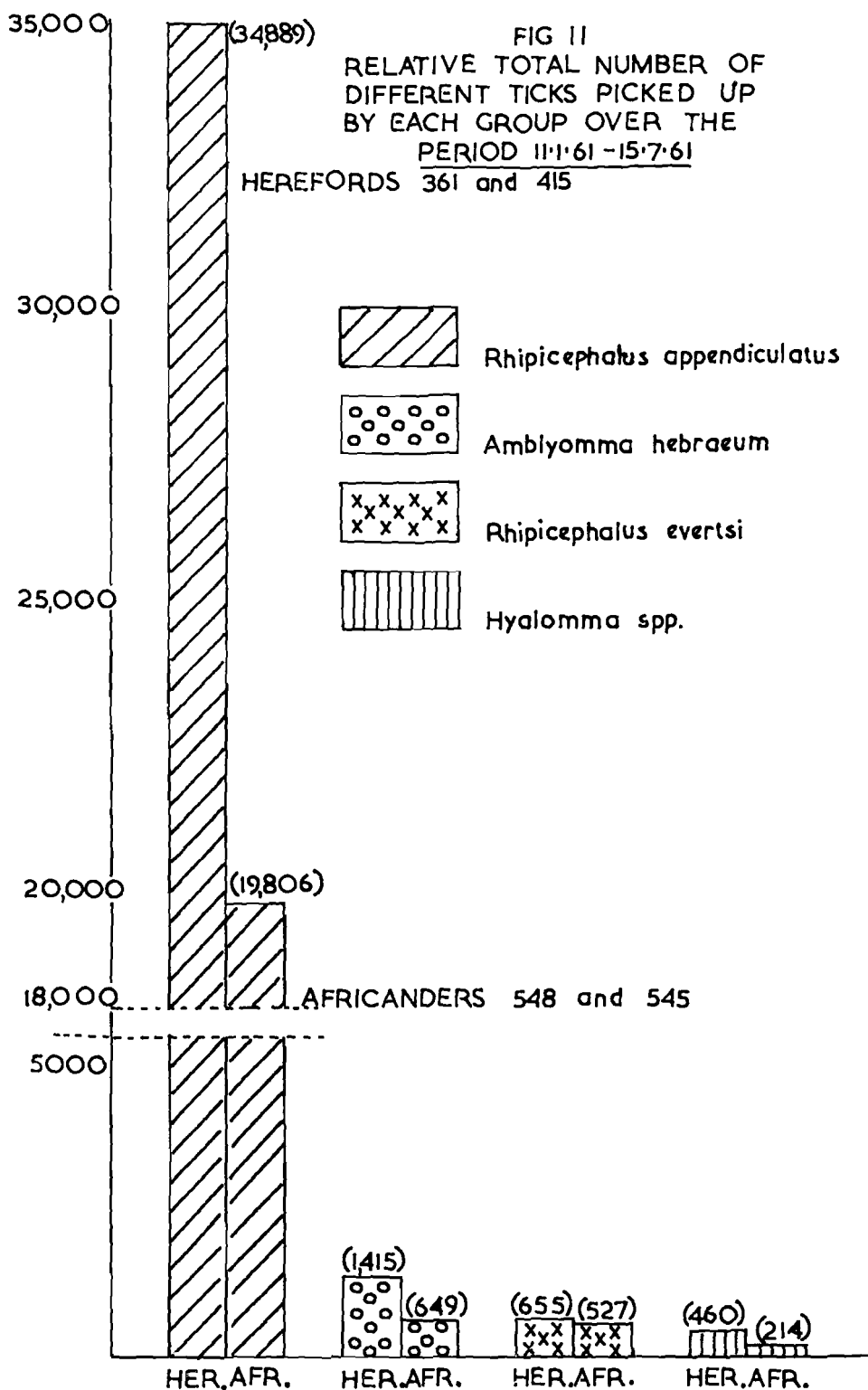


TABLE II.  
RED TICK (*RHIPICEPHALUS EVERTSI*)

Parts of the body	Total number of Ticks for each part of the Body				Total number of ticks on Each Part of the Body expressed as a percentage of the grand Total				Average Percentage for two Groups of Animals	
	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander	Hereford
Eyes .....										
Ears .....										
Head . ....										
Neck .....										
Axil .....										
Groin .....			31	25			7.3	10.9		9.1
Body .....				8				3.5		1.75
Foreleg .....										
Hindleg . ....				4				1.7		0.85
Prepuce . ....	1		6	1	0.3		1.4	0.4	0.15	0.9
Scrotum . ....			18	12			4.2	5.2		4.7
Perineum . ...	300	226	371	150	99.7	100	87.1	65.5	99.85	76.3
Tail .....				29				12.7		6.4

TABLE III.

BONTPOOT TICK (*HYALOMMA SPP.*)

Parts of the body	Total number of ticks for each part of the Body				Total number of Ticks on Each Part of the Body expressed as a percentage of the grand Total.				Average Percentage for two Groups of Animals	
	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander	Hereford
Eyes .....										
Ears .....										
Head . ....										
Neck ... ..										
Axil .....	3	1	27	4	3.2	0.8	8.8	2.6	2.0	5.7
Groin .....		17	4	2		14.3	1.3	1.3	7.1	1.3
Body .....		3	10			2.4	3.3		1.2	1.7
Foreleg .....	13	10	14	6	13.9	8.0	4.5	3.9	7.4	4.2
Hindleg . ....	9	38	152	83	9.6	31.6	49.3	53.9	20.6	51.6
Prepuce . ....	1	3	4	8	1.1	2.4	1.3	5.2	1.8	3.3
Scrotum . ....	3	4	2	9	3.2	3.2	0.65	5.8	3.2	3.2
Perineum ...	6	6	9	2	6.4	4.8	2.9	1.3	5.6	2.1
Tail .....	59	39	86	39	62.5	32.5	27.9	25.6	47.5	26.7

TABLE IV.  
BONT TICK (*AMBLYOMMA HEBRAEUM*)

Parts of the body	Total number of ticks for each part of the Body				Total number of ticks on each part of the Body expressed as a percentage of the grand Total				Average Percentage for two groups of Animals	
	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander	Hereford
Eyes .....										
Ears .....										
Head . . . . .	1		1	1	0.28		0.14	0.2	0.14	0.17
Neck .....	9		18	13	2.48		2.2	2.2	1.24	2.2
Axil .....	122	115	365	278	33.6	40.2	44.1	47.3	36.9	45.7
Groin .....	142	103	222	107	39.1	36.0	26.8	18.4	37.5	22.6
Body .....	4	2	6	8	1.1	0.7	0.72	1.3	0.9	1.01
Foreleg .....	9	2	20	7	2.48	0.7	2.4	1.3	1.6	1.8
Hindleg . ....	17	6	43	27	4.5	2.4	5.2	4.6	3.4	4.9
Prepuce . ....	7	7	46	68	1.9	2.4	5.5	11.5	2.16	8.5
Scrotum . ....	46	51	91	79	12.7	17.8	11.0	13.4	15.2	12.2
Perineum . ...	4	2	2	3	1.1	0.7	0.24	0.4	0.9	0.32
Tail .....	1				0.28				0.14	

TABLE V.  
BROWN TICK (*RHIPICEPHALUS APPENCICULATUS*)

Parts of the body	Total number of ticks for each part of the Body				Total Number of Ticks on each part of the Body expressed as a percentage of the grand Total				Average percentage For two groups of Animals	
	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander 548	Africander 545	Hereford 361	Hereford 415	Africander	Hereford
Eyes .....	299	238	759	642	2.3	3.3	3.6	4.4	2.75	4.0
Ears .....	10,242	6,418	13,686	11,120	81.5	89.1	66.7	77.8	85.3	72.3
Head . ....	1,115	123	1,418	605	8.7	1.7	6.9	4.2	5.2	5.5
Neck .....	508	166	2,709	860	4.1	2.3	13.2	5.9	3.2	9.5
Axil .....	21	5	449	258	0.1	0.07	2.2	1.7	0.085	1.9
Groin .....	10	12	160	99	0.05	0.16	0.78	0.68	0.105	0.73
Body .....	3	5	52	29	0.02	0.07	0.25	0.2	0.04	0.23
Foreleg .....	141	71	240	139	1.1	0.98	1.2	0.96	1.04	1.08
Hindleg . ....	251	162	417	122	1.9	2.2	2.0	0.84	2.05	2.1
Prepuce . ....	34	50	212	186	0.2	0.69	1.0	1.3	0.44	1.1
Scrotum . ....	12	8	69	87	0.09	0.11	0.33	0.62	0.10	0.48
Perineum . ....	2	17	67	46	0.01	0.23	0.32	0.31	0.12	0.32
Tail .....	75	15	226	67	0.5	0.21	1.1	0.46	0.35	0.78

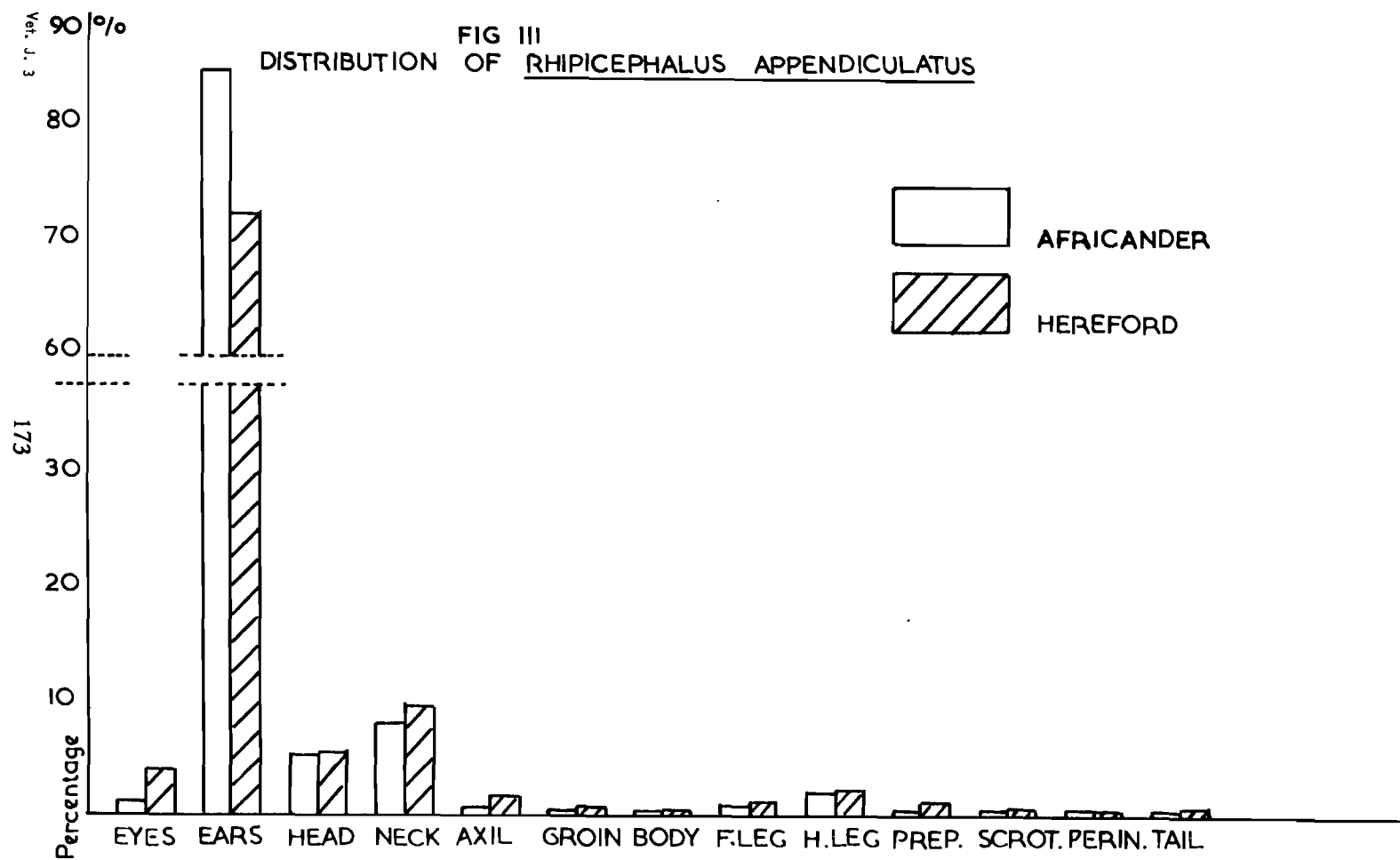




FIG IV  
DISTRIBUTION OF AMBLYOMMA HEBRAEUM

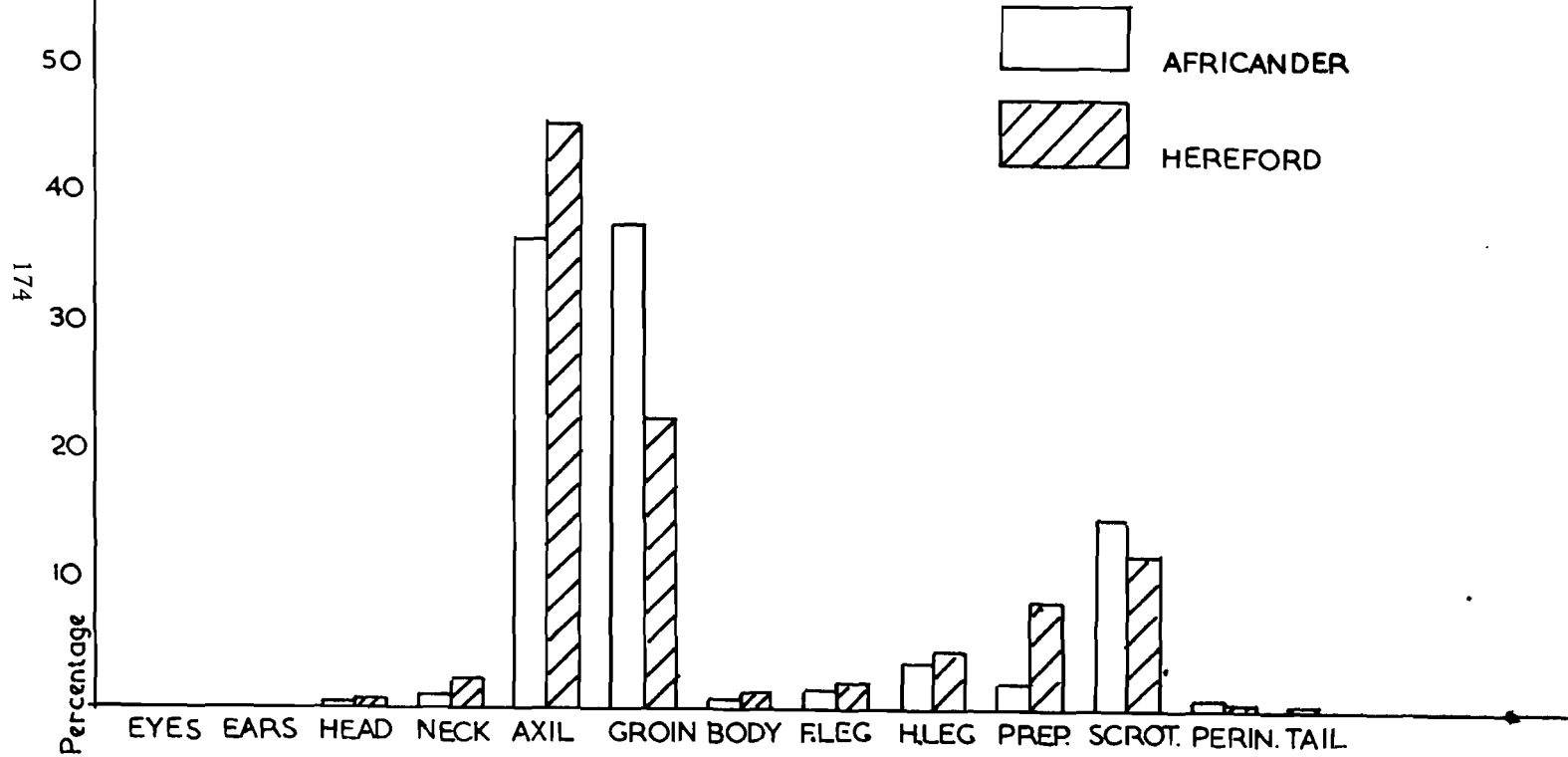


FIG V  
DISTRIBUTION OF HYALOMMA spp.

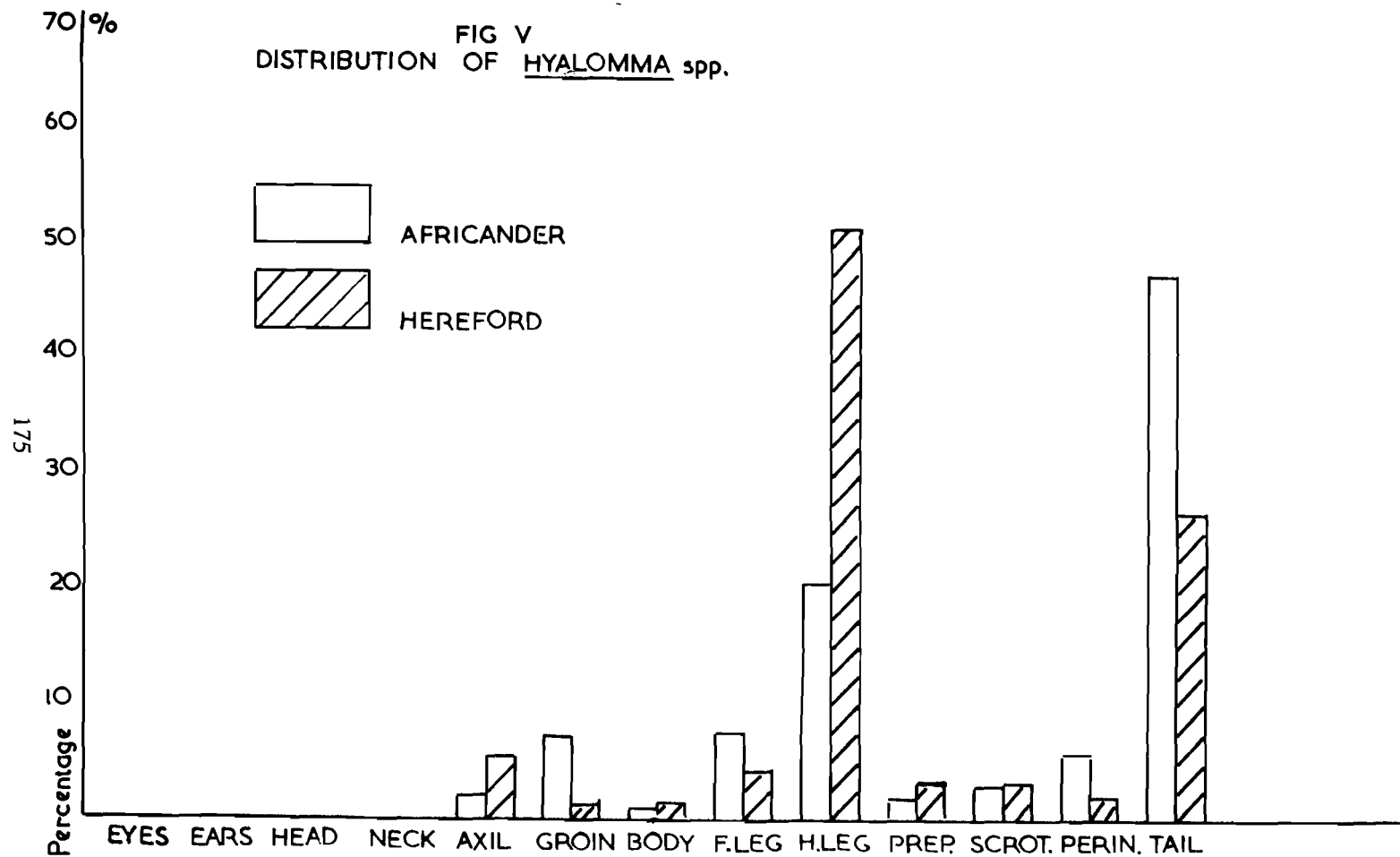
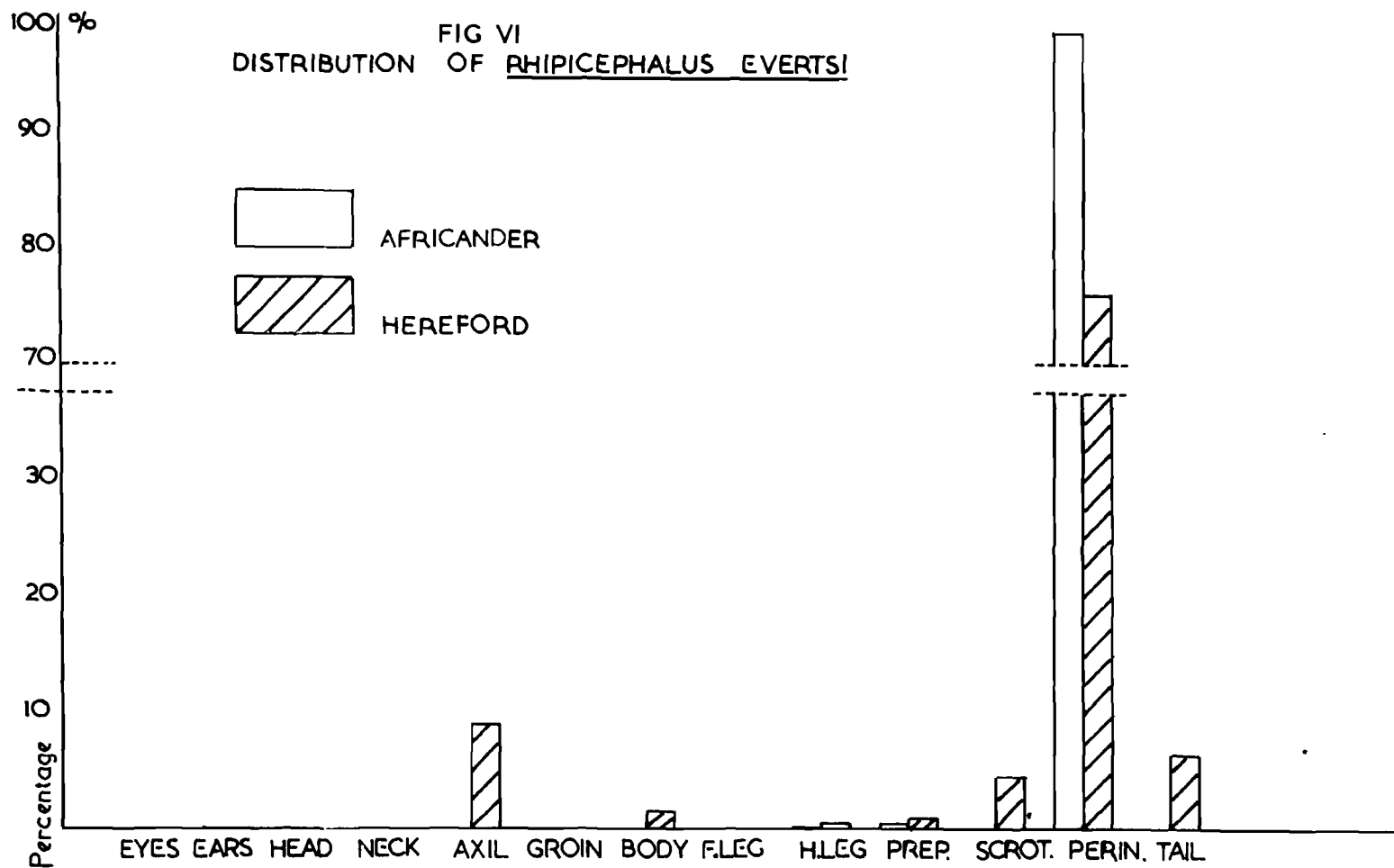


FIG VI  
DISTRIBUTION OF RHIPICEPHALUS EVERTSI



The total number of ticks on each body region is also expressed as a percentage of the total for that animal, and finally the average percentage for two animals of one breed, is compared with the corresponding figure for the other two animals.

This latter comparison is further given in graphic form in Fig. III, IV, V, and VI. It is of interest to note that 85% of the Brown ticks favour the ears in Afrikaner as against 72% in Hereford, but if one takes the head and neck as a whole the percentage rises to 96.5 and 91.4 respectively. In the case of *Amblyomma* (Bonttick), the lower aspect of the belly including axilla and groin, attracted 91% on Afrikaners and 89% on Herefords. The *Hyalomma* (Bontpoot) on the other hand seem to favour the hind legs and tail, while *Rhipicephalus evertsi* (Red tick) is almost 100% for the perineum on Afrikaners, but only 76% on Herefords — see note on Graph VI.

#### Relative Breed Susceptibility

It will be clear from all the foregoing tables and especially from Figs. I and II that the Hereford breed of cattle consistently picked up far more (up to double the number) of all four varieties of ticks counted.

No attempt will be made at this stage to explain this well marked predilection.

#### Other Peculiarities

*Amblyomma hebraeum* (Bont tick) was the only species of which the sexes were recorded separately. A cursory glance at Table I will show in the Ah column, that far more males than females were removed. The relevant figures are given in Table VIII below.

TABLE VIII

<i>Amblyomma</i> (Bont tick)	AFRIKANER		HEREFORD	
	A.548	A.545	H.361	H.415
Total male for period . . . . .	353	275	727	496
Percentage of male . . . . .	97.2%	96.1%	87.9%	84.0%
Total females for period . . . . .	10	11	100	92
Percentage of females . . . . .	2.8%	3.9%	12.1%	16.0%

This preponderance of male *Amblyomma* on animals has been confirmed by observation on numerous other cattle since. It is also a curious fact that a lone female of the species seldom attaches to the skin. It prefers to wander about until it reaches an attached male when it immediately attaches alongside it.

#### EXPERIMENT 2

##### Object

(a) to observe ticks during the total period they spend on the host, times of engorgement and fall off.

(b) to note any pathological effects (local or general) due to ticks.

Group C — No. 517 and 539 was intended for this experiment. After being freed of all ticks the two animals were allowed to graze with group A and B for one day only on 11.1.1961. Thereafter they were kept, fed and watered within the shed entirely.

The ticks on them were counted and recorded as to attachment sites — every other day — but were not removed.

Quite soon it became evident that the experimental set-up was quite unsatisfactory for the purpose in view. The tick counts were unexpectedly

erratic. It was noticed that the animals scratched against poles and other objects and so lost many ticks while fowls accounted for some more. There was also the possibility that fresh ticks could transfer from animals of groups A and B while housed together at night. In the absence of facilities and accommodation better suited to overcome these difficulties it was decided to discontinue the experiment.

### EXPERIMENT 3

*Object* to observe whether ticks would move towards and onto a host (ox) tethered in the veld.

TABLE VI

Date	A. 545				H. 415				REMARKS
	Ra.	Ah.	Re.	H.	Ra.	Ah.	Re.	H.	
20.4.61	27	7	1	1	84	—	—	1	To Start 415 was at Stable Arena To Start 545 was at Tree Arena
21	57	4	2	1	86	4	1	—	Tethered by day back to shed at night.
22	19	1	—	—	29	1	1	—	
23	—	—	—	—	—	—	—	—	Changed About 545 stable 415 tree
24	21	—	—	—	24	—	—	1	
25	16	1	—	2	18	4	2	1	Tethered day and night 415 stable 545 tree
26	11	1	2	1	15	—	—	1	
27	6	—	—	—	14	1	—	—	
28	1	—	—	—	6	1	—	1	
29	—	—	—	—	4	—	—	—	
30	—	—	—	—	—	—	—	—	31 R. app. + 1 Amb. Gathered in veld were freed onto grass periph- eral to each arena.
1.5.61	7	—	—	1	3	1	—	—	
2	1	—	—	—	—	—	—	—	
3	2	—	—	—	4	—	—	—	
4	1	—	—	—	1	—	—	—	
5	—	—	—	—	—	—	—	—	
6	—	—	—	—	—	—	—	—	
7	—	—	—	—	—	—	—	—	
8	—	—	—	—	2	—	—	1	
9	—	—	—	1	1	—	—	—	
10	2	—	—	—	—	—	—	—	29H., 4A., placed in grass near 415. 9A., 6H., 3 R app., 1 R e. placed in grass near 545.
11	—	—	—	—	—	—	—	—	
12	1	—	—	—	—	—	1	—	
13	—	—	—	—	—	—	—	—	
14	—	—	—	—	—	—	—	—	
15	—	—	—	—	—	—	—	—	
16	—	—	—	—	—	—	—	—	
17	—	—	—	—	—	—	—	—	
18	—	1	—	—	—	—	—	—	
19	—	—	—	—	—	—	—	—	
20	—	—	—	—	—	—	—	—	Animals changed over 545 stable 415 tree (Remained so to end of expt., i.e. 15.7.61. In that period H.415 picked up another 2 Rh. app., 3A, 3H, while A545 picked up 2 Rh. app., 2A, and 3H.)
21	—	—	—	—	—	—	—	—	
22	—	1	—	—	—	—	—	—	
23	—	1	—	—	—	—	—	—	
24	—	—	—	—	—	—	—	—	
25	—	—	—	—	—	—	—	—	
26	—	—	—	—	—	—	—	—	
27	—	—	—	—	—	—	—	—	

Oxen Nos. 415H and 545H (Group B) were withdrawn from Experiment I and used for this purpose. Each ox was tied to a pole planted in the centre of a circular arena about 16' in diameter from which all grass had been hoed and raked off. The two circles were sited at random, in tick infested veld, one in open grass veld 40 yards from the stable, the other about 300 yards away in another camp, was shaded in the centre by a small thorn tree.

Table VI reflects the daily tick counts made on these two animals. From the 20th to the 29th March, 1961 the two oxen were tethered by day and taken back to shed for the night. The brown tick found were obviously picked up from the grass while walking to and from the shed. The other ticks must have come from the trash left around the cleared area.

From 29.4.1961 onwards the oxen were left tethered continuously at their respective arenas, except that they were interchanged on 22.5.61

Adult ticks collected in the veld were released in the grass at the edge of arenas as shown. Although the animal was only a few feet away very few of these ticks seem ultimately to have found their way onto the animals.

The results obtained are not altogether conclusive, but they do suggest rather strongly that movement by ticks in search of a host is very restricted. In the case of the brown tick sitting on top of a grass blade — the only movement involved is that of becoming alert and putting out legs to catch or grasp the passing host by its hair.

For *Amblyomma* and *Hyalomma* the position is somewhat different. These ticks usually stay in hiding on the ground. They become alerted by vibrations caused by an approaching host. Then they quest at close range by very active and fast movement on the ground, towards and onto the animal. They usually make a bee line for the animals feet and must obviously be guided by perception of certain stimuli emanating from the host animal.

Further experiments would have to be carried out to establish definitely — up to what distances these ticks perceive stimuli from the host and respond to them.

It must be regarded as fortunate that ticks do not fly, and do not walk about to any extent in search of their host. This means that for dissemination they depend entirely on being carried around by their host.

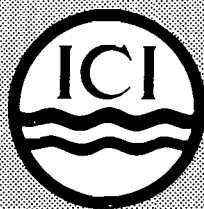
Obviously this fact is an advantage in favour of and to be remembered by those whose task it is to devise and apply control measures against ticks.

#### ACKNOWLEDGEMENT

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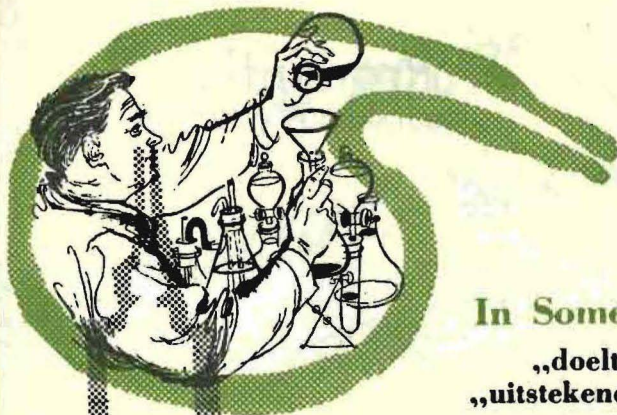
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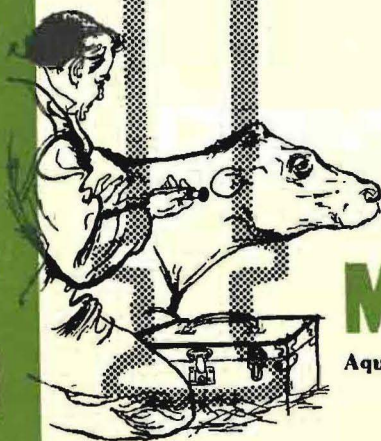
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## THE PATHOLOGICAL PHYSIOLOGY OF HEARTWATER

[COWDRIA(RICKETTSIA) RUMINANTUM — COWDRI, 1926]

R. Clark — Onderstepoort Laboratory

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### INTRODUCTION

Henning (1949) summarized the publications on heartwater. A study of the literature cited by him and subsequent publications indicated that little attention had been paid to the pathological physiology of the disease. The only report of work on the chemical pathology of the disease which could be found was that by Graf (1933) who confined himself to the determination of a limited number of blood constituents without finding any significant changes. It was, therefore, decided to follow the pathological physiology of the disease in as great detail as possible in the hope that the findings might indicate rational lines of supportive treatment. This aspect is of practical importance as many animals fail to recover in spite of specific chemotherapy which is judged successful on the criterion of body temperature.

### METHODS

In all, 15 adult Merino sheep and two Friesland oxen were infected with heartwater by the intravenous route, using infected sheep blood, and the ensuing reactions studied. The methods used for the blood analyses are listed below.

Plasma Na and K.....	Clark (1959).
Plasma Mg .....	Neil & Neely (1956).
Plasma Cl .....	Method of Shales and Schales: in Hawk, Oser & Summerson (1954).
Blood Ca .....	Ferro & Ham (1957).
Inorganic P.....	King & Wootton (1956).
Haemoglobin .....	King & Wootton (1956).
Plasma Proteins .....	Method of Kingsley: in Hawk, Oser & Summerson (1954).
Blood Sugar .....	Folin-Wu Principle: in Hawk, Oser & Summerson (1954).
Blood Urea N .....	Brown (1957).

In five of the sheep and both cattle the carotid artery on one side of the neck was exteriorized and placed in a tube of skin for a length of about three inches. This enabled the systolic and diastolic blood pressures to be taken by the conventional auscultatory method using a rubber cuff about 1½ inch in width. The reduced width of the cuff may have given somewhat high readings but this factor was standard throughout.

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## RESULTS

### A.—Sheep

#### Reactions

The incubation period varied from seven to 11 days (average nine), the onset of the disease being taken as from the first rise in temperature to above 104 F. The duration of the disease was three to eight days. All the cases produced were fatal. The body temperature remained between 104°F and 106°F until a final drop before death. None of the animals showed a biphasic temperature reaction, neither did they show the well-known nervous symptoms of heartwater. This may have been connected with the fact that the animals were housed throughout the experiment.

#### Food intake

The food intake was measured on four sheep. There was a rapid fall in food intake from the onset of fever with complete inappetence after the third day.

#### Water and sodium balance

The same four sheep were kept in metabolism cages and the water intake and urine volume measured. They were dosed 1 gm. NaCl per day.

TABLE 1.—*Water and sodium balance*

	Days of reaction					
	0	1	2	3	4	5
Water intake (l.).....	2.8	2.2	2.6	1.9	1.4	0.7
Urine volume (l.).....	0.76	0.74	—	0.72	0.43	0.33
Na output (M. eq.).....	27	44	—	40	26	11

(Figures represent average of four sheep)

The fact that, for the first three days, the water intake decreased more rapidly than did the urine output indicates a loss of total body water during the initial stages of the disease, if a constant extrarenal water loss is assumed. This is also indicated by the increased sodium loss recorded on days 1 and 3 despite a reduced food intake. The subsequent fall in urinary sodium output was probably a reflection of faecal loss as it coincided with the appearance of soft pultaceous faeces.

#### Post mortem findings

The post mortem findings were typical of heartwater except that there were only small amounts of fluid in the body cavities and moderate oedema of the lungs. The diagnoses were confirmed by the demonstration of *C. ruminantium* in impression smears made from the brain.

#### Haematology

The red cell counts and haemoglobin content fluctuated in accordance with the haematocrit, indicating that there were no marked changes in mean cell volume or haemoglobin content. (The haematocrits are discussed later).

No marked changes were found in total or differential leucocyte counts with the exception that the eosinophiles disappeared from the circulation even before the first rise in temperature.

### *Blood chemistry*

The large number of figures obtained have been condensed and given in Table 2 as averages.

TABLE 2.—*Blood chemistry*

	Days of reaction				
	Prior	1 & 2	3 & 4	5 & 6	7 & 8
Plasma Na (m. eq./l)...	139 (7)	135 (4)	138 (5)	136 (5)	132 (1)
Plasma K (m. eq./l)...	5.3 (7)	5.4 (6)	5.1 (6)	5.2 (3)	3.5 (1)
Plasma Mg (m. eq./l)...	1.9 (4)	1.3 (3)	1.2 (4)	1.1 (2)	—
Plasma Cl (m. eq./l)...	106 (7)	100 (7)	102 (7)	97 (4)	107 (1)
Plasma HCO <sub>3</sub> (m. eq./l)	25.8 (3)	28.6 (3)	20.5 (5)	10.28 (3)	—
Blood Ca (mg. %)....	11.4 (7)	12.3 (4)	12.9 (3)	9.4 (3)	—
Inorganic P (mg. %)...	4.6 (7)	3.8 (4)	2.6 (3)	5.1 (4)	—
Blood Urea N (mg. %)...	24 (4)	38 (1)	40 (3)	35 (1)	—
Creatinine (mg. %)....	0.8 (6)	0.5 (3)	0.5 (6)	0.5 (3)	0.5 (1)
Hb (gm. %).....	11.6 (5)	11.2 (4)	12.7 (5)	12.1 (1)	13.5 (1)
Total Plasma Protein	6.6 (13)	6.9 (8)	7.0 (9)	6.6 (5)	5.8 (1)
Albumen (gm. %)....	4.0 (13)	3.8 (8)	4.5 (7)	4.0 (5)	4.0 (1)
Globulins (gm. %)....	2.6 (13)	3.1 (8)	2.5 (7)	2.6 (5)	1.8 (1)

(The figures in brackets represent the number of readings)

None of the blood constituents listed in the table showed any constant changes during the course of the disease. The marked drop in plasma bicarbonate was probably due to the extreme hyperpnoea which accompanied the hyperthermia. As mentioned above, lung oedema was not marked in these cases. Should it occur, a respiratory acidosis could be expected.

The serum copper and magnesium contents were determined in four reacting sheep and found to be within normal limits.

The fact that the blood creatinine showed no rise indicates the absence of serious kidney damage. The slight rise in blood urea may be due to circulatory failure.

No evidence of liver damage was found. There was no bilirubinaemia and the "Bromsulphalein" retention test was negative in three sheep each in an advanced stage of the disease.

### *Blood sugar*

The blood sugar level was followed in ten sheep. The figures remained within normal limits (40-55 mg. per cent) until some 24 hours before death. Five of the ten sheep showed a marked terminal rise in blood sugar, from 60 to 93 mg. per cent being recorded. In the other five sheep the figures from samples taken within 24 hours of death were normal. A terminal rise in blood sugar in sheep is by no means peculiar to heartwater as it has been found in pregnancy disease, tribulosis and enterotoxaemia. In these diseases, as in heartwater, a concurrent terminal glycosuria is often seen.

### *Plasma colour*

It was noted that, after the onset of the reaction, the plasma became progressively darker in colour often reaching a deep orange. The van den Bergh test on these samples consistently showed the presence of less than 1 mg. per cent total bilirubin which was far too little to account for the plasma colour. The nature of the pigment is as yet unknown but its presence probably accounts for the yellow colour of the effusions often seen in heartwater.

### *Red cell sedimentation rate*

This was taken in Wintrobe tubes at an angle of 45° over one hour. The average sedimentation rate before infection was 3.5 mm. (2 to 6) which rose to 7.0 mm. (5 to 8) on the first day of infection and 17.5 (15 to 20) on the fifth day.

TABLE 3.—*Carotid artery systolic and diastolic pressures.*

	Day of reaction				
	Prior	2nd	4th	5th (a.m.)	5th (p.m.)
6.....	120/90	130/90	130/100	126/85	115/0
7.....	—	150/100	130/80	70/40	—
8.....	115/82	140/95	110/80	—	—
9.....	123/90	112/90	110/90	125/0	—
10.....	105/80	126/80	92/70	122/0	—

Sheep numbers correspond with those in Table 4

### *Arterial blood pressure*

In all the above cases the animal died within 24 hours of the last reading being taken. It will be noted that there was a very marked terminal drop in diastolic pressure in the terminal stages. By a diastolic reading of "0" is meant that a loud thumping sound was heard over the artery with no pressure being applied to the cuff. During this stage the pulse was bounding and typically "water hammer" in nature. So great was the pulse pressure that the exteriorized "arterial loops" could be seen bounding at each pulsation and the pulse rate could be counted from a distance with ease. At the same time the jugular veins were distended. When bleeding, a high venous pressure was indicated by the fact that the blood spurted from the needle and often forced back the plunger of the syringe without proximal occlusion of the vein. The heart sounds were exceedingly loud but normal in character.

### *Plasma and blood volumes*

The plasma volume was determined by the Evan's blue method. Sheep were given 15 mg. and the cattle 30 mg. of the dye. Samples were taken ten and 20 minutes after injection and zero concentration estimated by backward extrapolation, assuming a linear decline in concentration.

TABLE 4.—*Haematocrit, plasma and blood volume*

Sheep No.		Prior	Days of reaction						
			1	2	3	4	5	6	8
1	Hc.....	36	—	31	37	48	—	—	—
	P.V.....	1.46	—	1.92	—	0.50	—	—	—
	B.V.....	2.52	—	2.91	—	0.96	—	—	—
2	Hc.....	40	34	—	—	31	34	38	43
	P.V.....	1.55	—	—	—	1.03	—	0.87	1.07
	B.V.....	2.58	—	—	—	1.49	—	1.34	1.90
3	Hc.....	47	—	—	35	32	43	—	—
	P.V.....	1.69	—	—	—	1.63	0.24	—	—
	B.V.....	3.15	—	—	—	2.40	0.42	—	—
4	Hc.....	37	35	—	—	42	46	—	—
	P.V.....	1.50	1.82	—	—	1.38	0.74	—	—
	B.V.....	2.37	2.80	—	—	2.38	1.37	—	—
5	Hc.....	36	—	30	27	—	35	—	—
	P.V.....	—	—	2.22	1.40	—	1.90	—	—
	B.V.....	—	—	3.17	1.92	—	1.70	—	—
6	Hc.....	27	22	—	—	11	10	—	—
	P.V.....	2.46	2.50	—	—	2.17	2.02	—	—
	B.V.....	3.28	3.21	—	—	2.40	2.23	—	—
7	Hc.....	35	26	—	—	12	12	—	—
	P.V.....	2.17	2.14	—	—	2.46	1.97	—	—
	B.V.....	3.19	2.89	—	—	2.80	2.24	—	—
8	Hc.....	36	28	—	—	28	—	—	—
	P.V.....	2.08	1.97	—	—	1.97	—	—	—
	B.V.....	3.15	2.74	—	—	2.74	—	—	—
9	Hc.....	35	—	33	—	32	33	—	—
	P.V.....	2.27	—	2.38	—	1.95	2.38	—	—
	B.V.....	3.44	—	3.60	—	2.87	3.55	—	—
10	Hc.....	34	—	30	—	25	27	—	—
	P.V.....	2.05	—	2.30	—	2.00	2.14	—	—
	B.V.....	3.06	—	2.90	—	2.64	2.93	—	—

Hc = Haematocrit per cent.

P.V. = Plasma volume (litres).

B.V. = Blood volume (litres).

In all cases shown in Table 4 the animal died within 24 hours of the last readings shown. It will be seen that, with the exception of sheep No. 6, 8 and 10, there was a marked drop in plasma and blood volume shortly prior to death. That this occurred suddenly and terminally is well shown by the figures for sheep No. 3 and 4 where readings were taken on the day prior to death and shortly before death.

### Haematocrits

As will be seen from Table 4 there was a general tendency for the haematocrits to fall, often sharply, during the disease and rise suddenly terminally. This last rise coincided with the drop in plasma volume to which it can be partly attributed.

### B.→Cattle

The incubation periods in the cases of the two calves were 15 and 16 days respectively, death taking place in four and two days after the first rise in temperature. Both animals showed the typical nervous symptoms of heartwater.

TABLE 5.—*Clinical findings. Cattle*

Day of reaction	Bov. 1			Bov. 2	
	Prior	3rd	4th	Prior	2nd
Haematocrit (%).....	27	15	16	34	29
Red cell sed. rate (mm./hr.).....	5	13	10	2	5
Plasma Na (m. eq./l.).....	138	144	144	132	132
Plasma K (m. eq./l.).....	5.4	5.2	5.5	4.5	5.5
Plasma bicarb. (m. eq./l.).....	36	35	28	36	32
Total plasma proteins (gm. %)....	7.0	6.0	6.2	8.1	7.0
Albumen (gm. %).....	1.4	2.8	2.6	2.8	2.4
Globulins (gm. %).....	5.6	3.2	3.6	5.3	4.6
Blood sugar (mg. %).....	37	46	72	36	55
Creatinine (mg. %).....	0.8	1.1	1.1	1.0	1.2
Total bilirubin (mg. %).....	0	0	0	0	0
Plasma volume (litres).....	6.25	7.14	6.82	6.98	7.50
Blood volume (litres).....	8.59	8.40	8.12	10.58	10.56
Blood pressure.....	170	140	120	160	190
	120	0	0	110	110

The clinical findings are shown in Table 5. As will be seen, the findings on the two cattle were similar to those on sheep. There were no significant changes in the blood chemistry except a terminal rise in blood sugar and urea nitrogen and a fall in bicarbonate. These were also noted in the sheep.

There was again a fall in the haematocrit. In case of Bovine No. 1 the marked terminal drop in diastolic arterial blood pressure and rise in venous pressure, were encountered. Both animals died overnight, and, in the case of No. 2, the fall in diastolic pressure had not yet occurred at the time of the last examination. In neither case could a final fall in plasma volume be demonstrated.

### DISCUSSION

This investigation did not reveal any constant or specific changes in the blood chemistry which could be associated with heartwater.

The main findings were a marked drop in diastolic blood pressure followed by a severe fall in plasma and blood volumes. Both these events took place in the terminal stages of the disease.

The fall in diastolic arterial blood pressure together with a rise in venous pressure can only be ascribed to peripheral vaso-collapse as post mortem examination revealed no abnormalities of the heart valves.

It will be realised that it was extremely difficult to record all phases of this terminal circulatory collapse as it took place very rapidly and almost immediately before death. Furthermore, there was no method of predicting its onset and the majority of deaths took place at night.

That the fall in diastolic pressure could take place very suddenly is well shown in sheep No. 6 (Table 3) where it fell from 85 to 0 between 9 a.m. and 2 p.m.

Peripheral vaso-collapse could be attributed to a sympatholytic affect. That such an effect is present in heartwater is also shown by the haematocrit readings which showed a tendency to fall, often dramatically, during the disease. That this was not due to red cell destruction is suggested by the absence of bilirubinaemia. Turner & Hodgetts (1959) have demonstrated large variations in the haematocrits of sheep due to contraction and relaxation of the spleen and they concluded that the relaxed spleen can contain up to one-seventh of the total blood volume and one-quarter of the total red cell volume. This was confirmed during the present work where it was found that the haematocrit of sheep unaccustomed to being handled was invariably higher than it was when the animals were no longer nervous. The drop in haematocrit values seen in heartwater can, therefore, be ascribed to splenic relaxation following on sympatholysis which culminates in general vaso-collapse.

As would be expected, the haematocrit rose with the sudden reduction of plasma volume, but the extent of this rise was not proportional to the fall in plasma volume. Assuming the total volume of circulating erythrocytes to remain constant, the percentage plasma volume change indicated by a change in the haematocrit can be calculated by the formula—

$$\text{Percentage plasma volume change} = \left\{ \frac{\text{H. crit. 1}}{\text{H. crit. 2}} - 1 \right\} \times 100$$

In all cases the percentage decrease in plasma volume so calculated was considerably less than that shown by direct measurement. This would indicate that a further sludging of red cells takes place in the final stages of the disease. These conclusions are substantiated by the well-known finding of severe engorgement of the spleen at post-mortem examination of animals which have died of heartwater.

A study of the figures from sheep No. 9 and 10 (Tables 2 and 3) and from bovine No. 1 (Table 4) shows that the sudden decrease in plasma volume follows the peripheral vaso-collapse and fall in diastolic pressure. The decrease in plasma volume is not due to hypoproteinaemia as it is not preceded by any reduction in plasma total protein or albumin concentrations. The fact that the plasma protein concentration remains constant despite a sudden reduction in plasma volume indicates that the proteins escape from the vascular system as freely as does the fluid. It is therefore postulated that the decrease in plasma volume is due to a sudden increase in capillary permeability, allowing the escape of plasma proteins. This would naturally lead to severe oedema and shrinkage of plasma volume. This hypothesis is supported by the fact that the trasudates in heartwater frequently show coagulation, indicating that even so large a protein as fibrinogen (molecular weight 450,000) has passed through the capillary wall.

No suggestions can be made as to the cause of the nervous symptoms typical of heartwater as shown by the cattle. These symptoms cannot be ascribed to any changes in the blood constituent determined nor to the circulatory collapse which their appearance preceded.

The findings indicate that the main functional disturbance in the terminal stages of heartwater associated with sudden death is a sympatholysis followed by peripheral vaso-collapse, increased capillary permeability and a drastic reduction in blood volume which precipitates general circulatory failure. The indicated supportive treatment where this series



of events appears imminent would be the administration of sympathomimetics and the transfusion of whole blood or preferably packed red cells to maintain blood volume. Adrenaline or noradrenaline should be administered by constant intravenous drip, a procedure usually impracticable in veterinary practice. Therefore, the use of methylamphetamine as a sympathomimetic is suggested. In one of the experimental sheep showing peripheral vaso-collapse, 30 mg. methylamphetamine was injected subcutaneously. Within five minutes the diastolic arterial pressure rose from 0 to 80 mm. Hg. The effect lasted some two hours. Clinically, the indicated dosage and frequency of administration could be judged by the character of the pulse. A bounding "water hammer" type of pulse would indicate decreased peripheral resistance and the need for further treatment. The efficacy of blood or red cell transfusions could best be judged by the haematocrit, which should be kept above normal to supply blood volume. Where a centrifuge is not available, the haemoglobin level could be used as an indicator.

The unexpected finding of the disappearance of the circulating eosinophiles, even before the onset of a febrile reaction, may be of considerable practical importance. When immunizing against heartwater it frequently occurs that individual animals fail to show a febrile reaction. One is therefore left in doubt as to whether they have immunized or not. A second challenge with infected sheep blood into cattle may produce severe and often fatal shock, especially in Jerseys. Where such a challenge is carried out, it often happens that the animals again fail to react and subsequently prove to be immune. If it can be shown that the disappearance of the circulating eosinophiles is a constant phenomenon of the reaction to heartwater, it may well prove a useful technique in determining which animals have immunized. It may also act as an indication of an impending febrile reaction which may require treatment. Further work will be undertaken on these aspects.

A further aspect of the clinical manifestations of heartwater has been studied. It is not uncommon to find that sheep in which the febrile reaction has been controlled by chemotherapy, later pass into a state of collapse and may lie moribund even for days, showing a normal to sub-normal body temperature. Such cases invariably end fatally. This usually occurs when treatment has been delayed and nervous symptoms have been evident for some time. The period of survival is naturally prolonged if the animals are housed.

Three such sheep became available for study, which was carried out in collaboration with the Department of Internal Medicine. These animals appeared to be unconscious, the only outward signs of life being slow respiration and intermittent periods during which weak paddling movements were made. Examination proved that the plasma volume was not unduly reduced nor could any abnormalities be found in the blood constituents as determined in our experimental cases. The cerebrospinal fluid was examined and found to be normal.

It would, therefore, appear that in these cases specific therapy had eliminated the infection in time to avert death from circulatory collapse, but that irreversible brain damage had occurred. It can be said that the animals had the appearance of having undergone a functional decerebration. Death would naturally follow from dehydration and inanition. The treatment of such cases would appear to be hopeless.

## SUMMARY

The pathological physiology of heartwater has been studied in acute, untreated fatal cases in sheep and cattle. No significant changes could be found in the blood constituents. The main findings were (a) disappearance of circulating eosinophiles; (b) sympatholysis manifested firstly by a fall in haematocrit readings due to splenic relaxation followed by a subterminal peripheral vaso-collapse with a dramatic fall in arterial diastolic pressure; and (c) a terminal catastrophic drop in plasma volume due to an increased capillary permeability which allows of the passage of plasma proteins from the vascular system.

The use of sympathomimetics and intravenous infusions of packed red cells or whole blood are recommended as supportive treatment in advanced cases. The possible practical applications of the finding of the disappearance of the eosinophiles prior to the onset of the febrile reaction will be investigated further.

Three sheep showing collapse after having received chemotherapy late in the heartwater reaction were studied. These animals were showing general collapse. The condition is described as a functional decerebration with the vegetative functions of the body proceeding more or less normally. The cerebral damage would appear to be irreversible.

## ACKNOWLEDGEMENTS

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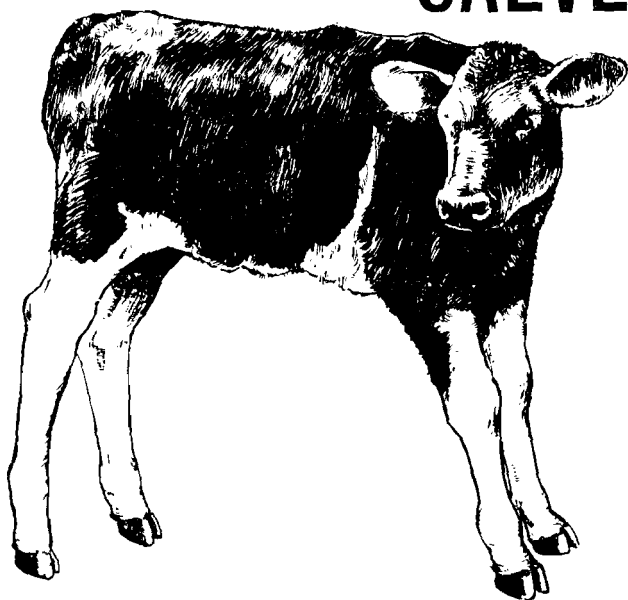
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# NEFTIN

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## TABLETS FOR RAPID & EFFECTIVE CONTROL OF PARATYPHOID AND BACTERIAL SCOURS IN CALVES



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## ANTHELMINTIC TRIALS WITH THIABENDAZOLE

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(Received for publication April 1962)

### SUMMARY

1. Anthelmintic trials with thiabendazole on calves, goats and horses are reported.

2. At 25 mg/K the drug was highly effective against the common nematodes of calves except *O. radiatum*, where a dose of 50 mg/K was 80 to 90% effective.

3. Both at 25- and at 50 mg/K the drug was highly effective against *H. contortus*, *Trichostrongylus* spp., *O. columbianum* and *Ostertagia* spp. in goats.

4. It was proved, both by differential egg counts and by examination of faeces for expelled worms after treatment, that this drug was highly effective against small and large strongyles and against ascaris of horses.

5. Thiabendazole is only mildly toxic in calves, even at 20 times the therapeutic dose.

6. Differential egg counts as a method of evaluating the efficacy of an anthelmintic are an essential prerequisite to serve as an indication of the necessity for slaughtering or not.

### INTRODUCTION

Brown *et al*<sup>1</sup> recently described a new drug 2-(4'-thiazolyl)- benzimidazole (generic name: thiabendazole) with a wide spectrum of activity against gastro-intestinal nematodes. Their claims have been confirmed by Müller<sup>2</sup>, Hebden<sup>6</sup>, Gardiner and Craig<sup>3</sup> Gordon<sup>4</sup> and Cairns<sup>2</sup>. This paper deals with the results of anthelmintic trials with this drug\*, on gastro-intestinal nematodes of cattle, goats and horses.

No animals were slaughtered in these trials. In three of the trials only differential egg counts were used to assess the efficacy of the drug: in the other, the worms expelled with the faeces after dosing, were also taken into account.

### EGG COUNTS

### MATERIALS AND METHODS

Differential egg counts were carried out by the McMaster technique of Gordon and Whitlock<sup>5</sup>, combined with larval differentiation on a percentage basis.

\*Kindly supplied by M.S.D. (Pty) Ltd., 142 Pritchard Street Johannesburg.

### *Trials on Calves*

Fifty calves with naturally acquired infestations were used as experimental animals. Faecal specimens were collected from a representative batch of 30 head seven days prior to dosing, and thereafter the 50 calves were sampled weekly for four weeks. Differential egg counts were carried out on each specimen; but the larval cultures of the final examination were unsuccessful, so that the efficacy of the drug could only be estimated for 14 days after treatment.

The herd was divided into five equal groups. Group 1 acted as undosed controls. Dosing with a suspension of thiabendazole took place on March 8 1961, groups 2 and 3 being dosed at 25 mg/K, groups 4 and 5 received 50 mg/K. In addition groups 2 and 4 were dosed with 100 ml. 10% NaHCO<sub>3</sub> solution, immediately prior to drenching with thiabendazole, to stimulate reflex closure of the oesophageal groove.

### *Trials on Goats*

A naturally infested flock of 30 Angora ewes and 15 kids was used. They were divided into six groups, groups 1, 3 and 5 consisting of ten ewes each and groups 2, 4 and 6 of five kids per group. Differential egg counts were carried out on faecal samples collected from each goat every week for five weeks. Groups 1 and 2 acted as undosed controls. At the second sampling on 26th May, groups 3 and 4 were drenched at 25 mg/K, while groups 5 and 6 received 50 mg/K. No prestimulation with 10% CuSO<sub>4</sub> solution was carried out.

### *Trials on Horses*

Faecal samples from four horses with symptoms of anal pruritus were examined. Egg counts were made (but no larval cultures) two days prior to dosing with thiabendazole at 50 mg/K, and on three subsequent occasions at irregular intervals over the next month.

## RESULTS

### *Trials on Calves*

Results are summarised in Table 1.

The egg counts in the control calves fell for all species with the exception of *Oesophagostomum radiatum* which increased markedly. In the latter species only those calves dosed at 50 mg/K showed an 80.5 to 89.9% fall in egg counts while there was no effect on calves dosed at half this rate. In spite of falling egg counts in the controls the drug was still effective against *Haemonchus placei* at 25 mg/K and *Cooperia* spp. at 50 mg/K. Since calves were very lightly infested with *Bunostomum phlebotomum* and *Trichostrongylus* spp. and the egg counts of the controls fell by 86.3 and 77% respectively during the trial period little note could be taken of the results.

TABLE 1  
MEAN EGG PER GRAM COUNTS IN CATTLE DOSED WITH THIABENDAZOLE

Group	Dose mg./K.	Date	Mean strong- yle e.p.g.	Mean differential e.p.g.				
				*H.p.	** Coop.	@ O. rad.	† Bphleb.	& Trich.
1	Controls.....	1/3	670	204	391	11	20	44
		8/3	940	280	550	7	34	77
		15/3	640	198	381	26	7	28
		22/3	438	161	158	108	0.4	11
		29/3	420	¶	¶	¶	¶	¶
Mean % reduction....			-38.1	-25.8	-42.6	†990%	-86.3	-77.0
2	25 After NaHCO <sub>3</sub>	1/3	747	322	370	7	23	25
		8/3	758	234	453	11	13	47
		15/3	103	8	68	26	0.1	0.1
		22/3	103	7	59	36	1	0
		29/3	215	¶	¶	¶	¶	¶
Mean % reduction...			-81.3	-97.3	-84.6	†244	-96.9	-99.8
3	25 No prestimula- tion	8/3	575	182	295	0	25	73
		15/3	43	2	31	9	0.7	0
		22/3	43	2	27	16	0	0
		29/3	98	¶	¶	¶	¶	¶
				‡				
Mean % reduction....			-89.3	-98.9	-90.2	?	-98.6	-100
4	50 After NaHCO <sub>3</sub>	1/3	818	206	529	10	25	48
		8/3	833	278	406	19	44	86
		15/3	33	2	29	1	0.4	1
		22/3	20	0.04	15	5	0.08	0
		29/3	45	¶	¶	¶	¶	¶
Mean % reduction....			-96.0	-99.5	-95.3	-89.9	-99.3	-99.3
5	50 No prestimula- tion	8/3	898	233	593	23	31	18
		15/3	20	0.5	15	4	0.6	0
		22/3	25	0.1	20	5	0	0
		29/3	53	¶	¶	¶	¶	¶
Mean % reduction....			-96.3	-99.9	-97.0	-80.5	-99.0	-100

\* H.p. *Haemonchus placei*

\*\* Coop. *Cooperia* spp.

@ O.rad. *Oesophagostomum radiatum*

† B.phleb. *Bunostomum phlebotomum*

& Trich. *Trichostrongylus* spp.

¶ Cultures unsuccessful

‡ Dosing took place on 8 March 1961.

### *Trials on Goats*

The results are summarised in Table 2.

Both at 25- and 50 mg/K this drug was highly effective against *Haemonchus contortus*, *Trichostrongylus* spp., *Oesophagostomum columbianum* and *Ostertagia* spp., averaging a 99% efficacy against a slight build-up in the controls.

TABLE 2.

MEAN EGG PER GRAM COUNTS IN GOATS DOSED WITH THIABENDAZOLE

Group	Dose mg./K.	Date	Mean strongyle e.p.g.	Mean differential e.p.g.			
				*H.cont.	**Trich.	@O.col.	†Oster.
Ewes	Controls.....	19/5	1,036	565	206	202	63
		26/5	720	278	165	226	51
		2/6	1,136	556	192	336	52
		9/6	792	361	222	122	87
		15/6	863	343	199	260	61
Mean % reduction.....			+5.9	-0.5	+9.7	+11.7	+15.8
Kids	Controls.....	19/5	925	481	120	296	28
		26/5	787	457	104	226	0
		2/6	760	458	122	146	34
		9/6	800	488	179	114	19
		15/6	533	349	104	61	19
Mean % reduction.....			-18.5	-7.9	+20.5	-59	+71.4
Ewes	25	19/5	963	600	126	220	17
		¶26/5	760	395	161	177	27
		2/6	0	0	0	0	0
		9/6	&0.3	0	0	0	0
		15/6	\$4	>1	1	2	>1
Mean % reduction.....			-99.8	-99.9	-99.8	-99.7	-98.6
Kids	25	19/5	1,800	1,534	91	144	31
		¶26/5	1,167	775	194	163	35
		2/6	0	0	0	0	0
		9/6	0	0	0	0	0
		15/6	0	0	0	0	0
Mean % reduction.....			-100	-100	-100	-100	-100
Ewes	50	19/5	1,087	535	111	380	61
		¶26/5	1,033	426	239	334	34
		2/6	0	0	0	0	0
		9/6	0	0	0	0	0
		15/6	&0.3	0	0	0	0
Mean % reduction.....			-99.99	-100	-100	-100	-100
Kids	50	19/5	1,153	934	18	194	7
		¶26/5	833	496	92	228	17
		2/6	0	0	0	0	0
		9/6	0	0	0	0	0
		15/6	&0.3	0	0	0	0
Mean % reduction.....			-99.99	-100	-100	-100	-100

\* H.cont. *Haemonchus contortus*\*\* Trich. *Trichostrongylus* spp.@ O.col. *Oesophagostomum columbianum*† Oster. *Ostertagia* spp.¶ Dosing took place on  
26 May 1961

&amp; Only one goat infested

\$ Only four ewes infested

## Trial on Horses

Results are summarised in Table 3.

TABLE 3.  
MEAN EGG PER GRAM COUNTS IN HORSES DOSED WITH THIABENDAZOLE  
AT 50 mg/k

Date	Name			
	Vrystaat	Johnson	Effie	Mary
29/3	1,600	2,750	1,900	4,400
1/4	Horses dosed at 50 mg/K.			
7/4	0	0	0	0
13/4	0	0	0	0
1/5	0	66	*N.S.	N.S.

\*No faecal samples available

Strongyle eggs were present at the first examination and disappeared entirely from all horses for at least two weeks after treatment. The worms were not identified on a species basis as cultures were not made. The *pruritus ani*, possibly due to *Oxyuris equi*, disappeared for at least a month after treatment.

## EGG COUNTS AND COLLECTION OF EXPELLED WORMS MATERIALS AND METHODS

Two Shetland foals with symptoms of diarrhoea were examined for worm eggs on the day of dosing and found to be heavily infested. A suspension of thiabendazole was dosed at the rate of 50 mg/K by mixing with the meal fed to each pony. For the following four days the total faecal output of both animals was collected, and placed in separate five gallon drums to which 200 ml. 10% formalin were added. After the removal of the large *Ascaris*, both collections of faeces were washed through sieves (44 mesh to the linear inch). Water was added to the sievings to make up a volume of 20 litres. A rubber tube attached to an air pump was placed in the suspension, and air blown through the suspension, mixing it vigorously.

Aliquots were collected by repeatedly dipping a small beaker (50 ml. capacity) into the bubbling suspension and transferring the fluid to a flask or jar marked at 500 ml. The aliquots were differentially stained with iodine and decolourised with sodium thiosulphate (Whitlock<sup>®</sup>). The reddish-brown stained worms were easily seen against the white background of a large (18" x 12" x 4") enamel tray. Parallel lines drawn with a blue grease-proof pencil on the base of the tray facilitated counting. If the difference in the number of worms recovered in two aliquots varied by more than 20% more aliquots were counted.

The total number of worms present = worms in the aliquots x 40.

Faeces were examined a week after treatment for worm eggs.

## RESULTS

The counts are summarised in Table 4.



TABLE 4

EGG PER GRAM COUNTS AND WORMS EXCRETED IN TWO FOALS TREATED AT 50 mg/K WITH THIABENDAZOLE

Horse	Date	Eggs per Gram		Worms recovered from faeces collected for four days after treatment
		Strongyle	Ascaris	
Santa.....	*14/4 21/4	6,200	1,000	5,020 small strongyles
		1,000	50	1 <i>Strongylus edentatus</i>
		—84%	—95%	4 <i>Parascaris equorum</i>
Victor.....	*14/4 21/4	5,266	833	8,580 small strongyles
		700	0	1 <i>Strongylus vulgaris</i>
		—87%	—100%	5 <i>Parascaris equorum</i>

\*The foals were dosed on 14 April, 1961

The fall in egg counts was accompanied by the excretion of large numbers of worms in the faeces, particularly small strongyles.

#### DISCUSSION

Although Brown *et al*<sup>1</sup> stated that 50 mg/K was the therapeutic dose of thiabendazole, in our tests, however, it was found that half this dose in calves was highly effective against all the nematodes present except *O. radiatum*. Even at dosage rates of 50 mg/K this drug was only 80 to 90% effective against this species, when tested during its period of seasonal build-up.

Both at 25- and 50 mg/K this drug was highly effective against the common nematodes of goats. This confirms the observations made on the same parasites of sheep<sup>7, 6, 3, 4, 2</sup>.

On the nematode parasites of horses its efficacy is equally encouraging. In one trial there was a fall in egg count of 100% lasting for nearly a month after treatment. The recovery of large numbers of small strongyles from the faeces of two treated Shetland ponies confirmed the anthelmintic effect of the drug. The paucity of large strongyles in the faeces can be explained by their long prepatent period and by the fact that the hosts were only 10 months old.

A decided advantage of this drug is its lack of toxicity, mild symptoms only being noted at 20 times the therapeutic dose. A small toxicity trial was carried out on three calves which were dosed at 600-, 800- and 1,000 mg/K respectively. The plasma transaminases in all three calves were normal for the following six days, indicating that no cell damage had occurred in the liver. The calf with the highest dose did, however, lie down for two days. (Lack of staining of mohair in Angora goats was a decided advantage when compared with phenothiazine).

The value of differential egg counts with or without the examination of faeces for expelled worms in determining the efficacy of an anthelmintic is briefly as follows:—

Many drugs are presented to the laboratory for screening. These can be checked fairly rapidly and at a low cost; the range of activity or so-called spectrum and the speed of their action can be determined by this

method. The anthelmintic effect of a drug on immature worms can be checked by carrying out egg counts on treated and control animals once the worms are adult. Finally, in the living animal a differential egg count is the only reliable present method of assessing the worm burden on a genus or species basis.

For these reasons it is felt that differential egg counts are not entirely without value as claimed by Müller<sup>7</sup> but that they serve as a lead to whether slaughter trials are necessary or not.

#### ACKNOWLEDGEMENTS

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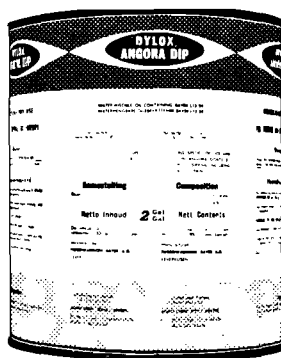
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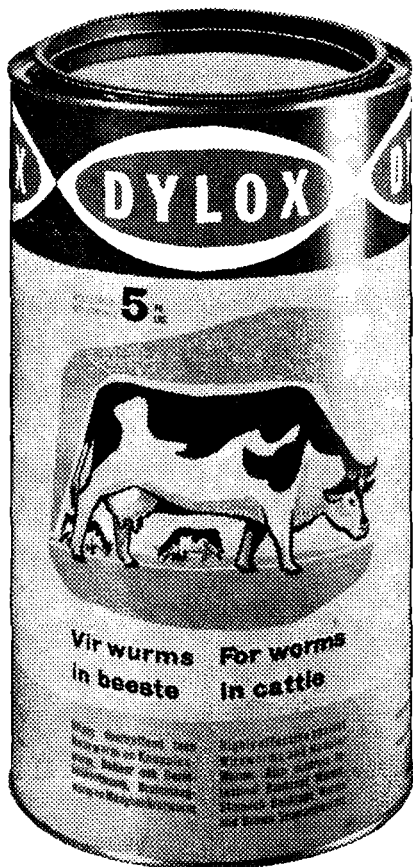
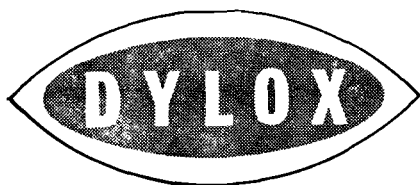
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## STUDIES ON PARAMPHISTOMIASIS

### PART IV MODIFIED CRITICAL AND CONTROLLED ANTHELMINTIC TESTS ON THE CONICAL FLUKE *Paramphistomum microbothrium*

(Parts I II and III will be published in the *Onderstepoort Journal of Veterinary Research* now in Press).

I. G. HORAK

Section of Helminthology,  
Onderstepoort Research Institute,

Received for Publication April 1962)

#### SUMMARY

1. Modified critical and controlled tests using four anthelmintics against *P. microbothrium* are described.
2. Critical tests showed that:—
  - (i) Freon was ineffective.
  - (ii) Single doses of hexachlorophene and Promintic were 43 to 45 % effective.
  - (iii) Multiple doses of these drugs increased their efficacy above 99 %.
  - (iv) Single dose of Lintex at 50 mg/K gave an efficacy greater than 94 % against paramphistomes in the abomasum and small intestine.
  - (v) Even at 75 mg/K a single dose of Lintex was only 15.9 % effective against these worms in the forestomachs.
3. Controlled tests with Lintex showed an efficacy in excess of 96 % against the worms in the abomasum and small intestine.

#### INTRODUCTION

Paramphistomiasis is a disease of sheep and cattle caused by certain genera of conical fluke belonging to the family Paramphistomidae. This condition is characterised by sporadic epidemics of acute parasitic gastro-enteritis and heavy mortality caused by the young flukes in the abomasum and small intestine. Examination *post mortem* reveals generalised emaciation of the carcass, hyperaemia and oedema of the first two metres of the small intestine and numerous immature paramphistomes present in and on the mucosa of the affected intestine<sup>4,9</sup>. Le Roux<sup>9</sup> describes two outbreaks of this disease in sheep in the Orange Free State, caused by the conical fluke *Cotylophoron cotylophorum*; the mortality in flocks of 275 and 300 sheep was approximately 30 and 50 % respectively. Lengy,<sup>8</sup> mentions sporadic epidemics in cattle in Israel and Butler and Yeoman,<sup>4</sup> in Tanganyika, recorded a mortality of 73 of a total of 76 young zebu cattle; the etiological agent in the latter cases was *P. microbothrium*.

Treatment of paramphistomiasis has always been unsatisfactory for although numerous drugs have been tried none have proved consistently effective. Le Roux<sup>9</sup> suggests large doses of carbon tetrachloride, Gretillat and Dumas<sup>6</sup> in Madagascar, used a dispersing agent, sodium alkyl-sulfate, against conical fluke present in the rumen. Bosman, Thorold and Purchase<sup>3</sup> recommend the use of hexachlorophene (2,2'-dihydroxy-3' 5, 5' 6—hexachlorodiphenylmethane) against adult fluke present in the

rumen, and record clinical recovery from acute paramphistomiasis, due to immature flukes in the small intestine, in three sheep after treatment, whereas two untreated controls died. In Tanganyika, Butler and Yeoman<sup>4</sup>, used Neguvon A (dimethyl-hydroxy-trichloro-ethyl-phosphonate 43.2% chloromethyl-coumarindiethyl-thiophosphate 4.3%) with inconclusive results. Reinecke and Swart<sup>11</sup>, were unsuccessful with sodiumflouride, hexylresorcinol, hexachlorethane, tetrachlorethylene and Neguvon A. Their results with hexachlorophene were erratic.

In an attempt to find an effective anthelmintic modified critical and controlled trials were carried out with the following drugs: hexachlorophene and tetrachlorodifluoro-ethane (Freon), which had proved effective against liver fluke<sup>5, 2</sup>. "Promintic"\* (2 — ( $\beta$  — methoxyethyl) pyridine), a highly effective anthelmintic against intestinal nematodes,<sup>10</sup> and N-(2' chlor 4 nitrophenyl)-5-chlorosolicylamid, hereafter referred to by the trade name "Lintex,"\*\* a cestocide with a high degree of efficacy<sup>12</sup>.

## CRITICAL TESTS

### Materials and Methods

(1) The modification of Hall and Foster's<sup>7</sup> critical test as described by Reinecke *et al*<sup>10</sup> was employed.

(2) Adult sheep were infested with single or multiple doses varying from 34,000 to 388,000 metacercariae of *P. microbothrium*. The animals were treated with the anthelmintic when the flukes were 21 to 36 days old, i.e. during the period of clinical symptoms of paramphistomiasis. Twelve sheep were used in this trial, nine receiving a single dose and the other three multiple doses of the anthelmintics.

(3) The anthelmintics used were:—

- (a) Fréon at 150 mg/lb per os to one sheep;
- (b) Hexachlorophene at 20 mg/K per os; one sheep receiving a single dose and another two doses.
- (c) Promintic at 200 mg/K per os or sub-cutaneously; two sheep were given a single dose each, a third two doses and a fourth
- (d) Lintex at 50 mg/K per os to four sheep and 75 mg/K per os to one sheep.

(4) After treatment a faecal collecting bag was attached and replaced daily until the sheep was slaughtered. The faeces in the collecting bags were soaked in water for an hour, the pellets broken manually, the faecal suspension sieved through a 100 mesh sieve and the worms present counted (*vide infra*).

(5) The procedure *post mortem* was examination of the whole alimentary tract from the rumen to the anus for immature flukes. The alimentary tract was tied off with double ligatures at the following places:—

- (a) The rumen, at the oesophagus and the ruminal-omasal juncture.
- (b) The omasum, at the juncture with the abomasum.
- (c) The abomasum, at the pylorus.
- (d) The first three metres of the small intestine.
- (e) The ileum, at the ileo-caecal valve.
- (f) The rectum, at the anus.

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The mesentery was then stripped and the gut divided between the ligatures.

The ruminal ingesta was emptied into a bucket, washed piecemeal through a coarse sieve (6 mesh to the linear inch) held above a 100 mesh sieve. The large grass fibres trapped by the coarse sieve were discarded and the material on the finer sieve retained for examination. The majority of the flukes in the rumen were adherent to its wall which was cut into pieces four inches square, and each of these was carefully washed over a 100 mesh sieve. The rest of the ingesta of the alimentary canal was washed through 100 mesh sieves.

The first three metres of the small intestine, where most of the flukes were present, was thoroughly washed and the mucosa scraped with the thumb-nail to remove all adherent paramphistomes. Where it was impossible to remove these flukes, they were counted *in situ*.

The sievings of the various portions were then transferred into glass vessels and water added to the nearest litre to make a very thin suspension. The procedures of mixing, sampling and counting were similar to those described in anthelmintic trials on nematodes<sup>10</sup>.

(6) The normal habitat in the sheep of immature *P. microbothrium*, is the first three metres of the small intestine, with lesser numbers occurring in the rest of the small intestine and abomasum. In infestations 15 days and older, paramphistomes may also be found in the omasum and rumen. Thus any paramphistomes found distal to the ileo-caecal valve after treatment were regarded as being expelled.

$$\text{Estimation of \% efficacy} = \frac{\text{No. of expelled worms} \times 100}{\text{Total No. of worms}}$$

## RESULTS

These are summarised in Table I.

It will be noted that Freon was ineffective and hexachlorophene and Promintic only 43 to 45% effective after administration of single doses. However, when multiple doses of either of the latter two drugs were given more than 99% of the worms were removed.

A single dose of Lintex, at 50 mg/K, on the other hand, was 94 to 99% effective in four sheep. In one sheep dosed at 75 mg/K the drug was only 15.9% effective. However, 2,838 worms recovered in this sheep, were in the fore-stomachs and only 105 elsewhere.

## DISCUSSION

The trials indicated that Lintex was the most effective drug on those immature worms still in the abomasum and small intestine. Once the parasite migrated to the fore-stomachs the drug was ineffective (cf. sheep 67 Table I).

Only if more than one dose of hexachlorophene or Promintic was administered did the efficacy of these drugs reach a satisfactory level.

Atony of the rumen was however observed after the second dose of hexachlorophene.

Many of the paramphistomes present in the faeces were wholly or partially digested. Only oral suckers, middle-pieces or acetabula were recovered in many cases and these could only really be distinguished microscopically. This made the process of doing worm counts unnecessarily



TABLE I  
CRITICAL TRIALS ON *P. MICROBOTHRIUM*

Sheep No.	Age of infestation in days	Treatment		Time between dosing and slaughter	Worms Recovered		% Efficacy
		Method	No. of doses		In normal habitat	Expelled	
(a) Freon 24	150 mg/lb 24—25	Oral	One	96 hours	23,059	84	.36
(b) Hexachlorophene 20 mg/K							
31	31—32	Oral	One	48 Hours	13,010	9,902	43.2
55	3—24	Oral	Two Day 1 & Day 8	10 days	92	23,238	99.6
(c) Promintic 200 mg/K							
40	24—25	Sub-cut	One	47 hours	13,476	11,215	45.4
631	3—29	Oral	One	6 days	67,991	52,271	43.5
26	14	Sub-cut	Two Day 1 & Day 8	16 days and 21 hours	73	14,747	99.5
68	3—24	Sub-cut	Three Day 1, Days 5 and 8	12 days	25	54,805	99.95
(d) Lintex 50 mg/K							
73	20	Oral	One	48 hours	110	20,833	99.5
46	21	Oral	One	48 hours	310	11,147	97.3
99	22	Oral	One	48 hours	488	7,960	94.2
25	32	Oral	One	70 hours	322	21,513	98.5
(e) Lintex 75 mg/K							
67	36	Oral	One	70 hours	*2,943	558	15.9

\* 2,838 pharamphistomes in fore-stomachs, 105 fluke elsewhere — see text.

laborious as the small (1 to 3 mm long), pink or white worms are readily seen macroscopically against the black background of the container, e.g. photographic developing tray. To assess the efficacy of a drug in critical tests, accurate counts of expelled and retained worms are essential. This method not being entirely satisfactory for the reasons mentioned the tests could only be regarded as pilot trials. They did, however, at low cost and with the use of a few sheep only, indicate the superiority of Lintex when compared with the other three drugs tested. To confirm the efficacy of Lintex controlled tests were necessary.

#### CONTROLLED TESTS

##### *Materials and Methods*

(1) The modified controlled test on immature worms described by Banks and Michel<sup>1</sup> was used.

(2) Six adult sheep were infested with doses of metacercariae varying from 70,000 to 77,000. They were divided into three pairs, each sheep of the pair having as far as possible received a similar dose of metacercariae.

(3) One member of each pair was treated on the 20th, 21st and 22nd day with Lintex at a dosage rate of 50 mg/K. Two days after treatment both sheep of the pair were slaughtered.

(4) The examination *post mortem* was the same as described previously.

(5) The number of paramphistomes present in the controls was taken as the probable number present before treatment and those still present in the treated sheep as flukes unaffected by the drug. The difference between these two figures represents the percentage-efficacy of the drug.

#### RESULTS

These are summarised in Table II.

TABLE II  
CONTROLLED TRIALS ON *P. Microbothrium* USING LINTEX  
AT 50 mg/K

Sheep No.	Number of Metacercariae dosed	Day		Paramphistomes Recovered	Percentage Efficacy
		Treated	Killed		
73	77,000	20	22	110	99.7
75	72,000	Control	22	37,818	
46	72,000	21	23	310	99.0
61	72,000	Control	23	29,791	
99	70,000	22	24	488	96.7
700	70,000	Control	24	14,816	

Lintex was 96 to 99% effective against immature flukes which confirmed the results of the critical tests.

Three sheep viz. Nos. 73, 46 and 99 were included in the critical and controlled tests. Faecal bags were attached after treatment, and expelled worms counted for inclusion in the critical trials. These same sheep were the treated sheep in the controlled tests of this trial. Results are shown in Tables I and II.

### DISCUSSION

It was obvious that anthelmintic trials by the critical method were unsatisfactory. Not only were the worms recovered in the faecal bags partially digested but many worms were completely lost after being killed by the drug.

The above statement is substantiated by comparing the number of worms recovered in the critical and controlled trials. In the former the average number of worms recovered, both from faecal bags and in the sheep *post mortem*, was 13,619 (cf. sheep Nos. 73, 46 and 99 Table I). An average of 27,451 worms was recovered from the controls (cf. sheep Nos. 75, 61 and 700-Table II). The average number of metacercariae dosed to the treated sheep was 73,000 compared with a mean of 71,333 for the control sheep (cf. Table II). Thus it is reasonable to assume that the smaller number of worms recovered from the treated group was due to digestion of worms after treatment.

### CONCLUSIONS

Controlled tests are the method of choice in anthelmintic trials on immature *P. microbothrium* in the abomasum and small intestine. Lin-tex in single doses was highly effective against these worms, whereas multiple doses of hexachlorophene and Promintic were necessary to achieve similar results.

### ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute is thanked for facilities to carry out these experiments and permission to publish the results, and Dr. R. K. Reinecke for help with the manuscript.

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## AN OUTBREAK OF PORCINE BABESIOSIS IN THE NORTHERN TRANSVAAL.

T. W. NAUDE — Toxicology Section, Veterinary Research Institute Onderstepoort.

(Received for publication April 1962)

### SUMMARY

1. An outbreak of porcine babesiosis in the Soutpanberg district of Northern Transvaal due to *Babesia trautmanni* is described. This is the second outbreak recorded in the Republic of South Africa.

2. The strain involved was very mild and deaths would probably not have occurred were it not for the state of malnutrition prevailing at the time of infection.

3. The infection was readily re-isolated the following season by exposing a splenectomized pig to natural tick infestation on the same farm.

### INTRODUCTION

Porcine babesiosis in Africa was first recorded by Trautmann in Tanganyika in 1914. This parasite was subsequently named *Babesia trautmanni* (Knuth and du Toit, 1918).<sup>1</sup>

According to Lawrence and Shone<sup>2</sup> Porcine babesiosis is quite common in Southern Rhodesia. In the Republic of South Africa, however, it has been diagnosed only once, by Canham and Osborne<sup>3</sup> at the Pongola Estates in the South Eastern Transvaal in 1947.

Hurrell<sup>4</sup> diagnosed porcine babesiosis at Gwanda, Southern Rhodesia in December 1957. This is the closest known outbreak to that now described.

### HISTORY

During December 1958 swine mortality was investigated on the farm Fleurfontein in the Soutpanberg district, Northern Transvaal.

Fleurfontein lies on the southern slope of the Zoutpanberg mountains, about six miles north east of Louis Trichardt in a sourveld area with a rainfall of approximately 40 inches per annum, and an altitude of 4,000 feet.

The owner is actually a tree-plantation farmer and his small herd of Large White pigs had consisted of a boar, ten sows and twenty-three weaners and piglets. It was kept as a small and unimportant side-line.

Apart from the swill obtained from Bantu workers porridge, (which was very little indeed) they were occasionally fed on mealie meal and given weeds from the garden and lands. Further they had to fend for themselves by grazing in a nearby vlei and consequently were in very poor condition.

At the time the rhipicephaline tick season was at its height and apart from a severe louse infestation the pigs were also moderately infested with ticks. The following species were identified by Theiler<sup>5</sup>:—

*Rhipicephalus appendiculatis*

*Rhipicephalus simus*

*Rhipicephalus sanguineus*

*Amblyomma hebraeum*

*Ixodes gandumus*

Indigenous forests and plantations in the area, shelter large numbers of African bush pig, *Potamochoerus porcus mashona*, Lonnberg.

#### COURSE OF OUTBREAK

On 5/12/58 a post mortem was performed on a Large White sow that had been dead for more than 24 hours. This was the second sow that had "died suddenly" and the owner suspected foul play.

Post mortem changes were advanced, but hyperaemia and oedema of the lungs with foam in the trachea, tumour splenis, constipation and a slight haemoglobinuria were in evidence.

A spleen smear was taken, which surprisingly showed numerous Babesia-like parasites on examination a few days later. Thomas<sup>6</sup> confirmed this suspicion and Neitz<sup>7</sup> identified the parasite as *Babesia trautmanni*.

After the death of this sow the weaners started dying. Post mortem examinations were carried out on five of them. Except for one (also decomposed) where the pathological changes were identical to that of the sow, only cachexia was seen. Furthermore only this one, out of the five, showed the presence of *Babesia trautmanni*.

Bloodsmears made by Thomas<sup>6</sup> from this group of weaners shortly after the diagnosis was made on the sow, showed that only a few harboured odd parasites.

The owner was advised to spray the pigs regularly with an acaricide, to inject "Phenamidine" (May and Baker) subcutaneously, to keep them confined to the pens, to avoid further tick infestation and above all to feed them properly.

In spite of this practically all the young pigs died.

#### TRANSMISSION EXPERIMENTS

During this outbreak citrated blood was collected from three pigs and dispatched to Onderstepoort where the infection was successfully transmitted to experimental pigs by Neitz<sup>7</sup>. It was however of such a mild nature — even in splenectomized animals, that it was lost after a few passages.

As this strain was required at Onderstepoort for research purposes, an attempt was made to re-isolate the infection from the farm during the summer of 1959 — 60.

A young pig, obtained from an area where no trouble with porcine babesiosis was experienced, was splenectomized. Three months later it was exposed to natural infection in a small paddock on Fleurfontein which was situated in the vlei where the pigs had grazed the previous season.

Exposure took place at the height of the rhipicephaline tick season and at approximately the same time that the others had taken ill, the previous season.

The pig's temperature and blood-smears were taken daily as from the seventh day. The latter were completely negative up to the fifteenth day, when a single parasite was noticed. The temperature was on the increase at this stage but still remained within the normal range on this day. On the sixteenth day however, the temperature had risen to 106°F. and parasites were easily found in the smears.

Blood was again dispatched to Onderstepoort at this stage and the infection successfully transmitted to experimental animals by Neitz<sup>7</sup>.

His previous observations as to the mildness of this particular strain was confirmed.

The experimental pig at Louis Trichardt showed no untoward effects except for the fever, a pale skin and slightly anaemic mucous membranes and the fact that it tired easily on being handled. Even its appetite was never seriously impaired.

On the eleventh day of the reaction the blood-smears were still positive although the fever had abated. At this stage the pig was treated with 40% "Phenamidine" (May and Baker) followed by 5% "Pirevan" (Evans) two days later.

It made an uneventful recovery and when slaughtered by the local butcher six weeks later, was in excellent condition.

#### DISCUSSION

The mildness of the strain encountered here was an outstanding feature. Had it not been for the complicating factors of severe malnutrition and bad management, the outbreak would probably have passed unnoticed.

This is in contradistinction to the virulence of the strain described by Lawrence and Shone<sup>2</sup> in Southern Rhodesia.

Shone and Phillips<sup>8</sup> proved that the African bush pig, *Potamochoerus porcus maschona*, Lonnberg, which occurs over large areas of Southern Africa, can serve as carrier of *Babesia trautmanni*.

This suggests the possibility that the disease can be expected over large areas of the Republic where the vectors and reservoirs occur. It will however only be observed where domestic pigs in such areas are allowed to become infested with ticks. Furthermore, if the strain is as mild as that described above, it will easily be missed if blood or spleen smears are not examined.

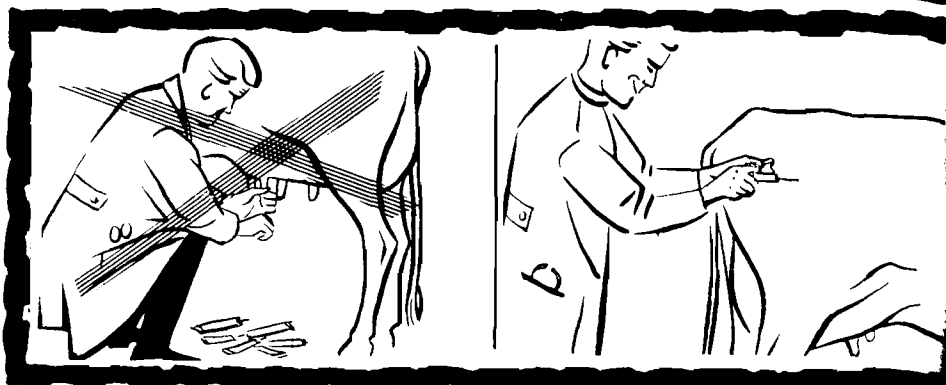
#### ACKNOWLEDGEMENTS

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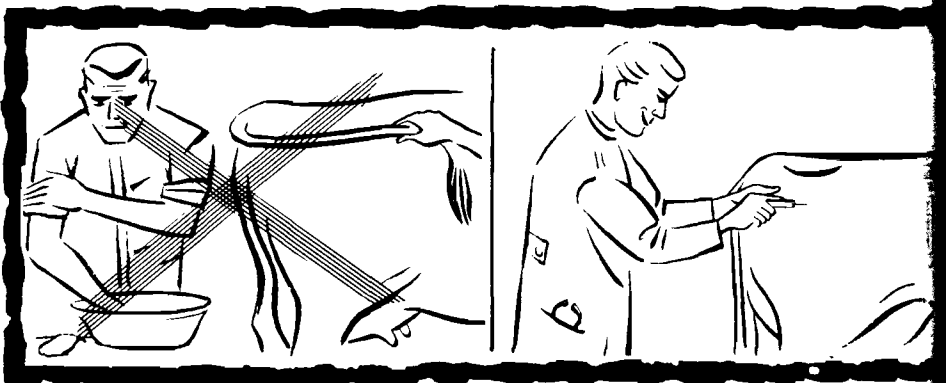
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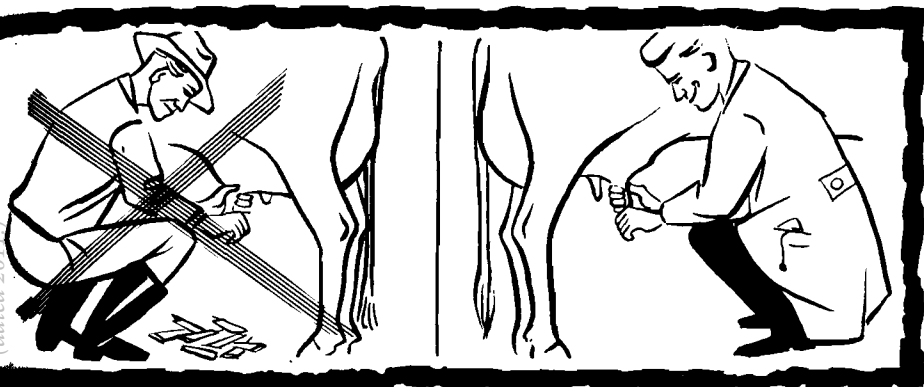
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## SEROLOGICAL IDENTIFICATION OF MEAT FROM DIFFERENT SPECIES BY THE AGAR GEL DIFFUSION METHOD

L. W. VAN DEN HEEVER — Senior Lecturer, Veterinary Food Hygiene and Public Health, Faculty of Veterinary Science Onderstepoort.

(Received for Publication May 1962)

### SUMMARY

The application of the agar gel diffusion techniques to the identification of meats from sheep, cattle and horses is described and details of the precipitin reaction lines obtained are provided and illustrated with photographic plates. Successful application of the technique to biltong (air-dried salted strips of meat) is described.

### INTRODUCTION

In South Africa the commonly known and accepted food animals are the bovine, sheep, goat and pig. Poultry is also accepted as a source of "white" meat. Rabbits, hares and game are also consumed by a section of the population, as is meat from equine animals. By and large, however, it is safe to state that the average person who buys meat from a butcher expects firstly to find only pork, beef and mutton in the shop, and secondly expects to receive the type of meat he orders. Substitution would be an offence against the purchaser in terms of the Food, Drugs and Disinfections Act of 1929.

Local authority by-laws in most instances demand that equines be slaughtered separately from other animals, and their meat shall not be sold from ordinary butcher shops. Equine or horse meat is also usually stamped as such for the purposes of identification.

There seems, therefore, little doubt that in terms of both common and enacted law, substitution of meat from one type of animal for that of another would not be tolerated.

Whereas the characteristic appearance and taste of beef, pork and mutton respectively, would make substitution unlikely, there is also little real objection to such substitution except possibly on religious grounds in the case of pork. On the other hand, there is a very real aesthetic objection by most members of the community to the consumption of horse meat.

The difference in cost might also create the temptation for certain unscrupulous persons in the trade to sell horse meat for beef, either as fresh meat, as biltong, or as mince or sausage meat.

By the use of the precipitin test, using antisera prepared against certain animal species and extracts of the meat under test, it is possible to identify the animal species concerned, provided the protein has not been denatured by subjecting the meat to heat exceeding 80°C. (Tammemagi<sup>1</sup>). Due to the presence of common antigenic factors, e.g. between

ox and sheep and to some extent between horse and bovine, and also due to the care necessary to obtain a satisfactory and undisturbed interface between antigen and anti-serum in the tube, this test presents practical difficulties in routine application and requires care to ensure reliable interpretation.

Various workers, notably Weitz<sup>2</sup> have shown that the problems of non-specific precipitin reactions are readily overcome by absorbing the heterologous antibodies from an anti-serum with heterologous serum, thereby leaving the specific homologous antibodies intact and unreduced in titre. Pinto<sup>3</sup> has put this to practical application in distinguishing closely related spp. such as the ox, buffalo, goat and deer.

However, these procedures further complicate the routine testing procedure required to screen and test meat from different species.

The agar gel diffusion techniques described by Oudin<sup>4</sup> and Auchterlonie<sup>5</sup>, now in common use in serological procedures, suggested a means of simplifying the test. Accordingly the following experiments were undertaken:

## MATERIALS AND METHODS

1. *Antisera* were produced against cattle, pigs, sheep and horses using potash alum-precipitation sera as antigens and the technique described by Proom<sup>6</sup>.

*Antigen:* Meat extracts were produced by storing and shaking finely cut meat in sterile normal saline of equal weight at 4°C as recommended by Ginsberg<sup>7</sup>. Eventual clarification was obtained by filtration.

*Diffusion plates* were prepared by pouring sterile 1% plain (non-nutrient) agar into standard Petri-dishes, positioning a perspex mould and allowing to cool and set. The mould produced "cups" or hollows in the agar 15 m.m. apart, 8 m.m. deep, 13 m.m. in diameter. The hollows did not extend to the bottom of the agar layer and so did not require sealing off to prevent under-run. Each cup had a capacity of about 20 drops, and each cup was half-filled with antigen or anti-serum. The plates were held at 40°C or at room temperature for up to six days and read daily.

## EXPERIMENTAL TESTS

1. *Using anti-horse serum* in the centre cup and extracts of beef, mutton and horse flesh in the surrounding cups: *After 2 days* at either temperature one heavy and two narrower but definite lines of precipitation, were to be seen between the anti-horse serum and the horse meat extract. No clear reaction was detectable between the anti-horse serum and the beef or mutton extracts.

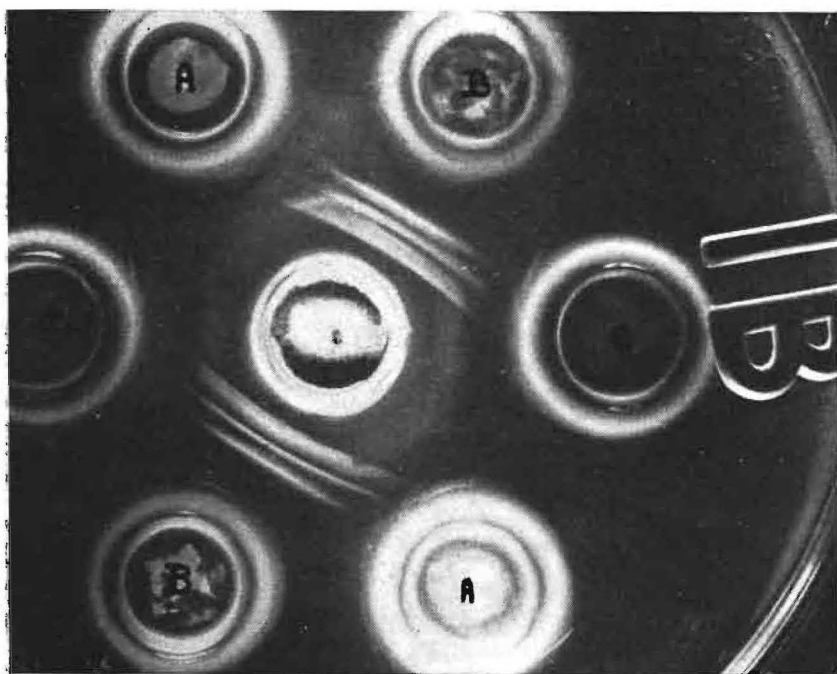
After the 3rd day the incubated plates started showing a more definitive broad line between the antiserum and the beef and mutton extracts. This line intersected the horse-meat-anti-horse serum lines. The reaction in the plates held at room temperature remain poorly defined. (See Plate I).

2. *Using anti-beef serum* in the centre cup, the above test was repeated. Two narrower and one broad reaction line developed between the anti-serum and the beef and mutton extracts by the second day, and these lines all connected at their junction between the two cups, showing that the precipitins were the same in beef and mutton.

On the 3rd and 4th day a clear reaction line which intersected the ruminant lines, developed between the anti-beef serum and the horse flesh extract. The two temperatures of incubation had little effect on these plates (See Plate II).

3. *Using anti-mutton serum*, the experiment was again repeated. Up to the 5th day no reaction developed between the anti-mutton serum and the horse flesh extract.

A single broad band developed between the antiserum and each of the cups containing beef and mutton extracts respectively. These bands intersected where they met. On the 3rd day the incubated plates showed a clear narrow line of specific reaction between the anti-mutton serum and the mutton extract.



*Plate I: 4th day at 40°C. incubation*

Centre: Anti-horse serum

A: mutton extract

B: horse-meat extract

C: beef extract

TABLE I.

Extract of	Horse meat	*	Beef	*	Mutton
Antiserum— Horse.....	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="background-color: black; height: 10px;"></div>	†  †	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="background-color: black; height: 10px;"></div>	1  1  †	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="background-color: black; height: 10px;"></div>
Beef.....	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div>	†	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="background-color: black; height: 10px;"></div>	1  1  †	<div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="border-bottom: 1px solid black; height: 10px; margin-bottom: 5px;"></div> <div style="background-color: black; height: 10px;"></div>
Mutton.....	0		<div style="background-color: black; height: 10px;"></div>	†	<div style="background-color: black; height: 10px;"></div>

\* Relative positions of reaction lines at junctions.

† = intersected and different.

1 = linked up and apparently identical.

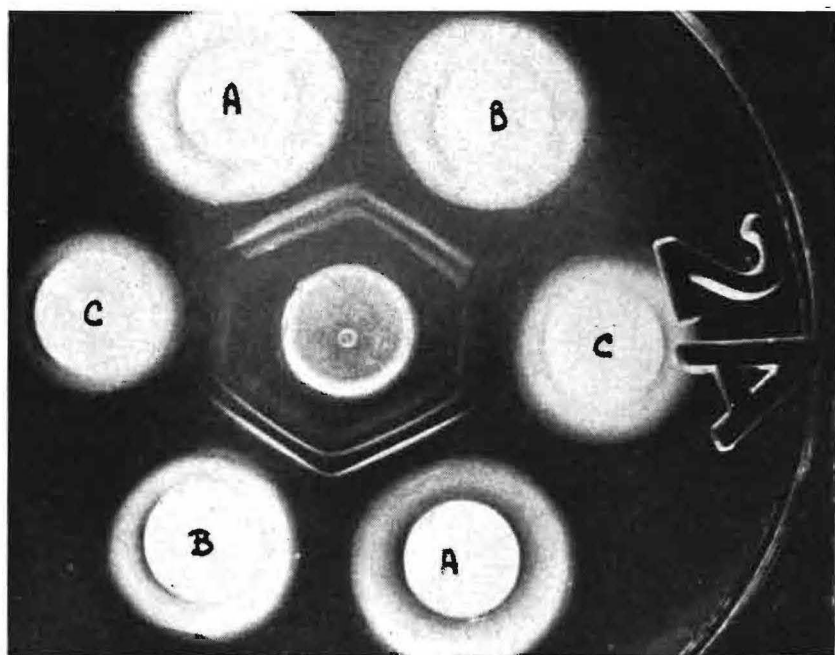


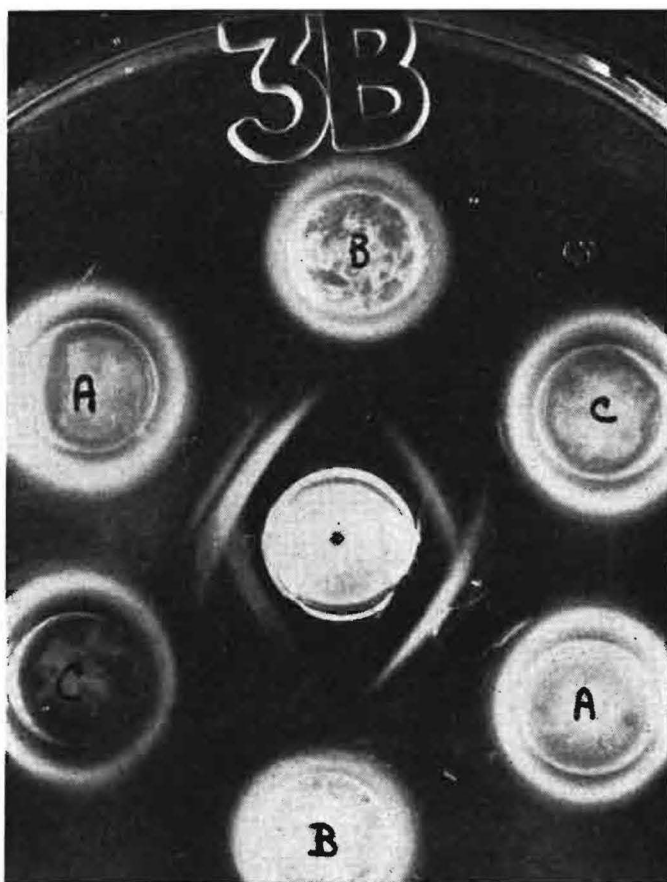
PLATE II: 3rd day at room temperature:

Centre: Anti-bovine serum

A: beef extract

B: mutton extract

C: horse-meat extract.



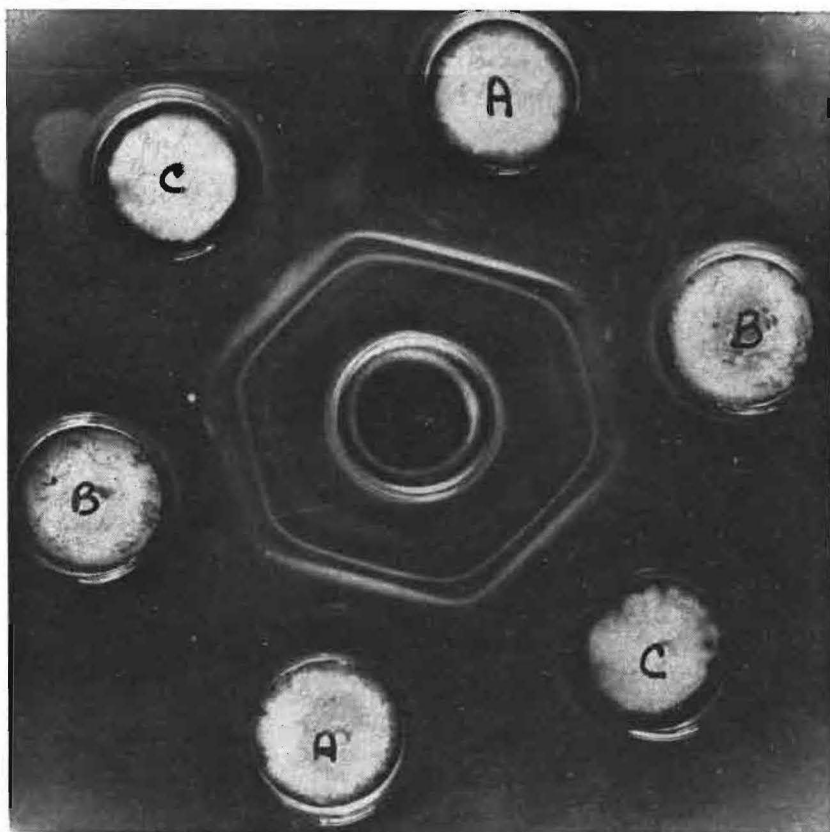
*PLATE III: 3rd day at 40°C:*

Centre: Anti-sheep serum  
 A: mutton extract  
 B: horse-meat extract  
 C: beef extract.

#### SUMMARY OF TEST RESULTS.

4. *Application to Biltong:* Strips of sealed air-dried meat, known to South Africans as "biltong", made from horse meat and beef, were tested. Fresh and old biltong with salt-in-water phase percentages of 22.8 and 41 respectively, was used. Pieces of the biltong were cut into fine pieces and extracted in sterile distilled water.

The tests were carried out as already described . Plate IV shows that the lines of reaction obtained with old biltong were not as clearly defined as those obtained with fresh biltong, the latter again being less clear than those obtained with meat. However, no difficulty was experienced in distinguishing beef from horse meat biltong, when known antigens were used as controls.



*PLATE IV: 3 days incubation at 40°C*

Centre: Anti-beef serum  
A: Fresh beef extract  
B: Old beef biltong extract.  
C: Fresh beef biltong extract.

#### CONCLUSIONS

The agar gel diffusion method appears to be well suited to the serological tests utilised in the distinction of meats from cattle, horses and sheep. Incubation of the plates at 40°C and daily readings up to the 5th

day results in clear readings being obtained even in respect of late reactions. Specific reaction lines are obtained when using anti-sera and homologous meat extracts, and absorption of heterologous antibodies appears unnecessary in dealing with horses, cattle and sheep. The method is suitable for application to "biltong" even in its very dried state. The technique was also of value in the assessment of the degree of success with which non-specific antisera-fractions had been removed by the absorption-technique using heterologous sera of different related species.

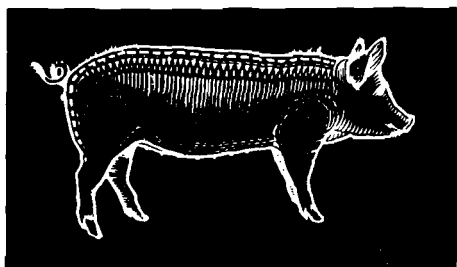
#### ACKNOWLEDGEMENTS

1. To MR. F. VISSER for carrying out the tests.
2. To DR. R. A. ALEXANDER for suggestions and advice.
3. To the Chief, Veterinary Research Institute for assistance, material and permission to publish.
4. MR. DE BRUIN for the photographs.

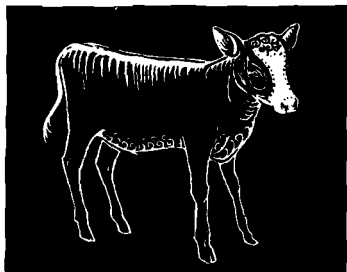
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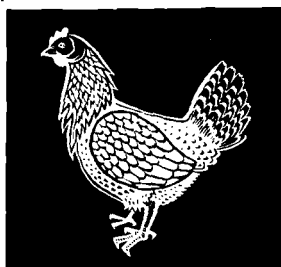
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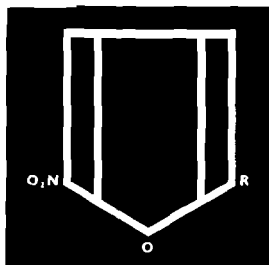


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**PENICILLIN TREATMENT OF FOOT CANCER OF THE HORSE**

J. H. MASON

— The South African Institute for Medical Research, Johannesburg.

Received for publication April 1962

**SUMMARY**

Canker of the feet of five horses was cured in from two to six weeks by the injection of large doses of procaine penicillin intramuscularly, plus minor surgery and the application of a penicillin-containing medicament locally. A mildly infected frog was cured in ten days by the daily local application of a penicillin-containing base.

**INTRODUCTION**

In 1946, I<sup>1</sup> showed that canker could often be cured by minor surgery and the local application of a paste containing glycerine, zinc oxide, kaolin and boric acid, and in 1951 I<sup>2</sup> found that the intramuscular injection of large doses of penicillin had a marked beneficial effect. Because penicillin was then expensive and a complete cure was not brought about in a few days, I stopped using it. During the past few years, procaine penicillin has been reasonably cheap and I have used it to treat the disease in six horses with very satisfactory results. Thirteen feet were infected, all four in each of two horses, both front in one and one front in each of the others.

**TREATMENT**

The dosage depended on the severity of the disease. Into a horse with a badly infected foot, I injected three million units of procaine penicillin intramuscularly daily, until improvement was obvious. Thereafter the injections were given every second day and, towards the end of the course of treatment, every third day. On the other hand a mildly infected frog responded favourably to *local* treatment with a penicillin-containing base in seven days and was healthy in ten days. The infected feet were inspected daily, diseased horn removed, a penicillin-containing medicament applied on cotton wool, and the hoof encased in a leather boot.

In the accompanying table, a summary is given of the treatment of the six horses.

**TABLE**

Horse	Site and Degree of Infection	Treatment		Result
		Parenteral*	Local	
118	1 frog, severe.....	42	C.w.	Cure in 3 weeks**
267	4 frogs, severe.....	90	Pen	Cure in 6 weeks
374	4 frogs, severe.....	84	Pen	Cure in 5 weeks
172	1 frog, severe.....	57	Pen	Cure in 4 weeks
404	2 frogs, mild.....	27	Pen	Cure in 3 weeks
408	1 frog, mild.....	0	Pen	Cure in 10 days**

\* Mega units of penicillin, intramuscularly

\*\* See Text

C.w. — Dry cotton wool

Pen — Penicillin in aluminium stearate — arachis oil base

The treatment of two horses is worthy of special mention. One, number 118 with one frog severely infected, received 130 grams of streptomycin sulphate intramuscularly over a period of four weeks; diseased horn was removed daily and absorbent cotton wool only, used as a dressing. At the end of four weeks, the frog was cleaner and firmer but definitely not healthy. At this stage, a change was made to penicillin and, over a period of three weeks, 42 million units were injected, and cotton wool only was applied locally. Cure was complete after three weeks. The mildly infected frog of another horse, number 408, was dressed daily for ten days with aluminium stearate in arachis oil. The frog was then cleaner and drier, an effect that could have been expected from daily paring and cleaning alone, but it was not normal. Complete cure was obtained in a further ten days by daily treatment with penicillin in an aluminium stearate-arachis oil base.

### DISCUSSION

There is a dearth of articles on the treatment of canker by antibiotics in English and American literature but Mr. M. Crawford, Director of the Commonwealth Bureau of Animal Health was able to find four for me in Continental journals. Erkurt<sup>3</sup> found that daily intramuscular doses of two million units of procaine penicillin for three to four weeks, combined with the local application of a sulphonamide dusting powder, cured canker without surgical interference, and Schultes<sup>4</sup> used a depot penicillin in one horse with good results. Kakanovic & Maksimovic<sup>5</sup> found penicillin treatment to be more expensive and not markedly superior to other methods, and Banic & Skušek<sup>6</sup> used aureomycin with good results but found that relapses occurred in 10.9% of cases.

Although, in my opinion, there is no doubt that treatment with penicillin cures canker, further work is called for to find the smallest effective dose. Possibly the injection of three million units every second or third day, instead of daily, would be sufficient. Again, broad spectrum antibiotics or a combination of penicillin and streptomycin might give quicker results. Although parenteral antibiotic therapy would appear to be of first importance, local treatment — the frequent removal of diseased horn and the application of an antiseptic non-irritating ointment or antibiotic containing medicament — is necessary.

### ACKNOWLEDGEMENT

I have pleasure in thanking Mr. R. Brown of Glaxo-Allenburys (S.A.) (Pty.) Ltd. for supplying to me the aluminium stearate in arachis oil, and penicillin in this base

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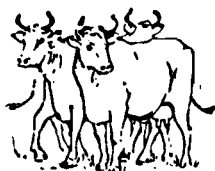
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## COMMENT ON ARTICLE: PENCILLIN TREATMENT OF FOOT CANKER OF THE HORSE BY J. H. MASON

C. F. B. HOFMEYR. — Department of Surgery: Faculty of Veterinary  
Science Onderstepoort.

As Dr. Mason indicates, there is a dearth of articles in recent veterinary literature on newer treatments of hoof canker. His article is therefore of particular interest and importance and will put new heart into those who have had frustrating experiences with this condition. On being informed of Dr. Mason's work in this regard, I used his methods in a case, mention of which may be of interest. A gelding was presented with advanced canker of all four feet, involving the whole of the hoofs and spreading to the soles. With conventional treatment it was thought that if recovery could be secured, this would take up to six months, if not much longer. Only loose horn was removed, the feet dusted with sulphanilamide powder, then enveloped in Unna's Sticky Paste bandages, and encased in leather boots. Six mega units of penicillin and three grams streptomycin were injected intramuscularly. This treatment was carried out daily for just over 2½ weeks. After a week there was marked improvement and after three weeks all the feet were dry. The local treatment of the feet was continued altogether for a month. The gelding was kept under observation for six weeks after the feet were dry, and then discharged.

A great deal requires to be clarified of the predisposing (or constitutional) causes of hoof canker. It would be interesting if Dr. Mason would record in future any tendency towards recurrence. He has eliminated the active (presumably microbial) cause during his treatment. The question arises whether horses treated by his methods, would be more prone to relapse than those which recovered after older methods of treatment, particularly those receiving what may be called vaguely "systemic build up" as well.



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## **THE PREPARATION OF TISSUE VACCINES FOR USE IN THE TREATMENT AND PREVENTION OF BOVINE PAPPILOMATOSIS**

JOHN MARE

— Virus Section  
Veterinary Research Institute,  
ONDERSTEEPOORT

(Reproduction of a Newsletter prepared by the Division of Veterinary Services for its personnel)

It has become evident in recent years that the disease bovine papillomatosis, or warts, can be a very real problem in areas where the disease has become endemic. The use of expensive imported vaccines, as also the host of "quack remedies" available, has done little to improve the situation. On the other hand, it has been proved conclusively that with the use of autogenous vaccines, very good results may be expected.

The procedure for wart vaccine preparation as set out below, is based on the proved method as described by Pearson and co-workers in the *Vet. Record* 70, pp. 971 (1958). It is desirable that vaccine should be prepared from fresh warts obtained from several animals on the farm where the vaccine is to be used. (Should more than one type of wart occur, material from each type should be included.)

*Materials:* Fresh wart material, sterile alundum (or sterilised sand), pestle and mortar (sterile), 50% glycerol saline and butter muslin.

*Method:* plus-minus 5 gm. of fresh growth is cut fine with scissors and then ground with the pestle and mortar; alundum or sterile sand facilitates grinding. This finely ground wart material is then added to 50 ml. glycerol saline, mixed thoroughly, and allowed to stand for 10 minutes to allow coarse particles and sand to settle. Decant the supernatant fluid, and bottle. Leave at room temperature for 2 days, filter through fine butter muslin, and the "vaccine" is ready for use.

*Dosage:* This "vaccine" may be used as a preventative measure or for treatment. The initial dosage of 10 cc subcut. should be followed by a booster dose of 15 cc subcut. plus-minus 2 weeks later.

The recovery following administration of autogenous vaccine prepared as described, was 86% in the experiments done by Pearson and co-workers.

Teat wart vaccines should be prepared separately, but results with these vaccines have been disappointing.

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# A NOTE ON ORAL INFECTION IN THE KANGAROO (*MACROPUS RUFUS*) AND WALLABY (*MACROPUS RUFICOLLIS*) (DESMAREST).

D. H. G. IRWIN

C. CAMERON

Department of Surgery Faculty of  
Veterinary ScienceSection of Bacteriology Division  
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ONDERSTEEPOORT

Received for publication May 1962

The anatomy of the mouth in kangaroos and wallabies favours accumulation of feed there, which enables infection to gain a foothold when the mucosa is damaged. Fox<sup>1</sup> recognised this in captive marsupials and Watts<sup>2</sup> states that this train of events occurs even in nature. Sharp-spined grass seeds, hard fodder and gritty material in the ration, predispose to feed accumulation and breach of the buccal mucosa, resulting in oral infection. Neither of these authors describe conjunctivitis as a sequel to oral infection.

The case histories of nine kangaroos and wallabies are summarized in a table. These animals were from the National Zoological Gardens, Pretoria, and were seen in the clinic of the Faculty of Veterinary Science, Onderstepoort in the autumn of 1961.

Although the number of cases reported is small, it seems that in cases of conjunctivitis among these species, oral examinations should be undertaken. This is not very easy because of the short distance the jaws open. However, thiopentone sodium anaesthesia ( $\pm 0.5$  gm intravenously) is well tolerated<sup>2</sup>, and facilitates examination.

Case	Species	Oral Pathology	Conjunctivitis	Outcome
1	Kangaroo	Alveolar periostitis upper molars	Present	Died 6 weeks later
2	Wallaby	Alveolar periostitis of premolars and first molar in right maxilla, purulent sinusitis	Present, plus panophthalmitis dextra	Died on arrival
3	Kangaroo	None	Present	Recovered
4	Kangaroo	Alveolar periostitis one lower incisor	Present	Recovered
5	Kangaroo	None	Present	Recovered
6	Kangaroo	Gross purulent alveolar periostitis, osteitis and fracture of left mandible	Absent	Recovered
7	Wallaby	Alveolar periostitis of lower incisors and intermandibular abscess	Absent	Died 3 days later
8	Wallaby	Purulent alveolar periostitis, haemorrhagic rhinitis	Conjunctivitis and ophthalmitis	Died 4 days later
9	Wallaby	Necrotic glossitis	Absent	Died 2 days later

Case 6 is interesting, in that lower jaw infections are usually more serious than maxillary infections<sup>2</sup>.

#### BACTERIOLOGICAL ASPECTS

Fox<sup>1</sup> incriminated *Nocardia* species and other members of the family Actinomycetaceae as being largely, if not primarily, responsible for oral infection in the kangaroo and wallaby. Although these organisms were specially sought, they were not found by culture nor recognised in direct smears from specimens submitted.

The following organisms were isolated:—

Cases 3, 4 and 5:

*Streptococcus faecalis*.

*Streptococci viridans*.

*Staphylococcus aureus* (var. *albus*) (koagulase negative).

*Staphylococcus aureus* (koagulase positive).

*Neisseria sicca*.

*Neisseria cariae*.

Small gram positive pleomorphic rods were seen in some smears, but no such organisms could be isolated on culture.

Case 6:

*Corynebacterium striatum* (pure culture).

Case 9:

Specimen: Tongue

Isolate: *viridans Streptococci*

Most of these organisms may occur normally in the nasal and oral cavities of domestic animals<sup>3</sup>.) Therefore, no significance can be attached to any of the above isolates without further investigation and information. These organisms should only be considered to be secondary parasites invading tissues already damaged by some other noxis.

It is, however, interesting to note that *Streptococci viridans* were repeatedly isolated and usually also the predominant organism present. *Streptococci viridans* is very closely related to *Streptococcus bovis*.

#### SUMMARY AND CONCLUSIONS

1. Oral infection is common in kangaroos and wallabies.
2. Conjunctivitis sometimes occurs concurrently with oral infection on the same side.
3. It is suggested that there may be a relationship between the two.

#### ACKNOWLEDGEMENTS

The Chief, Veterinary Research Institute is thanked for kindly allowing publication of this note.

Our thanks are due to Drs. Koornhof and Schrire of the South African Institute for Medical Research who kindly assisted in the identification of the *Neisseria* sp.

We also wish to thank Dr. R. Bothma who kindly referred these cases to the Faculty Clinic, Onderstepoort, and to Mr. Brand, Assistant Director of the National Zoological Gardens, Pretoria, who found the reference to Fox, and contacted the Adelaide Zoo.

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## OSTEODYSTROPHY IN KITTENS

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Received for publication — 10 April, 1962

### INTRODUCTION

This condition is met with almost daily in practice in Salisbury, Southern Rhodesia. The majority of cases noted have been in Siamese, but other mixed breeds are often presented for examination and in one case the kitten suffering from osteodystrophy was a pedigree Chinchilla. One such case was sent over to the Royal College after destruction, where the Histological Division very kindly performed a detailed post mortem, confirming that was the typical Osteodystrophy described by other workers.

Unfortunately the condition has acquired the misleading and frightening nickname of "cat polio", among the public, and the first task upon making a diagnosis is to set the owners minds at rest and instill upon them that this term is only a nickname which unfortunately has come to stay, and that it has no connection with the condition of poliomyelitis.

### SYMPTOMS

The condition is first noticed from about 8 — 15 weeks of age. The symptoms in most cases are very similar, but the degree of paralysis involved varies a great deal. There is usually pain on movement of the hindlegs and a degree of paraplegia. Pain is also marked over the lumbo-sacral area. Abdominal distention is present often with a cystitis and a rise in temperature. Quite often the faeces are black and gelatinous in nature. The kitten has difficulty in retracting the claws, its movements are slow and it cries when lifted or handled. Affected kittens differ greatly from the normal boisterous kitten at that age. Owners usually approach the veterinarian thinking the cat has injured or even broken a leg, or perhaps bruised a limb in some accident.

The post mortem of the specimen sent over to the Royal College revealed typical findings of a long standing calcium deficiency. The scapulae were misshapen and paper-thin, and there was a severe scoliosis in the lumbo-sacral region. Radiography revealed severe rarefaction in all parts of the skeleton, but there was no sign of rickets. Typical impacted fractures of the bodies of the lumbar vertebrae were present. In short, the entire picture was one of severe osteoporosis. Furthermore there was an acute atrophy of the thyroid gland, suggesting some long-term iodine deficiency or a continuous intake of some anti-thyroid substance. The direct cause of death was due to a marked septicaemia with gross infection of the heart, lungs and alimentary canal. Scott and Scott, who were kind enough to conduct the post mortem, commented that when depleted of their calcium reserves the kittens resistance to infections drop severely, hence the septicaemia is only the terminal disaster in this condition. The histopathology of the thyroid gland is interesting and most workers believe this to be the prime cause in the symptoms that develop later.

## AETIOLOGY

Here opinions vary a good deal. Scott and Scott (Royal College Histology Division) feel that the real cause is really environmental in origin, and suggest a long-term intake of anti-thyroid substances. Features of the mineral content of the soil, water or vegetation, may be relevant e.g. trace elements such as cobalt, iodine, or organic anti-thyroid substances. Barton (*American Journal of Veterinary Medicine*) states that the condition occurs in defined scattered geographical areas and that the condition varies in degree from year to year. It is interesting to note that this condition, so very common in Salisbury, hardly occurs in other parts of the country. Another view on the aetiology (Henderson and Keywood) is that diet plays the all important role and is by far the most significant factor. A similar syndrome has been produced in kittens by feeding an entirely meat free diet after weaning, and the most severely affected kittens were those fed no milk but only water, after weaning. The atrophy of the thyroid gland comes into the picture but its significance is rather obscure. This lack of thyroid development has been noted at birth and in aborted foetuses, hence it is reasonable to conclude that these factors occur *in utero*, and are perhaps aggravated later by the incorrect diet. These two workers can furnish no positive evidence of an hereditary factor being involved.

Barton also records evidence of disorders of the endocrine glands, such a hyperfunction of the pituitary and adrenals, and later the parathyroid. He declares the origin to be partially related to a congenital condition that may be hereditary and is aggravated by a faulty diet.

## TREATMENT

Treatment before severe endocrine changes have occurred, can be successful (Barton, Scott and Scott, Henderson and Keywood). Coop writes that he cured 26 out of 28 cases by confining them and using hormone therapy. Many mild cases will recover spontaneously while many go unnoticed. Usually cats attaining the age of 12 months, when the bones solidify, can then lead a normal healthy life. Workers in America have used sexual hormones, added milk to the diet and Vitamins A, D, B12 and B1, plus synthetic pituitary extract by injection along with antibiotics to lessen the chances of a secondary infection.

In practice in Salisbury I have found that injections of Calcium and Vitamin A, plus supplementing the diet with Calcium (tablets or powder) brings about very rapid and gratifying results. Attention is always paid to the diet which we try to vary as much as possible. Milk is essential in the diet. The lameness gradually wears off and the distended abdomen goes back to normal. The marked tenderness all over the cat's body disappears. The affected female, if allowed to become pregnant, usually has great difficulty in producing kittens and one has to resort to caesarian section. Hence owners are advised to have these cases spayed to prevent such complications. In this respect the breeder is faced with problems but for the ordinary owner, treatment in the majority of cases proves effective and the kitten can grow up to prove a reasonable pet.

From first hand clinical observations of cases presented to me in Salisbury, together with the low incidence of the condition elsewhere in Southern Rhodesia, it would appear that environment especially plays a large role. Investigation into the existence of such anti-thyroid substances described by Scott and Scott, is certainly called for in this country.

Personally I doubt if a breed susceptibility exists. Siamese are generally more susceptible but this I think is due to their fussy eating habits and a certain degree of inbreeding which has probably lowered resistance.

## **A CASE OF SCHISTOSOMIA REFLEXUM IN A COW**

O. H. J. MEHNERT — State Veterinarian,  
GREYTOWN

### **SUMMARY**

A case of *Schistosomia reflexum* in a cow, and the removal of the foetus by Caesarean operation is described. Preference to this was given as there was no possibility of performing an embryotomy.

### **HISTORY**

On the afternoon of November 9th 1961, I was called out to a farm in the Kranskop district. The anamnesis revealed that a Friesland cow had been in labour from the morning, and that part of the intestines were protruding from the vagina.

I found the cow in a recumbent position in the paddock, with the small intestines of the calf protruding from the vagina and spread on the grass.

By careful vaginal examination, the liver of the calf could be located in the pelvic cavity. It was removed by twisting movements of the hand, after removal of the intestines with scissors. By further examination the inner parts of the ribs and part of the spine of the calf could be made out. No head could be found and the legs of the calf were thought to be further anterior and pointing in a ventral direction. The foetus proved to be indurated and rigid and could not be moved, as the wall of the womb was enclosing it very tightly.

There was no possibility of directing a wire saw round the foetus and the owner was informed that the only way of removing the deformed foetus was by means of a Caesarean operation. As the cow was a good milker bearing her second calf, and her general condition satisfactory, he agreed to the operation.

### **OPERATION TECHNIQUE**

The cow was taken into a shed and its left flank prepared for the operation: 10 ml. 2% Planocaine being injected into the epidural space to prevent the cow from pressing out the rumen, when operating on her in a standing position. The skin and the muscle layers were infiltrated with 90 ml. 2% Planocaine. The skin and M. obl. abdom. ext. and int. were incised. When cutting through the M. rectus abdom. and the peritoneum, the cow went down, and could not get up again. The fore legs and right hind leg were tied, and the left hind leg pulled back and secured. By now the light was failing and we had to make use of the lights of a tractor.

Having opened the abdominal cavity, it was extremely difficult to bring the tip of the uterus into the operation wound owing to its shortness and conical form. One foot of the calf was gripped through the uterus, which was then incised to a length of 10 inches. Two forceps were fixed

to the uterus wall. Two sterile calving ropes were tied to two of the four feet, which were lying close together. Even now it was impossible to extract the foetus, and the operation wound had to be extended in a dorsal direction. Two men had to apply considerable force to remove the deformed calf and during this operation the uterus received a tear of about 4 inches in length, thus extending the wound to a length of about 14 inches.

The afterbirth was easily removed and was of a somewhat jelly-like consistency. Four Hibitane pessaries were inserted. The suturing of the torn uterus had to be hastened owing to its rapid involution. As first suture, Schmiedens technique with Chromic Catgut IV was applied. It was followed by a Cushing right angle suture, which could not be completed due to the fact that about 4 inches of the posterior uterus wound could no longer be reached. One and a half million units of Mylipen were spread on the uterus after having cleansed it. Some Sulphonamide powder was applied to the abdominal cavity. The peritoneum and M. rectus abdom. were joined with a continuous suture using Chromic Catgut VI. The same technique was applied to the M. obl. abdom. int. and ext., and Sulphonamide powder was applied freely between the muscle layers: the last named muscle being supported by three Wolfs sutures with strong silk. One and a half million units of Mylipen was administered i.m. and the skin closed with strong silk. Iodoform powder was dusted over it, and next day it was covered with Stockholm tar. The operation lasted 2 hours 20 minutes.

On the following two days, 3 million units of Mylipen were injected, and 2 Hibitane pessaries inserted daily into the womb. The cow made a good recovery and the skin sutures were removed on the 22nd November, 1961.

## DISCUSSION

The foetus proved to be a typical *Schistosomia reflexum* with ankylosis of all 4 legs. There was no heart or lungs; the head was enclosed by the four legs.

*Benesch* points out that a *Schistosomia reflexum* should be removed solely by foetotomy and operation methods adapted according to the contractions and ankylosis of the legs, which may occasionally occur.

*Gotze* states that *Schistosomia reflexum* is met with frequently and that an apparently large foetus can be removed quickly and easily by embryotomy.

I may point out that this is the first case that I have had to deal with in many years.

An attempt to place a wire saw would have failed, a priori, because there was no way of directing it round the deformed calf; the only way out being the Caesarean operation.

Regarding the operation technique, a relatively high chosen site on the left flank proved to be the right approach. Even by doing so it was difficult to secure one foot through the uterine wall, and to fix it, because of the short conical form of the uterus. The epidural injection of 10 ml. 2% Planocaine was too large a dose as the cow went down after some minutes. Six to eight ml. should be sufficient to keep a cow standing, and prevent her from pressing out the rumen; an experience I have had with a Dexter cow when performing the same operation. The second suture of the womb could not be completed owing to the rapid involution of this organ, and

about 4" were closed by a single Schmieden suture, although there was considerable doubt that the posterior part of the uterine wound would heal completely. Fortunately no complications were encountered.

#### ACKNOWLEDGEMENT

I wish to thank the Chief, Veterinary Field Services for his kind permission to publish this case, and to A. S. I. Barnard who assisted with the operation.

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## A NOTE ON THE USE OF SUCCINYL CHOLINE CHLORIDE IN CATTLE

R. C. BIGALKE — Alexander McGregor Museum,  
Kimberley.

Received for publication April 1962

### SUMMARY

The reaction of four young Afrikaner-type bulls to intramuscular injection of succinyl choline chloride at various dosage rates is described. The results suggest that cattle have a very low tolerance of this drug, which makes it unsuitable for general use.

### INTRODUCTION

Since a team of American workers (Crockford *et alia*<sup>1,2</sup>) described a technique for immobilising large mammals by delivering a projectile type syringe from a gas-powered rifle, much interest has been shown in drugs suitable for use with this method. Initially nicotine salicylate was favoured (Crockford *et al.*, *op cit*) but Buechner, Harthoorn and Lock<sup>3, 4, 5, 6</sup> have all but discarded this substance for wild African mammals and have turned to the muscle relaxants, in particular succinyl choline chloride.

The author became interested in the technique because of its value as a tool for capturing and marking free-living antelope, an essential preliminary for many types of field study. In order to gain more practical experience of the action of succinyl choline chloride, he took advantage of an opportunity for testing the drug on cattle. Although the experiment was only carried out on a small scale, it is felt that the results may be of some interest to veterinarians, especially in view of Hofmeyer's<sup>7</sup> rather unfavourable report on the drug when used in horses.

### METHOD

Four Afrikaner-type bulls, in excellent condition, two estimated at between 1½ and 2 years old and the other two at about three years old, served as experimental animals. The weight of each was obtained by means of a weight-band and from this the appropriate dosage of the drug ('Scoline', Allen & Hanbury's preparation of succinyl choline chloride) was calculated. After an intramuscular injection had been given in the haunch, the animal was released from the crush and allowed to walk away undisturbed.

### RESULTS

*Bull A.* Estimated age: 1½–2 years. Weight: 507 lbs. Dosage rate: approx. 0.04mg/Kg. Dose 0.2ml Scoline (50mg/ml)

Five-and-one-half minutes after the injection slight muscular paralysis appeared. The legs and tail became stiff and the animal flexed its fore-legs once or twice as though wanting to lie down. It did not lie down however and commenced grazing 15 minutes after receiving the injection. By this time it had completely recovered from the transitory effects of a dose apparently just too low to throw it.

**Bull B.** Estimated age:  $1\frac{1}{2}$ –2 years. Weight: 600 lbs. Dosage rate: approx. 0.05mg/Kg. Dose: 0.3ml Scoline (50mg/ml).

<i>Time after Injection</i>	<i>Effects of Drug</i>
2m.15sec.	First sign of muscular paralysis.
2m.40sec.	Lies down. Defaecates.
3m.10sec.	Head drops.
3m.36sec.	Has collapsed, is stretched out on its side quite immobile. Salivates fairly profusely.
7m.15sec.	Vasectomy by means of a burdizzo has been completed. No reaction.
15m.30sec.	Respiration has stopped. Artificial respiration given.
18m.30sec.	Breathing — did not notice exactly when it started. Legs are moved spasmodically.
20m.30sec.	Breathing has stopped again and despite resumption of artificial respiration the animal dies.

**Bull C.** Estimated age: 3 years. Weight: 900 lbs. Dosage rate: approx. 0.03mg/Kg. Dose : 0.25ml. scoline (50mg/ml.)  
No reaction could be observed.

**Bull D.** Estimated age: 3 years. Weight: 950 lbs. Dosage rate: approx. 0.04 mg/Kg. Dose 0.34 ml. scoline (50mg/ml).

Apart from a slight indication that the animal might be a little more tranquil than usual (7 mins. after injection), no reaction could be observed.

## DISCUSSION

It would appear that while a dose of 0.04 mg/Kg produces only a mild paralysis insufficient to immobilise a beast, (*Bull A* and perhaps also *D*) the very slight increase to 0.05 mg/Kg. results in complete paralysis, in the case of *Bull B* leading to death. Quite possibly a veterinarian would not have lost this animal. Nevertheless it seems clear that cattle have a low tolerance of succinyl choline chloride and that the drug's margin of safety is low.

It is interesting to note that several antelope species show tolerance of quite a different order. Thus Buechner *et al.*<sup>4</sup> found 0.35 mg/Kg. to be an effective immobilising dose for Uganda Kob (*Adenota kob thomasi*) and Waterbuck (*Kobus defassa ugandae*) and 0.30mg/Kg. for Jackson's Hartbeest (*Alcelaphus buselaphus lelwel*). The author has found the former quantity satisfactory for Springbok (*Antidorcas marsupialis*) and is informed (Prof. K. van der Walt, pers. comm.) that it also works well in Impala (*Aepyceros melampus*). The margin of safety also appears to be high in these species since, despite the impossibility of assessing an animal's weight accurately in the field, Buechner *et al*<sup>6</sup> were able to capture 46 Uganda Kob with no fatalities.

Yet in the case of Buffalo (*Syncerus caffer*) the same authors<sup>4</sup> report "After the first four attempts to determine the correct dose for buffalo, we concluded that these animals, for lack of sufficient plasma cholinesterase or for some other reason, simply could not hydrolyse the succinyl choline chloride". They have subsequently<sup>3</sup> confirmed that 0.055mg/Kg., given together with atropine (0.11 mg/Kg., to counteract excessive bronchial secretion which otherwise causes death), is the effective dose. The results of the experiment described in this paper suggest that the close phylogenetic relationship between Buffalo and Domestic Cattle extends to at least some aspects of their physiology.

#### ACKNOWLEDGEMENTS.

I am much indebted to Mr. B. Humphreys, who generously allowed me to experiment on his cattle. Advice and assistance given by Dr. A. J. Williamson and Dr. L. Stonier is also gratefully acknowledged.

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Decided sedative effects were apparent after three minutes. After five minutes, the anaesthetic was administered — Pentobarbitone-Sodium being used. Fifty to seventy per cent of the normal dosage was required for complete anaesthesia. Induction was invariably smooth and recovery uneventful. There were no serious side effects.

In 19 cases "RO 4-0403" was used as a narcotic without a general anaesthetic. Using 2 mg/lb o.v. there was pronounced sedation and analgesia. Wounds could be painlessly sutured and severe ear canker treated.

**DISCUSSION**

"RO 4-0403" was found to be an excellent tranquilizer with pronounced sedative narcotic and analgesic properties. As a pre-anaesthetic, the drug is extremely useful and appears safe to use in debilitated animals and poor operative risks, as well as healthy dogs.

**CASE REPORT**

Four semi-wild and uncontrollable Brahman hiefers of approximately 600 lbs live weight were to be loaded on a truck but could not be approached or handled

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R. K. REINECKE — Section of Helminthology Veterinary Research Institute, Onderstepoort.

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##### (b) *Trematodes*.

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##### (c) *Nematodes*

This drug is not effective against these worms.

#### *Dosage*

It will be sold as a wettable powder and the dosage rate is 50 mg/K of the active ingredient. Even at 10 times the therapeutic dose this drug showed no evidence of toxicity.

#### THIBENZOLE

Chemically this is 2-(4'-thiazolyl)- benzimidazole (generic name thiabendazole) also known as M.K.360.

##### (a) *Nematodes*

A highly effective, safe and rapid acting drug against all the common nematodes of the gastro-intestinal tract of ruminants with the exception of *Trichuris* spp. (Whip worm). In horses it is equally efficient in the treatment of large and small strongyles and ascaris; in pigs it is effective against ascaris and also effective on the common nematodes of poultry. Although effective on the nematode parasites of dogs it causes vomiting and is, therefore, unsuitable.



In trials on immature worms it was more than 99% effective against 7 and 14 day old *Haemonchus contortus* (wire worm) and *Trichostrongylus colubriformis* (bankrupt worm) parasites of sheep. This applies to a wide variety of immature nematode parasites of other hosts.

It inhibits the production of helminth eggs and development of larvae and is thus suitable for low level dosing. The drug must be incorporated in a lick to which the sheep have free access and at such a concentration that each sheep receives an average of 5 mg/K active ingredient per day.

Trials on two experimental stations one in the summer the other in the winter rainfall areas with three flocks were carried out as follows:

(a) A full dose of 50 mg/K followed by low level dosage of thibenzole by means of a lick.

(b) Drenching every six weeks with thibenzole at 50 mg/K.

(c) A full dose (500 mg/K) followed by low level dosage (0.5 g per sheep per day) of phenothiazine by means of a lick.

Regular autopsies every month showed that all the groups had a mild infection of *Trichuris* spp.; group (a) was more or less free of other parasites; group (b) was lightly infested but group (c) was fairly well infested especially with *Trichostrongylus* spp. Weight gains in the live sheep followed the same pattern, group (a) gaining the most and group (c) the least weight.

This remedy is practically non-toxic even at 20 times the therapeutic dose.

(b) and (c) *Trematodes and Cestodes*

This drug has no effect on these parasites.

### Dosage

The drug will be sold as a wettable powder and the dose is 50 mg/K active ingredient.

In treating horses and pigs the dry powder can be mixed with the cereals or mash at the dosage level of 50 mg/K live weight.

### MINTIC

Chemically this is 2-( $\beta$ -methoxyethyl) pyridine also known as methyridine or "promintic". The latter is an injectable preparation and only available to the profession.

#### (a) *Nematodes*

A highly effective very rapid acting drug which removes all adult intestinal parasites within 16 hours. Its effect on the abomasal parasites *Haemonchus* and *Ostertagia* spp. (brown stomach worm) is erratic due to the acid pH of the ingesta. It has some effect on *Dictyocaulus* spp. (lung worms).

The efficacy on immature *Haemonchus* and *Trichostrongylus* worms varies. It is more than 99% effective against 7-day old but only 92 to 93% effective on 14-day old worms.

#### (b) *Trematodes*

Single doses showed an average efficacy of 44% against immature *P. microbothrium* in the abomasum and small intestine of infected sheep. If the dosage was repeated 5-8 days later efficacy increased to 99%. This was not affected by the mode of dosage.

(c) *Cestodes*

Although the drug has some effect on tapeworms it is not regarded as a cestocide.

*Toxicity.*

Unfortunately this drug is toxic in some cases when double the therapeutic dose is administered. Symptoms of inco-ordination, stumbling and posterior paresis are noted within eight hours and death is due to respiratory collapse. This process is irreversable and there is no antidote; the drug itself is excreted within 24 hours of dosage.

When injected subcutaneously with strict aseptic precautions in Merino's and goats, promintic may cause extensive oedema, necrosis of the underlying muscle and cutaneous sloughing. Lameness of a fore limb injection in the brisket is common. On the other hand no reactions are noted after intraperitoneal injection.

*Dosage*

The dose is 200 mg/K of the active ingredient which is incorporated in a fluid medium. Mintic is dosed orally and promintic intraperitoneally or subcutaneously.

CONCLUSIONS

(1) The drugs mentioned are more effective than other anthelmintics against both mature and immature worms.

(2) The effect on immature worms of thibenzole and methyridine means that sheep need only be drenched every 6-8 weeks and not every 3 weeks as was the case in the past.

(3) The extremely rapid action ensures that if stock are drenched and moved to clean paddocks 24 hours later, they are in fact free of parasites and will take a long time to infest the new paddock.

(4) Lintex is not only an effective and safe cestocide but a major advance in the treatment of immature paramphistomes.

(5) Finally, if the *cost is not prohibitive*, lintex thibenzole and mintic should take the place of the older anthelmintics.

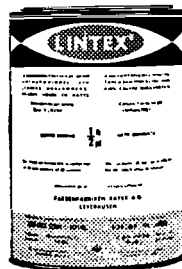


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## DRIE NUWE WURMMIDDELS

R. K. Reinecke — Afdeling Helminnologie Navorsingsinstituut vir Veeartsenykunde, Onderstepoort.

Hiermee word u ingelig aangaande drie uitstekende wurmmiddels wat tans geregistreer word en heelwaarskynlik in Julie 1962 aan die publiek beskikbaar gestel word.

### LINTEX

Die chemiese samestelling is N-(2-chlor 4-nitrofeniel)-5-chlorsalicylamid wat ook bekend staan as Bayer 2353 of Yomesan.

#### (a) *Cestode*

Hierdie is 'n hoogsdoeltreffende snelwerkende wurmmiddel teen die lintwurms van huisdiere en is daarby feitlik nie giftig nie. Dit word veral vir die behandeling van alle lintwurms van lammers en kalwers aanbeveel met die uitsondering van *Stilesia hepatica*. Die doeltreffendheid teen lintwurms van honde, katte en selfs duiwe het algemene praktisyns beïndruk.

#### (b) *Trematode*

Proewe te Onderstepoort in skape besmet met onvolwasse peer-vormige slakwurms *Paramphistomum microbothrium*, was indrukwekkend. 'n Enkele dosering *per os* van Lintex teen 'n dosis van 50 mg/K het gemiddeld 98.9% van die onvolwasse wurms in die abomasum en dunderm verwyder. Die doeltreffendheid het geweldig gedaal nadat die wurm na die voormae migreer het. Aangesien die wurm aldaar min uitwerking op sy gasheer het is behandeling van minder belang.

#### (c) *Nematode*

Die middel is ondoeltreffend teen hierdie wurms.

#### *Dosering*

Die middel sal as benatbare poeier verkoop word en die dosis is 50 mg/K van die aktiewe bestanddeel. Selfs teen tien-maal die behandelingsdosis was daar geen tekens van vergiftiging nie.

### THIBENZOLE

Die chemiese samestelling is 2-(4<sup>1</sup>-thiazolyl) benzimidazole (geneesiese naam thiabendazole) ook bekend as M.K.360.

#### (a) *Nematode*

'n Hoogsdoeltreffende snelwerkende en veilige middel teen al die gewone wurms in die maagdermkanaal van herkouers met die uitsondering van *Trichuris* spp. (Sambokwurms). Dit is net so doeltreffend teen die groot en klein strongilide en ascaris van perde. In varke is dit doel-

dunderm van besmette skape. Die doeltreffendheid het na 99% gestyg in gevalle waar die dosis 5 of 8 dae daarna herhaal was. Die resultaat was nie deur die metode van toediening beïnvloed nie.

(c) *Cestode*

Alhoewel daar 'n mate van doeltreffendheid is word die middel nie as lintwurmmiddel beskou nie.

*Toksisiteit*

Ongelukkig is hierdie middel in sekere gevalle giftig teen tweemaal die behandelingsdosis. Binne agt uur na dosering word inkoördinasie, struikel en verlamming van die agterlyf gesien. Die dood tree in as gevolg van verlamming van die asemhalingsstelsel. Die proses is onomkeerbaar en daar bestaan geen teëmiddel nie. Die middel self word binne 24 uur uitgeskei.

Selfs wanneer streng aseptiese maatreëls getref word met die onderhuidse inspuiting van promintic in Merinos en bokke, mag die middel uitgebreide edeem, nekrose van die onderliggende spier, asook afsterwing "sloughing" van die vel veroorsaak. Mankheid van 'n voorheen na onderhuidse inspuiting in die borsstuk is dikwels sigbaar. Daarenteen inspuitings in die buikholte "intraperitoneal" verwek geen reaksie nie.

*Dosering*

Die dosis is 200 mg/K van die aktiewe bestanddeel wat in 'n vloei-bare vorm verskaf word. Mintic word *per os* toegedien en promintic in die buikholte of onderhuids ingespuut.

#### GEVOLGTREKKINGS

(1) Die middels alreeds gemeld is meer doeltreffend beide teen volwasse en onvolwasse wurms as ander middels.

(2) Die doeltreffendheid van thibenzole en methyridine teen onvolwasse wurms lei daartoe dat diere pleks van drie-weeklikse behandelings, slegs elke 6 tot 8 weke hoef doseer te word.

(3) Die uiters snel werking van hierdie middels beteken dat vee 24 uur na dosering wel vry van wurms is. As hulle dan na skoon weidings verskuiwe word sal die nuwe kamp vir 'n lang periode redelik skoon van wurmbesmettings bly.

(4) Lintex is nie net 'n doeltreffende en veilige lintwurmmiddel nie maar 'n groot stap vooruit in die behandeling van onvolwasse peer-vormige slakwurm.

(5) Ten slotte mits die *middels nie te duur is nie* behoort lintex, thibenzole and mintic die plek in te neem van die ouere middels.

treffend teen *ascaris* en ook effektief teen die gewone nematode van pluimvee. Nieteenstaande die doeltreffendheid teen rondewurms in honde, veroorsaak dit braking en is dus nie geskik vir behandeling nie.

Die doeltreffendheid teen onvolwasse *Haemonchus* en *Trichostrongylus* wurms van skape was hoër as 99% beide op 7 en 14 dae oud wurms. Hierdie is ook van toepassing op 'n groot verskeidenheid onvolwasse wurms in ander gashere.

Dit werk wurmeierproduksie en larfontwikkeling teen en is dus geskik vir laevlakdosering. Die middel moet met 'n lek gemeng word waarna daar vry toegang is in so 'n konsentrasie dat elke skaap, gemiddeld 5 mg/K per dag kry.

In proewe op twee proefplase een in die somer- die ander in die winter-eënvalstreek is drie troppe as volg behandel:

(a) Een volle dosis 50 mg/K gevolg deur laevlaktoediening thibenzole deur middel van 'n lek.

(b) Dosering elke ses weke met thibenzole teen 50 mg/K.

(c) Een volle dosis (500 mg/K), gevolg deur laevlaktoediening (0.5 gm per skaap per dag) van fenotiasien deur middel van 'n lek.

Gereelde lykskouings elke maand het bewys dat al die groepe matige *Trichuris* spp. besmettings gehad het. Groep (a) was so te sê vry van ander wurms; groep (b) was liggies besmet maar groep (c) was redelik goed besmet veral met *Trichostrongylus* spp. Die gewigstoename in die lewendige skape was in dieselfde volgorde d.w.s. groep (a) het die grootste en groep (c) die minste toename gewys.

Hierdie middel is feitlik nie giftig selfs teen 20 maal die behandelings-dosis nie.

(b) en (c) *Trematode* en *Cestode*.

Hierdie middel is ondoeltreffend teen hierdie wurms.

### Dosering

Die middel sal as 'n benatbare poeier verkoop word. Die dosis is 50 mg/K aktiewe bestanddeel. Vir die behandeling van perde en varke kan die poeier met die graan of meel gemeng word teen 50 mg/K lewendige gewig.

### MINTIC

Die chemiese samestelling is 2-( $\beta$ -methoxyethyl) pyridine ook bekend as methyridine of promintic. Laasgenoemde is die inspuitbare vorm wat net aan die professe beskikbaar is.

(a) *Nematode*

'n Uiters doeltreffende baie snelwerkende middel wat alle volwasse dermparasiete binne 16 uur verwyder. Die doeltreffendheid teen die abomasale wurms *Haemonchus* en *Ostertagia* spp. is wisselvallig tewyte aan die suur pH van die maag inhoud. Dit het 'n mate van uitaerking op *Dictyocaulus* (longwurms).

Die doeltreffendheid teen onvolwasse *Haemonchus* en *Trichostrongylus* varieer. Dit is meer as 99% doeltreffend teen 7 dae oud maar net 92 tot 93% teen 14 dae oud wurms.

(b) *Trematode*

Na een dosis was die middel gemiddeld 44% doeltreffend teen onvolwasse *P. microbothrium* (peervormige slakwurms) in die abomasum en



## DOODBERIG

### MICHIEL WILHELM HENNING

Met die heengaan van Prof. M. W. Henning op 1 Maart 1962 het die Veterinêre Professie 'n kollega van uitstaande formaat en persoonlikheid verloor.

Van die eerste 5 Afrikaners wat as veterinêre studente in 1915/16 na Dublin gegaan het, is hy een van vier wat nie meer by ons is nie.

Die vier van hulle was algar briljante studente en as Sheppard lank genoeg gelewe het sou hy net soos C P. Naser, Parkin en Henning diep spore in die veeartsenykunde gemaak het.

Prof. Henning was die enigste van die vier wat nie alreeds 'n akademiese graad in die wetenskap gehad het, toe hulle oorsee gegaan het om in die veeartsenykunde te studeer, maar hy het by die Grey Universiteitskollege, Bloemfontein, sy intermediêre eksamen in die vier basiese wetenskaplike vakke met sukses afgelê.

Hy kwalifiseer in 1919 en verower die Fitzwygram-prys aldaar.

Na sy terugkoms in Suid-Afrika was hy eers aangestel in die velddiens en daarna op Onderstepoort, waar hy lektor was in Anatomie in die Fakulteit Veeartsenykunde. Later is hy aangestel as Professor in Veeartsenykunde in die Fakulteit Landbou van wat nou die Universiteit van Pretoria is. Hy het die pos beklee totdat hy enige jare uit die diens getree het, waarna hy 'n tydelike pos in die Departement Landbou beklee het tot 1 Maart 1962.

Prof. Henning het meer as net 'n werkende kennis van plantkunde gehad. Getuienis hiervan is die pragtige tuin by sy huis en sy belangstelling in giftige plante. Op laasgenoemde het hy alreeds navorsingswerk gedoen toe hy in die velddiens werksaam was.

As Professor in Veeartsenykunde in die Fakulteit Landbou, was hy in 'n mate geïsoleer van fundamentele veteriniere navorsingswerk. Ten spyte hiervan het hy dit reggekry om gereeld uit te gaan na Onderstepoort waar hy werk gedoen het, veral op kalwersiektes en die Salmonellas. Hy was later selfs erken as 'n internasionale outoriteit op die gebied.

In 1938 behaal hy die graad D.V.Sc in die Universiteit van Suid-Afrika Sy tesis het gegaan oor "The Antigenic Structure of Salmonellas Obtained from Domestic Animals and Birds in South Africa".

Met al hierdie verantwoordelikhede was sy tyd taamlik vol in beslag geneem en tog het hy met buitengewone werksvermoë dit reggekry om 'n boek te skrywe oor "Animal Diseases in South Africa". Hierdie boek is van besondere waarde vir veteriniere studente in die wydste sin van die woord, omdat dit die huidige kennis (oorsee sowel as binnelands) oor die betrokke siektes volledig gee.

Hy het die beskikbare materiaal so behandel dat volle krediet gegee word aan die werkers op die verskillende siektes en op die manier het hy buitengewoon daarin geslaag om nie die indruk te skep dat die werk deur homself gedoen is nie.

Hy het ook 'n uitstekende boek oor die kalf, sy versorging, voeding en siektes geskrywe.

Hy was verder verantwoordelik vir by die 35 publikasies oor verskillende siektetoestande en die belangrike werk oor die lewe van Theiler is reeds voltooi en sal eersdaags gepubliseer word.

Die Akademie het hom verder vereer deur die toekenning van die Havenga-prys.

Prof. Henning was 'n gedugte vegter en waar hy dit nodig gevind het, het hy onverskrokke opgetree, maar by dit alles was hy 'n getroue en opregte vriend. Ons betreur sy heengaan wat 'n groot verlies vir die Veteriniere Professie is. Ons betuig ons opregte simpatie met sy vrou Sarie en die twee dogters Helene en Lucia.

Die van ons wat vir hom en sy familie gedurende die laaste dae gesien het, bewonder die stil en moedige onderworpenheid aan die onverbiddelike uur wat hy en hulle bewus moes afwag.



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### WETENSKAPLIKE KONGRES \*962 EN SEWE-EN-VYFTIGSTE JAARVERGADERING

### SCIENTIFIC CONGRESS \*962 AND FIFTY-SEVENTH ANNUAL GENERAL MEETING

Die Jaarlikse Kongres van die Vereniging sal op Onderstepoort op 25—28 September 1962 gehou word.

Sy Edele mnr. P. M. K. le Roux, Minister van Landbou Tegniese Dienste het vriendelik ingestem om die kongres te open, en Sy Edelaagbare mnr. Ernest Smit, Burgemeester van Pretoria, het toegestem om die gaste en afgevaardigdes te verwelkom.

*'n Interessante program—gelleg en leersaam—is gereel.*

Besprekings by die Hotel Boulevard sal deur die kantoor van die Sekretaris gereel word vir die wat dit verlang.

The Annual Congress of the Association will be held at Onderstepoort on 25—28 September 1962.

The Hon. Mr. P. M. K. le Roux, Minister of Agricultural Technical Services has kindly consented to open the Congress and His Worship The Mayor of Pretoria, Mr. E. Smit, has kindly agreed to welcome the guests and delegates.

*An interesting program—both socially and scientifically—has been arranged.*

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2nd Ed. 282 pages. Illus. Price 52s. 6d.  
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## SMITH & JONES:

### *Veterinary Pathology*

By Hilton A. Smith, D. V. M., M. S. Ph. D. and  
Thomas D. Jones, B. S., D. V. M.  
2nd Ed. 1068 pages. Illus. Price 130s.  
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## BOOK REVIEWS

### DIAGNOSTIC METHODS IN VETERINARY MEDICINE

Geo. F. Boddie.

5th Edition. 1962, pp. 420. Published. Price 30/-

Published by Oliver and Boyd. Edinburgh & London.

In the eighteen years since its first publication, "Boddie" has now entered the Fifth edition. This sustained popularity is largely due to the small but important changes in every edition which bring the latest information and emphasis to the student and practitioner.

The Fifth edition is no exception. The clear and readable style is maintained throughout. Important symptom complexes are conveniently summarized in the form of differential diagnostic tables. The digestive system of the bovine, for instance, is revised extensively and includes a scheme for differential diagnosis of causes of acute abdominal distension, as well as a scheme for digestive disturbances without distension of the abdomen.

The growing importance of clinical pathology has not been overlooked and this section has been revised and extended. A new chapter on the application and interpretation of clinical laboratory methods as an aid to diagnosis, has been added. This serves to bridge the important gap between the clinical and the all too prevalent, purely laboratory method of diagnosis. From the clinician's point of view this chapter is perhaps the most important improvement in the Fifth edition.

As in the previous editions there are chapters on clinical bacteriology, post mortem technique, and poultry diseases, and this new edition will again be regarded by both student and practitioner as a cover to be kept available for reference at all times.

K. v.d.W.

### TICKS AND DISEASE

DON R. ARTHUR 1962. — International series of monographs on Pure and Applied Biology — Zoology Division Vol 9: 445 pages 95 figs. A reference list to each chapter. Pergamon Press. Price 90/-

Dr. Arthur has done the general biologist a good service by gathering together the literature and presenting him with a most readable digest of the present knowledge of ticks.

The chapters on the cuticle, on the capitulum and feeding mechanism; on feeding, excretion, circulation and respiration; on the Sense-organs, behaviour and regulating mechanisms of the unfed ticks; on the reproduction and development, that is chapters based on laboratory work and dealing mainly with histology, gross structure and function, are extremely well presented.

Some of the other chapters suffer somewhat in comparison; the author having been caught up in the trap of "over-generalisation", a fate which befalls many others who centre their work on a "type species" and who then draw general conclusions from the specific example, and end up by oversimplifying actuality. Thus he leaves the reader with the impression

that the species in other countries behave much in the same way as does *I. ricinus* in the British Isles. Throughout but scant, or no attention, is given to ticks in Australia, Asia, South America and Europe, other than the British Isles.

It was a happy idea to include a chapter on some economically important ticks; the brief descriptions (adults only) are adequate; unfortunately the accompanying drawings are oversimplified; and too often, in the text, there is no indication on which continent, leave alone on which country, the tick plays its economic rôle.

The chapters on diseases transmitted by ticks give a very good outline of the distribution of the main present-day accepted tick-born diseases and their vectors. The value of the chapter would have been enhanced had more attention been given to the hosts of the immature stages, in view of their possible rôle as disease reservoirs; the omission — of birds as intermediate hosts for the *Hyalomma*s is a grievous one. In the discussion on tick paralysis, also, one is surprised that but scant mention is made of the work done in Australia on *I. holocyclus* and the production of a serum.

Anyone attempting to condense the Control of Ticks into fourteen pages is foredoomed to failure, especially in these days of everchanging formulations of the ever increasing number of synthetic insecticides. It is to be hoped that, in future editions, instead of attempting the impossible, this chapter be approached from a different angle; that is from the historical one, showing how the methods have changed according to the acaricides available, and how present day workers are beginning to realize that the *ad hoc*, rule of thumb, application of chemicals is not the final answer, but that a detailed knowledge of the habits of the ticks themselves is essential to control programmes. This approach would be more readable and would have more meaning.

In future issues, it is also to be hoped, that the synonymy of ticks will be brought up to date, as also their accepted local popular names.

Nevertheless, all in all, this book is a remarkable piece of work, and the author needs to be congratulated at having had the courage to undertake it, as also upon succeeding to integrate the various zoological disciplines.

G.T.

## MEAT HANDLING IN UNDERDEVELOPED COUNTRIES

### I Slaughter and Preservation

I MANN.

Pp. XI, Figs. 88 and Plans 14. F.A.O. Agric. Development Paper No. 70.  
FOOD & AGRIC. ORGANISATION OF THE UNITED NATIONS,  
ROME

The declared object of this publication is the promotion of better methods of slaughter and the fuller utilisation and preservation of the various products so obtained with elimination of wastage of proteins and fats in view. Primarily intended for use in countries such as most of Africa where conditions are fairly primitive, it nevertheless aims at showing the way to improving methods and facilities and takes the reader up to the most modern developments which may be utilised provided throughout, interest and financial resources are adequate. For example, reference is made to the production of sterilised meat meal in mobile and relatively primitive abattoirs. At the same time simple and yet effective suggestions, well illustrated, are offered as to how even open-air slaughtering on a



small scale may be made more hygienic and generally satisfactory. Emphasis is placed on preservation by means other than refrigeration. Practical suggestions are offered for dealing with abattoir waste and for treating "measly" carcasses when facilities for freezing are non-existent.

In a methodical fashion the publication deals with approaches to the problem of improving existing systems and facilities, the basic requirements for slaughter, factors in abattoir design and equipment, slaughter-house procedure, designs of various types, poultry slaughtering, mobile and stationary field units, environmental sanitation, the handling and preservation of meat, fats and oils, etc.

The booklet is well illustrated and printed on quality paper and is recommended to those concerned with improving slaughtering conditions particularly in primitive rural areas.

The only criticism offered is that whereas DDT or Gammexane powder is recommended for prevention of insect infestations of preserved meat products such as biltong, the use of pyrethrins in pea flour would be more acceptable in view of the non-toxicity of the latter towards mammals.

L. v.d.H.

## DIE NARKOSE DER TIERE.

BAND I — LOKALANASTHESIE

M. WESTHUES UND R. FRITSCH

1960. p.p.192 AND FIGS. 95.

DM 25.80.

VERLAG PAUL PAREY, BERLIN AND HAMBURG.

Vol. II of this book deals with general anaesthesia and has been reviewed in the March issue of this Journal.

The fact that anaesthesia is dealt with by these authors in two volumes gives an indication of the tremendous development in this field during recent years.

The first 46 pages are devoted to the fundamentals of local anaesthesia or local analgesia, to use a term that is replacing the former. The various types of local analgesia are discussed, the advantages, disadvantages, toxicity, side effects and groups of drugs. An interesting exposition is given of the way which local analgesics act.

The technique of local analgesia is divided into the following main sections.:

- (1) Terminal analgesia.
- (2) Conduction analgesia of head and extremities.
- (3) Paravertebral analgesia.
- (4) Spinal analgesia.
- (5) Analgesia for abdominal operations.
- (6) Analgesia of perineum, vulva and penis.
- (7) Analgesia of the udder.
- (8) Analgesia for castration.
- (9) Sympathetic block.

From the above list it is evident that the whole field received attention. All of the common procedures are described. The joints are given adequate attention e.g. the technique of analgesia of a comparative inaccessible joint in the horse, the hipjoint, is well described. This is of considerable importance in diagnosis.

Unless the reviewer descends to trivialities this book can be recommended without reserve. The descriptions are brief without being inadequate. The figures are clear with good artistic quality. The book is a compliment to the printer's art: the headings and subheadings are distinct and well spaced thereby facilitating rapid reference.

The last few pages are occupied by an impressive bibliography.

Perhaps, in a future issue, the authors may consider giving something of the history of local analgesia.

C.F.B.H.

### THE VETERINARY ANNUAL — THIRD ISSUE 1961

Edited by W. A. POOL, M.R.C.V.S.

JOHN WRIGHT AND SONS Ltd., Bristol

Price 42c.

To Veterinarians and those concerned with animal welfare wishing to keep abreast of recent developments in Veterinary Science, this book can be recommended without hesitation. The field worker and general practitioner who have limited library facilities will find the work particularly useful, and the research worker who desires information on subjects other than his own will also find the book valuable for rapid reference purposes.

The book is divided into two main sections; special articles and a review of the current literature. In the former are included articles such as "The Ecological Approach to Veterinary Medicine in the U.S.A." which will be of value to country practitioners interested in preventative veterinary medicine on a herd basis and "Recent Advances in the Physiology of the Udder" which is a simplified account of the recent trends in research into normal mammary function. The articles are contributed by leading workers in their fields on subjects of topical veterinary interest.

The second and more important section of the book is devoted to a review of current progress in virtually every aspect of veterinary science. Subjects such as the following are included: Diseases related to bacteria, fungi, viruses and protozoa, parasitology, nutritional and metabolic disorders, reproduction and reproductive disorders, veterinary surgery, obstetrics. Numerous references are given.

R.T.

### THE MERCK VETERINARY MANUAL SECOND EDITION

The Second Edition of The Merck Veterinary Manual, a Handbook of Diagnosis and Therapy for the Veterinarian, is published by Merck & Co., Inc., Manufacturing Chemists of Rahway, New Jersey as part of a program of service to the veterinary and allied professions. The price in U.S.A. is \$9.75. Orders should be addressed to Publications Department, Merck & Co., Inc., Rahway, New Jersey or placed with local book dealers.

#### *General Content*

The new edition of the Veterinary Manual contains about 1,600 pages of text covering diagnosis and treatment of virtually all disorders commonly encountered by the veterinarian.

As with the previous edition, the objective is to provide veterinarians with well-organized, up-to-date facts, so as to facilitate accurate diagnosis

and promote the employment of effective treatment. Sufficient etiologic, physiologic, pathologic and other background material is included to insure a rounded, thoughtful approach to each entity presented.

An outstanding feature of the new book is a separate section comprising several hundred carefully selected prescriptions embodying the most up-to-date medicinal advances. Along with four special therapy chapters, they are conveniently grouped in one selection. All prescriptions are categorized according to clinical indications or mode of action.

Comprising a total of 422 chapters are 20 main sections, each thumb-indexed, each covering a specific field of practice. More than 48 completely new subjects and numerous added tables combine to keep this edition thoroughly in step with recent developments.

#### *Other Outstanding Features*

Broadened coverage offered by the Second Edition embraces a wide variety of new findings related to well-known diseases, and the latest reliable information regarding several newly discovered clinical entities. The number of exotic diseases discussed has been increased for the information of those interested in these problems in the U.S.A. and the convenience of veterinarians who live elsewhere

A number of special sections deal with aspects of veterinary medicine that often present their own unique problems. Much new material has been added to the sections Fur, Laboratory, and Zoo Animals : Poultry and Toxicology. The Nutrition section has been extensively rewritten and brought up-to-date, with added emphasis on deficiency states. The section on Parasitic Diseases has been greatly revised and enlarged.

In addition to the diagnostic and therapeutic text already mentioned, Part II of the book presents informative advice on office laboratory procedures and selected immunization methods. In this section information on veterinary radiology, oxygen therapy and practical reference tables are quickly available.

#### *Preparation and Format*

More than 250 authorities in various fields of veterinary science served as authors or consultants in preparation of the new book. It was compiled under the editorial direction of the Merck Sharp & Dohme Research Laboratories Division.

The Merck Veterinary Manual Second Edition is printed in handbook size on strong light paper with dark red, gold-stamped durable Sturdite binding. All copies are thumb-indexed.

(for) H. P. de B.

## UNITED STATES ARMY VETERINARY SERVICE IN WORLD WAR II

The Historical Unit U.S.A. Army Medical Service  
Washington 12, D.C. U.S.A. .

Lieutenant General Leonard D. Heaton, The Surgeon General, Department of the Army, has announced the recent publication of "United States Army Veterinary Service In World War II."

While this is an official history in the series, "History of the Medical Department, U. S. Army In World War II," the author, Lt. Col. Everett B. Miller, VC, USA, and the Editor, Colonel George L. Caldwell, VC, USA (Ret) have rightly retained the human element. For this and other reasons, the book is interesting and enlightening to read.

During World War II, the United States Army Veterinary Service fulfilled a vital mission in safeguarding the health of the Army by compiling an outstanding record of veterinary medicine on a worldwide basis.

The global aspects of World War II, particularly in the Tropics, brought complexing and varied problems to the Veterinary Corps. New diseases that could spread from animal to man were encountered and many well-known diseases took on new importance, especially such entities as leishmaniasis, leptospirosis, rabies, ornithosis, and others.

Although food inspection was the Veterinary Service's principal activity, they were also responsible for all animal health and efficiency. This included the professional care of the more than 56,000 Army horses and mules, over 10,000 K-9 Corps dogs, almost 55,000 birds of the Signal Corps Pigeon Service, the undeterminable thousands of laboratory animals, and hundreds of captured military animals.

Every Veterinarian and every military man who is interested in veterinary medicine will want to read this important and interesting book. Its 734 pages, 178 illustrations, maps, charts, and tables are handsomely bound into a book that will make a valuable addition to all personal, veterinary society and graduate school professional libraries.

Copies of this publication can be purchased for the modest cost of \$7.00 each from the Superintendent of Documents, Washington 25, D. C.

The book will be reviewed in the September 1962 issue by two members of the Onderstepoort staff.

## LEHRBUCH DER ANATOMIE DER HAUSTIERE

Nickel, Schummer & Seiferle

Vol. I. Pp. 502. 2nd Ed. 1961. Price approx. R20.

Verlag Paul Parey, Berlin and Hamburg.

After the second world war publication of the classical and internationally known textbook of the comparative anatomy of the domesticated animals by Ellenberger and Baum was stopped. Since that time a great need was felt for a detailed textbook on comparative veterinary anatomy that would comply with the requirements of student, practitioner and scientist. A work of this nature demands dissections and illustrations of a high standard. This difficult task was performed by the anatomists of Hannover, Giessen and Zürich who have succeeded admirably in producing a textbook which satisfies those demands.

The first volume, of which the second edition recently appeared, deals with the locomotor system. The authors are to be congratulated on their approach to the subject matter. As a preliminary to each section of the system a chapter is written dealing with the basic pattern and the morphological, physiological and clinical aspects common to all species. Then a detailed description is given of each animal. The horse as a type-species has been entirely abolished and the relations in the other animals are consequently more evenly distributed. The tendency is to start with the dog as the least specialised and end with the horse in which specialisation has reached its maximum — a logical approach. A most informative chapter is given on the statics and dynamics of the locomotor system. The value of the book is further enhanced by numerous illustrations which are of a very high standard. The book is bound to become a standard text on veterinary anatomy and can be recommended without reservation.

J. M. W. LE R.

## ONDERSTEEPOORT JOURNAL OF VETERINARY SCIENCE

Volume 29, Number 1 (March 1962) of the above journal has been published and contains the following articles:

*Studies of Rift Valley fever.*—Passive and active immunity in lambs. K. E. WEISS.

*On two new Catenotaenia tapeworms from a South African rat* with remarks on the species of the genus. R. J. ORTLEPP.

*Observations on Rojotaenia gerbilli Wertheim, 1954, and anoplocephalid from gerbils.* R. J. ORTLEPP.

*The pathological physiology of heartwater* (Cowdria (Rickettsia) ruminantium Cowdry, 1962). R. CLARK.

*Pathological studies on neoplasms of dogs in South Africa.* GILLES DE KOCK.

*Ovulatory failure in bovines.* S. W. J. VAN RENSBURG and W.H. DE VOS.

*Studies on specific oculo-vascular myiasis of domestic animals* (uitpeuloog). 1. Historical review. P. A. BASSON.

*Blood groups in bovines* II. Normally occurring isoantibodies in cattle blood. D. R. OSTERHOFF.

*Ovine ketosis* II. The effect of pregnancy on the blood ketone body levels of wellfed ewes. J. PROCOS.

*A preliminary report on the occurrence of selenosis in South Africa* and its possible roles in the aetiology of tribulosis (geeldiklop), enzootic icterus and some other disease conditions encountered in the Karoo areas. J. M. M. BROWN and P. J. DE WET.

The Chief, Veterinary Research Institute, Onderstepoort, intimates that the expiry periods of some Onderstepoort Vaccines have been revised. The maximum periods for which these vaccines can now be kept are shown below.

The expiry dates are stamped on the vaccine containers on the day of issue.

## INTIMATION

VETERINARY RADIOLOGY — W. D. CARLSON, D. V. M.,  
Ph.D.

MESSRS. BAILLIERE, TINDALL and COX advise us that the Book, VETERINARY RADIOLOGY by CARLSON, reviewed in this Journal on page 123 of Vol. 33 No. 1 (March, 1962) is, by arrangement with MESSRS. HENRY KRMPTON and SONS, now available from 7 & 8 HENRIETTA STREET, COVENT GARDEN, LONDON.

## BOOK NEWS

*Latest additions to our stock include:—*

- BLACK'S VETERINARY DICTIONARY**, W. C. Miller & G. P. West; the sixth completely revised edition (1962); 1016 pp; 36 illus.; 321 diagrams; R4-95.
- PHYSIOLOGY OF REPRODUCTION AND ARTIFICIAL INSEMINATION OF CATTLE**; G. W. Salisbury & N. L. Van Demark; 637 pp; profusely illustrated; the most recent and greatest of all aids to students, teachers, research workers and others interested in reproduction; R9-55.
- VETERINARY RADIOLOGY**; W. D. Carlson; 460 pp; over 1000 illus.; the most comprehensive book in English on the subject; R13-95.
- RADIOGRAPHY FOR THE VETERINARY SURGEON**; R. N. Smith; 80 pp; 35 illus.; presents all the information a practising veterinarian needs to use diagnostic X-ray apparatus successfully. R1-55.
- VETERINARY DIETETICS**; J. O. L. King; 230 pp; many refs.; a new manual dealing with the relationship between nutrition and disease in animals; R3-30.
- JERSEY CATTLE**; E. J. Boston; 232 pp; 45 illus., presents the history, distribution and all other relevant information on the breed; R2-55.
- SHEEP AND PROPERTY MANAGEMENT**; E. H. Pearse; 612 pp; many illus.; the seventh edition of the former well-known **SHEEP, FARM AND STATION MANAGEMENT**; R3-95.
- PRINCIPLES OF DAIRY SCIENCE**; E. Vanstone & B. M. Dougall; 238 pp; 22 illus.; it deals concisely with all aspects of dairy science; R2-75.
- STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS**; the approved publication of the American Public Health Association; 11th edition; 448 pp; R6-40.
- ANIMAL BREEDING**; L. M. Winters; 5th edition; 420 pp; III figs.; R5-60.
- THE MERCK VETERINARY MANUAL**; enlarged second edition; R7-90.

Prices quoted include postage.

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## PUBLIC RELATIONS SERVICE

### NEWS OF MEMBERS

#### GOOD TIDINGS

*Dr. B. C. Jansen and Dr. M. C. Lambrechts* left for Paris during the first week of May 1962 and attended a series of meetings of O.I.E. Dr. Jansen also visited a number of institutes in Europe and Britain and attended a meeting of the Organizing Committee of the World Veterinary Association. Dr. Lambrechts had a number of assignments from the Department of Agricultural Technical Services to attend to. They returned early in June.

*Dr. H. P. A. de Boom* may remain in U.S.A. until December 1962.

*Dr. G. H. R. Bisschop* flew out of Canada recently to attend his brothers wedding at Duiwelskloof. He has returned to Canada and will probably stay there another year.

*Dr. W. H. Chase*, retired Chief Veterinary Officer Bechuanaland Protectorate, now living in Pretoria, recently underwent a serious operation but is now greatly recovered. We wish him a speedy and complete recovery.

*Dr. John Mare* leaves by boat on July 20th, 1962 for the U.S.A. via England. He has been granted an American Research Fellowship which aided by South African grants will enable him to undertake research studies in Virology at Iowa State University, Ames. He expects to be away for 3 years. His wife Margaret and son Carl will accompany him.

*Dr. L. W. van den Heever* left on 20th. May 1962 and attended the Third Symposium of the International Association of Veterinary Food Hygienists at Nice. He visited Institutes and Faculties in Switzerland, Germany, Belgium, France and Italy where research and teaching in connection with meat and milk and other foods of animal origin is undertaken.

*Dr. I. F. H. Purchase* who is doing research work at Cambridge on Anaesthetics has been awarded a Wellcome Fellowship of the Animal Health Trust.

*Dr. J. M. M. Brown* of the Physiology Section at Onderstepoort attended the meeting of the New York Academy of Science during May, 1962. This meeting was concerned with the Metabolic Diseases of Man and Animals and the Conference Chairman was Dr. W. D. Malherbe of Onderstepoort. Dr. Brown presented a paper on Geeldikkop and Enzootic Icterus. He later visited the United Kingdom and was absent from the Republic for fourteen days.

### DIARY OF EVENTS

*International Congresses on Fertility:* Two International Congresses on fertility are to be held within the next two years.

The first is the *IV World Congress on Fertility and Sterility* which will take place in Rio de Janeiro, Brazil on 8 — 15th August 1962. This is

sponsored by the International Fertility Association, and will be a meeting of specialists from all parts of the world. All aspects of reproduction, fertility and infertility in both humans and animals will be discussed.

The second is the *Fifth International Congress on Animal Reproduction and Artificial Insemination* which will be held in Trento, Northern Italy on 6 — 13th. September 1964.

The preliminary programme of this Congress is already available. There will be four sections namely:

1. Biology of reproduction
2. Morphology and Physiology of reproduction
3. Artificial Insemination
4. Pathology of reproduction

Full details of these two Congresses can be obtained from the Secretary: Reproduction Group of the S.A.V.M.A., P.O. Onderstepoort.

### *Meetings of the Veterinary Board*

The Veterinary Board has held two meetings in connection with the proposed amendments to the Veterinary Act. These suggested amendments will be in the hands of the Minister by the time the Parliamentary Programme for next year is considered, and it is hoped that they will be introduced at the next Session of Parliament.

### *The Witwatersrand Branch*

This Branch held its Annual Meeting on May 22nd. 1962.

This businesslike branch concerned itself with things that matter and settled the question of professional fees, as well as making the occasion a memorable one for its members.

### *Western Cape Branch*

The Western Cape Branch held its Annual Meeting on March 30th. 1962. The Dinner on the previous evening, the interesting talks and the closing cocktail party all contributed to a very pleasant and well organized occasion.

### *Eastern Cape Branch*

The Eastern Cape Branch held its Annual Meeting on 16th. June 1962 which was attended by the President. This Branch has the happy knack of successfully combining business with pleasure as the President no doubt personally experienced.

### *The Natal Branch*

The Natal Branch held its Annual Meeting on 13th. June 1962. The Programme was most interesting and resulted in the dissemination of some valuable information to practitioners by Professor van der Walt. The Secretary opened the Proceedings. The lunch and the cocktail party further enhanced the prestige of this, the oldest branch of the S.A.V.M.A.

### *The O.F.S. Basutoland and Northern Cape Branch*

This Branch held its Annual Meeting on 23rd. of June 1962 when a very pleasant and informative gathering of the "Vrystaters" took place.

The traditions of hospitality enjoyed by this Province were well attended to by the Veterinary fraternity and everyone felt that the meetings were held far too infrequently.



### *Reproduction Group*

The Reproduction Group plan to present three very interesting demonstrations during the forthcoming Congress on 25th — 28th September 1962 viz

1. Pudential nerve block for relaxation and anaesthesia of the penis in the bovine.
  2. The rectal massage of the ampulla for collection of semen from the bull.
  3. Transfer of fertilized ova in the ovine.
- 

### **BRITISH COUNCIL SCHOLARSHIPS**

TWENTY-TWO British Council Scholarships are available from 1 October 1963 to residents of the Republic of South Africa.

The Scholarships are normally tenable at Universities and other educational institutions in the United Kingdom, and provide opportunities for advanced study and research. They are not primarily intended to enable the holders to obtain University Degrees or other professional or practical qualifications, although with special permission they may be allowed to do so if this does not necessitate an extension of the Scholarship.

The awards are mainly for men and women between 25 and 35 years of age, who have already successfully completed University Degree Courses or who hold equivalent professional qualifications.

The Scholarships are normally tenable for one or two academic years, according to the duration of the approved course of study. Scholarships for shorter periods may be awarded in special cases.

The emoluments include any or all of the following, as may be appropriate: return fares to and from the United Kingdom, tuition fees, a personal maintenance allowance, and certain other expenses.

APPLICATION for these Scholarships must be made through:

**THE CULTURAL ATTACHÉ**

**BRITISH EMBASSY**

**6 HILL STREET, PRETORIA**

from whom further particulars and application forms may be obtained.

THE CLOSING DATE for the receipt of completed applications in Pretoria is 30 SEPTEMBER 1962

## **VERSOEK VAN DIE HOOF VAN VEEARTSENY (VELD) OOR DIE HEEN-EN-WEER VERVOER VAN HONDE EN ANDER TROETELDIERE TUSSEN SUID-WES-AFRIKA EN DIE REPUBLIEK VAN SUID-AFRIKA.**

Die ondervermelde afskrif van 'n brief gedateer 22 Maart 1962, is van die Direkteur van Veeartsenydiens ontvang en word vir algemene inligting herhaal:

Meneer,

'n Steeds toenemende aantal gevalle kom onder aandag waar reisigers vanuit Suidwes-Afrika, die Republiek met honde en ander troeteldiere betree sonder dat hulle oor die vereiste gesondheidssertifikate beskik.

Vanweë die gevaar wat sodanige bewegings ten opsigte van verspreiding van hondsdoelheid inhou, is die saak na die Direkteur van Landbou, Windhoek verwys wie, onder andere, as volg skryf:—

„ . . . Die anderkant van die saak is dat daar baie honde met besoekers vanaf die Republiek hierheen kom. Die honde kom so dikwels as nie, vergesel van 'n Gesondheidssertifikaat uitgereik deur 'n privaat praktisyn. Die privaat praktisyn waarsku nie dat die mense 'n voorafgaande invoerpermit van die Gebied moet hê nie en waarsku hulle ook nie dat hulle moeilikheid gaan ondervind met die terug vervoer van die hond nie. Moontlik sal dit ook 'n goeie doel dien indien u 'n omsendbrief laat uitstuur, aan u kant, aan alle privaat praktisyns.”

Soos u bewys is bestaan daar in albei gebiede wetgewing wat neerlê dat tussengebiedse bewegings met diere, wat ook voëls insluit, onderhewig is aan permitte uitgereik in die geval van bewegings vanaf Suidwes-Afrika deur hierdie kantoor en na Suidwes-Afrika deur die Direkteur van Landbou, Windhoek, waarin die voorwaardes van vervoer neergelê word.

Dit sal besonder waardeur word as u by wyse van 'n sirkulêrende brief hierdie aangeleentheid onder die aandag van alle lede van die S.A.V.M.V. sal bring sodat hulle hul kliënte kan inlig oor die vereistes wanneer by hulle om gesondheidssertifikate aangeklop word.

Dankend,  
Die uwe,  
HOOF VEEARTSENY (VFLD)

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### **SUMMARY**

The Chief of Veterinary Field Services draws attention to the legal requirements by which Veterinary removal permits are necessary for the transportation of dogs and other pets between South West Africa and the Republic.

## VETERINARY HEALTH CERTIFICATES

Printed and offered for sale by the Association in both languages and obtainable from the Secretary, P.O. Box 2460, Pretoria.

Books of 50 Veterinary Health Certificates bearing the crest of the South African Veterinary Medical Association and printed in Afrikaans and English are available at the following prices.

Members desiring these books of certificates should remit the necessary amount to the Secretary, P.O. Box 2460, Pretoria.

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Certificates for Bull .....	75c.
Certificates for Horse .....	75c.
Certificate for Ram .....	R1.00

## EXPIRY PERIODS OF SOME ONDERSTEPSPOORT VACCINES.

Bluetongue . . . . .	3 months
Horsesickness . . . . .	3 months
Fowl Pox . . . . .	3 months
Rift Valley Fever & Wesselsbron Disease)....	3 months
Lumpy Skin Disease . . . . .	3 months
Rabies . . . . .	3 months
Newcastle Disease . . . . .	3 months
Distemper . . . . .	1 month*
Enterotoxaemia . . . . .	6 months
Lamb Dysentery . . . . .	6 months
Lamsiekte. . . . .	6 months
Black Quarter . . . . .	6 months
Anthrax . . . . .	6 months
Calf Paratyphoid. . . . .	6 months
Brucella (Str. 19). . . . .	1 month
Brucella (Rev. I) . . . . .	1 month

\* The expiry period of 1 month for Distemper vaccine is applicable when the vaccine is stored at 4°C in an ordinary refrigerator. If the vaccine is stored in a deep-freeze at 18; the vaccine can be kept for 3 months.

Although the expiry periods for the other vaccines have been calculated for storage on the shelf, it is nevertheless advisable to store all vaccines, much are not used immediately, at temperatures ranging from 4 to 8°C.

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3. The general order of sequence of headings should be —  
Summary  
Introduction/History  
Methods  
Results  
Discussion  
Acknowledgements  
References
4. *The position of all Tables, line drawings, illustrations and photographs must be clearly indicated in the manuscript.*

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1. *The Summary* should not exceed 3 per cent of the submission.
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4. *The Results.* Full findings should be clearly and concisely described.
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6. *Acknowledgments.* These should be brief, courteous and include any authority which granted the investigation.
7. *References.* These should be indicated in numerical sequence at the end of the contribution and relate to the authors or statements referred to in the text.

References must be identified in the text by Superscript numerals placed above the names of the authors or the statements made. Where more than one such numeral is used in the text they must be separated by commas and the full stop placed at the end of the line and not at the end of the last numeral thus . . . . that the vaccine was successful <sup>234</sup>.

All publications referred to in the compilation of references must:

- (a) *In the case of articles* state the names of the authors, the date and title of the work referred to, the accepted abbreviation of the Journal in which it appeared, the volume number in Arabic numerals (which must be underlined for printing in heavy type; the word "Volume" being omitted) and the numbers of the first and last pages of the article quoted, e.g.: Thompson James and Benedict R. J. (1959). The Effects of Alcohol on *Glossina austeni*, *J. Parasit. Sud.* 26 249-58.

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- (e) *Each class of illustrative copy* must be consecutively and independently identified, e.g.:  
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### *Abbreviations and Numbers*

Only conventionally accepted abbreviations may be used, e.g. c.c. ml. Kg. etc. Avoid such terms as S.A. for South Africa. *Numerals*. Numbers used in the text should be written except where exact measurements are indicated e.g. Twenty sheep were given 5 mg. per Kg. live weight. All numerals to be spelt out in full at the beginning of a sentence.

### *Biological Nomenclature*

With the first reference to a species the full generic and specific names must be given e.g. *Boophilus decoloratus* (underlined). Subsequently *B. decoloratus* may be used.

### *Names of Diseases*

Popular names of diseases should not be started with capitals except where proper nouns are included e.g. heartwater, bluetongue but Rift Valley fever or John's disease.

### *General Hints*

Keep your sentences short, the average sentence should not contain more than 10 to 12 words.

Use English as against American spelling e.g. oestrus, haemoglobin etc.

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A book entitled *Medical Writing* by Dr. M. Fishbein, former Editor of the American Medical Association is recommended by the Editor of the South African Medical Journal.

The Government Printer issues a publication known as Circular No. 1 of 1959 "*Requisitions for Printed Matter and the preparation of Copy*".

### *Correspondence to the Editor*

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SIEKTES VIR DIE  
TYDPERK APRIL — JUNIE 1962

REPORT OF OUTBREAKS OF NOTIFIABLE STOCK DISEASES  
FOR THE PERIOD APRIL — JUNE 1962

SIEKTE/DISEASES	DISTRIK/DISTRICT
<i>XB.W.D.—B.W.D.</i> .....	Stellenbosch.
<i>Brandsiekte—Skaap</i> .....	Nil.
<i>Sheep Scab</i> .....	Nil.
<i>Episootiese Limfangitis</i> .....	Nil.
<i>Gonderiose—Milde Bees</i> .....	Entonjaneni.
<i>Gonderiosis—Benign Bovine</i> .....	Ingwavuma.
	Kranskop.
	Lower Umfolozi.
	Mahlabatini.
	Mzinga.
	Nelspruit.
	Ngotshe.
	Nongoma.
	Pilgrimsrest.
	Port Shepstone.
	Soutpansberg.
	Ubombo.
	Umtata.
	Umvoti.
	Umzinto.
<i>Gonderiose—Kwaadaardige Buffel</i> .....	Hlabisa.
<i>Gonderiosis—Malignant Cynerine</i> .....	Mahlabatini.
	Soutpansberg.
<i>Hoendertifus</i> .....	Bethlehem.
<i>Fowl Typhoid</i> .....	Dewetsdorp.
	Kliprivier.
	Lionsriver.
	Pietermaritzburg.
	Pinetown.
	Potchefstroom.
<i>Hondsdotheid</i> .....	Bloemfontein.
<i>Rabies</i> .....	Bothaville.
	Babanango.
	Entonjaneni.
	Hlabisa.
	Lower Umfolozi.
	Letaba.
	Mafeking.
	Mahlabatini.
	Mtunzini.
	Ngotshe.
	Nongoma.
	Posmasburg.
	Pietermaritzburg.
	Rouxville.
	Sibasa.
	Soutpansberg.
	Theunissen.
	Vryburg.
	Wesselsbron.



SIEKTE/DISEASE	DISTRIK/DISTRICT
<i>Knopvelsiekte</i> .....	Barberton.
<i>Lumpy Skin Disease</i> .....	Bethlehem.
	Bethal.
	Bloemfontein.
	Bloemhof.
	Boshof.
	Bothaville.
	Ermelo.
	Eshowe.
	Fouriesburg.
	Germiston.
	Herbert.
	Hoopstad.
	Indwe.
	Kroonstad.
	Kempton Park
	Klerksdorp.
	Kimberley.
	Kuruman.
	Ladybrand.
	Lichtenburg.
	Mafeking.
	Mtunzini.
	Mahlabatini.
	Ngotshe.
<i>Knopvelsiekte (vervolg)</i> .....	Nongoma.
<i>Lumpy Skin Disease (contd.)</i> .....	Piet Retief.
	Pretoria.
	Rustenburg.
	Taungs.
	Vryburg.
	Warmbad.
	Waterberg.
	Winburg.
	Witbank.
	Wesselsbron.
	Wolmaranstad.
<i>Laringotracheitis Aansteklik</i> .....	Nil.
<i>Laryngotracheitis Infectious</i> .....	
<i>Miltsiekte</i> .....	Babanango.
<i>Antrax</i> .....	Bloemfontein.
	Bronkhorstspuit.
	Entonjaneni.
	Fouriesburg.
	Johannesburg.
	Klerksdorp.
	Middeldrift.
	Mahlabatini.
	Stutterheim.
	Ventersburg.
	Vryburg.
<i>Newcastlesiekte</i> .....	Nil.
<i>Newcastle Disease</i> .....	Nil.
<i>Pappagaaisiekte</i> .....	Nil.
<i>Psilla Cosis</i> .....	Nil.
<i>Slapsiekte</i> .....	Posmasburg.
<i>Dourine</i> .....	Vryburg.
	Worcester.

SIEKTE/DISEASES		DISTRIK/DISTRICT
<i>Skurfte</i> . . . . .	Bok	Hlabisa.
<i>Mange</i> . . . . .	Goat	Keiskamahoe.
		Middeldrift.
		Uggeleni.
		St. Marks.
		Ventersburg.
	Perd	Nil.
	Vark	
	Vark	Kempton Park.
<i>Tuberkulose</i> . . . . .	Bees	Barberton.
<i>Tuberculosis</i> . . . . .	Bovine	Ceres.
		Kingwilliaamstown.
		Klerksdorp.
		Lower Tugela.
		Nelspruit.
		Ngotshe.
		Parys
		Piet Retief.
		Queenstown.
		Riversdal.
		Stellenbosch.
		Swellendam.
		Soutpansberg.
		Taungs.
		Touws River.
		Volksrust.
		Viljoenskroon.
		Vryheid.
		Vryburg.
	Hoender	Nil.
	Haan	