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The Preservation of Horsesickness Vaccine with 'Merthiolate'

By J. H. MASON and R. A. ALEXANDER, Onderstepoort.

Horsesickness vaccine, as issued from Onderstepoort, is an emulsion, in 10 per cent. (horse) serum-saline, of the brains of mice killed *in extremis* after infection with selected strains of neurotropic virus. A detailed description of the method of vaccine production in use in 1936 has been published [Alexander, Neitz, and du Toit, (1936)], but since then several improvements that merit description have been introduced.

Six antigenically different strains are now used (O, 449, 464, Vryheid, K.A., and O.D.) and a total of 124 infected mouse brains are necessary to make a batch of vaccine (6000 cc., 600 doses). The dose for the horse is 10 cc. injected subcutaneously; 0·05 cc. contains at least 100 minimal infecting doses of each strain for the mouse (intracerebral inoculation).

The vaccines issued in 1937, 1938, and 1939 were made in the following way. The brains of the mice were removed and emulsified mechanically* for half-an-hour in 300 cc. of 10 per cent. serum-saline. The emulsion was clarified by centrifugation, and the supernatant fluid diluted to 6000 cc. with serum-saline. Ether, to make a 2 per cent. concentration, was added as preservative. Recently we have introduced a few modifications. The brains are emulsified in 1000 cc. of serum-saline in a tall glass cylinder provided with an outlet situated 3 cm. from the bottom. A further 1000 cc. of serum-saline is added to this emulsion and thoroughly mixed with it. A sterility test is carried out, 'Merthiolate'** added to make a concentration of 1:20,000, and the emulsion stored in the refrigerator for 20 hours. At the end of this period most of the brain particles have settled out, so that a relatively clear supernatant fluid may be tapped off. This is diluted with 4000 cc. of serum-saline containing 'Merthiolate,' 1:20,000.

The 1936 vaccine contained four strains (O, 449, 464A, 464B), and only 48 brains were used to prepare a batch. The brains were emulsified in serum-saline in 50 cc. centrifuge tubes by drawing fluid into and ejecting it out of a 1 cc. tuberculin syringe, the final disintegration of the brains being assisted by rapidly freezing and thawing the emulsion. The emulsion was clarified by centrifugation and the vaccine prepared by diluting the supernatant with the required amount of serum-saline. Ether was added as a preservative.

* A "turbo-generator," made by the Moritz Chemical Engineering Company, Ltd., 14, Palmer Street, London S.W.1, and kindly brought to our notice by Dr. W. S. Gordon of Moredun Institute, Midlothian, Scotland, is used.

** Sodium Ethyl Mercuri Thiosalicylate (Eli Lilly & Co.).

The vaccine, as prepared by the method used in 1937, 1938, and 1939, gave excellent results as a prophylactic, and, to the best of our knowledge, caused only a very small number of local reactions or abscesses at the site of inoculation. However, opportunities for the entrance of bacteria into the vaccine are afforded at almost every step of the preparation — on inoculation of the mice, at the removal of the brains, and during emulsification, syphoning, and bottling. Further, the mice themselves may be inapparently infected with some bacterial disease.

Realizing the relatively poor germicidal properties of ether, we decided to investigate the possibility of replacing it with another bactericide. The result of the investigation has been an appreciation of 'Merthiolate' (Eli Lilly & Co.). It should be understood clearly that we did not set out to test the germicidal or other properties of 'Merthiolate' — the results of such research have been reported in detail by other workers — but to investigate its value as a preservative of horsesickness vaccine.

Experimental Procedure.

General. — Throughout the work, mice *in extremis* as a result of neurotropic horsesickness virus infection were killed by ether anæsthesia, the brains removed and emulsified in 10 per cent. serum-saline. The supernatant fluid obtained by centrifugation at 3000 revolutions per minute was diluted to what we term "vaccine strength" and this product was treated as detailed in the following experiments. Virus titrations were carried out by inoculating mice intracerebrally with falling two-fold dilutions of the fluid under investigation, the dose being 0·05 cc. Sterility tests were carried out by seeding 0·25 cc. amounts into 15 cc. of broth and on to agar slants. Because of the known bacteriostatic power of 'Merthiolate,' even in very low concentration, the broth was subinoculated into fresh broth after incubation for 48 hours, and the final reading made after a further 72 hours' incubation.

Experiment 1.

Strain 464; 3 bottles of vaccine were prepared and treated as follows:—

(a) ether added to produce a concentration of 2 per cent.

(b) ether, and a drop of dilute bacterial suspension (a salmonella and a diphtheroid) added.

(c) 1 : 50,000 'Merthiolate'* and a drop of the same bacterial suspension added.

The bottles were then placed in an incubator at 37° C, and the further investigations are recorded in tabular form (Table 1).

* Throughout the text, the phrases "1 : 50,000, 1 : 10,000 'Merthiolate' added" mean that sufficient 'Merthiolate' was added to produce the indicated concentration.

TABLE 1.

The Effect of Ether and of 'Merthiolate,' 1:50,000 at 37° C.

Material	Treatment	Titre	Sterility	
	No. of days at 37°C		Agar	Broth
Original: control	None	At least 1:256	—	—
Plus ether (a)	1	„ „ 1: 64	—	—
Plus ether and Bk (b)	1	„ „ 1: 64	+	+
Plus 'M' and Bk (c) ..	1	„ „ 1: 64	—	+
Plus ether (a)	3	„ „ 1: 16	—	—
Plus ether and Bk (b)	3	„ „ 1: 16	+	+
Plus 'M' and Bk (c) ..	3	„ „ 1: 16	+	+

(Bk = bacteria; 'M' = 'Merthiolate'; — = no growth; + = growth).

Conclusion. — As end points were not reached in the virus titrations, the relative effect of ether and 'Merthiolate' is not apparent. In the concentrations used, neither was bactericidal.

Experiment 2.

Strain 449; similar to experiment 1 except that a white staphylococcus and a salmonella were added, and that 'Merthiolate' in a concentration of 1:25,000 was used. Sterility tests of those bottles to which bacteria were added showed growth on agar and in broth when tested prior to incubation. The results are shown in Table 2.

TABLE 2.

Effect of Ether and of 'Merthiolate', 1:25,000 at 37°C.

Material	Treatment	Titre	Sterility	
	No. of days at 37°C		Agar	Broth
Original: control	None	1:64	—	—
Plus ether only (a)	1	1:64	—	—
Plus ether and Bk (b)	1	1: 8	—	—
Plus 'M' and Bk (c)	1	1:64	—	—
Plus ether only (a)	3	> 1: 2	—	—
Plus ether and Bk (b)	3	1: 8	—	—
Plus 'M' and Bk (c)	3	1:32	—	—

(Bk = bacteria; 'M' = 'Merthiolate'; — = no growth; + = growth).

Conclusion. — 'Merthiolate', 1:25,000, had no virucidal effect after 1 day at 37°C and very little after 3 days at 37°C, whereas after 3 days at 37°C the ether reduced the virus titre significantly. (This big reduction in titre is exceptional, and should not be regarded as typical of the effect

of ether.) Apparently both preservatives were equally bactericidal, because no growth on agar or in broth was obtained from any of the bottles although these bottles had been shown to contain living bacteria before the commencement of incubation.

Experiment 3.

Strain 449; similar to experiments 1 and 2 except that 3 drops of a dense suspension of a mixture of a staphylococcus, a streptococcus, *Ps. pyocyanea* and *Br. abortus* were added. In addition, 'Merthiolate' 1 : 10,000 was included. Before the commencement of incubation, all bottles to which bacteria had been added were found to contain living organisms. The results are shown in Table 3.

TABLE 3.

Effect of Ether and of 'Merthiolate,' 1:10,000 and 1:25,000 at 37°C.

Material	Treatment	Titre	Sterility	
	No. of days at 37°C		Agar	Broth
Original: control	None	1:256	—	—
Plus ether only (a)	1	1:128	—	—
Plus ether and Bk (b)	1	1: 64	+	+
Plus 'M' 1:10,000 and Bk (c)	1	1: 64	—	—
Plus 'M' 1:25,000 and Bk (d)	1	1: 64	+	+
Plus ether only (a)	3	1: 64	—	—
Plus ether and Bk (b)	3	1: 32	+	+
Plus 'M' 1:10,000 and Bk (c)	3	1: 64	—	—
Plus 'M' 1:25,000 and Bk (d)	3	1: 64	+	+

(Bk = bacteria; 'M' = 'Merthiolate'; — = no growth; + = growth).

Conclusion. — 'Merthiolate' in a concentration of 1 : 10,000 was able to sterilize a bacterial contamination that was not controlled by either a 1 : 25,000 concentration or by 2 per cent. ether. Both the merthiolated and the etherized emulsions showed a definite fall in virus titre under the conditions of the experiment but it is not possible to say whether this fall was due to the virucidal action of the preservative or to inactivation caused by temperature.

Experiment 4.

Strain O.D.; similar to experiments 1, 2 and 3 except that one drop of a dilute suspension of the bacteria noted in experiment 3 was used. Before incubation all bottles that received the suspension were shown to be contaminated. The results are given in Table 4.

TABLE 4.

Effect of Ether and of 'Merthiolate,' 1:10,000, 1:14,000 and *1:20,000 at 37°C.*

Material	Treatment	Titre	Sterility		Treatment	Sterility		Titre
	No. of days at 37°C		Agar	Broth	No. of days at 37°C	Agar	Broth	
Original: control	None	1:256	—	—	None	—	—	
Original	1	1:128	—	—	3	—	—	a.l. 1:64
Plus ether and Bk.	1	1:128	+	+	3	+	+	a.l. 1:64
Plus 'M' 1:10,000 & Bk.	1	1:128	—	—	3	—	—	a.l. 1:64
Plus 'M' 1:14,000 & Bk.	1	1:128	—	—	3	—	—	a.l. 1:64
Plus 'M' 1:20,000 & Bk.	1	1:128	+	+	3	+	+	a.l. 1:32

(Bk = bacteria; 'M' = 'Merthiolate; — = no growth; + = growth; a.l. = at least).

Conclusion. — The conclusion drawn from experiment 3 that 'Merthiolate' in a dilution of 1:25,000 is incapable of dealing with the gross experimental bacterial contamination is confirmed. The suspensions were sterilized by concentrations of 1:10,000 and 1:14,000. The higher concentrations of 'Merthiolate' were no more virucidal than ether.

Comment.

The results of the four experiments show that 'Merthiolate,' in concentrations of from 1:10,000 to 1:20,000, was superior to 2 per cent. ether as a bactericide, and was no more virucidal than ether. However, the conditions of the experiments were far more rigorous than any to which routine vaccine would be subjected. Firstly, it is rare for vaccine to be exposed to a temperature of 37°C for a period of 3 days; secondly, the degree of experimental contamination was much greater than would be encountered in any batch of vaccine even if it were produced in a grossly careless manner under the worst possible conditions. Further, the results of the sterility tests as noted in tabular form do not reflect the true picture. The number of colonies appearing on agar inoculated with a merthiolated vaccine was always much less than on agar inoculated with an etherized vaccine. As a rule, only two to ten colonies grew on the slant seeded with the merthiolated product, whereas, on that inoculated with the etherized material, a confluent growth or an uncountable number of colonies was the result. We decided therefore to imitate more closely the conditions that might prevail in a very carelessly produced batch of vaccine issued in the ordinary way during the hot summer months.

Experiment 5.

A serum-saline emulsion (strain 449) of vaccine strength was pipetted in 30 cc. amounts into a number of bottles. A very dilute suspension of a mixture of a staphylococcus, a streptococcus, *Ps. pyocyanea* and *Br. abortus,*

was prepared. One drop of this suspension was added to each of a number of these bottles of vaccine; to some of these ether, to make a 2 per cent. concentration, was added and to others 'Merthiolate' 1:10,000, 1:15,000 or 1:20,000. A few bottles, without the addition of either bacteria or preservative, were retained as controls. Before sealing the bottles sterility tests were carried out. The controls all proved to be sterile; growth in broth was obtained from all others, but only the etherized samples showed growth on agar slants.

The control bottles were stored in a refrigerator at approximately 5°C and were tested on the 10th, 24th, and 41st days. The others were divided into two groups and treated in the following manner.

A. Vaccine prepared on 2:2:40 and stored in the refrigerator until 12:2:40, on which day it was packed in sawdust and forwarded by passenger train to Allerton Laboratory, Pietermaritzburg. It was received at Allerton on 14:2:40, and placed on a shelf for 5 days at room temperature (27° to 30°C). It was then returned by passenger train to Onderstepoort and arrived there on 21:2:40, *i.e.* 9 days after despatch and 19 days after preparation. The parcel was placed in the refrigerator until the following day when the tests were carried out.

B. Forwarded by passenger train to Windhoek, South West Africa on 12:2:40; received in Windhoek on 16:2:40; held at room temperature (24° to 27°C) for 5 days and then returned to Onderstepoort where it arrived on 24:2:40, *i.e.* 12 days after despatch. The package was stored overnight in the refrigerator, and the tests were carried out the following day. The results are given in Table 5.

Conclusion. — The results show that vaccine, untreated, treated with ether and infected, or treated with 'Merthiolate' and infected, and stored at refrigerator temperature (*ca.* 5°C) did not deteriorate in value over a period of 41 days. The untreated and the merthiolated samples were bacteriologically sterile when tested on the 24th day; the etherized samples still contained living bacteria at this time. The merthiolated samples sent to either Allerton or Windhoek had, after a period of 20 and 23 days respectively, approximately their original titre and were bacteriologically sterile; the control samples were badly infected but were nearly of the original value; the etherized Allerton sample was grossly infected and had dropped in value, and the etherized Windhoek sample was so badly infected that a virus titration was impossible.

These results show that a vaccine, contaminated to a degree that could not happen in this laboratory, and treated with 'Merthiolate,' 1:20,000, could be issued with safety for immunization purposes.

TABLE 5.

Effect of Ether and of 'Merthiolate' on Contaminated Vaccine issued to the Field.

Material.	Storage (5°C)						A. to Allerton		B. to Windhoek	
	10 days		24 days		41 days					
	Titre	Sterility	Titre	Sterility	Titre	Sterility	Titre	Sterility	Titre	Sterility
Untreated control	1: 64 – 1:128	—	1:256	—	1:256	N.D.	1:128	Badly contaminated	1:128	Badly contaminated
Ether and bacteria	1:256	+	1:128 – 1:256	+	1:128	N.D.	1: 32 – 1: 64	Grossly contaminated	×	Grossly contaminated
'M' 1:10,000 & bacteria	1:128	—	,,	—	1:256	N.D.	1:128 – 1:256	—	1:128	—
'M' 1:15,000 & bacteria	1:256	—	,,	—	1:256	N.D.	1:128 – 1:256	—	1:128	—
'M' 1:20,000 & bacteria	1: 64 – 1:128	—	,,	—	1:128	N.D.	1:128 – 1:256	—	1:256	—

(Abbreviations as in table 1; N.D. = not done; × = so grossly infected that a titration in mice was impossible.)

Comment.

We considered that our method of carrying out sterility tests (see under "General Experimental Procedure") would dilute the 'Merthiolate' below its effective bacteriostatic range. Further work proved, however, that this preservative in a dilution of 1:1,200,000 (0·25 cc. of infected vaccine containing 1:20,000 'Merthiolate' in 15 cc. of broth) sometimes prevented the growth of microbes. Marshall, Gunnison and Luxen (1940) made a similar observation and recommended the use of Brewer's (1940) medium (glucose broth, containing 0·05% agar and 0·1% sodium thioglycollate) for sterility tests of substances containing 'Merthiolate.' We were unable to obtain sodium thioglycollate locally, but found that Robertson's meat broth (horse flesh infusion peptone broth plus minced meat particles) supported the growth of bacteria from merthiolated vaccines that failed to grow in ordinary broth or on agar*. For this reason we repeated a portion of the word and record the details in Experiment 6.

Experiment 6.

To each of 4 bottles containing 10 cc. of 1:20,000 merthiolated vaccine 1 drop of a dilute suspension of a mixture of a staphylococcus, a pasteurella, and a salmonella was added. Immediately after this, 6 drops (0·25 cc.) from each bottle were seeded into 15 cc. amounts of meat broth, 15 cc. amounts of ordinary nutrient broth and on to nutrient agar. The tubes were incubated at 37°C for one week. Two bottles of infected vaccine were left at room temperature (17°C to 23°C) and two were left at refrigerator temperature (*ca.* 5°C). At intervals thereafter, sterility tests and virus titrations were carried out. The results are recorded in table 6.

Conclusion: The results confirm those of previous experiments. 'Merthiolate,' 1:20,000, after 43 days contact at either 5°C or at between 17°C and 23°C had no demonstrable harmful effect on the virus. We have ample evidence, from routine experience, that the drop in titre, particularly at room temperature, is to be ascribed to the storage and not to the 'Merthiolate.' As with most stored horsesickness vaccines, difficulty was experienced in obtaining a sharp endpoint in the titrations, hence the value, '1:500 – 1:2000,' given for '1 R.T.' sample.

After 4 days, the vaccine left at room temperature was sterile when tested in meat broth, but between 34 and 43 days were necessary to sterilize

* While the above article was in press, we received, through the courtesy of Dr. W. A. Jamieson of the Lilly Research Laboratories, a supply of Thioglycollate medium (Baltimore Biological Laboratory). In the single test we have been able to carry out, on the lines detailed in the article, the thioglycollate medium, as a detector of bacterial contamination in merthiolated horsesickness vaccine, proved much superior to broth and nutrient agar, but considerably inferior to meat broth.

TABLE 6.

Effect of 'Merthiolate,' 1:20,000, on Infected Vaccine, at 5°C and at between 17°C and 23°C.

Bottle.	Interval (Days). 0 Titre.	0 Sterility. Ag.	0 Sterility. Br.	0 Sterility. M.B.	4 Titre	4 Sterility M.B.	12 Sterility. Ag.	12 Sterility. Br.	21 Titre	21 Sterility M.B.	34 Titre	34 Sterility M.B.	43 Titre.	43 Sterility. Ag.	43 Sterility. Br.	43 Sterility. M.B.
1 R.T.	1:4,000	+	—	+	1:4000	—	—	—	1:2000–1:4000	—	1:500	—	1:500–** 1:2000	—	—	—
2 R.T.	N.D.	—	—	+	N.D.	—	—	—	N.D.	—	N.D.	—	1:1000	—	—	—
3 Refrig.	N.D.	+	—	+	1:4000	+	+	+	1:2000–1:4000	+	1:2000–1:4000	+	1:2000–1:4000	—	—	—
4 Refrig.	N.D.	—	—	+	N.D.	+	—*	—	N.D.	+	N.D.	—	1:2000–1:4000	—	—	—

(R.T. = room temperature; Refrig. = refrigerator temperature; N.D. = not done; Ag. = agar; Br. = broth; M.B. = meat broth; + = growth; — = no growth; * = this bottle left at room temperature during days 6 to 8; ** = see text.)

those samples stored at 5°C. Further work however has shown that bacterial sterility of a lightly infected vaccine is brought about by 'Merthiolate,' 1 : 20,000, in 48 hours at room temperature and usually in 24 hours.

Thus, on the assumption that sterility tests in meat broth are reliable, there is much evidence for stating that the results of sterility tests recorded in the earlier portion of this article are true.

Experiment 7.

A batch of vaccine, containing all the 6 strains of virus usually included, was prepared and divided into two equal portions. To one ether, and to the other 'Merthiolate,' 1 : 20,000, was added. Both were bacteriologically sterile and the titre was 1 : 1024. Each of 12 guinea-pigs received 1·0 cc. of the etherized vaccine intraperitoneally, and each of another 12 received 1·0 cc. of the merthiolated vaccine intraperitoneally. After an interval of approximately 1 month, each guinea-pig was tested for immunity by the intracerebral inoculation of 0·2 cc. of a 10 per cent. emulsion of infected mouse brain. The results are given in Table 7.

TABLE 7.

Immunizing Power of Etherized and of Merthiolated Vaccine.

(Guinea-pigs inoculated i.p. 8.4.40; tested i.c. 10.5.40.)

Test Virus.	Ether Vaccine.						'Merthiolate' Vaccine						Controls.			
Vryheid	9	9	9	12	—	—	10	10	—	—	—	—	9	9	10	10
464	—	—	—	—	—	—	—	—	—	—	—	—	7	7	10	10

(7, 9, etc. = day of death; — = lived).

The immunity resulting from the inoculation of the merthiolated vaccine was as good as, if not better than, that produced by the etherized vaccine.

Experiment 8.

While the above-mentioned experiments were in progress 'Merthiolate,' in a concentration of 1 : 10,000, was added to stock emulsions of strains 449, O, 464, and Vryheid, and the emulsions were stored in the refrigerator at about 5°C for use as antigens in serum-virus neutralization tests. Considerable experience has been gained of the keeping qualities of unpreserved antigens stored under identical conditions. The potency of the stored merthiolated antigens was as good in every case, and they could be relied upon to retain full infectivity for periods up to 3 months.

Experiment 9.

The innocuousness of 'Merthiolate' was proved by injecting subcutaneously as much as 20 cc. of a 1 : 10,000 dilution into horses. No reaction other than a slight, transient swelling was produced.

Since the completion of these experiments we have had the opportunity of inoculating over 20,000 horses with vaccine containing 'Merthiolate,' 1 : 20,000. Up to the present, no adverse reports have been received.

Conclusions.

1. 'Merthiolate,' in concentrations of from 1 : 10,000 to 1 : 20,000, has no appreciable adverse effect on horsesickness virus during a period of 3 months at 5°C, and of 43 days at between 17° and 23°C.

2. In these concentrations, it is a much better bactericidal and bacteriostatic agent than ether.

3. A concentration of 1 : 20,000 would appear to be sufficient to destroy the chance contaminant that might gain access to horsesickness vaccine.

4. 'Merthiolate' 1 : 20,000 is an excellent preservative for neurotropic horsesickness vaccine, and is being used as a routine.

Acknowledgments.

We have pleasure in acknowledging our indebtedness to Mr. J. R. Stamper, South African representative of Messrs. Eli Lilly and Company for supplies of 'Merthiolate,' and to Mr. A. S. Canham of the Allerton Laboratory and Mr. J. L. Williams of Windhoek for receiving and despatching supplies of experimental vaccine.

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The Value of Bone Marrow and Tail Smears in Facilitating Diagnosis in Decomposing Carcases.

By K. SCHULZ, Onderstepoort.

INTRODUCTION.

Ever since the introduction of smear examination as a routine method for the better control of certain diseases, particularly anthrax and East Coast fever, it has been felt that a way of making smears should be devised that would, to some extent, (i) eliminate the hampering effects of decomposition on proper diagnosis, (ii) eliminate the necessity for opening the carcase to make spleen smears and (iii) produce a smear in which Koch's bodies should be fairly frequent and evenly distributed. Any new technique must be suited to our field conditions and must be simple, so that any person could make a satisfactory smear.

At the beginning of 1938 an investigation aiming at improved methods of smear preparation in East Coast fever areas was started. The use of the smear from the ear, though recommended in the regulations dealing with the control of anthrax, was not considered, owing to the extreme difficulty — and often the impossibility — of obtaining a suitable smear from this source some time after the death of an animal.

Although the taking of a spleen smear has been adopted as a routine procedure in East Coast fever areas, the desirability of superseding this method by a more suitable one had to be considered seriously for the following reasons:—

i. The safety of persons handling carcases of animals, which may have died of anthrax, is endangered.

ii. Natives carrying the spleen or portions of it to stations specially provided for making smears may spread anthrax over considerable areas.

iii. Putrefactive changes may set in in the spleen comparatively shortly after the death of an animal, depending on the outside temperature, and the resulting smear may be completely useless for diagnostic purposes. Another disadvantage to be considered is the nuisance caused to the persons who have to deal with, and later destroy, these putrid organs.

As numerous Koch's bodies are always present in the lymph-gland smears of affected animals, and as these bodies appear in gland smears much earlier than in those made from the blood, it seemed quite likely that smears from a superficial lymph gland would suit the purpose best. A technique for the removal of a lymphatic gland, such as the parotid, was developed;

but it soon became apparent that the lymph gland putrefied much sooner than the spleen. This method had therefore to be abandoned.

Attention was then turned to the tail, a readily accessible organ that did not dry so quickly as the ears, and that did not putrefy so quickly as spleen and lymph glands. Its use eliminated the necessity for opening the carcase and for transporting organs liable to spread anthrax. However, for reasons to be enumerated later, the tail smear, although useful in many ways, did not come up to expectations, and attention was turned to another organ, the bone marrow. This appears to give more satisfactory results than any other organ examined.

History.

On several occasions Government Veterinary Officers stationed in East Coast fever areas said that the disease could sometimes be diagnosed from tail smears, when the usual lymph-gland and spleen smears were too decomposed, and suggested that the tail smear should be introduced as a routine measure for controlling the disease.

On examining smears taken from the ear, the jugular vein, the spleen, the bone marrow, the coronet, and various levels of the tail of a cow, which had been injected with *T. congolense* some time prior to its death, the writer found a few trypanosomes in the coronet and tail smears only. In the smear taken about 5 cm. from the tip of the tail they appeared to be more numerous than at other levels. On account of these observations it was decided to see whether the tail smear would be suitable for the diagnosis of East Coast fever in the field.

EXPERIMENTS.

The Material Used.

Arrangements were made with Mr. van Drimmelen, then Government Veterinary Officer at Ermelo, Transvaal, for the forwarding of duplicate sets of gland, spleen, and tail smears from all cattle which died in East Coast fever areas under his control. The one set was used for the ordinary routine examination, and the other was examined by the author. The results of the latter examination are fully described below under heading 1.

As comparatively few positive smears were obtained from the field in this way, other material (smears from the ear, the jugular vein, a lymph gland, and the tail) was obtained from live animals infected with East Coast fever at Onderstepoort. These smears were supplemented with smears from the spleen, the liver, the kidney, the coronet and bone marrow taken after the death of the animals. The organs were kept at room temperature and smears taken from them at intervals of 24 hours until the organs were useless for further diagnostic purposes.

These smears served the dual purpose of controlling the field experience to some extent and of demonstrating the distribution of Koch's bodies in the general circulation before and after the death of the animal. The results are discussed under heading II.

Undoubtedly the material collected at Onderstepoort could not be strictly compared with that obtained under field conditions; therefore, to study the effects of putrefaction, a similar set of smears was made at random from decomposed carcases of animals which had either died at Kaalplaas or which had been brought in by farmers for post mortem. The results appear under heading III.

To determine histologically whether any active lymphoid tissue was present in the tail during the course of the disease, transverse and longitudinal sections were made from different levels of the tails of some nearly full-term foetuses, and from the tails of normal, and of East Coast fever infected animals. After fixing the material in 10% formalin solution it was embedded and stained with the usual routine stains. The details of the examination are described later (Heading IV).

Results.

The results obtained are reviewed briefly under the headings I – IV.

I. *Smears from East Coast Fever Area (Carolina).*

(a) *Negative Smears* examined from animals either killed or died.

Spleen smears	250	(55 of which were useless).
Ear smears	2	
Tail smears	250	(45 of which were useless).
Gland smears	71	(7 of which were useless).
Total	573	

Although there is no appreciable difference between the number of useless smears from the spleen and tail (11 of the useless spleen smears were sent without a corresponding tail smear) yet, on the whole, the tail smears were less putrefied than the spleen and gland smears.

(b) *Smears from Positive East Coast Fever Cases.* — These were made from 19 animals and were distributed as follows: 18 tail smears, 10 gland smears, and 9 spleen smears, making a total of 37 smears. A summary of the findings is given in table 1.

TABLE 1.

Number.	Result of Examination.		
	Gland.	Tail.	Spleen Smears.
1	+	—	0
2	+	0	0
3	+	+	0
4	+	+	0
5	+	Useless	0
6	Useless	Small piroplasms*	0
7	+	+	0
8	+	+	0
9	+	+	0
10	+	Useless	0
12	0	Small piroplasms only	+
18	0	Small piroplasms only	+
13	0	+	+
14	0	+	+
15	0	+	+
16	0	+	+
17	0	+	+
11	0	Small piroplasms only	+
19	0	+	+

+ = positive (smear); — = negative (smear); 0 = no (smear).

East Coast fever could, therefore, be diagnosed definitely from tail smears in only 11 out of 18 positive cases (in one case no corresponding tail smear was forwarded); in four other cases only small piroplasms were noted, two were useless for diagnostic purposes, and in one neither Koch's bodies nor small piroplasms could be found owing to the scarcity of blood cells.

Koch's bodies were numerous in the smears taken from the gland and spleen, whereas they were extremely rare in the tail smears and their distribution there was irregular. However, in one tail smear small piroplasms could be found, whereas the corresponding gland smears (including the routine smear) were unsatisfactory and no diagnosis could be made.

II. *Smears from Animals infected with East Coast Fever at Onderstepoort.*

(a) After the death of an animal East Coast fever could be diagnosed from the bone marrow smear in 10 out of 11 cases for 2–4 days after death and from the tail smear in 20 out of 21 cases for at least one day longer than from the corresponding lymph and spleen smears. (In one case no material was collected at post mortem.)

(b) Of 21 infected animals 8 were killed and 13 died. The carcases of three of the latter were in an advanced state of decomposition before smears could be taken and were perhaps the only East Coast fever cases which could be compared with cases from which smears are taken in the field. In two of these the tail smears were much better than the corres-

* The piroplasm-line stage of *Theileria parva* found in the erythrocytes.

ponding parotid lymph gland and spleen smears, although Koch's bodies were less frequent than in the latter. In the remaining case bone marrow smears were taken and proved to be the most satisfactory.

(c) If smears are taken from an animal shortly after death, then East Coast fever can be diagnosed much more easily from spleen, lymph-gland and bone-marrow smears than from tail smears, owing to the comparative scarcity and irregular distribution of Koch's bodies in the last. The comparative frequency of Koch's bodies in the smears from the various organs is as follows:— (1) lymph gland, (2) spleen and bone marrow, (3) tail, ear, and large vein (jugular) and (4) coronet.

(d) Small piroplasms and Koch's bodies could be demonstrated for a longer period after death in tail and bone-marrow than in spleen and lymph-gland smears.

(e) If organs are kept under similar conditions, putrefaction sets in earliest in the lymph gland, then in the liver and spleen, then in the tail and lastly in the bone marrow. This observation was substantiated by taking smears from these organs from carcases too decomposed for post-mortem examination. The sooner an organ is removed from a carcase after death the slower it putrefies. The rate depends on the temperature. When ribs were kept at room temperature during a cold spell no putrefactive organisms were found in the smears made from the bone marrow seven days after post mortem, whereas following a rise of temperature, suitable smears could be made only up to three days post mortem. Bone-marrow smears were serviceable at least two days after the lymph-gland and spleen smears had become useless. When the ribs were wrapped in paper to prevent their drying, suitable smears could be obtained for a longer period than when the ribs were exposed to the air. If they were enclosed in a flap of their cutaneous and muscular covering, the value of the smear was reduced owing to putrefactive changes setting in much earlier.

(f) The bone-marrow smear was far more satisfactory than the tail smear, as Koch's bodies were frequent (up to 9 and even more in one field) and signs of putrefaction were less apparent. Considerable difficulty was experienced in finding Koch's bodies in smears from the tips of tails kept for 2 – 4 days owing to the large amount of serum and comparatively few blood cells at this site. When smears were taken from portions enclosing larger blood vessels and more blood elements, Koch's bodies appeared to be more frequent.

(g) There was no apparent difference in the results of the tail, ear, jugular vein, and coronet smears. In all smears, small piroplasms and Koch's bodies were present. Koch's bodies were also found in the smears of the brain, kidney, liver, and testes, but as these smears offered no advantages over those from the other organs they were discarded at an early date.

(h) In some cases the presence of a number of small piroplasms in the tail smear of an animal suspected of having died of East Coast fever made possible a tentative diagnosis of this disease, even though the corresponding lymph-gland and spleen smears were useless for diagnostic purposes.

(i) In the marrow smears, Koch's bodies appeared to be more susceptible to the effects of putrefaction than the erythrocytes.

(j) From smears made over the whole course of the disease, it seemed that Koch's bodies appeared in the blood fairly early and that their number increased from extremely rare to fairly frequent as the disease progressed. Their distribution in the smears, however, was somewhat irregular, and Koch's bodies may be overlooked if the smears are examined casually.

III. *Results of smears taken from Carcases too Decomposed for post-mortem examination.*

On examining the smears taken from three carcases too decomposed for post mortem, it was found that the results corroborated those already described under heading II, i.e. the bone-marrow smears remained satisfactory for a considerably longer period than those of the lymph gland, liver, spleen, and tail.

IV. *Results of the Histological Examination.*

The histological examination of the sections from various levels of the tails of nine animals (four of which originated from East Coast fever cases, three taken from animals at random; and two from nearly full-term foetuses obtained from the Pretoria abattoir) revealed no appreciable differences between sections from the various levels of the tail of the same animal and from the other animals. In no section could any lymph follicles or lymphoid tissue be found.

Smears taken at various levels were examined and the results compared with those obtained from smears taken at similar levels of the tails of East Coast fever cases. In no case was the number of lymphocytes increased to such an extent as to suggest the presence of ordinary or proliferating lymphoid tissue.

Discussion.

The conditions under which these smears were taken were of course more favourable than those prevailing in the field, and therefore the results under heading II cannot strictly be compared with those enumerated under heading I. However, the tail smears sent in were, on the whole, in better condition than those of the corresponding spleen and lymph-gland smears. The observations also showed clearly that bone-marrow smears were more

satisfactory than other smears taken at the same time, under similar conditions. This has been substantiated to some extent by the results described under heading III (smears from decomposed carcases).

The difference in the number of Koch's bodies present in smears made from a tail shortly after death and some time later, probably depends on the state of coagulation of the blood, as these bodies were quite frequent in the earlier smears and extremely rare or even absent in the later; i.e. after the blood had coagulated. Only serum with comparatively few cellular elements could then be obtained. Presumably the cells containing Koch's bodies become fixed to or are enclosed by the blood clot and are therefore not easily detached. This phenomenon, and possibly also faulty technique, may be reasons why tail smears from field cases of East Coast fever have not been so good for demonstrating Koch's bodies.

Conclusions.

Final conclusions cannot be drawn at this stage, but it would appear that the bone-marrow smear taken from a fairly decomposed carcase at the same time as the tail, lymph-gland or spleen smears, is far more satisfactory than the latter three and could probably supersede the lymph-gland or spleen smears as a routine method for diagnosing East Coast fever.

The tail smear, it may be added, will not supersede the lymph-gland or spleen smears, but there is no doubt that it will be of value where the carcases are in a fairly advanced state of decomposition. Care should, however, be taken to make a smear from blood, and not from the serous fluid which oozes from the cut surface.

It is generally admitted that Koch's bodies and small piroplasms appear in the blood stream during the course of the disease, and the distribution of the parasites apparently becomes uniform throughout the blood stream. When the carcase is still fairly fresh there is thus no point in making a bone-marrow or tail smear since smears from the ear and any other part (lymph gland) will give as good results. When decomposition has taken place, however, there is undoubtedly an advantage in taking a bone-marrow and a tail smear, since the blood here will not hæmolyse nor decompose so rapidly.

Finally by combining the ear smear with a superficial lymph-gland smear in a fresh carcase, or the bone-marrow smear with a tail smear in a decomposed one, East Coast fever could probably always be diagnosed. This procedure would eliminate to a considerable extent the necessity for opening the carcase of an animal that might have died of anthrax.

Summary.

Series of smears from various organs of animals which died of East Coast fever, taken under similar conditions in the field and at the laboratory,

have been examined to determine their suitability for diagnosing East Coast fever in fairly decomposed carcases. Bone-marrow smears prepared from ribs proved to be the most suitable. Such smears were easily made and showed many Koch's bodies evenly distributed throughout the smear. The bone marrow decomposed relatively slowly, and opening of the carcase was avoided.

Acknowledgments.

I wish to express my sincere thanks to Mr. van Drimmelen for the trouble he took in supplying me with smears from animals which died in an East Coast fever area, and to Mr. Neitz for letting me have smears and material from animals in an East Coast fever experiment at Onderstepoort.

Appendix.

The tail smear is made as follows:—

In a fresh carcase, a transverse cut is made about 5 cm. from the tip of the tail and from the blood oozing from the cut surface a smear is made. In some cases it is necessary to apply pressure just above the cut to obtain sufficient blood for smears. During warmer weather, however, owing to the drying out of the tail and the coagulation of the blood, considerable difficulty is experienced in obtaining a satisfactory smear from this site. Usually only serum is obtained which is useless for diagnostic purposes. The incision should then be made some distance below the root of the tail in order to sever a larger blood vessel from which sufficient blood may be obtained for the smears.

The bone-marrow smear can be made as follows:— After the skin and muscles covering one of the last three ribs have been removed, cuts are made along both edges of the exposed rib to sever the intercostal muscles, and to expose the costochondral junction. A cut is made through the cartilage and the rib bent by taking hold of the free end, lifting and pushing it over towards the vertebral column until it snaps. The cartilage at the sternal end is now cut away with a knife, and, by applying pressure just above the cut end or squeezing it in an ordinary pair of pliers, sufficient bone marrow can be obtained to make several smears.

In the decomposed carcase, however, as hæmolysis and putrefaction seem to appear earlier in the vascular sternal end than in the shaft of the rib, smears should be made from the latter instead of the former portion.

If the smears have to be made some distance away from the carcase, excessive drying out of the rib may be prevented by wrapping it up in paper immediately after its removal. The skin and muscle covering should not be used, as the moisture of these structures accelerates putrefactive changes.

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Parotid Abscesses in Cows due to Tuberculosis and Other Causes.

By E. J. PULLINGER, the Municipal Abattoir, Johannesburg.

The object of this note is to draw attention to the incidence of naturally-occurring tubercular and non-tubercular abscesses of the parotid lymphatic gland of cows, in view of the report by Thorburn and Thomas (1940) of the occurrence of similar lesions in kudu.

I have recently investigated the cause of such parotid abscesses encountered in 17 cows during the course of routine inspection of dairy herds. Of these, 10 were due to infection with *Corynebacterium pyogenes*, 4 to *M. tuberculosis*, 1 to *Actinobacillus lignieresi*, and in 2 instances the abscesses were too heavily contaminated for a satisfactory diagnosis to be made.

In all cases the parotid abscesses appeared to be primary and not metastatic in origin, and this was almost certainly so in one of the tuberculous animals which showed a parotid abscess containing creamy yellow pus, the only other lesion being a small tubercular focus in the bronchial gland. The second tuberculous cow showed parotid and submaxillary abscesses containing creamy pus and in addition caseating foci in the bronchial and mediastinal glands. The third tuberculous cow proved on post-mortem examination to be heavily infected, whilst no post-mortem report was available on the fourth cow.

In addition to the cases noted above, Martinaglia and Robinson have records of 7 cases of tuberculosis in cows in which a parotid abscess, containing creamy pus, was one of the chief clinical signs of infection.

Except in the case of Actinobacillus infection, satisfactory diagnoses were made from smear examination of pus obtained by gland puncture. Corynebacteria were recognizable when stained by Gram's stain, but smears from the tuberculous glands were negative when stained in this manner; that is to say, neither gram-negative, nor gram-positive organisms were to be seen, nor was there any sign of gram-positive debris which sometimes indicates the presence of degenerated gram-positive organisms in pus. The finding of negative smears from cases such as these, is in itself highly suggestive of tuberculosis, and when duplicate smears were stained for acid-fast organisms, typical tubercle bacilli were found. In the one instance numerous characteristic tubercle bacilli were seen, and the diagnosis was simple, but in the other three cases, typical organisms were found only after a very prolonged search. The valuable indication given by the negative Gram smears was well illustrated in the latter cases, for these negative

findings strengthened the suspicion of tuberculosis gained from clinical examination, and more effort was expended upon the microscopical search for acid-fast organisms than might otherwise have been the case.

In every instance the diagnosis of tuberculosis was confirmed by biological test, and strains of tubercle bacilli were obtained having normal pathogenicity for guinea-pigs.

Discussion.

From the foregoing data it would seem that parotid abscesses occur in cows rather more commonly than is realised, and that these abscesses are sometimes tubercular in origin. Moreover, since this constitutes a sign of tuberculosis not generally recognised by the farmer, it is one which may quite often be encountered during veterinary inspection of dairy herds. That superficial lymphatic glands usually contain creamy yellow pus when tubercular, instead of caseating material, should be remembered; and it is worth noting that, contrary to orthodox teaching, a diagnosis of tuberculosis can generally be made by the careful microscopical examination of pus obtained by simple gland puncture and not scraped off the abscess wall.

The hypothesis advanced by Thorburn and Thomas, that tubercular infection of the parotid gland of the kudu gains entrance through lesions in the ear, is an extremely attractive one in the light of the data they present. When applied to the question of parotid abscesses of cows, it still appears to be valid, so far as *Corynebacterium pyogenes* infection is concerned, for this organism is fairly ubiquitous in South Africa, and might well be introduced into the ear directly or indirectly as result of tick bites. When, however, one considers the occurrence of primary tuberculosis of the parotid gland of well-kept dairy cows, it is difficult to imagine how tubercular material is likely to reach the ear of such an animal.

An obvious alternative portal of entry is the conjunctiva, for it is easy to visualise the eyes being subjected to continuous droplet infection when a cow is kept in close contact with an open case of pulmonary tuberculosis. Some, at least, of the organisms coughed into the eye may survive the destructive action of the lysozyme and invade the tissues successfully, to be carried away in the lymph stream to the regional lymphatic glands. The regional glands receiving lymph from the conjunctiva must be either those situated in the region of the throat or else be the parotid lymphatic gland; and it seems probable that although the bulk of the lymph takes the lower route to the throat region, a certain proportion may drain through the parotid gland, thus accounting for the tuberculous lesions sometimes found there.

Summary.

The incidence and common causes of parotid abscesses in cows are noted and the pathogenesis of these lesions is discussed.

ACKNOWLEDGMENT.

I wish to thank Dr. G. Martinaglia and Mr. M. C. Robinson for giving me permission to use their data.

REFERENCE.

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An Epizootic in Seabirds: A Visit to Dassen and Malagas Islands.

By J. D. W. A. COLES, Onderstepoort.

On Monday, 28:11:38, Mr. Howie, government veterinary officer of Cape Town, and I called on Mr. Patterson, superintendent of the guano islands, and discussed the news from Malagas Island. He could tell us very little beyond the fact that the Malagas birds were dying and that the situation was viewed with a certain degree of alarm. He asked me to be at the docks next morning at ten, and we sailed shortly afterwards in the ss. *Harrier*, 193 tons (Capt. Olsen). Dr. Jackson accompanied me in an unofficial capacity and subsequently rendered great assistance in conducting the investigations. Our guide was Mr. Fourie and he was most companionable and helpful.

Passing Robben Island we saw a seal, but a fresh breeze, a heavy swell and much rolling put an abrupt end to any studies in natural history. We learnt instead what it meant to have green seas washing over the gunwales, how the water rushed out afterwards through the scuppers, and how the most important thing was to hang on and not slide overboard. I regret to have to record that my colleague came near to death's door. Yet, most nobly, he never reproached me for inducing him to join the expedition.

At the conclusion of (for a landlubber) a hectic passage, we anchored off Dassen Island about 2.30 p.m. and the headman, Mr. Traut, rowed us ashore. After his good wife had given us some food we set about exploring part of the island. We could not roam at will, in case we frightened the brown duikers off their nests. When they leave them, they do not return.

Dassen is a penguin's paradise. At least a million roam the tiny bit of land. They were both comical and friendly and their antics afforded a feast of light entertainment. We had not gone to study penguins, but in a few minutes we were thoroughly engrossed. The jackass penguins, *Spheniscus demursus,* live in pairs. On the sheltered parts of the land they dig shallow holes for their homes. Elsewhere they tunnel almost horizontally into the ground, so that their nests resemble miniature caves. Very often they make pan-shaped excavations under the numerous low sage bushes; the foliage forms a pleasant roof. Casting the eye over the island, one sees hundreds of Lilliputian volcanoes emitting steam. These are actually little pale yellow clouds of dust, thrown upwards by the penguins as they deepen their nests by kicking the sand out backwards with their feet.

If the eggs are stolen from the nest, the female may go on laying up to about twenty, although the usual number is only two or three. This psychological stimulation of the ovary is something worthy of careful study.

I believe that a scientific investigation might show how the present sale of 100,000 eggs annually from Dassen could be doubled or trebled. The demand far exceeds the supply and the retail price in Cape Town is 3/6 per dozen. The incubation period is 28 days. There are usually two chicks in a nest, seldom three. The parent penguins waddle down to the sea before dawn and live on fish. The babes are fed when the old folks return.

DASSEN ISLAND.—*Adult and young Jackass Penguins on nest.*

The jackass is, of course, a most curious bird. When sensing possible danger he (or she) advances with outstretched neck, rotating the head from side to side and showing a good deal of the white of the eye; during these actions he keeps on saying ho-ho, with emphasis on the aitches. Periodically he lifts his beak to high heaven and, by violently constricting the throat, forces out blasts of air that enable him to bray like a donkey. Hence his name. If you shut your eyes and imagine ten thousand asses braying, and as many calves bellowing, and as many jackals crying in the Kruger Park, and as many asthmatics wheezing, you will have some idea of what the noise is like all night and all day on Dassen Island. People get used to it.

We examined some nests and found them all swarming with *Argas talaje*. We therefore examined blood smears of four chicks. All had marked anæmia. One had acute spirochætosis: parasites, mononuclears, and neutrophiles swarmed in the blood. Another had a few piroplasms in the erythrocytes and I fancy they consti-

DASSEN ISLAND.—*Jackass Penguins on shore.*

tute a species of *Aegyptianella.* I do not for one moment think the spirochaete is *S. anserina.* It gave a thrill to see these organisms. I have no doubt now what kills a lot of the young penguins. Moreover, I think measures could be devised for saving their lives.

We walked to the lighthouse and from there got a magnificent view of the island. Patches of brown birds were nesting on the higher rocks between the penguins. These birds were duikers, *Pseudocarbo capensis,* excellent guano producers, but not always putting in an appearance to deliver the manure. Here and there were clusters of accursed gulls. They tease the duikers and penguins, and then confederates dart in from behind and steal the chicks and eggs. The gull is fit for one thing only: a bullet. An equally despicable bird is the chimney sweep, *Threskiornis aethiopica aethiopica.* This terror has a long sharp beak which is thrust through the vent of its victim. In this way it pulls out the entrails on which it lives.

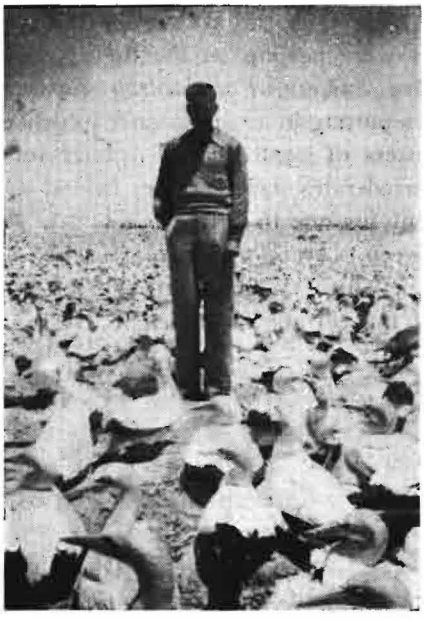
MALAGAS ISLAND. — *White-breasted duikers* (Phalacrocorax lucidus) *nesting and brown duikers in foreground.*

Mr. Traut keeps Swiss goats for fresh milk. Occasionally a number die at once, and I suspect geilsiekte. It is possible that poisonous plants grow on the island. We examined one sick nanny and considered it was suffering from helminthiasis. I would like to recommend that the few goats on the islands be tested for *B. melitensis.* The islanders put up with a lot of hardships and discomforts, not to mention loneliness, and I think everything should be done to keep them happy and healthy.

After dinner we sat and talked to Mr. and Mrs. Traut. He showed us a photo of a white duiker that had pink eyes and yellow legs and beak. Albinism is encountered very rarely in fowls and is inherited as a simple mendelian recessive. The fact that such a condition should be seen in the dark brown duiker is most interesting.

There are 23 islands off the coast of South Africa and 19 of them are under the guano islands administration. Mr. Traut told us of some. I must confess we sat and listened like spellbound schoolboys. There is no doubt that not one South African in ten thousand knows what a fairyland lies at his very door. Even our national parks have a rival in these islands, which constitute an ornithologist's paradise.

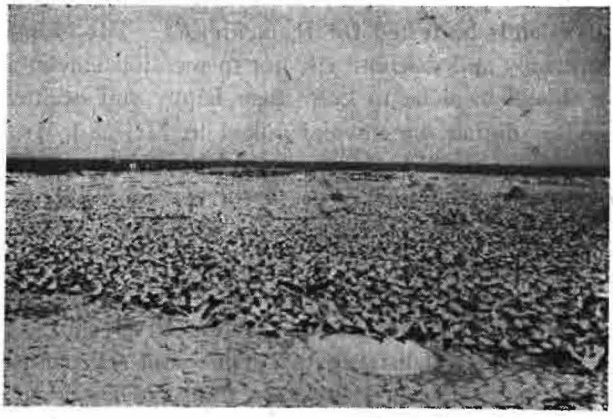
What to me is even more wonderful is that the Union Government is the biggest poultry farmer in the world and does not realize it. Fowls produce eggs, meat and manure; turkeys and ducks produce meat, eggs and manure; seabirds produce manure and eggs. The seabirds are not wild animals like our kudu or giraffe; they may roam the waters in search of food, but they are the most charmingly domesticated of all our poultry. They have no fear, and do not shun man. They can, if necessary, be inoculated with the utmost ease, and I have no doubt that scientific studies of their troubles will repay us handsomely.



MALAGAS ISLAND. — *Nesting gannets are not shy.*

Then Mr. Traut told us about the Malagas and the penguins on Possession Island. They nest in colonies, but the birds on the fringe of one colony will not face the birds on the fringe of the next. They are most unsociable and the two species turn their backs on each other. Ten years ago or more, thousands of penguins died on this island. Their legs got paralysed. Was this *Argas* paralysis ? Only the future can tell. We heard that *Argas talaje* is so troublesome on some islands that human beings are wont to stand the bedposts in paraffin.

We heard of the northern islands where it never rains. Drinking water is taken up from Cape Town. Mercury seems to be remarkable. To all intents and purposes it is a giant rock with a tunnel running from side to side, through which the sea surges. The vibration caused by the waves led to the name of the island. There is a care-



MALAGAS ISLAND. — *Gannets or Malagas nesting.*

taker on Mercury, as there is on each of the other islands, and I do not envy him his post. He takes his exercise on a wooden platform, as the rock is too steep for walks.

At 6 a.m. on 30:11:38 we embarked once more in the *Harrier* and headed for Saldanha Bay. The seas were still fairly heavy and we failed to make contact with the Foundlings, which is a tiny rocky islet. During the morning we saw flocks of Malagas for the first time. We pushed on, with Jutten lying to starboard, and anchored off Malagas Island about 10.30 a.m. We were taken off in a dinghy by the headman, Mr. de Jager. We saw dead birds floating on the water.

On arrival at his homestead, Mr. de Jager told us his story. The island is about a quarter of a square mile in extent, and affords a home for a million or more Malagas, *Morus capensis*. Dark brown duikers, white-breasted duikers, and a few penguins also have their abode there, but none of them were ill.

MALAGAS ISLAND. — *Brown duikers nesting.*

The Malagas is a beautiful bird and is the most valuable producer of guano. Its migrations are regular and each year the amount of guano produced on any of the three islands it inhabits (Malagas, Ichaboe, and Possession) can be estimated fairly accurately in advance. There are stories that the Malagas also patronised other islands once upon a time. Why did they go ? Was it disease ? To me it seems not a far-fetched possibility that some of these islands would become repopulated if eggs were taken there, artificially incubated, and the chicks fed by man and then allowed to go to sea. I think they would return to breed on the new islands, just as the salmon return years later to breed in the rivers from which they went to the ocean. Experiments would settle these speculations one way or the other.

The Malagas seeks a flat bare rocky spot for its nest. It lays down a few twigs and these are cemented firmly together by guano. The finished nest is a smooth shallow pan, rather like a spitoon, grey in colour and as hard as soft plaster, if one can imagine what that is like. The female lays only one egg, which hatches in 42 days. The baby chick is bare and black. Later it grows white down, then black down, and then black feathers shot

with white. At this stage it flies to sea; on its return it is dressed in adult plumage. No banding has ever been done, so we do not know where the Malagas goes when away from the islands.

Due to the moulting of so many chicks, the air over Malagas is full of white eider. The picture suggests a light snowstorm. The eider gets into the nostrils and is irritating. The air too is full of wheeling birds. They circle round and land like autogiros between the nests, which are packed ever so closely together. Only one parent bird leaves the nest at a time, and the family reunion is both affectionate and raucous. The mates are so happy at seeing each other that they lift their heads and squawk, and rub their beaks together in the way that sticks are tapped in hockey. Then they rub their necks together — and I have no doubt that sedate etymologists will one day admit that this action inspired the verb " to neck." The greeting lasts a minute or two. But we found evidence to indicate that all is not virtue and perfect bliss on Malagas. Whether absent-mindedly or not, some birds obviously return to the wrong nests. Since they all live so huddled together and in full view of their neighbours, it is only natural that the intruder should be bitten severely by the victim of such an outraged sense of modesty.

MALAGAS ISLAND. — *Young Malagas showing black plumage.*

The Malagas appears to subsist solely on fresh fish and seawater. This fact suggested that a diagnosis of botulism should be made only on irrefutable evidence.

Mr. de Jager considered two thousand birds had died out of a population of a million or more. We could not even estimate the number of sick, as it meant walking between hundreds of thousands of birds and making them move to see if their legs were paresed. We had to confine our attention to the periphery of the huge colony.

Affected birds get weak in the legs, and their wings droop. They develop a greenish diarrhoea. The head often hangs. The pharyngeal region gets very itchy in about one case in ten, and the bird may scratch so incessantly that blood flows and soils the feathers. Some sick birds drag themselves down to the sea and then have not the strength to get ashore again. Occasionally a victim does manage to leave the water and struggle up the rocks.

I have no doubt that many sicken when flying at sea and never reach land. Death seems to be the general end to the disease, and it supervenes in a day or two. At autopsy we found only a catarrhal to a haemorrhagic duodenitis. A fair number of lice were also on the bodies, and one bird had a solitary worm far down in the oesophagus.

MALAGAS ISLAND. — *Sick Malagas in early stage scratching its neck.*

The affection closely resembles the mysterious condition that broke out in the Pretoria Zoological Gardens in November, 1931, when the Stanley crane, fruit-eating pigeon, ibis, coot, guinea-fowl, pheasant, partridge and other birds died. I feel sure it must be an infectious disease, and infection probably occurs *per os* when the liquid faeces are scraped up by the bird with its beak for the purpose of plastering its nest. Young Malagas were also dying, but I think they were orphans succumbing to starvation. At the time of the Pretoria outbreak, I observed that patients recovered when nursed carefully. For this reason we arranged accommodation in a guano shed for a number of affected birds. I showed the headman how to feed the sick twice daily on sweetened condensed milk, poured into the gullet through a rubber tube and glass funnel. The milk was diluted fivefold with water, and a cupful was given at a time. I regret to say these efforts were in vain.

MALAGAS ISLAND. — *A gannet (Malagas) bleeding from the throat after scratching itself severely.*

The *Harrier* lay off Malagas all day unloading stores and materials. The captain very kindly invited me to an excellent lunch on

board. Towards evening we returned to the ship and sailed into Houtjies Bay (a part of Saldanha Bay) where we were landed. Next day we travelled 133 miles by train to Cape Town at the alarming speed of exactly 11·9 miles per hour, the tedium of the journey being relieved only by the explosions of mirth set off by reading Peter Fleming's inimitable *Brazilian Adventure.*

In the two days we had for our investigations we were simply amazed at the wealth of problems calling for immediate attention. A biologist should assume scientific control of the islands, and he should be able to investigate diseases as well as take a deep interest in ornithology. What makes this doubly important is that conditions lend themselves so extraordinarily well to disease control by inoculation or other methods. Then again, the islands produce penguin eggs and guano, about the only two agricultural commodities that are required in greater quantities by our markets.

Finally, let me say just one more thing. I think the possibilities of the islands should be explored from the educational and tourist points of view. I fancy they could be made as compelling and attractive as our parks. Small pleasure boats could do the round trip in a week, and might be very well patronised in summer by those for whom the Cape rollers have no terror.

Jl. S.A.V.M.A.
XII(1): 30 - 34
1941.

POSTSCRIPT

(to Mr. Coles' Article).

The Editor, Jl. S.A.V.M.A.

Sir, — Since in the foregoing article some aspersions have been cast on my qualities as a sailor, it is only fair that I should be allowed to add a few words in self-defence.

The fauna of Dassen (Cherry Kearton's unnamed "Island of Penguins") cannot fail to be of absorbing interest to any visitor and it is hard to tear oneself away from this fascinating and humorous spectacle. The outlandishness of the penguins contrasts with the commonplaceness of the many thousands of humdrum rabbits which dart about among them. Writers are never tired of emphasising the extraordinarily human qualities of the penguin, which elicit an immediate sympathy from all human observers. At first sight (like sheep) they look as like as the backs of playing cards. But soon one comes to recognise the prototypes of the varieties of one's fellow-men: the patient and domesticated father, the overworked and irate housewife, the ne'er-do-well who scrounges around the dwellings of others bent on theft or worse forms of immorality; the humble *bywoner* content with a primitive dwelling and with scarcely a roof over his head, the more ambitious sapper whose tunnelings enable him to get almost out of sight and from

the depths of which he gazes mournfully upwards with one white-rimmed eye, the elite, who (whether in accordance with some penguinish class-distinction or whether by sheer opportunism) have come to acquire veritable mansions in the form of old soap-boxes or discarded petrol-tins. Families parade, flipper to flipper, abreast down the well-worn paths which thread their way in turn through the fashionable suburbs and the slums. They are most reluctant to be hurried by the threat of being overtaken by the visitor, but cannot resist constantly looking behind them to see how close he is getting. This almost inevitably ends in their falling over a boulder through not looking where they are going.

The braying, as Mr. Coles has remarked, is absolutely indistinguishable from that of a donkey, from the preliminary long drawn-out tuning-in signal, through the working up of speed and confidence in the succeeding hee-haws, to the sobbing and resigned subsidence in the finale of the concerto. One stands appalled at the coincidence that an aquatic bird should produce from its syrinx a noise identical with that delivered by a domesticated mammal from its larynx. Surely, one thinks, there must be enough possible combinations of timbre, rhythm, and pitch of sound to make it unnecessary for totally unrelated phyla of animals to trespass upon each other's patent rights.

The rapidity of the head rotations which accompany the "ho-ho" greeting of the visitor is almost alarming. One fears that the birds will twist their necks off, so fast and so exaggeratedly do they pivot their heads. The object is undoubtedly to get an assured view of the intruder, since birds do not have binocular vision and penguins, being cautious customers, will not, when confronted with the apparition of a human-being, trust the evidence of either eye for longer than a split second. I fully shared Mr. Coles' sympathy for the lonely life of the wardens of such islands. But I confess that I left Dassen with a feeling of supreme regret at having to abandon also the worthy and honest penguins to their hard and patient existence.

The malagas birds, handsome though they are, make no appeal of that kind. They reminded me of the gigantic family of some super-Joseph Kennedy, all looking much alike and complete with horn-rimmed spectacles. I found the noise at Malagas definitely depressing. One wondered how people get used to it. The birds emit characteristic but raucous screams, very difficult to imitate but not unlike what I imagine the cries of the Gryphon in *Alice in Wonderland* to have sounded. Carroll conveyed this noise by "HJCKRRH," and that about covers it. One missed the Mock Turtle from a littoral and rocky setting in which his appearance would not have occasioned the slightest surprise. I considered the Malagas to be, by comparison with the penguins, somewhat unnecessarily aggressive. They peck without warning, the penguins only when all reasonable expostulation — from Munich to Gödesberg — has gone unheeded. And they peck sore. But I

may be doing them an injustice, since they sit so tightly packed that one has literally to force several away with each step, and then stir up a few more to get room to put one's foot down.

It was a memorable trip, somewhat overshadowed by a certain squeamishness which put me off my food. Mr. Coles insists that I was suffering from the sixteenth (or would it be the seventeenth?) variety of the group of diseases that in this country go under the name of galsiekte. Perhaps he was correct. At any rate he was extremely sympathetic and time and again dragged me back from the brink of the watery grave of whose terrors I had long since become oblivious.

The end was dismal, since after saying goodbye to our excellent acquaintances on board, we had to face an evening and a night at Houtjies Bay, surely the last place on earth. The bugle band of its coloured boy scouts rivals the braying of the Dassen penguins, and like the latter performs during the hours of darkness. Its mosquito population puts the bird-density of the islands in the shade. Why is it that the inhabitants of pest-ridden places are not merely resigned to but so totally unconscious of their environmen ? It seems to be the rule that where flies are worst, there nobody bothers to destroy them; where mosquitoes swarm, mosquito-nets are unknown; where heavy infestations of larval ticks contaminate the veld, picnics are the order of the day; while swimming never reaches such heights of popularity as in bilharzia-infected streams and shark-ridden seas. I dreamed all night that I was back in Malagas, that the gannet had become transformed into gigantic mosquitoes with blood-stained throats and that they were trying to " neck " me as I battled my way among them.

Next morning we faced " The Worst Journey in the World " — Saldanha Bay to Cape Town, not Cherry-Garrard's — with swollen faces and somewhat heavy hearts. We had been thoroughly chastened in spirit by a railway official at Saldanha Bay, and this was the way of it.

We wanted tickets to Cape Town and innocently inquired the fare from Houtjies Bay. Mr. Coles claimed afterwards to have solved the problem of the difference between Saldanha and Houtjies, but to me it remains as academic a distinction as that between Onderstepoort and Laboratory. However, the official certainly spared no pains to rub this subtle difference into us. He was genuinely aggrieved that we should assume the name of his station to be Houtjies, and I suppose one can't blame him for that. He further indicated that he found it unreasonable for passengers to board a train at a place whose name they did not know. We pointed out, in extenuation, that we had never arrived in this part of the world by train. We were, in fact, anxious only to leave it. We had arrived by sea, we explained. From where ? From Cape Town. But if we had set out by sea from Cape Town, how was it that we had left the ship and now wished to return to our starting place ? Further, if we insisted, there was a much better train

leaving in exactly two days' time, nobody in his experience ever travelled by this one. We pointed out that, however bad the train might prove, it could not be worse than the prospect of another two days in Saldanha Bay. His brows beetled as if he had finally lost all patience, and we realised we had once more trodden on his pet corn in the matter of place-names.

" So sorry," we apologised belatedly, " we really did mean to say ' Houtjies Bay ' that time. No-one," we went on rapidly and feasting our eyes upon its manifold scenic attractions, " could possibly say a word against Saldanha Bay, except by a slip of the tongue and while actually intending to refer to some entirely different part of the world, such as Houtjies Bay or, for that matter, Devil's Island."

The official did not appear mollified. There is no doubt we appeared suspicious customers. Houtjies depends entirely on a meagre rainfall for its supply of water. It is a liquid which is there carefully conserved for use as a culture medium for the chief local product, the mosquitoes of which I have already written. Nobody thinks of setting a mugful or so aside and heating it up for shaving purposes. If you watch a man trying to shave in Houtjies you find it difficult to decide whether he is attempting to work up a lather or work out a new method of exterminating wrigglers with a brush.

Thus we were ill-shaven. The contents of addled penguin eggs, long since crushed between boots and hairbrushes during the voyage in the *Harrier*, seeped remorselessly and embarrassingly from our suitcases, turning patches of the coal-dust platform into a putrid, tarry liquid. Fruit-jars oozing bloodstained 50% glycerin peeped from our bulging pockets. Evil-looking post-mortem knives, stained with the ochre-coloured muscle-juice of scores of dead and decomposed gannets, had found the weak spots in our hastily-packed and ill-fastened gladstones. Our clothes were soiled with penguin guano, malagas guano, duiker guano, gull guano, and chimney-sweep guano, not to mention the gastric contents of our fellow-passengers in the ss. *Harrier*. We looked fishy, and upon my word I believe we smelled worse.

J. D. W. A. C. would from time to time absentmindedly flick an overlooked *A. talaje* from his coat, forgetting that the official was no systematic entomologist and would jump to wrong conclusions. Auditory memories — even of the most characteristic sounds — fade with surprising rapidity unless constantly recalled, and I was determined to return with a clear recollection of the bird cries, even if it did mean practising them aloud from time to time in public places. Worst of all, we had been overheard to converse in a strange tongue — who knew, a thinly disguised gibberish code? — in which words of potentially sinister strategic implications (Aegyptianella, Aethiopica, Capensis) constantly recurred. Perhaps we had landed from an alien country without passports. You can't be too careful: it was soon after the September Crisis.

It seemed that another had arisen. There was only one hope. Fixing the official with one (still jaundiced) eye, I ejaculated "Ho-ho" several times in succession, at the same time rotating the atlas upon the *dens* of the axis as rapidly as possible. Like even the best-conceived strategic operations, this manoeuvre had as its basis a plan which contained one grave element of chance. Had the railwayman ever visited Dassen, he would no doubt have thought such actions on the part of a biped the most natural thing in the world. But fortune was with us, and after a breathless moment of suspense it became apparent that his travels had not embraced the Islands. A look came into his face as of one who has arrived at a sudden but irreversible decision. Tickets and change appeared, and he was seen no more.

But that train! In sluggishness of pace and irresoluteness of progress it reminded me of a supravitally stained lymphocyte when the concentration of Janus green has been somewhat overjudged. And the landscape in this dismal and depressing "tail-end of the Kalahari Desert" is of so monotonous a character that when you awake from an hour's sleep you have no means of judging whether or not you have moved meanwhile.

Arrived eventually in Cape Town, the scientific personnel of the expedition dispersed.

"Were you sick? And did you have to return by the slow train from Saldanha?" enquired my host.

I could deny neither.

"Well," he gloated, "didn't I warn you that you wouldn't enjoy going to the Islands in a south-easter and coming back by rail?"

"Nonsense, I wouldn't have missed it for worlds. HJCKRRH to you," I retorted stoutly.

Yours, etc.,

C.J.

Jl. S.A.V.M.A.
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1941.

A Water-Miscible Livestock Spray containing Pyrethrum Extract.

By R. DU TOIT, Onderstepoort.

Of late an increasing amount of attention has been directed to the plant extract insecticides, particularly those containing the active principles Pyrethrins 1 and 2, and Rotenone. These principles, apart from being extremely active insecticides, have the great advantage of being to all intents and purposes entirely harmless to warm-blooded animals.

In the livestock industry some attention has been directed towards their use as sprays, but the fact that they are not soluble in water but only in oils has largely precluded their use, the reason being that in order to produce a suitable spray an oil of low viscosity must be used as a vehicle and, generally speaking, such oils are far too irritating when applied to the hides of animals.

The writer has directed his attention to this question for some time and the object of this short article is to draw attention to a method of overcoming this difficulty.

In recent years the theory of emulsions has reached a great deal of prominence, particularly with the high degree of specialisation which industry has undergone, and a great deal of study has been devoted to the elaboration of various emulsificants, of which previously the various "alkaline" soaps made up by far the greater number. There are now on the market a large number of emulsificants which have enormously widened the various uses to which emulsions can be put. With this wealth of literature and experience available it has been possible to apply this knowledge to the special requirements one is faced with in the eradication and control of insects.

The soluble or miscible oil is a well-known method in the preparation of emulsions. Briefly, it consists of an oil and an emulsificant in intimate solution, which, upon the addition of water, immediately forms a milk-white stable emulsion. This principle is embodied in many of the coal-tar derivative dips and disinfectants in which various emulsificants are used, among which soaps figure prominently.

In order to apply this principle in the preparation of a miscible oil containing pyrethrum, it is necessary entirely to exclude any emulsificant which is alkaline in reaction, since, in the presence of alkali, pyrethrum rapidly loses toxicity. A solution is offered by the various sulphonated oils, of which sulphonated castor oil is readily available, and saponified triethanolamine, *e.g.* triethanolamine oleate, which are either neutral or slightly acid in reaction.

When preparing a soluble oil as outlined above, using as a base a mineral or plant oil, it is frequently necessary to incorporate a third substance known as the "auxiliary solvent" or "aid to dissolution," which renders possible the solution or molecular interspersion of the emulsificant into the oil and *vice versa* and prevents separation into layers. There appears to be no guide to the selection of such an "auxiliary solvent" as different oils and emulsificants require different substances: trial and error alone must be resorted to.

In order, therefore, to prepare a suitable soluble or miscible oil embodying pyrethrum for use as a livestock spray it is necessary that the following conditions be observed:—

1. The resulting product must be neutral or slightly acid in reaction to prevent deterioration of the pyrethrum.

2. The oils must be in true solution, which necessitates the use of a suitable "auxiliary solvent."

3. The emulsion produced by the addition of water should be stable even should a "hard" water be used. This depends upon the emulsificant selected.

There is an obvious advantage in such a soluble oil-pyrethrum spray, viz., the elimination of a great deal of bulk, thus minimising transport costs and difficulties. Furthermore, as pyrethrum is deleteriously affected by light, the soluble oil method has the advantage that emulsions need not be made up until immediately before use, thus minimising the absorption and refraction of light which normally occurs in the minute oil droplets making up an emulsion.

A soluble oil-prytherum spray has been prepared by the writer which fulfils the above-mentioned requirements and for which the name "Pyresol" is proposed. A typical example of this is as follows:

Mineral oil (J.D.2) .... } Pure Pyrethrum extract (2·5% Pyrethrins 1 and 2) .... }	equal parts .....100 parts (of the mixture) by volume.
Sulphonated castor oil (emulsificant)	..... 15 parts by volume.
Oleic acid (auxiliary solvent)	.....6·3 parts by volume.

For use this is diluted with water in the proportion 1 to 9 parts by volume.

The proportions of the various ingredients may be altered to suite individual requirements and no hard and fast rule can be laid down as to the quantities of the individual ingredients which will be required. As a general rule it may be stated that the emulsificant should not exceed 25% of the whole, but the auxiliary solvent will vary in amount according to the amount of emulsificant and and the nature of the oil. Triethalolamine oleate may be used in place of sulphonated castor oil and is particularly useful when a vegetable oil is employed. In mineral oil triethanolamine has been found to dissolve without the addition of an auxiliary solvent.

Pyresol has been designed particularly with the view towards the control of horn fly along the eastern coastal areas of the Union. Experimentation has been directed towards covering the back and upper portions of the neck and head of cattle with a heavy oil containing pyrethrum extract, the object being not to repel the flies but to paralyse and kill them when their feet come into contact with the spray and the pyrethrum is absorbed through the pulvilli.

So far, results have been encouraging, but a further article will appear dealing with the practical application of Pyresol.

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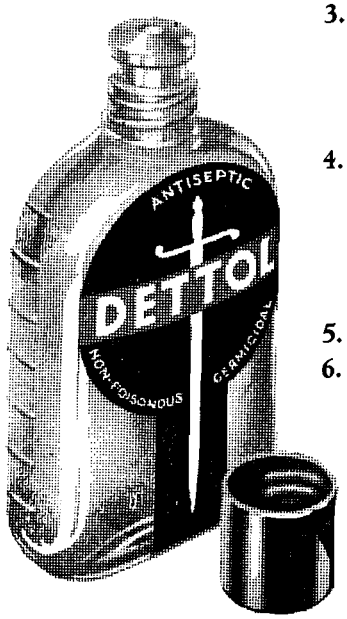

3. Dogs and other animals usually remain quiet during treatment with Dettol as the solutions neither irritate nor sting raw wounds.
4. It has been shown that the film of Dettol which is left when solutions of it dry on the skin or wounds possesses antiseptic protective properties for several hours subsequently.
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Jl. S.A.V.M.A.
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1941.

Some Preliminary Experiments on the Survival of Heartwater "Virus" in Rats.

J. R. HUDSON and RUTH M. HENDERSON,
Division of Veterinary Research, Kabete, Kenya.

The desirability of finding a small animal in which to maintain strains of heartwater has been recognized both at Onderstepoort and at Kabete for many years. The upkeep of the "virus" in sheep is expensive and, in the experience of Kabete workers, uncertain, unless transmissions by one of the vecting ticks are interposed at frequent intervals.

In a recent publication, Mason and Alexander (1940) have reviewed briefly their attempts to infect small laboratory animals with heartwater. These attempts were made on guinea-pigs, rabbits, rats, and mice, using almost every conceivable type of inoculum and method of inoculation. Efforts were also made to lower the resistance of guinea-pigs by deep X-ray treatment and of guinea-pigs and mice by blockade of the reticulo-endothelial system. While all their attempts to cause true infection of these animals were unsuccessful, the authors mention two occasions when "there was a translocation of the virus to the brain and its survival therein, once in a guinea-pig and once in a rat." The experiments to be recorded in this paper were made before the publication of Mason and Alexander's article. Occasional attempts to infect laboratory rodents with heartwater had been made previously at Kabete without success. These experiments were by no means as comprehensive as those carried out at Onderstepoort, but they were sufficient to show that laboratory rodents were relatively insusceptible to this disease. The report of Pinkerton and Bessey (1939) that a riboflavin deficiency greatly lowered the resistance of the rat to endemic typhus suggested that the susceptibility of similarly deficient rats towards heartwater should be investigated. At the same time the opportunity to study the effects of inoculating certain wild rats with heartwater blood was taken.

Vitamin-deficient Albino Rats.

As the object of the experiment was to determine whether vitamin-deficient rats were susceptible to heartwater, rather than to determine the exact type of deficiency which rendered rats susceptible, a diet was used which was complete except for the whole of the B_2 complex. The diet was essentially that used by Pinkerton and Bessey; but modified slightly in that vitamin B_1 was given as Goldberger's concentrate [Hoagland and Snider (1930)]. As this preparation consists of the concentrate absorbed on

cassava starch, the amount of maize starch in the diet was reduced proportionately.

The diet had the following composition:

Maize starch	210 parts
Casein (alcohol extracted)	180 ,,
Dextrose	320 ,,
Goldberger's concentrate	160 ,,
Cod liver oil	40 ,,
Lard	50 ,,
Salt mixture	45 ,,

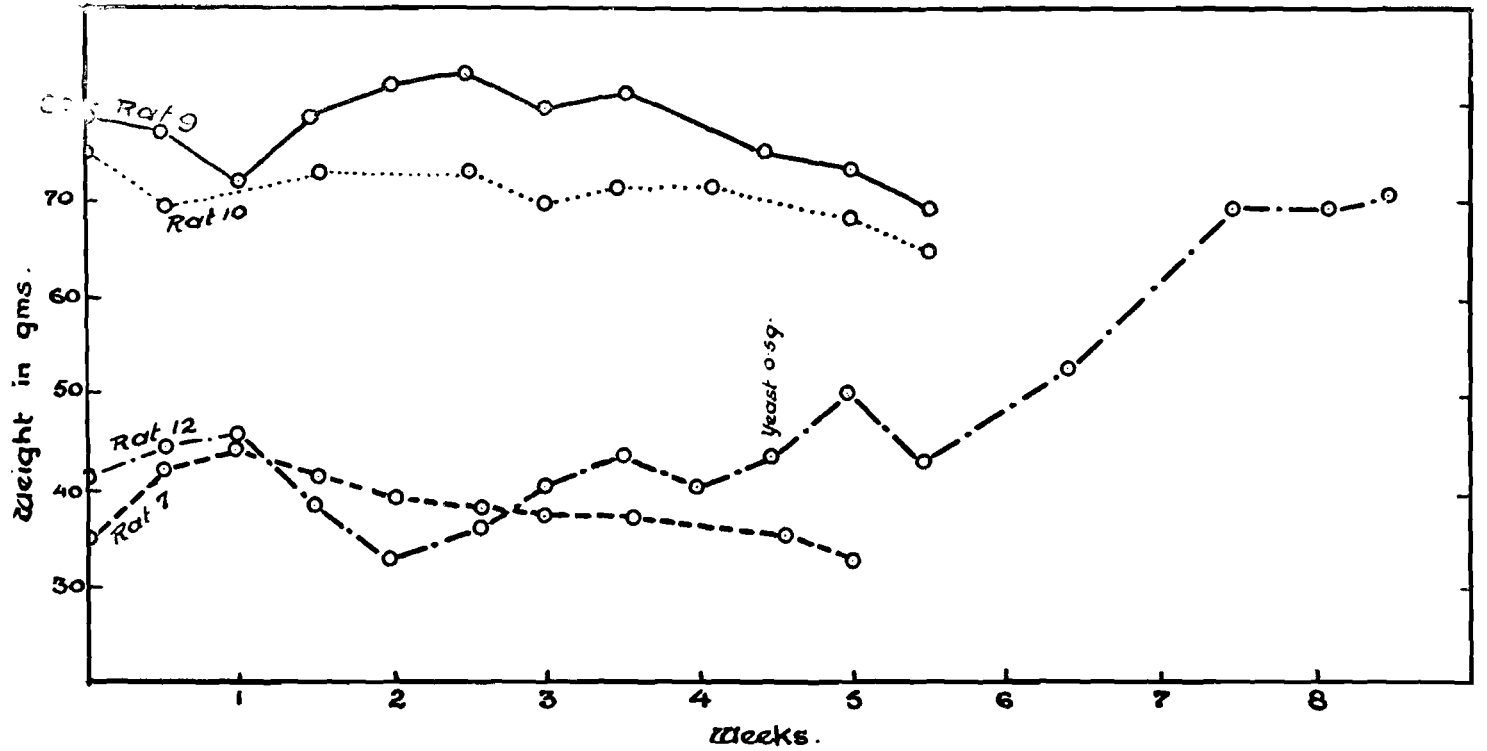
The rats were kept in separate metal cages on wire-netting floors, the food being placed in small metal hoppers to prevent refection. The weights of the rats and of the amounts of food consumed were recorded twice weekly, the technique being that found satisfactory during previous investigations at this Institute into the nature of the B_2 complex. Young rats, when transferred to the diet ceased to increase in weight. The diet, when supplemented by a daily dose of 0·5 gm. of brewers' yeast that had been autoclaved for four hours at a pH of 4·8, allowed of normal gains in weight (see Chart 1, rat 12). Thus it may be assumed that, apart from the B_2 complex, the diet was complete. Unfortunately, only a limited number of rats of 30 – 40 gm. weight were available and it was necessary to employ a few rats of 70 – 80 gm. These latter, however, did not increase in weight on the diet.

After a period of two to three weeks to allow of depletion of the body-reserves of the B_2 complex, the rats were inoculated with two cc. of citrated blood obtained from sheep which were giving a thermal reaction to heartwater. About 14 days after inoculation the rats were killed, the liver, kidneys, heart, and lungs were removed aseptically and, by grinding with sand and physiological saline, an emulsion was prepared. This emulsion was inoculated into sheep intravenously.

In the first experiment, rats 7, 9 and 10 (see Chart 1) were inoculated intraperitoneally with two cc. of citrated blood of sheep 5507 on 21:12:39. Two cc. of the same sample of blood was inoculated intravenously into sheep 5502 and 5504, both of which reacted and died of heartwater. Rat 7 was killed on 4:1:40 and an emulsion of the organs was inoculated intravenously into sheep 5872. Four days later rats 9 and 10 were killed and their organs were emulsified and inoculated into the same sheep. Sheep 5872 gave a peracute type of heartwater reaction (see Chart 2) and died on the tenth evening. The post-mortem lesions were suggestive of heartwater. On section the brain showed histological lesions of heartwater; but prolonged search failed to reveal the presence of rickettsias. Blood from sheep 5872 was collected in citrate on the ninth day and inoculated intravenously into

CHART 1.

Weights of Vitamin-B2-deficient rats.



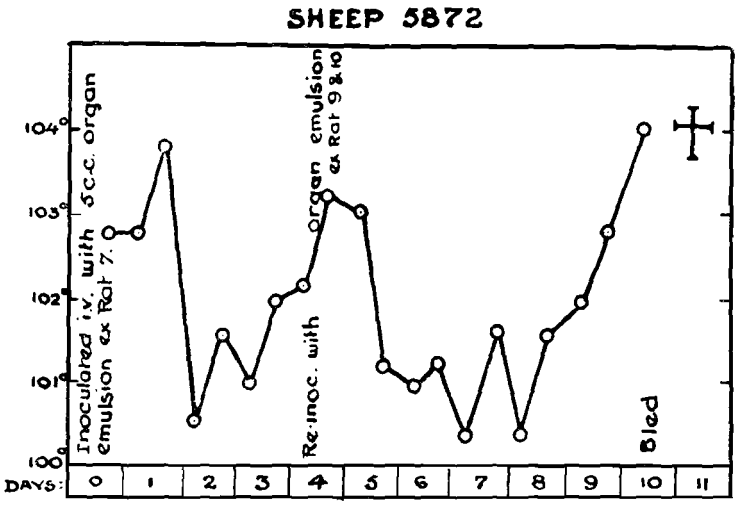
sheep 5948 (Chart 2) and 5963 . The former reacted from the eighth day to the fifteenth day, when it was killed *in extremis*. The latter reacted from the seventh to the eleventh day when it was killed. Both sheep showed typical lesions of heartwater at post mortem. The former showed rickettsias in jugular intima smears and the latter in sections of the brain. Further subinoculations from these two sheep also produced heartwater. (See Chart 3 which summarizes experiments in vitamin deficient rats).

In the second experiment rats 20 and 21, which when placed on the diet weighed 30 and 33 gm. respectively, were inoculated intraperitoneally on 27:2:40 and again on the following day with blood from sheep 5986. The rats were killed on 12:3:40 and an emulsion of their organs was inoculated intravenously into sheep 5971 and 5973. Neither of these sheep gave any thermal reaction; but blood was collected on the fifteenth, sixteenth and seventeenth days after inoculation and subinoculated into sheep 5976 and 5980. Twenty-seven days after inoculation, sheep 5971 and 5973 were tested for immunity by the inoculation of blood from a reacting sheep. Unfortunately sheep 5971 died fifteen days after this test inoculation. There were no lesions to suggest heartwater, the cause of death being a large *Corynebacterium pyogenes* abscess in the neck at the site of the original inoculation. Sheep 5973 aborted and died of sepsis four days after the test inoculation. Sheep 5976 and 5980 showed thermal reactions suggestive of heartwater, the latter dying on the seventeenth and the former on the nineteenth day after inoculation. On post-mortem examination both sheep showed lesions suggestive of heartwater. The histological examination of sections of brain from each case showed lymphostasis, round-cell infiltration and satellitism, lesions which we associate with heartwater. Rickettsia, however, could not be found. Sheep 5976 and 5980 were bled on the ninth and thirteenth days after inoculation and their blood was inoculated into sheep 5889 and 5891. Sheep 5889 gave a reaction strongly suggestive of heartwater and was killed *in extremis* on the thirteenth day. Post-mortem lesions were poor. The brain showed mild lesions suggestive of heartwater; but no rickettsias could be found. Sheep 5891 reacted and died of heartwater, rickettsias being found in the brain. (See Chart 3.)

The third attempt to infect vitamin B_2-deficient rats was unsuccessful. Rats 11 and 14, which weighed 36 and 45 gm. when placed on the diet, were inoculated on 10:2:40 with blood from sheep 5952 and 5968. The blood was collected during the temperature reaction and should have been virulent. As a control, sheep 5967 was inoculated. This sheep gave no detectable thermal reaction, but proved immune on test. Rats 11 and 14 were killed on 24:2:40 and their organs were inoculated into sheep 5950 and 5951. Neither of these sheep reacted and both proved susceptible on test.

Sections of the brain of rats 7, 9, 10, 20, 21 and 11 were examined microscopically. That of rat 7 showed an abnormal number of lymphocytes in some of the smaller capillaries. There was an increase in glial nuclei

CHART 2.



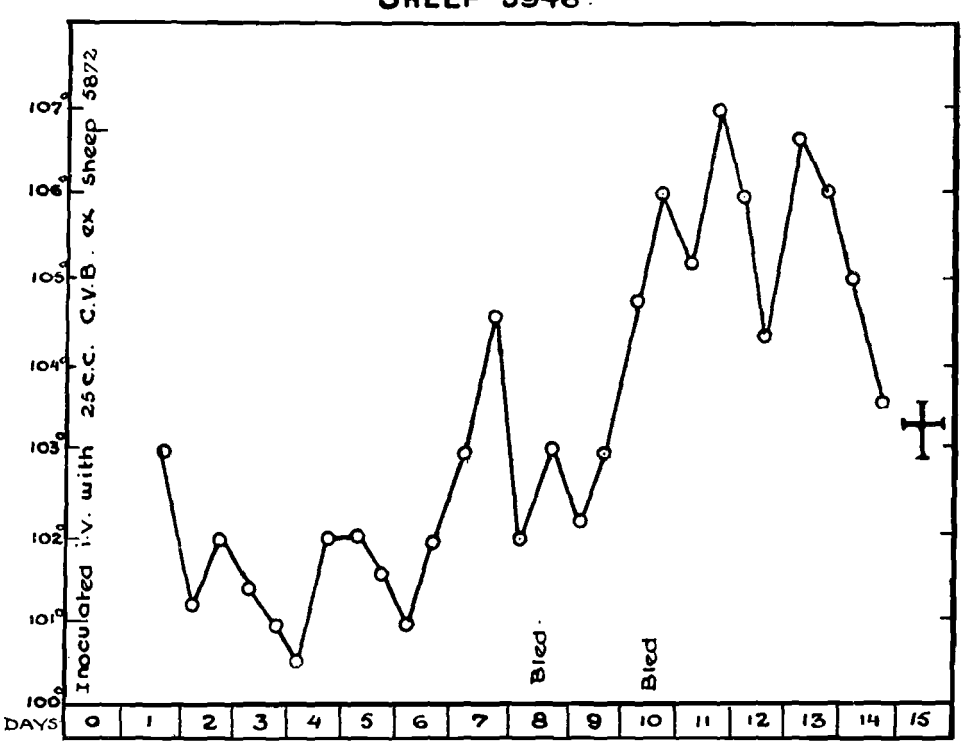
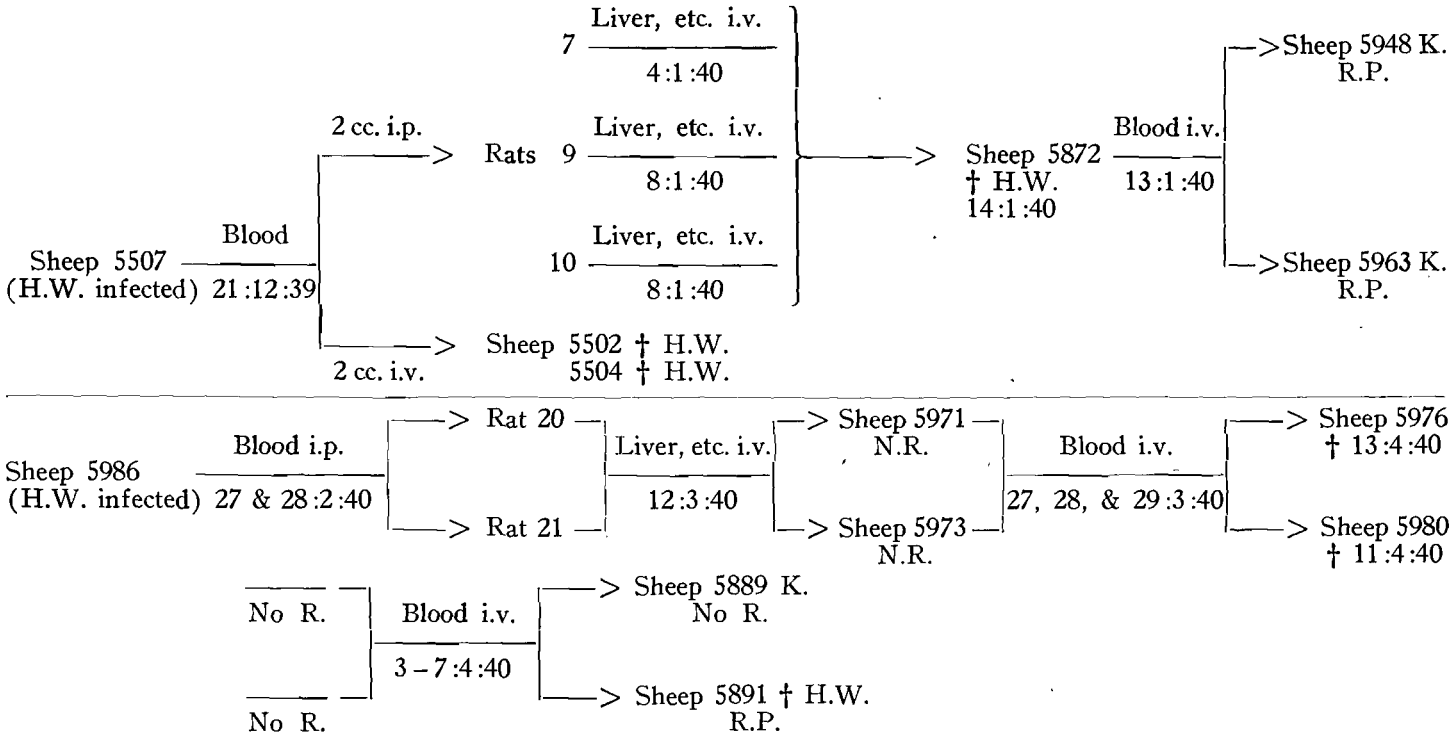


CHART 3.

Summary of experiments on transmission of heartwater to vitamin-deficient rats.



(H.W. = heartwater; † = died; K = killed while reacting; R.P. = rickettsias present); No R = rickettsias not found).

in the lower regions of the cortex and some swelling of endothelial nuclei of the larger vessels near the hippocampus. Rat 9 showed a migration of round cells to the Virchow-Robin space. The brains of the other rats were normal. In no case were rickettsias demonstrated. The results of the inoculation experiments show, however, that in two out of the three attempts, the " virus " persisted in the bodies of the rats for fourteen days. (Chart 3.)

Wild Rats.

Similar experiments to ascertain whether heartwater could survive were made with *Rhabdomys pumilio, Otomys angoniensis elassodon, Lophuromys aquilus aquilus* and *Rattus rattus kijabius* trapped at Kabete and kept on a normal diet. The rats were inoculated with two cc. of citrated blood intraperitoneally, killed after fourteen days, and a suspension of liver, kidneys, heart and lungs inoculated intravenously into sheep.

Rhabdomys pumilio.

Two *Rhabdomys* were inoculated on 31:1:40 with blood from sheep 5940. They were killed on 13:2:40 and their organs inoculated into sheep 5957. This sheep gave a thermal reaction suspicious of heartwater (see Chart 4), and was bled on the fourteenth day for the inoculation of sheep 5917 and 5896. Five weeks after inoculation the immunity of 5957 was tested by the inoculation of blood. The sheep gave no reaction although the control sheep 5959 reacted and died. Of the subinoculated sheep, 5917 gave no reaction and was proved susceptible on test. The other sheep, 5896, however, died (see Chart 4) on the twelfth day with lesions of heartwater. Rickettsias were present in the brain. Such results, although rare, are not unknown when working with heartwater. Blood from 5917 and 5896, collected on the ninth and tenth days after inoculation was transferred to sheep 5919 and 5924. Both reacted and died with heartwater lesions although rickettsia were only found in the brain of 5919. On 12:2:40 one *Rhabdomys* was inoculated with the blood of sheep 5952 and 5968. It was killed on 27:2:40 and an emulsion of its organs inoculated to sheep 5900. This sheep gave a rather indefinite temperature reaction from the tenth to fifteenth days. It was bled on the eleventh, thirteenth and seventeenth days to inoculate sheep 5972 and 5958 and died on the twenty-ninth day before its immunity could be tested. Post-mortem examination revealed an interesting case of verrucose endocarditis, a rather rare condition in adult sheep, and pyæmic abscesses. Sheep 5972 showed an indefinite temperature reaction from the fourteenth to twenty-fifth days and died before its immunity test was completed. This animal was an indigenous, hairy sheep which had been reared under tick-free conditions at a Veterinary Training Centre, and death appeared to be due to anaplasmosis. Sheep 5958 started to react on the ninth evening and died on the fourteenth day from heartwater. Rickettsias were present in the brain.

In the two experiments, therefore, heartwater persisted in *Rhabdomys* for fourteen and fifteen days respectively.

Otomys angoniensis elassodon.

Two *Otomys* were inoculated on 22:1:40 and again on the following day with blood from sheep 5945 and 5946. The infectivity of the blood was controlled by the inoculation of sheep 5981 which reacted and recovered. The *Otomys* were killed on 2:2:40 and their organs inoculated into sheep 5953 and 5954. Sheep 5953 gave no reaction. It was tested on the twenty-second day; but the control sheep did not react. Retested after six and a half weeks, it proved immune to a controlled dose of virulent blood. Sheep 5954 also failed to react. It was tested on the eighteenth day with controlled blood and gave a mild reaction. On retest it proved immune. Unfortunately no subinoculations were made from sheep 5953 and 5954 and it is possible that they received a sub-infective, but immunizing dose of heartwater in the rat-organ suspension. In a second experiment, three *Otomys* were inoculated with blood from sheep 5952 and 5968 on 12:2:40. This blood was used in the second *Rhabdomys* experiment which controls its infectivity. The *Otomys* were killed on 26:2:40 and their organs inoculated into sheep 5903. This sheep did not react; but the inoculation of infective blood on the twentieth day led to a fatal attack.

Therefore, out of two attempts to infect *Otomys* one gave a negative result and the other was inconclusive.

Lophuromys aquilus aquilus.

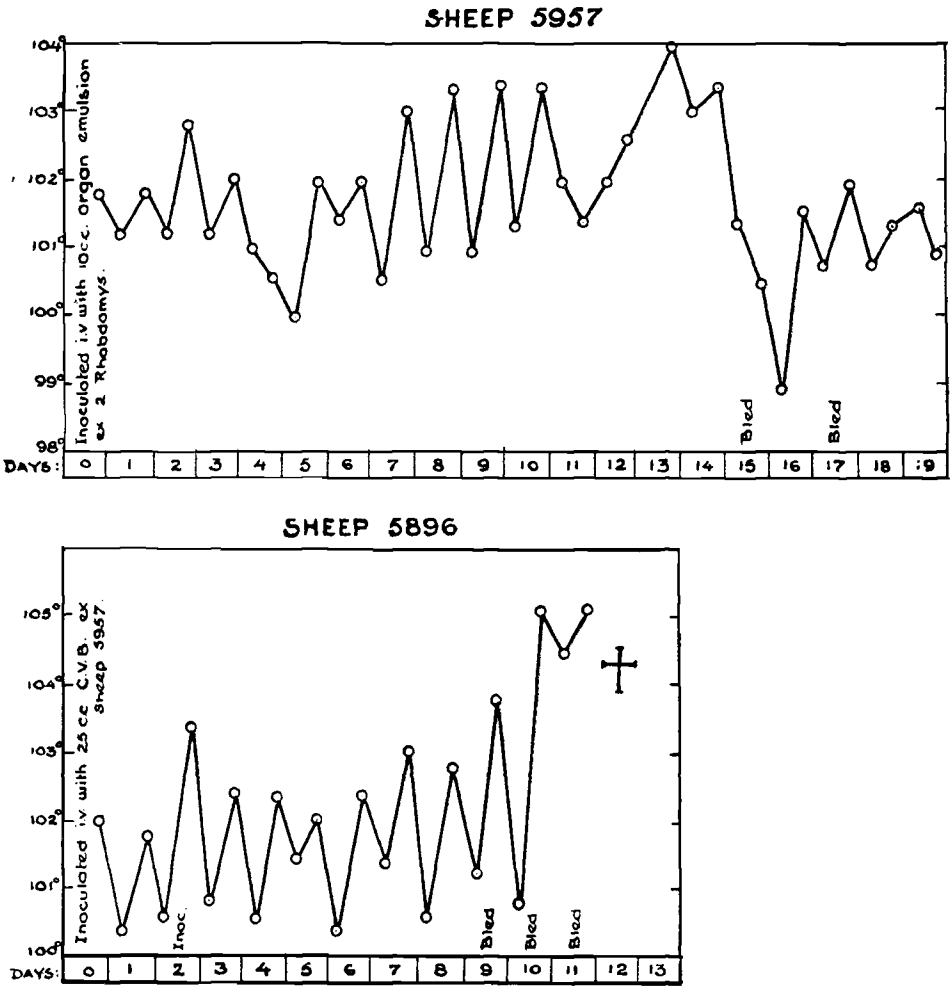
Only one specimen of this rat was obtained. It was inoculated on 31:1:40 with blood of the same sample as that used in the first *Rhabdomys* experiment. Killed on the 13:2:40, its organs were inoculated into sheep 5961. This sheep appeared to give a mild reaction beginning on the seventh evening. It was bled on the eighth day to inoculate sheep 5959, and on the fourteenth day to inoculate 5969. Five weeks after inoculation its immunity was tested with blood. It died of heartwater, rickettsias being present in the brain. Of the two subinoculated sheep, 5959 gave no reaction, but reacted and died on test. Sheep 5969 also gave no reaction and when tested reacted, but recovered.

The one experiment with this species was therefore unsuccessful.

Rattus rattus kijabius.

One specimen of the local subspecies of the black rat was inoculated with blood from sheep 5945 and 5946 on 22:1:40 and again on the following day. This blood was used in the first *Otomys* experiment. On 2:2:40 the rat was killed and an emulsion of its organs inoculated into sheep 5955. This animal gave no reaction. It was tested by the inoculation of blood on the eighteenth and nineteenth days. Eight days later it began to react and subsequently recovered. The nature of the reaction was confirmed by subinoculation.

CHART 4.



Discussion.

Although the experiments reported do not prove that true infection of rodents with the "virus" of heartwater can occur, they show that the "virus" can survive in the body of rats for a fortnight. Alexander (1931) gives the maximum period for survival in defibrinated blood *in vitro* at room temperature as 38 hours and states that usually the virus will not survive for 24 hours. It is difficult to see how so delicate an organism can persist for fourteen days in rats without multiplying. Unfortunately our stocks of rats were insufficient to allow us to explore fully the possibility of serial passages. Two attempts to obtain a second transfer were made but both failed. On the first occasion, organ emulsion of rats 11 and 14

was inoculated intraperitoneally to three rats at the same time that the two sheep were inoculated. As reported, this organ emulsion was apparently devoid of "virus." On the second occasion the subinoculated rats died of a *Pasteurella* septicæmia 36 hours after inoculation. Such accidents are difficult to avoid when working with rats on a deficient diet.

Having obtained survival of the "virus" for fourteen days we feel that there is reason to hope that true infection might be secured if a larger number of animals were inoculated. In Mason and Alexander's (1940) first series of ferrets the virus survived for one passage; but could not be recovered from the second transfer. In their second series the virus failed to survive, whereas in the third it multiplied and the infection was successfully passaged five times. It is hoped to make further observations with *Rhabdomys* in the future as it is felt that this species will give a better chance of success than vitamin-depleted rats. When attempting to transfer the "virus" in the latter, there is always the risk of a "flare-up" of an organism of low pathogenicity for normal rats.

The survival of the "virus" in a common field rat may prove of practical importance. Neitz (1935 and 1937) demonstrated that antelope could be responsible for the infection of ticks and we have recently obtained confirmatory evidence from the field suggesting that antelope were responsible for the maintenance of infection in an area kept free from domestic ruminants. It is obviously desirable to determine whether, if virus multiplies in *Rhabdomys*, it circulates in the blood and so can serve for the maintenance of infection in *Amblyomma*.

It is interesting to note that on no occasion were we able to demonstrate rickettsias in sections of brain from sheep inoculated with material from rats. Rickettsias were, however, demonstrated in the next sheep passages.

Summary.

1. In two out of three experiments the causal agent of heartwater was shown to persist for fourteen days in the bodies of albino rats fed a vitamin B_2-complex-deficient diet.

2. In two experiments the causal agent survived for fourteen and fifteen days in the bodies of *Rhabdomys pumilio* kept on a normal diet.

3. Of two similar experiments with *Otomys angoniensis elassodon*, one gave a negative and the other, an inconclusive result.

4. Experiments with *Lophuromys a. aquilus* and *Rattus r. kijabius* were negative, but only one rat of each species was used.

Acknowledgments.

The authors wish to express their gratitude to Dr. D. MacInnis of the Coryndon Memorial Museum, Nairobi, for the identification of *Lophuromys aquilus aquilus*, a rat previously unknown to them.

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'BAYER" GOLF TROPHY COMPETITION, 1941.

In 1939 Messrs. Bayer Pharma (Pty.) Ltd., presented a trophy for annual competition amongst members of the Veterinary, Medical, Dental, and Pharmaceutical Professions in Pretoria.

This year twenty-eight competitors entered the field at Zwartkop Country Club on March 30th, and were fortunate in having ideal weather throughout the day. Play was keen and the winners are to be complimented on coming out on top in such a strong field.

The donors were hosts at luncheon and during the day, and the Club Secretary, Mr. Frith, is to be congratulated on his efficient organization which ensured a most enjoyable day.

The list of prize-winners is as follows:—

Winners. — Dr. A.J. Broughton and Dr. A. R. Davison.

Runners-up. — Capt. J. Stead and Dr. F. J. Joubert.

Third Prize. — Major R. Culverwell and Sgt. Miller.

Best Morning Round. — Dr. C. H. K. Coetzee and Mr. Louis Schwartz.

Best Afternoon Round. — Dr. J. G. A. Davel and Dr. Leonard Sachs.

Special Prize (18 and over). — Dr. J. H. Erlank and Mr. W. O. Neitz.

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XII (2): 50 - 58
1941.

Resistance to Arsenic as Displayed by the Single Host Blue Tick *Boophilus decoloratus* (Koch)* in a Localised Area of the Union of South Africa: Preliminary Report.

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Onderstepoort.

Early in 1940 reports were received from the veterinary staff of the East London area indicating that great difficulty was being experienced in the control of the single host blue tick, *Boophilus decoloratus* (Koch), by means of the sodium arsenite dipping solutions commonly employed in this country for the control of ticks. The reports showed that, at the time, this difficulty was only being experienced in a comparatively localized area on the west bank of the Buffalo River in the East London district.

Investigations were instituted and a preliminary survey made which revealed that the total area involved appeared to be no more than 30 by 15 miles in extent. Gross infestations of cattle with ticks were encountered, and on all sides the farmers in the area complained that whereas previously they had no difficulty in controlling the blue tick by means of sodium arsenite solutions, these dipping solutions now appeared to have lost practically all efficacy.

It was learned from the local veterinary staff that various measures had been taken to ascertain whether the dipping procedure itself was not at fault. Dipping was watched by members of the veterinary staff; tank strengths (i.e. concentrations of sodium arsenite) were carefully checked. Where a dipping tank was small and it was feared that the animals had not been immersed for a sufficient time the animals were driven through twice. Concentrations of sodium arsenite were increased, both where plain sodium arsenite as well as where proprietary brands of arsenite dips were being used. These investigations seemed to indicate that the dipping itself was not at fault, but that some change had taken place in the tick which rendered it more resistant to arsenic than the same species in other parts of the country.

We were not inclined to accept this hypothesis until the problem had been investigated from all angles, as nowhere in the literature available to us could we find any reference to a similar experience with this or allied species of tick. Investigations revealed that the problem was more complex than appeared on the surface. Within the area mentioned, many anomalies

* According to a recent classification of the genus *Boophilus*, Curtice, Minning (1934) has placed this species in the new sub-genus *Palpoboophilus* created by him.

were encountered. Several of the dipping tanks were used by a large number of stock owners who dipped their animals either on a fixed date at intervals of seven days or fourteen days, or on different days, and it was found that the infestations of ticks in the herds varied enormously, some herds being comparatively free from ticks, while others showed gross infestations. Even on individual farms a similar state of affairs was encountered, where the herd of milk cows might be grossly infested and the oxen and dry animals almost tick free, or vice versa. So far as could be ascertained this did not appear to be correlated with the type of veld on which the animals were grazing, as animals running on well-bushed low-lying valleys were often found to be comparatively "clean," whereas those from the high-lying, grass-covered and unbushed hills were grossly infested, although more often than not the reverse was the case.

After a careful consideration of the problem we appeared to be faced with three possibilities.

(a) The dipping fluid was not making proper contact with the parasite, due to some physical factor operating on the skin of the animal or on the tick itself.

(b) The tick itself had developed a tolerance or a resistance to arsenite of soda.

(c) The tick had changed its habits in the area in question and instead of reaching maturity on a single host might drop from the animal at completion of the larval or nymphal stage or both, moult on the ground, and crawl on to another host animal as an unengorged nymph or adult and in this way escape contact with the dipping fluid.

To obtain some information about the wetting power of the dipping fluid employed, we collected samples of the water used on a number of farms in the area, analysed the degree of hardness and expressed this as temporary hardness in terms of N/10 HCl per 100 cc. water. The results are shown in table 1. It will be noted that the various samples differed considerably. It was found that the waters employed in making up the dipping solutions were obtained from a variety of sources: streams, wells, boreholes, springs, dams for the collection of surface storm water, etc.

TABLE 1.

The Hardness of Waters used in the Preparation of Dipping Solutions.

Farm.	Nature of dipping fluid used	Degree of hardness of water
Prospect	Cooper's K Dip.	2
Chalumna	Arsenite of Soda.	9·8
New Hope	Tixol.	7·4
Lilly Valley	Arsenite of Soda.	4·4
Windy Ridge	,, ,, ,,	10·4
Ernsheenie	,, ,, ,,	2·6
Gulu	,, ,, ,,	6·4
No Trumps	Stogol.	7·0

No definite conclusions could be arrived at from these values, which appeared to bear no relation to the degree of tick infestation present, and it was decided to attempt to increase the wetting power of the dip by the addition of substances which could be obtained locally.

A quantity of soft soap (potassium soap) was added, in the proportion of one pound per 100 gallons of dipping fluid, after dissolving by heating in a small quantity of water, to the tank on the farm Ernsheenie, where the water was shown to be fairly soft (degree of hardness 2·6). About 80 head of cattle were then dipped, and it appeared as if these animals were more thoroughly wetted than on previous occasions. A fair amount of foaming took place in the tank during dipping. Adult fully engorged female ticks were collected from these animals immediately before and after dipping and brought back to Onderstepoort. Each was placed in a separate glass tube and kept in a room held at 26°C and 80% relative humidity. Those ticks collected before dipping all laid eggs which hatched, while nearly all those collected after dipping died. It must be noted that these cattle had been dipped seven days previously in this tank at the usual seven-day strength of sodium arsenite, namely 0·16% expressed as As_2O_3. Subsequent observations showed that this dipping had practically no effect on the severity of the tick infestation on these cattle, and samples of tank fluid collected one, two, and three weeks later showed that the soap had practically all been precipitated, as judged by shake tests to note the amount of foaming which resulted. This rough field test seemed to indicate that wetting power in itself was not a factor which need be stressed. In the light of the observations made on the cattle themselves, the laboratory observations made on the ticks collected appear to have no significance.

The only other substance which suggested itself at the time was a well emulsified carbolic dipping fluid. This was added to two tanks in the proportions of 1 : 550 and 1 : 600 parts by volume respectively. We wished to see whether these additions would increase wetting power by virtue of the emulsificants contained in the carbolic dip and whether the addition of phenol derivatives would have any effect upon the ticks. However, the desired result was not obtained as judged by observation both on the cattle, and on ticks collected before and after dipping and treated similarly to those in the soft soap addition test.

The third possibility, namely, a change in the life cycle of the tick, was investigated by three methods. (1) Engorged female ticks were collected from cattle in the affected area and brought to Onderstepoort where they were allowed to lay eggs and the resulting larvae placed on two tick-free cattle in an isolation stable. On the 22nd day after the infestation one adult engorged female was obtained from one animal, and from the 23rd to 29th days approximately 40 engorged females were recovered from the two. No further ticks were found during the following two months that

these animals were kept under observation. The period of 23 days represents the average life cycle from larva to adult for the blue tick under average South African summer conditions.

(2) Two young cattle were brought from a farm on the eastern extremity of the affected area where tick infestation was extremely light and thoroughly sprayed with a spray containing pyrethrum, thoroughly washed with soap and water, and subsequently again scrubbed and carefully examined for ticks. These animals were then exposed to infestation on a grossly infected farm and examined daily for ticks. On the 22nd day the first engorged females were found and thereafter the infestation steadily increased.

(3) On two occasions calves were born in herds on badly infected farms and the dates of birth noted. Daily observations were made and again only on the 23rd day were the first engorged females found. The observations made on these calves show the gross infestation of ticks present in the pasture as, in the light of the previous observations, these calves must have become infested with blue tick larvae on the day of birth in order that the life cycle could be completed in the period of 23 days.

These three experiments show that the life cycle for the blue tick in the affected area conforms to that of this species as observed over the remainder of the Union.

It was now realized that a systematic investigation would have to be made into the question of effective additions to the existing dipping fluid, or a search made for chemical substances capable of destroying this strain of blue tick and which could be substituted for sodium arsenite.

We were immediately faced with certain practical considerations which limited the number of parasiticides that could be considered, e.g., cost, availability, harmful effect upon the host, and durability or effectiveness over long periods. However, since the problem was so pressing and the farmers' need and public opinion demanded as rapid a solution as possible, it was decided to approach the problem from two angles by undertaking (1) field trials of various substances as additions to the usual arsenical dips and (2) laboratory trials of a large number of chemical substances of known toxicity, where immersion tests on ticks would be made. Preliminary immersion tests with sodium arsenite in the concentrations ordinarily employed in the field were made upon blue ticks of known susceptibility to arsenic. These indicated that this method gave results quite comparable to those achieved in the field by dipping.

As this article is merely a summary of the investigations made up to date and a discussion of the results so far obtained, the details of the experiments undertaken will not be discussed, and a more comprehensive report will appear later.

For the field trials of various additions to the existing dipping fluids,

12 farms were selected and the following substances used: paraffin (kerosene) emulsion, coal tar derivative dip, copper sulphate, which reacted with the sodium arsenite to give copper arsenite, and nicotine sulphate. The twelve dipping tanks selected were divided into two groups, the tanks in the first group being emptied and fresh sodium arsenite solutions of a strength of 0·16% (expressed as As_2O_3) made up, and the second group left untouched or " dirty." The paraffin was emulsified by means of a Hurrel Homogeniser, the emulsificant consisting of a resin-casein soap of sodium hydroxide which gave a smooth creamy emulsion, which we designated " Parem."

Three tanks were selected, one of which had been cleaned out and filled with fresh sodium arsenite solution and the other two left " dirty." " Parem " was added to give a 1% suspension of paraffin in the clean tank, and a 1·5% and 1% in the two " dirty " tanks respectively.

Three tanks were selected for the copper sulphate additions, two being cleaned out and one left dirty. To the clean tanks 0·5% and 1% copper sulphate were added respectively, and 1% to the dirty tank. Due to the insolubility of a considerable portion of the resulting copper arsenite, some difficulty was experienced in maintaining a suitable concentration of arsenic in solution, and this varied between 0·12% and 0·15% (expressed as As_2O_3). Furthermore, the concentration of As_2O_3 was found to decrease gradually with the passage of time.

Carbolic dip was added to one cleaned out and one dirty tank in a concentration of 0·5%. Forty per cent. nicotine sulphate (tobacco extract) was added to a cleaned out tank in a concentration of one part per 1000 by volume, corresponding to 0·04% nicotine, and to a dirty tank in a concentration corresponding to 0·02% nicotine. Two tanks, one cleaned out and one left untouched, were included as controls.

Two methods of estimating the results were adopted: (a) direct observation of the general incidence of tick infestation in the various herds made by the same observer over a number of dippings at seven-day intervals; and (b) ticks were collected before and after each dipping and at various intervals between dippings. These were forwarded to Onderstepoort where ten ticks were selected from each lot and placed in separate glass tubes in a room held at 26°C and 80% relative humidity. The mortality, egg laying, and subsequent hatching of eggs were noted. The results of these tests are summarised in table 2.

In considering the above table it becomes apparent immediately that the results of the laboratory observations on the ticks themselves were rather inconclusive, with the possible exception of those obtained in the case of farm number nine where nicotine sulphate had been added to the dip in a concentration corresponding to 0·04% nicotine. In comparison with the other figures obtained, the percentage of female ticks dead and of females which laid eggs that subsequently failed to hatch, if not by any means

TABLE 2.

Effect on Blue Ticks of Arsenite Dipping Solutions to which Different Chemicals have been added.

Farm No.	Test Substance added to dipping solution.	No. of Collections of ticks from dipped cattle.	Total ticks collected.	Percentage dead.	Percentage which laid eggs which hatched.	Percentage which laid eggs which failed to hatch	Field observations of incidence of tick infestation in herds.
1	Paraffin 1%	17	170	0	95·3	4·7	Slight improvement after 5th dipping, but ticks again increased later.
2	Paraffin 1·5%	25	250	2·0	93·6	4·4	Heavy infestation until 4th dipping. Thereafter sudden and marked improvement, but ticks (immature stages) increased later.
3	Paraffin 1% later changed to 2%	27	269	0·7	95·5	4·1	Slight improvement after 5th dipping. Thereafter ticks again increased, but decreased again after 5th dipping in 2% paraffin.
4	Copper sulphate 0·5%	16	160	1·3	94·4	3·8	Some improvement noted, but engorged females still present after 6th dipping.
5	Copper sulphate 1%	14	140	0·7	96·4	0·7	Improvement only after 5th dipping, and engorged females to be found right through.
6	Copper sulphate 1%	27	270	0	94·8	5·2	Ticks plentiful until 4th dipping, thereafter considerably reduced, but increased again later.
7	Carbolic 0·5%	14	138	0	92·8	7·3	Original degree of infestation low. Improvement after 5th dipping, but results unsatisfactory.
8	Carbolic 0·5%	25	247	0·4	97·6	2·0	Fluctuations in tick infestation. Oxen heavily infested after 6th dipping. Not satisfactory.
9	Nicotine 0·04%	26	256	3·5	63·1	29·3	Marked improvement after 1st dipping which was maintained. Immature stages the first to disappear.
10	Nicotine 0·02%	5	50	0	98·0	2·0	Infestation not heavy at start, but apparent improvement noted. Time not sufficient.
11	Control	21	210	1·9	89·0	4·3	Ticks remained at more or less low level, but engorged females always present.
12	Control	13	130	0	96·2	3·8	Infestation fluctuated. Engorged females always present.

spectacular, at any rate appear to us to be significant, especially when considered in conjunction with the field observations.

It must be stated that there was a tendency to weight results in favour of tick survival, for the adult engorged females taken from the marked animals were generally the best specimens that could be found. Upon arrival at Onderstepoort a point was made of again selecting the ten best specimens for placing in tubes for subsequent observation. In this way the ticks on which the above results were based were not an average sample of those present upon the cattle. Furthermore, all ticks which laid eggs, no matter how few in number, were classed as living and as having laid eggs, because egg laying varied considerably and it was impossible in many cases to state with certainty whether or not a female had laid an average number of eggs. The subsequent hatching of the eggs was noted, as it is generally accepted that arsenic in its action upon ticks of known susceptibility has the property of sterilizing the eggs of most of those ticks that escape actual destruction.

The results obtained from the observations on the ticks themselves should be correlated, therefore, with the field observations on the general level of tick infestation on the cattle, in order to arrive at any conclusion regarding the efficiency or otherwise of the dipping fluids tested. As has been pointed out previously, the infestations varied enormously on different farms and even within different herds on individual farms. Coupled with this, many practical difficulties were encountered which considerably complicated the interpretation of the results.

Throughout the duration of the field trials drought conditions prevailed in the area, which tended to keep tick life at a comparatively low level in comparison with what had been the case towards the latter portion of the summer (i.e. about February to May) of 1939-1940. In spite of this, fluctuations in tick incidence occurred, but here again it was not possible to correlate this with any constant factor, as the fluctuations did not occur uniformly but varied almost from farm to farm. On a few occasions light to heavy showers of rain fell, which caused considerable dilution of dipping fluids and it was not possible in all cases to make the necessary readjustments at the time, due to shortage of materials.

The paraffin emulsion dips had a tendency to become more concentrated at the surface and showed a certain amount of consolidation here. Such masses of emulsion were difficult to break up and were inclined to be carried out of the tank by the first few animals, which resulted in a lowering of the concentration and consequent decrease in effectiveness on ticks infesting animals that followed.

Considerable difficulty was experienced in keeping the arsenic content of the copper arsenite dips at what was considered to be an effective level, namely 0·16% As_2O_3. This was partly because the copper arsenite is only partially soluble and considerably less soluble in an alkaline than in an acid

solution. All dipping fluids which are in use tend to be alkaline and though the addition of copper sulphate tended to acidify the dip there was usually sufficient alkali present partially to offset this effect. A gradual decrease in the concentration of arsenic was noted and the actual values of copper arsenite expressed as As_2O_3 obtained varied between 0·12% and 0·15% in spite of the addition in some cases of a considerable excess, over the calculated quantities, of both sodium arsenite and copper sulphate.

From the chemical point of view the addition of nicotine sulphate gave by far the best results. We were gratified to note that whereas nicotine in water, when used as a dip, loses practically all its effectiveness within a very short time, the presence of sodium arsenite prevented this deterioration. The break down of nicotine in dipping fluids is apparently due to bacterial action, which seems to be almost inhibited by the presence of sodium arsenite in a concentration of 0·16% As_2O_3. Dipping solutions containing 0·04% nicotine when made up showed almost negligible alterations in concentration after several months, apart from that brought about by dilution with water added to make up for the solution removed during the process of dipping.

On farm number nine the concentration of nicotine was kept between 0·04% and 0·035% from the middle of September to the middle of December, 1940. From the middle of December, 1940, until the early part of February, 1941, no nicotine was added; and though water plus sodium arsenite was added to maintain the tank strength at 0·16% As_2O_3 and to compensate for the fluid removed by the regular weekly dipping, the concentration of nicotine was found to be 0·02%. This concentration was what could be expected due to the dilution which had occurred. Under laboratory conditions it was found that samples of a sodium arsenite-nicotine solution of the same concentration as that used on the above farm retained practically the same concentration for a period of five months.

From the foregoing it will be realized that at this stage only general conclusions, based largely on field observations, are possible. These may be summarised as follows:

1. A high degree of resistance or tolerance to sodium arsenite solutions at the concentrations used for dipping, namely 0·16% As_2O_3 (or seven day strength) and 0·24% As_2O_3 (or fourteen day strength) is shown by the single host blue tick, *Boophilus decoloratus* (Koch), in the East London district.

2. Sodium arsenite at a concentration of 0·16% As_2O_3 exerts a limited effect upon this strain of blue tick as tick infestation rapidly increases where dipping has been neglected.

3. The other common species of ticks present in the area, namely, *Rhipicephalus evertsi*, *R. appendiculatus*, *R. simus*, *R. capensis*, and *Amblyomma hebraeum*, do not display this resistance to sodium arsenite and are effectively controlled by dips containing it.

4. The addition to the usual seven day strength sodium arsenite dip of a well emulsified paraffin-resin-casein soap emulsion in such a concentration as to give 1% paraffin in the dip, enhances the parasiticidal effect of the dip to some extent, but is not an effective means of controlling this species of tick.

5. Copper sulphate when added to the seven day strength dip reacts with the sodium arsenite to produce copper arsenite which produces a dipping fluid somewhat more effective than sodium arsenite alone, but insufficiently parasiticidal to justify its use as a means of control. In addition, difficulty is experienced in maintaining the arsenic content at a satisfactory level.

6. A well emulsified carbolic dip added to sodium arsenite dip of seven day strength is not effective in controlling this strain of blue tick.

7. 40% nicotine sulphate added to the arsenical dip in the proportion of one part per 1000 by volume to produce a 0·04% nicotine solution effectively controls this strain of blue tick. A small proportion of the ticks escape, as only after three to four dippings at seven day intervals are cattle entirely freed from ticks. It would appear that the immature stages of the tick on cattle show a greater susceptibility than the adults, which, however, are themselves visibly affected.

8. Nicotine sulphate in the presence of sodium arsenite retains its effectiveness over long periods and seems to offer an immediate solution to the problem of controlling the arsenic resistant strain of blue tick encountered in the East London area.

In conclusion it must be stated that a considerable amount of experimental data has been collected which it has not been possible to report upon here. A large number of immersion tests with various substances of a toxic nature are at present being undertaken and have yielded valuable and interesting information; but, as these experiments are not yet complete, the results will be published later.

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XII (2): 59 - 61
1941.

Measles in Cattle and Pigs : Ways of Infection.

H. O. MöNNIG,
Onderstepoort.

As in the case of all infectious diseases, the successful control of measles necessitates a thorough understanding of its epizootology. Although we are far from knowing everything about this aspect of the measles problem, some important facts stand out quite clearly and indicate the direction control measures should take.

What we want to know is where, how, and when cattle and pigs ingest viable eggs. The possibilities would seem to be unlimited, because the ripe segments passed by infected persons expel their eggs or break up through autolysis, etc., so that the eggs are liberated and may be disseminated by wind, water, flies, dungbeetles, and other agents. Now exact knowledge of the viability of the eggs becomes important and on this point we have very scanty information. In water or moist surroundings in which decomposition of organic matter is restricted the eggs may remain viable for at least three months; desiccation kills them rapidly. However, conditions in nature are made up of a multitude of factors that may act in one way or another on the eggs and it is the sum of all these that produce the practical results, which may be very different from those obtained in laboratory tests. We are therefore unable to assess the importance of infection spread by wandering natives, road gangs, and railway gangs, who will, as a rule, void their infective material some distance from farm buildings and therefore be a greater danger to cattle than to pigs.

We find more definite indications by examining the evidence found in pigs and cattle and by correlating this with the habits of the human and animal hosts and of the parasites themselves. It is usually stated that pigs and cattle become infected by ingesting the eggs or ripe segments passed in human faeces and that the use of latrines will stop the spread of infection. But this statement is far too simple to reflect what really happens and it is also partly untrue. An examination of freshly passed ripe segments shows that those of *Taenia solium* are rather fragile, flabby and inactive, while those of *T. saginata* are comparatively firm and active, crawling about somewhat like caterpillars and expelling their eggs along the way. The *T. solium* segments therefore probably remain in or close to the faeces until they disintegrate and then only do the eggs become disseminated. Pigs are scavengers and so the chances of their finding and ingesting the eggs are much better if no dissemination occurs. The result is also well known, viz., that an infected pig is usually heavily infected, and so we may probably

conclude that the classical picture of infection is true in the case of *T. solium* and that the use of latrines would be very effective.

Cattle, however, are not scavengers, although a marked pica may lead them to deviate from their normal feeding habits and the general phosphorus deficiency may play some part in the measles problem in the Union. As a rule cattle do not graze close to the ground where most disseminated *Taenia* eggs will be and where they will remain viable for the longest time. It is therefore very desirable, from the point of view of the parasite, that dissemination of its eggs should occur as soon as possible, for there is a race between the acquisition of a host and loss of viability, and the host will not come to the eggs as the pig does. Also in this case the result is well known, viz., that cattle are, as a rule, only lightly infected. But that is not always the case, especially in calves.

This exception is very probably accounted for by the active nature of the ripe *T. saginata* segments. Their activity does not begin only after evacuation, but even before that, with the result that, as is well known, the ripe segments leave the host spontaneously. A *T. saginata* produces about five ripe segments every 24 hours and two to three of these are not passed at defecation, but crawl out of their own accord. They may therefore be "lost" by an infected person anywhere and this provides ample and daily opportunity for calves in a pen or paddock, or other cattle, to become infected through the agency of an infected native attendant. This is an important factor in the epizootology of bovine measles, the realization of which has led the writer to lay much stress on the necessity for treating human carriers of tapeworms as the only final solution to the problem, because it is obvious that even the strictest use of latrines will not stop the spread of infection. This point has recently also been emphasized by W. C. Ph. Meyer, Government Veterinary Surgeon in Bali, in a report read in May, 1938, before a meeting of veterinary officers in Batavia.

The viability of measles in cattle and pigs appears to differ markedly and may influence the degree of infection found at slaughter. Man is also susceptible to infection with *Cysticercus cellulosae* and some cases of epilepsy are caused by cysticerci in the brain. It has been found that this occurs when the cysts die and become turgid and that this usually happens about eight to nine years after infection. Man is an abnormal host for *C. cellulosae* and if they live so long in man they probably also do so in the pig, so that heavy infections in pigs may be the result of an accumulation.

In cattle the case appears to be different, for it is found generally at abattoirs that there is a higher incidence of measles in cattle under three years than in older cattle. This can only mean that the bovine measle dies off about two years after infection and that the calcified remains disappear to such an extent that they cannot be recognised after some time. Penfold, Penfold and Phillips in Australia found that an experimental infection with

measles produced in cattle an immunity to subsequent infections which lasted a considerable period. As a practical means of immunizing cattle it would not appear to be very suitable on account of the rather long period of life of the measle. However, the occurrence of an immunity and the periods of viability of measles in pigs and cattle are important points to consider in estimating the value of various epizootological factors on the basis of the results which they produce.

Gastrotomy in a Dog.

J. SPREULL,
East London.

The following clinical record of a dog that swallowed and retained a ball in its stomach for approximately one hundred days and made a wonderful recovery after operation may be of interest.

On August 21, 1939, I examined a small dog of the fox-terrier type said, by a boy, to have swallowed a " sorbo " rubber ball eight days previously. It had vomited very frequently ever since. The dog was fat and the belly very full and nothing could be felt by manipulation.

Dietary and symptomatic treatment was started and the dog improved somewhat. During the course of the next three months the state of health of the animal fluctuated, but improved whenever treatment was given. Palpation of the abdomen failed to reveal the presence of a foreign body.

On November 20, three months after the dog was first brought in, it was starved for 24 hours and deeply anaesthetized by the injection of three grains nembutal intravenously. The ball could then be distinctly felt through the belly wall. On the following day an operation was performed by Mr. Allchurch, Government Veterinary Officer, East London.

Anaesthesia was induced with three grains nembutal intravenously and continued with A.C.E. mixture. On opening the stomach, Mr. Allchurch removed a rubber ball 1·5 inches in diameter and weighing seven drachms. The stomach, peritoneum, and abdominal wall were stitched and dressings applied. As there was some bleeding, 60 cc. saline was injected subcutaneously.

The dog vomited a number of times during the first four days after the operation, but by the sixth day its appetite was good and it was picking up well. The stitches were removed on the ninth day, and the patient discharged on the tenth day.

The delay in operating was due to scepticism of the boy's story about the dog's swallowing the ball. After the operation the boy admitted that the ball had stuck in the dog's throat and that as he could not pull it out he had pushed it down to save the dog from suffocating.

Jl. S.A.V.M.A.
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1941.

The Occurrence of *Babesia bovis* in South Africa

W. O. NEITZ,
Onderstepoort.

Introduction.

The records dealing with redwater in South Africa mention one parasite only, namely, *Piroplasma bigeminum (Babesia bigemina)* (Smith and Kilborne 1893). The existence of the disease was first noticed in 1870, but the micro-organism responsible was only recognized by Koch (1898) in the Cape Province and Transvaal and by Hutcheon (1898) in Natal and the Orange Free State. Subsequently frequent references to piroplasmosis have been made in the reports of the Government Veterinary Bacteriologist and in the Annual Reports of the Onderstepoort Veterinary Laboratories.

It seemed strange that *Babesia bovis* (Babes 1888) was never observed, although cattle were introduced into South Africa from Europe, where this parasite occurs. One of the reasons given why *B. bovis* had not established itself was that the known vector, *Ixodes ricinus* L. 1804, does not occur here. This reason is not adequate because Lignières (1903) showed that *Boophilus microplus* Canestrini 1888 was the transmitter of *B. bovis (B. argentina)* in the Argentine and Filmer (1931) concluded, from his field observations, that *B. microplus* was the vector in Australia. In 1935 Legg was able to confirm Filmer's conclusion experimentally under laboratory conditions.

About two years ago, Mr. B. van der Vyver, the Government Veterinary Officer of the Pretoria district, reported that on several farms in the Pretoria district severe outbreaks of redwater were observed in cattle that had been born and bred on these farms or that had been introduced from known redwater areas, such as the Middelburg and Lydenburg districts in the Transvaal. The stock-owners who were well acquainted with the value of trypan blue as a specific remedy for piroplasmosis complained that this drug did not cure the sick animals.

Experiments.

1. *Isolation of* Babesia bovis.

As the real cause of the trouble was obscure, it was decided to study the disease under laboratory conditions. At the time when the investigations started it was believed that variations in the virulence of strains of *P. bigeminum* would explain the peculiar nature of the disease. Arrangements were

therefore made to collect blood from a sick animal on one of the farms where outbreaks of this form of redwater had been observed.

Towards the end of February, 1941, an outbreak of redwater was reported from Bynespoort in the Pretoria district. During the first week in March, blood was collected from one of the sick animals and injected subcutaneously into a year-old calf reared under tick-free conditions at Onderstepoort. This animal reacted severely, the temperature rising to 105°F on the eighth day following inoculation. Blood smears were examined daily and, on the day on which the temperature rose, parasites which were morphologically identical with *B. bovis* were found in the peripheral blood. Clinically the calf showed inappetence, general weakness, haemoglobinuria, and a marked anaemia. On the fifth day of the illness the calf was treated with an adequate dose of acaprin. No improvement was noticed 24 hours later, and therefore blood was subinoculated into a susceptible calf to maintain the strain for further experimental work. In addition the calf was again treated with acaprin, but died three hours later. At autopsy it was found that the calf was suffering from a marked anaemia, icterus, tumor splenis, hyperaemia of the kidneys, haemoglobinuria and a swollen liver.

The inoculated calf also reacted to *B. bovis,* but recovered without treatment. Since it was not known whether the original reservoir harboured *Anaplasma marginale* Theiler 1910 or *Theileria mutans* (Theiler 1906), it was decided to eliminate these parasites by subinoculating blood from the reacting animals early during the disease. The infection was passaged through five generations. Six calves were used but no parasites other than *B. bovis* were found in the experimental animals. Of these six calves, one which was treated in the later stage of the disease died, two recovered after being treated with acaprin, one recovered without treatment, and one, which had not been treated, died.

2. *Morphology of* Babesia bovis.

The accompanying photomicrographs show the morphological differences between *P. bigeminum, B. bovis,* and *Theileria mutans.*

B. bovis may be pear-shaped, elliptical, round, or irregular. As a rule one or two parasites are found in an erythrocyte, but sometimes three or four may be present. Frequently two parasites whose long axes form a wide angle have been observed. Occasionally extracellular forms have been seen. *B. bovis* is smaller than *P. bigeminum.* The former is about 1·5 μ in diameter but sizes up to 2 μ may also be found, whereas the latter is from 2–4 μ in diameter.

In blood films prepared from living animals little difficulty should be experienced in differentiating between the two species of parasites. Difficulty arises, however, when blood and organ smears are prepared from animals some time after death, when parasites are contracted. It may then be difficult to make a differential diagnosis.

3. *Cross-immunity Experiments with* Piroplasma bigeminum *and* Babesia bovis.

Since *B. bovis* resembles *P. bigeminum* morphologically, and produces a disease which cannot be distinguished clinically from piroplasmosis, cross-immunity experiments were considered essential.

In the first experiment, animals premunized against *P. bigeminum* were inoculated with *B. bovis* and in the second experiment cattle premunized against *B. bovis* were inoculated with *P. bigeminum*. These tests showed that *P. bigeminum* premune cattle were susceptible to *B. bovis* and that *B. bovis* premune animals were susceptible to *P. bigeminum*. Only a few young animals were used for these tests and it was therefore not possible to decide whether the one parasite produces any resistance to infection with the other.

4. *Transmission.*

The vector of *B. bovis* in South Africa is not known. On the farm Bynespoort the cattle were chiefly infested with *Boophilus decoloratus* (Koch 1844). It is possible that the three known vectors of *P. bigeminum* in South Africa may transmit *B. bovis*.

5. *Treatment.*

The stock-owners on the farms on which this form of redwater has been observed, reported from time to time that trypan blue did not cure the affected animals. The use of acaprin was recommended and they stated that the results were satisfactory. Up to the present, only a limited number of animals have been treated with acaprin. The animals were young and at this stage it is not possible to say whether the recoveries were due to the drug. Further experiments are being carried out to ascertain the value of trypan blue, trypaflavin, and acaprin as treatments for cattle suffering from babesiosis. *

Acknowledgments.

The author wishes to thank his colleague Mr. B. van der Vyver for the information supplied, and Mr. T. Meyer for preparing the photomicrographs.

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* **Note added in proof.** Since writing the above, Gonacrine (May & Baker) has been found to have a specific action on *Babesia bovis.*

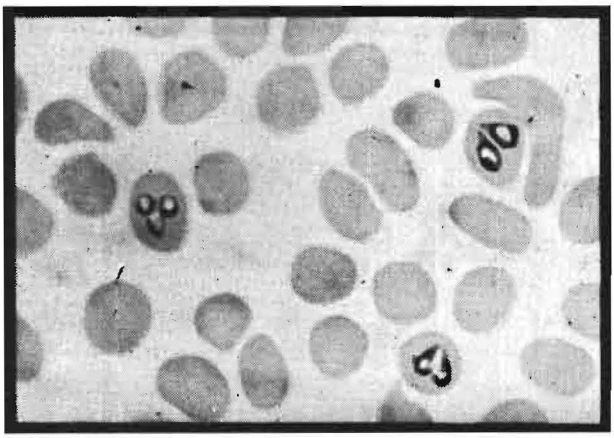

Fig. I. — *Piroplasma bigeminum in a blood smear.*
Magnification 2000 ×.

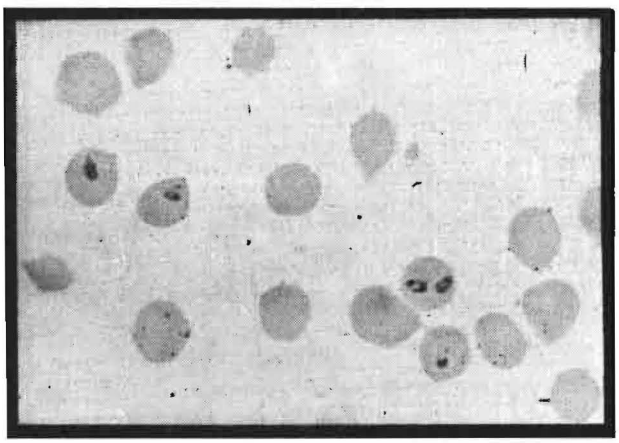

Fig. II. — *Babesia bovis* in a blood smear.
Magnification 2000 ×.

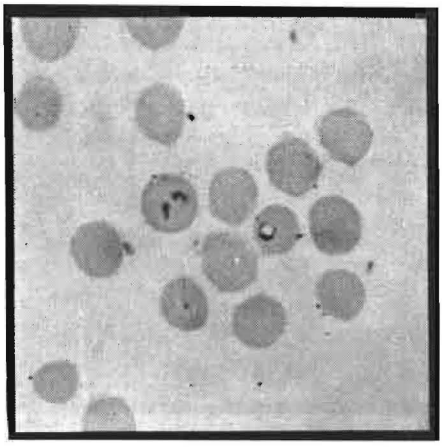

Fig. III. — *Babesia bovis* in a blood smear.
Magnification 2000 ×.

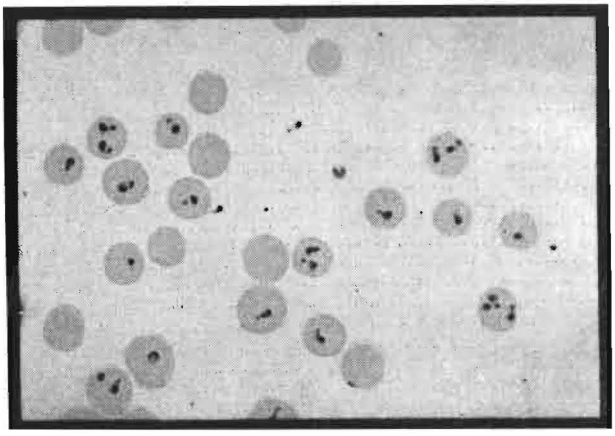

Fig. IV. — *Theileria mutans* in a blood smear of a splenectomized calf.
Magnification 2000 ×.

An Atypical Case of Strychnine Poisoning.

C. F. B. HOFMEYR,
Umtata.

The subject of this note was a ten-year-old half-bred cocker spaniel, which, up to the time of its death, was in good health and very active for a dog of this age. The occurrence was unusual as strychnine rarely sets up so severe a gastro-intestinal irritation.

History.

The two dogs of the house were allowed out for a few minutes at 10 o'clock on the night of the death of the cocker spaniel. The dogs did not leave the house again, since all doors were closed because it was mid-winter. Nothing untoward could be noticed in the behaviour of the animals, excepting that the cocker spaniel refused sweets of which he was very fond. At twelve o'clock everyone went to bed. Before this, however, a member of the family found that a dog had vomited in the house, but this was attributed to the terrier which was subject to periodic attacks of gastritis.

At about one o'clock the cocker spaniel was heard to run down the stairs. At four o'clock he was found dead in the sternal position surrounded by a pool of vomitus and liquid faeces.

No poison was kept in the house.

Post-mortem Examination.

Rigor mortis was present throughout and the animal had stiffened in the sternal position. The skin was contaminated with faecal and vomited material. Severe gastritis was found, with enteritis, haemorrhagic in patches, throughout the intestine. Stomach and bowel contents were scanty. Pulmonary emphysema was present in patches and a small worm — most probably a migrating ascarid larva — was found in one of the lungs. The kidneys were hyperaemic, whilst the rest of the examination showed nothing unusual.

Diagnosis.

Arsenical poisoning was suspected. Liver and stomach with contents were sent separately to Onderstepoort for analysis. The specimens were negative for arsenic, and positive for strychnine.

Discussion.

The symptoms of acute gastro-intestinal irritation certainly lead one to suspect acute arsenical poisoning rather than strychnine poisoning. The fact

that the animal died in the sternal position suggests that death was due to acute heart-failure or, more probably, to asphyxiation caused by a fatal spasm of the diaphragm and intercostal muscles.

This case clearly illustrates the danger of not confirming a clinical diagnosis of poisoning by means of a chemical analysis.

ACKNOWLEDGMENT.

I wish to express my indebtedness to the Section of Toxicology, Onderstepoort, for the chemical analysis of the specimens submitted.

BOOK REVIEWS.

Man's Greatest Victory over Tuberculosis. J. Arthur Myers.

The author who is Professor of Medicine, Preventive Medicine and Public Health in the University of Minnesota has written a book* which will make an appeal to all veterinarians engaged in tuberculosis control work. The first five chapters are devoted to the control of animal diseases in general, and detail some of the achievements of the veterinary profession in this direction, the author being very complimentary to the members of our profession who took part in this work. Chapters are devoted to the work of the United States Bureau of Animal Industry, quarantine measures enforced in the United States of America, and the federal inspection of meats. Chapters nine to seventeen deal with such subjects as the geographic distribution and prevalence of tuberculosis in cattle, the nature of tuberculosis, the tubercle bacillus, tuberculin, the tuberculin test, the diagnosis of tuberculosis in cattle, immunity, and bovine tuberculosis in man.

To those familiar with the study of tuberculosis there is very little which is new in these chapters, but the subject matter is dealt with in a very able and comprehensive manner and summarizes very well the information available at the present time.

Chapters eighteen to twenty-five are devoted to the progress of the eradication campaign in the United States. It is these chapters which will interest the reader most, as they describe in detail the magnificent achievement of the Bureau of Animal Industry in almost completely eradicating the disease in the course of twenty-three years. In 1917, when the campaign commenced, only 20,000 cattle were subjected to the tuberculin test, but by 1929 this number had risen to 11,000,000, and the peak was reached in 1935, when 25,000,000 cattle were tested. The incidence of reactors decreased from 3·2% in 1917 to ·5% in 1939. The amount paid in compensation in 1917 was 75,000 dollars and reached its maximum of 26,792,197 dollars in 1935. By August 1st, 1940, all the states except California were rated as modified accredited areas, and

* "Man's Greatest Victory over Tuberculosis," by J. Arthur Myers, Ph.D., M.D., F.A.C.P., Professor of Medicine and Preventive Medicine and Public Health, University of Minnesota, and chief of medical staff, Lymanhurst health centre, Minneapolis. p. ix + 419. Charles C. Thomas, Springfield, Illinois. Price $5.

only two counties in California were unaccredited. The description of the educative and legislative measures adopted at the commencement and during the course of the campaign make very interesting reading, as does the chapter on the opposition which was encountered, varying from press campaigns to legal actions.

A summary of the most important points in each chapter is given at the end of it, a very valuable aid to the reader. If any criticism may be offered, it is of the last chapter on the lessons which physicians in human medicine could learn from this campaign. In man the high percentage of infection, the social problems involved, and many other almost insuperable difficulties, make it difficult to compare the work of the veterinarian in tuberculosis eradication to that of the practitioner of human medicine.

A comprehensive bibliography is given at the end — the bulk of the references, as would be expected, being to American publications. There is a very interesting photo at the commencement of the chapter on the tubercle bacillus showing Robert Koch with two of his brothers who were living in the United States. The photo was taken in 1908, two years before his death, and as he stipulated that only three prints should be made and the negative then destroyed, the photo is probably unique.

E. M. R.

Practical Notes on Pharmacology, Prescription Writing and Therapeutics. J. M. Watt (1940).

These notes* by Watt, who is professor of Pharmacology in the University of the Witwatersrand, Johannesburg, are neatly printed by the Replika Process and are very well bound.

As the author states, they are not intended to take the place of a textbook on pharmacology, but they merely present very useful information on the choice of suitable drugs in the treatment of the various diseases. Anybody who has to prescribe treatment, especially with the more recent and proprietary drugs, will appreciate the great usefulness of this booklet. The notes on the writing of prescriptions, antiseptics, and vitamins are of great value. The index is very complete. The author can be congratulated on the very concise way in which he has placed most useful information at our disposal.

Although this publication is primarily intended for those concerned with the treatment of human diseases, they will also prove to be of great value to the veterinarian. It can be obtained from the Librarian, Witwatersrand University, Milner Park, Johannesburg.

D. G. S.

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* "Practical Notes on Pharmacology, Prescription Writing and Therapeutics," by J. M. Watt, M.B., Ch.B. (Edin.), M.R.C.P.E., F.R.S.E., F.R.S.S.Af., S.B.St.J. Professor of Pharmacology, Witwatersrand University. 272 pages. Witwatersrand University Press, 1940. Price 7/6.

Diseases of the Pig and Its Husbandry. Dàvid J. Anthony (1940).

The author in his preface expresses the wish that this book* "will not only be of some use to veterinary surgeons and students, but to all who are interested in that most useful animal the pig, from the agriculturist, the large-scale breeder, to the cottager and the enthusiastic amateur anxious to try his hand and to help his country by keeping a pig or two at the bottom of his garden." As in most instances in which a book is written to meet such a varied group of readers, this little book is somewhat disappointing from the purely veterinary point of view. I have no doubt that the book would be an invaluable guide to the layman.

The greater part of the book is devoted to diseases of the pig. These are briefly described and this portion of the text should be of some value to veterinarians. The discussion on the management and feeding is only a guide and is very brief and incomplete. The deficiency diseases are rather poorly described.

H. P. S.

* "Diseases of the Pig, and Its Husbandry," by David J. Anthony, M.R.C.V.S., D.V.S.M. pp. xii + 272. 48 illus. Baillière, Tindall & Cox, London. Price 10/6.

THE ASSOCIATION.

Council Meeting held at Polley's Hotel, Pretoria, on 16 April, 1941, at 8 p.m.

Present: S. T. Amos (President), A. C. Kirkpatrick, A. D. Thomas, D. G. Steyn, R. Alexander, M. Sterne, J. H. Mason (representing Capt. J. L. Dickson), and S. W. J. van Rensburg, Hon. Sec.-Treas.

Apologies for absence: P. J. du Toit, C. J. van Heerden, J. L. Dickson and N. F. Viljoen.

(1) *Minutes of Meeting* held on 12th November, 1940, were taken as read and were confirmed.

(2) Arising from these minutes —

(a) *Lecturer in Anatomy.* — The Secretary submitted a letter from Dr. Alexander regarding this appointment, and reported that no further information had been received since the last meeting. The matter had apparently not yet been settled by the Department of Agriculture and the Council of the University. Several members strongly deprecated the great delay, and it was agreed that a deputation of Council, consisting of Drs. P. J. du Toit, D. G. Steyn and R. Alexander should interview the Secretary for Agriculture on this matter.

Dr. Sterne pointed out that the veterinary profession had no direct representation on the Faculty of Veterinary Science, and it was decided that this question be placed on the agenda for the next meeting of Council.

(b) *Benevolent Fund.* — The President drew attention to the distressing position of the widow of a late member. It was decided that the pension granted to her be increased from £2 to £4 per month.

The President stressed the necessity for strengthening the Benevolent Fund in order to render substantial assistance to all deserving cases among dependants of colleagues, and it was decided that the Secretary should take the necessary steps to draw the attention of members to the Fund, in an endeavour to increase the revenue.

(3) *Council Member.* — Dr. P. S. Snyman was unanimously elected as a member of Council to fill the vacancy created by the death of the late Dr. Cloete.

(4) *New Members.* — Messrs. G. P. Bishop, F. Hempstead and G. L. Faull were proposed, and it was unanimously decided to recommend their acceptance to the next General Meeting.

(5) *Resignation.* — The Secretary read a letter from Mr. E. T. Perossi in which this member submitted his resignation on account of advancing age and indifferent health. This was accepted with regret. In view of his long term of membership and the circumstances which necessitated his resigning, Council decided that the Journal be sent to him free of charge in future.

(6) *Representation on Meat and Dairy Control Board.* — The Secretary read a letter from Dr. J. I. Quin, drawing attention to the fact that the veterinary profession had no representatives on the Meat Control Board, and asking Council to make representations to the authorities concerned, with a view to getting the profession represented on the Board.

During the ensuing discussion, the need for veterinary representation on the Dairy as well as on the Meat Control Board became evident, and it was decided that the Secretary should request the Secretary for Agriculture to submit to the Minister of Agriculture the desirability of having Veterinary representation on both these Boards with the suggestion that such representatives be appointed by the Council of the Association.

(7) *General.* — The Secretary informed the Meeting that Mr. v. d. Wath had resigned as Assistant Secretary and that Mr. de Boom had also indicated that he could not continue with the work of the Book Fund. It was decided that a letter of thanks be sent to each of these two, that no Assistant Secretary be appointed for the time being, and that the Secretary, with the Committees concerned, make the necessary arrangements for the work of the Book Fund to be carried out.

The Meeting closed at 11 p.m. with a vote of thanks to the President for having come up from Durban specially for this Meeting.

S. W. J. van Rensburg,

HON. SECR.-TREAS, S.A.V.M.A.

Jl. S.A.V.M.A.
XII (3): 73 - 80
1941.

An Unusual Case of Rickets in a Dog

R. CLARK, A. D. THOMAS, and K. C. A. SCHULZ
Onderstepoort

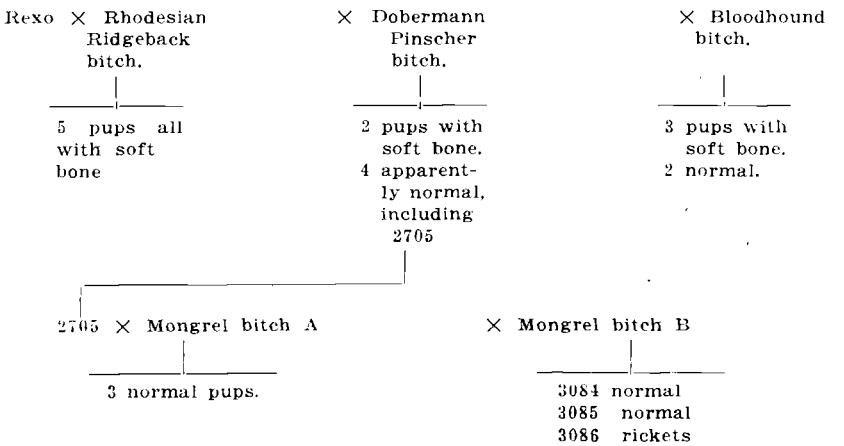
Introduction

During recent years the authors have encountered several cases of soft bone, complicated by very poor development of the bone marrow, in dogs. These had most of the deformities usually associated with clinical rickets, but the absence of the typical histopathological features of this disease always left the diagnosis in doubt.

Mainly from cases seen at the South African Police dog depot, a suspicion arose that this condition was in some way hereditary. One animal at the depot, a Rhodesian Ridgeback dog named "Rexo," was particularly suspected, because when he was mated with three different bitches, pups of all the litters developed deformities of the skeleton. These were the only cases of this kind seen at the depot for many years. One of Rexo's pups (2705) was presented to Onderstepoort and has been used for breeding. The case of rickets to be described is a son of 2705, the mother being a nondescript bitch purchased locally.

The following chart gives the history of the family.

Table 1.



It will be noted that the wider the out-cross the lower the proportion of actual cases, which is in accordance with the ideas of heterozygocity and adds weight to the theory of an inherited factor.

Clinical History

The litter (3084, 3085 and 3086) all appeared normal at birth and all remained healthy and grew well till they were three months old. The only male of the litter (3086) was then noticed to be lagging behind in growth and there was a slight swelling of the carpal joints. These symptoms became gradually more pronounced until the pup was four months old, when marked changes occurred very suddenly. Within three or four days a marked sagging of the hocks and bowing of the fore legs developed. Up to the time when the dog was nine months old, these changes became progressively more pronounced. The head was comparatively well developed and appeared too big for the body. The teeth were well formed and hard, but there had been very little general body growth and the pelvis was especially poorly developed. The fore legs were bowed to a remarkable degree and the carpals sagged so that practically the entire volar aspect of the metacarpals was on the ground. The carpals and toes were widely separated from each other. The hocks sagged and the hind feet resembled the fore. There was a pronounced rickety rosary. The marked bending of the bones and lack of growth are shown in Fig. 1.

The weight increase in the three dogs is shown below:—

TABLE 2.

Date	3084 ♀ Normal	3085 ♀ Normal	3086 ♂ Rickets
31:12:40	29 lb.	26 lb.	16 lb.
21: 1:41	32 lb.	31 lb.	18 lb.
25: 3:41	36 lb.	41 lb.	20 lb.

On 30:5:41 the dog 3086 was noted to be very weak and listless. The temperature was subnormal and the pulse feeble and irregular. No parasites could be found in blood smears, but there was a yellow muco-purulent discharge from the nose and eyes. The dog went into periodic coma from which it would partially recover now and again. The condition became progressively worse until the animal died on 2:6:41. The post-mortem findings will be described later.

Previous to this fatal illness the dogs had been in good general health since birth and all had been on the same diet. The two bitches are well grown out and are in good condition and there have been no intercurrent diseases such as biliary fever or distemper. Intestinal parasites have been controlled by faeces examinations and treatment applied where necessary.

Feeding

The pups were weaned gradually and placed on whole cow's milk and then given small pieces of raw meat as well. They were then put on to a

diet of raw meat, whole raw milk, and maize porridge with meat cooked in it. There is an open run to each kennel and the dogs are usually in the sun for most of the day. As soon as the retardation of growth was observed, 3086 was separated from the two bitches and the diet carefully controlled. The actual daily amount consumed averaged a pound of fresh raw meat, a pint of whole raw milk, and a small amount of the maize porridge. Liver and uncleaned tripe were given when available, and soft raw bones such as ribs were always available and readily eaten. Despite this excellent diet the bone lesions not only developed, but became progressively worse.

The only abnormality in the appetite of the dog was a tendency to pick up and swallow stones and sand when allowed out of the run.

Histopathology of the Bone

A small piece of the distal end of a rib together with the costochondral joint was resected from each dog on three occasions. These biopsies took place four months, four months and three weeks, and eight months after birth. The first biopsy was therefore made as soon as the bending of the bones had become apparent.

From these samples sections were prepared, using the double embedding process as described by Thomas, Clark and Schulz (1940) and the combined Silver-haemalum-eosin staining method recently introduced at this institute [Moolman (1941)].

As no marked change occurred in the histological picture over this period it is not necessary to describe each of the sections in detail. The condition became progressively worse as time went on.

The bone presented the classical histological picture of rickets. The hypertrophic zone of the cartilage was much widened, spread out, and jumbled, there being practically no sign of the formation of primary trabeculae. The line of preliminary calcification was very irregular and tongues of cartilage extended into the bone. Islands of cartilage appeared deep in the diaphyseal region. The chondrocytes were large and swollen and towards the outer edge of the cartilage the cartilaginous matrix was often poorly calcified forming a loose lace-work in which degenerate chondrocytes were still embedded. The trabeculae were fine and fragmented and towards the epiphysis showed no definite arrangement. Towards the diaphysis they became arranged parallel to the long axis of the rib, but were very narrow and delicate. Broad seams of osteoid lined practically all the trabeculae. These changes are well illustrated in Fig. 2. Whereas, with the silver staining used, the trabeculae of the normal dogs (3084, 3085) appeared solid brown to black with perhaps a few discrete dots of calcium in the narrow band between the bone and the osteoplastic layer, those of the affected dog (3086) showed quite a different picture. Here, under high power, discrete dots of black stained calcium could be seen scattered through-

out the matrix and these seldom coalesced to form the dense even black colour of normal bone tissue when stained by this method. This phenomenon has often been observed in sections of poorly calcified bone when these have been prepared by the method now used.

The osteoblasts were small and lay with their long axes parallel to the edges of the trabeculae, but in places sheets of swollen osteoblasts, four or five deep, were encountered. Osteoclasts were numerous and active.

The intertrabecular spaces contained very few marrow cells, consequently the reticulum and blood vessels appeared very prominent. The few marrow cells present were nearly all of the granulocyte series, the erythrocyte precursors being almost absent. (See Fig. 3). This scarcity of marrow was very well demonstrated by smears made from a small piece of bone cut from the proximal end of the portion of rib removed at biopsy. This was squeezed in a pair of pliers and smears made from the extruded marrow. The smears from the normal bitches (3084-3085) contained numbers of marrow cells of all types, but one had to hunt through those of 3086 in order to find a cell other than reticulum cells, endothelials, or granulocytes.

As mentioned previously this lack of marrow was noted in all the cases of soft bone described in the first part of this article.

The Blood

The absence of erythrocytopoietic cells in the marrow was directly reflected in the blood, there being a marked oligocythaemia in the affected dog. The following is a summary of the blood counts made.

TABLE 3.

Dog	3 months old		7 months old		8 months old	
	Erythrocytes		Erythrocytes		Erythrocytes	
	% Precipitate	Count 10^6	% Precipitate	Count 10^6	% Precipitate	Count 10^6
3084	42	7.2	47	10.9	45	7.6
3085	36	8.7	41	7.4	47	7.9
3086	20	2.7	14	1.9	15	1.5

The leucocytes were in all cases normal both as regards total and differential counts and morphology. It is of interest to note that despite these extraordinarily low red blood counts, the dog appeared quite lively and the only clinical symptom was a paleness of the visible mucous membranes. It is also noteworthy that the biopsy wounds healed just as readily and quickly, by first intention, as in the other dogs. The morphology of the erythrocytes was at all times normal, no immature erythrocytes having been seen in the bloodsmears, a further indication that the anaemia was aplastic in nature rather than due to the destruction of blood cells.

On 21:1:41 the blood of all three dogs was analysed for inorganic phosphorus with the following results.

TABLE 4.

Dog	P_2O_5 in mgm. per 100 cc.
3084	4.0
3085	4.1
3086	6.6

On 25:3:41 a more complete analysis gave the following figures.

TABLE 5.

Dog —	3084	3085	3086
Blood inorganic phosphorus	4.20	3.40	7.20
Serum inorganic phosphorus	7.04	7.29	9.24
Serum inorganic phosphorus plus liberated phosphorus	9.86	10.03	17.95
Blood Calcium	9.20	7.40	8.40
Haemoglobin	14.38	14.03	4.88
Blood Phosphatase	2.82	2.93	8.71
Bone Phosphatase	——	——	14.30*

N.B. — Phosphatase expressed as Bodansky units, other figures mgm per 100 cc.

* 3—5 considered normal.

Post-Mortem Findings

The carcass was extremely emaciated and the blood very watery. There was no abnormal collection of fluid in any of the body cavities. The right ventricle was dilated and the wall appeared thin.

Of the soft tissues the kidneys were the only organs showing any abnormality. They were small and very firm and, with the capsule still *in situ*, showed numerous protuberances on the surface, mostly about five mm. in diameter and two to three mm. in height. They were extremely tough to cut and the capsule stripped with some difficulty, but its removal did not damage the surface of the organ. With the capsule removed, the protuberances mentioned above were seen to be light grey in colour while the depressions between them were white. On section grey rays could be seen running through the almost solid connective tissue mass of the medulla to the pelvis.

On histological examination the cortex of the kidney is seen to consist of radially arranged broader bands of renal tissue alternating with narrower ones of practically solid connective tissue. The outer surface is depressed over the latter areas and bulges out markedly between them, thus forming

the protuberances already mentioned. In the connective tissue bands, renal corpuscles and isolated tubules lie scattered here and there, mostly in a state of compression, distortion and obliteration. The areas between these bands are filled with renal corpuscles and tubules of very irregular diameter. Even here the connective tissue stroma is very exaggerated, tubules often being separated from one another by collagenous strands as wide as themselves. Smaller bands of connective tissue often run radially through the cortical substance.

The medulla consists almost entirely of connective tissue through which run widely separated and constricted tubules, many of which contain plugs of desquamated cells. In addition to the great decrease in functional renal tissue present, there is obviously a renal stasis in the cortical tubules, which has resulted in their irregular dilation, and which has been caused by the constriction of the medullary elements.

No abnormality could be found, either macroscopically or histologically, in any of the other organs, including the endocrines.

Examination of the skeleton showed the rickets to have been generalized. All the bones are exceedingly light and friable and the epiphyseal lines are broad. The long bones of the limbs show bending and distortion of the shafts and flattening of the articular surfaces. The epiphysis is also often wider than the adjacent end of the diaphysis, causing a projection which accounts for the enlarged joints seen during life. The surface of the cleaned bones is porous and rough to the touch. This is especially noticeable in the maxillae, mandibulae and vertebrae. Smooth hard-looking bone is found in the centre portion of the shafts of the long bones.

Histological examination of the costochondral junction taken after death shows that the lesions of rickets are still present, but there is some improvement in the general picture. The hypertrophic zone of the cartilage is narrower than at the time of the last biopsy and the formation of primary trabeculae is present. There is, however, very little increase in the marrow. Sections from the distal and proximal extremities of the humerus and femur show a similar picture. The apparent improvement can probably be ascribed to the cessation of growth.

Discussion

Rickets is generally regarded as being a disturbance of the phosphorus metabolism due to a deficiency either of phosphorus or of vitamin D. The vitamin may either be supplied in the diet or by the action of sunlight on the body. Some authors (e.g. Marek) include a calcium deficiency and an imbalance in the acid-base ration of the diet as aetological factors. It is also well known that certain mineral poisons, e.g. fluorine, may cause disturbances in ossification resembling rickets.

Fig. 1. — *Litter mates, dog (3086) left and bitch (3084) right, at the age of nine months.*

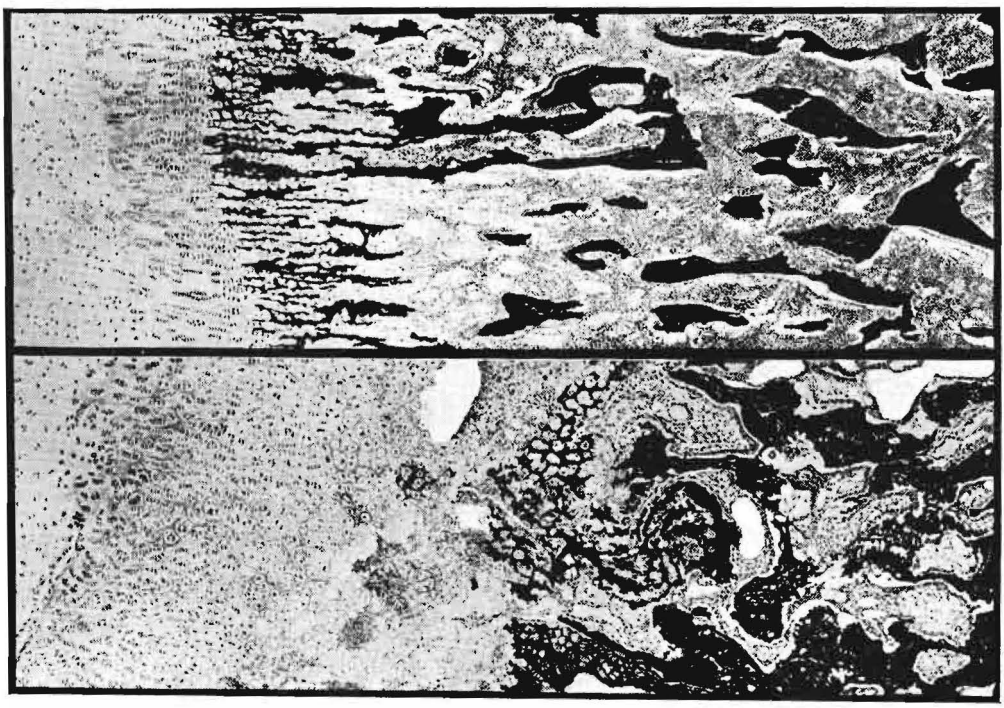


Fig. 2. — *Rib sections of 3085 (top) and 3086 (below) as resected at the age of five months.*

Note the normal structure of that of 3085 and the marked typical rickets in the case of 3086.

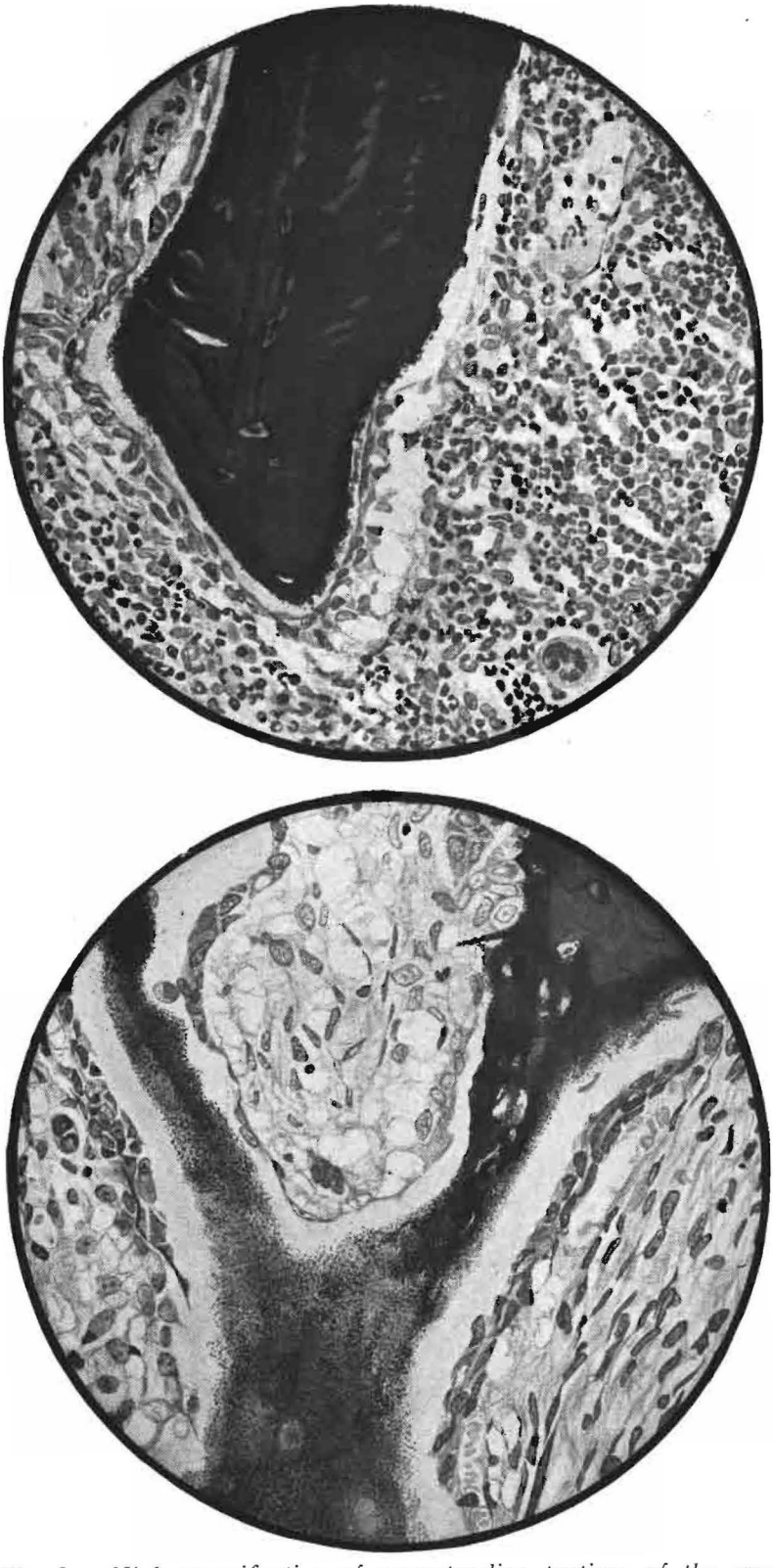


Fig. 3. — *High magnification of corresponding portions of the same sections as in Fig. 2.*

Top (3085) Showing well calcified trabecula and abundant marrow cells.

Below (3086) Showing poor calcification, wide osteoid border and scarcity of marrow cells.

In the case described, however, the diet appears to have been more than adequate in all respects. The high figure for blood phosphorus proves not only that this element was available but also that it was absorbed. The fact that the dog was exposed to the bright South African sunshine almost daily should alone ensure that there was no lack of vitamin D. Furthermore, the fact that the two bitches developed normally on the same diet and under the same conditions makes it difficult to conceive that the aetiology in this case was either dietary or environmental.

It would appear therefore that the basic cause of the condition was inherent in the animal itself. Hess and Blackberg (1932) have shown that in dogs there is a "constitutional factor" in the aetiology of rickets and indicate that this factor is hereditary.

Hunt (1927) reviews a disease of children known as "renal infantilism" in which chronic interstitial nephritis is associated with lack of growth and ricket-like changes. In some respects this disease resembles the condition seen in the subject of this article, but "renal infantilism" is not considered to be hereditary nor does the aplastic anaemia, which was so striking a symptom in the dog, appear to be present.

The case described raises the interesting possibility of an inherent and possibly hereditary factor which, directly or indirectly, influences phosphorus metabolism and is probably associated with haemopoiesis. It is as yet too early to make any but tentative suggestions as to the cause of the condition, but further breeding along the same line may enable us to carry out more work later.

Summary

(1) A case of canine rickets is described which developed on an apparently adequate diet and in the presence of abundant sunlight.

(2) The rickets was complicated by a marked aplastic anaemia.

(3) The dog was bred from a family of which members had previously shown paucity of marrow and bending of bones.

(4) A marked fibrosis of the kidney was discovered at autopsy.

Acknowledgements

The authors are greatly indebted to Captain Claassens, Officer Commanding the South African Police Dog Depot, for his help in supplying information on the condition among the dogs under his care and especially for arranging for the gift of the dog 2705 to this institute. They also wish to thank Mr. P. K. van der Merwe for the chemical analyses and Miss G. E. Laurence who did the camera-lucida sketches.

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An Improved Method of Staining Bone Sections

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A method for preparing sections of bone without the necessity for prior decalcification has been described by Thomas, Clark and Schulz (1940). By this means the degree of calcification, an important feature in bone pathology, otherwise lost by ordinary methods of preparation, is preserved and can be studied together with other histological changes. Moreover since such sections can be cut to a uniform thickness of 8μ or less, the comparison of the ossification process in the various bone diseases is made relatively easy.

To demonstrate the calcified tissue in these sections the silver impregnation staining method of von Kossa has hitherto been used, while duplicate sections were stained with haemalum-eosin. A combination of these two procedures has now been devised and has proved so useful that it has been adopted as the method of choice for all our histological preparations of bone material. The method could be used advantageously in other fields of research and teaching, as for example in embryology.

The essentials of the method are outlined below.

1. Bone material should be cut or sawn into slices about 3 mm. thick and fixed in formol.
2. Wash well in tap water to remove *all traces* of formol.
3. Dehydrate and proceed with double embedding in celloidin-cedarwood-oil-paraffin as described by Thomas, Clark and Schulz.
4. Shave off and discard about 0·5-1 mm. of the surface bone which may contain sawdust in the marrow spaces. Then cut sections at suitable thickness, say 8μ.

5. Fix section on to slide by means of glycerin-albumen in oven at 37° for 24 hours or, preferably, longer.
6. Deparaffinate in xylol and bring to distilled water through descending alcohols of 100%, 90%, 70% strength.
7. Place in 5% aq. solution of silver nitrate and expose to sunlight until brownish-black colour is well developed (usually ½ hour or longer if light weak).
8. Return to distilled water (section may tend to lift off slide and should be pressed down with blotting paper whenever necessary).
9. Fix silver nitrate in 10% aq. solution of sodium hyposulphite (thiosulphate) for 10-20 minutes (longer if sections become cloudy).
10. Return to distilled water.
11. Stain with Böhmer's haemalum 5-10 minutes.
12. Pass through tap water and differentiate with acid alcohol (1% HCl) very rapidly and return to tap water until blue (10 minutes or longer). (Do not overstain with haemalum as acid alcohol has to be used very sparingly and rapidly in differentiation since it dissolves the lime in the bone. The use of Mayer's haemalum obviates this necessity but does not give as good results).
13. Pass through 60% alcohol, then eosin (0.5% in 70% alcohol) and so through ascending alcohol, carbol, xylol, and mount in canada balsam.

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THOMAS, A. D., CLARK, R. and SCHULZ, K. (1940). A method for preparing sections of bone without decalcification. *Onderstepoort Jl. of Vet. Sc. and Anim. Industry.* 15(1/2).

THE BLOODLESS PHLEBOTOMIST.

We have just received an advance copy of "The Bloodless Phlebotomist", Vol. VIII, No. 6, which is usually issued in 15 languages with a total circulation among members of the medical and allied professions throughout the world of 1,500,000 copies. This year, however, due to the War, it will be sent to members of the professions only in English, Spanish and Portuguese speaking countries outside of the War zone.

This little journal published by the Denver Chemical Manufacturing Company of New York is replete with interesting articles written by physicians who are located in different countries and while the purpose of the publication is to acquaint its medical readers with Antiphlogistine and Galatest, the physicians will find a number of items and illustrations which will excite their curiosity and interest.

If you do not receive a copy write to the Denver Chemical Mfg. Company, New York, who will place your name on their list. The journal will be supplied you free of all charges.

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The Determination of the Antigenic Values of Botulinum Formol-Toxoids by their Total-Antitoxin-Combining-Powers.

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Onderstepoort

Mason, Steyn, and Bisschop (1938) showed that cattle could be immunized against lamsiekte (botulism) by the injection of *Cl. botulinum* type C and type D formol-toxoids. Bennetts and Hall (1938) in Australia successfully protected sheep and cattle against botulism with formol-toxoids made in a similar way.

The determination of the immunizing power of such toxoids has presented some difficulties. The toxicity of the parent toxin is too unreliable an indication to be of much practical value. A reliable method is the injecting of toxoid into guinea pigs and the testing of their sera for antitoxin a few weeks later. By using a large number of animals and doing many titrations one can make this test as accurate as one pleases. It is, however, time-consuming and laborious.

The total-antitoxin-combining-power of a toxoid should give a good indication of its immunizing value, but this test does not seem to have been tried for botulinum formol-toxoids. In the following experiments the total-antitoxin-combining-powers of toxoids were compared with their ability to immunize guinea pigs. The immunity of the guinea pigs was estimated directly by their ability to withstand an injection of toxin, and indirectly by the neutralizing power of their sera.

Experiments

Total-antitoxin-combining-power of Botulinum Formol-toxoids

The tests were carried out as follows.—Falling dilutions of toxoid were mixed with an arbitrarily chosen unit of antitoxin and the mixtures allowed to stand at room temperature for two hours. To each mixture in the series half a test dose * of toxin was added and this mixture allowed to stand for a further hour at room temperature. It was then injected into mice which were observed for five days to see whether they lived, became paralyzed, or died. Table 1 gives an example of such a test.

* The test dose of toxin used was that amount of dry stable toxin that caused no symptoms when mixed with the unit of antitoxin and injected into mice (the Lo dose).

TABLE 1.

Total-antitoxin-combining-power of a Botulinum Formol-toxoid

Toxoid cc.	Antitoxin (units)	Toxin	Results of injecting mixture into mice							
1/10	1	1/2 test dose	†	†	†	†	†	†	†	†
1/20	1	„ „	†	†	†	†	†	†	†	†
1/40	1	„ „	†	†	†	†	†	†	†	†
1/80	1	„ „	†	†	†	P	P	P	L	L
1/160	1	„ „	L	L	L	L	L	L	L	L

† = died. P = paralyzed. L = lived.

The Lo point in this example is 1/160 cc. toxoid plus 1/2 test dose of toxin. As 1/2 test dose of toxin is neutralized by 1/2 unit of antitoxin, 1/160 cc. is also neutralized by 1/2 unit of antitoxin. Therefore 1·0 cc. of this toxoid is neutralized by, or is equivalent to, 80 units of antitoxin, and is said to have a total-combining-power (T.C.P.) of 80 units.

The total-combining-powers of four type C and three type D toxoids were determined in this way. Then four groups of six guinea pigs were immunized with the C toxoids, 1C, 2C, 3C and 4C respectively; and three groups of seven guinea pigs with the D toxoids, 1D, 2D and 3D respectively. Each guinea pig received 0·5 cc. of one of the toxoids subcutaneously. Two months later the antitoxin content of the serum of each guinea pig was measured by mixing falling dilutions of the sera with a test dose of toxin, allowing the mixtures to stand for an hour at room temperature, and injecting them into mice. The results are summarized in table 2.

TABLE 2.

Comparison of Total-antitoxin-combining-powers of Botulinum Formol-toxoids with their Ability to Elicit Antitoxin Formation in Guinea pigs

Toxoid	M.L.D. of parent toxin for mice, cc.	T.C.P. (units)*	Units of antitoxin per cc. in sera of guinea pigs immunized with Botulinum toxoids							
			1	2	3	4	5	6	7	G.M.
1C	0.001	10	8	1	8	32	8	2		5.7
2C	1.0	<5	<1	<1	<1	<1	<1	<1		<1
3C	0.0001	40	8	8	16	16	32	8		14.1
4C	0.005	40-80	8	32	16	16	32	8		17.8
1D	0.001	40	<1	<1	<1	<1	<1	<1	<1	<1
2D	0.001	40	<1	<1	<1	<1	<1	<1	<1	<1
3D	0.001	320	2	160	1	20	160	20	160	20.8

T.C.P. = total-combining-power. G.M. = geometric mean.

* See table 1 for definition of "unit of toxoid."

Clearly, C toxoids 1C, 3C, and 4C, that contained enough antigen in one cc. to combine with 10, 40, and 40-80 units of antitoxin respectively, were able to elicit the formation of considerable amounts of antitoxin in guinea pigs. This ability increased with increasing combining power. Toxoid 2C, which bound no antitoxin at the lowest dilution tested, did not provoke the formation of antitoxin in guinea pigs.

The D toxoids bound more antitoxin than the C toxoids. Toxoid 3D, one cc. of which bound 320 units of antitoxin, elicited the production of considerable antitoxin in guinea pigs, while 1D and 2D, which showed a combining power of 40 units, did not elicit any antitoxin production.

It should be noted that the toxicity of the parent toxins was a poor guide to the efficacy of the toxoids.

Another experiment was then carried out to compare the immunity produced in guinea pigs by toxoids of different combining powers. The resistance of the guinea pigs was tested directly by the injection of 2 to 3 lethal doses of toxin. The results are given in detail in table 3.

TABLE 3.

Comparison of Immunity produced in Guinea Pigs by Toxoids of different Combining Powers

Number of guinea pigs	Each guinea pig immunized with	Immunity of each guinea pig tested with 2-3 M.L.D. of	Results
8	one dose 0.5cc. toxoid 2C	C toxin	Died within 2 days.
8	* two doses 0.5cc. toxoid 2C	,, ,,	Died within 2 days.
8	one dose 0.5cc. toxoid 1C	,, ,,	Lived. Showed no symptoms.
4	nil (controls)	,, ,,	Died within 2 days.
8	one dose 0.5cc. toxoid 1D	D toxin	Died within 4 days.
8	two doses 0.5cc. toxoid 1D	,, ,,	Died within 4 days.
8	one dose 0.5cc. toxoid 3D	,, ,,	Lived. Showed no symptoms.
4	nil (controls)	,, ,,	Died within 4 days.

* The interval between the two doses of toxoid was 25 days and the test with toxin was made 14 days after this and 39 days after the first (and single) injection of toxoid.

It is clear that neither one nor two injections of toxoid 2C (T.C.P.<5) produced demonstrable immunity in guinea pigs, while a single injection of 1C (T.C.P. 10) immunized solidly against 2 to 3 lethal doses of toxin. The results with the D toxoids were similar; neither one nor two injections of 1D (T.C.P. 40) protected against the D toxin, while one injection of 3D (T.C.P. 320) protected the guinea pigs fully. No D toxoids of T.C.P. between 40 and 320 were examined, so one cannot say at what level of

binding power the D toxoids would become efficient. The results shown in table 3 confirm those shown in table 2.

Summary and Conclusions

The experiments show that the total-antitoxin-combining-powers of botulinum formol-toxoids can be determined, and that these give a good indication of their immunizing efficiency.

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Quintuplets in a Cow

A. TARR

Ixopo

Quintuple pregnancy is seldom observed in the cow. For this reason the following observations from Ixopo are interesting.

Subject. A grade Friesland cow, the property of Mr. R. J. Shuttleworth of Port Shepstone.

History. The above cow is about six years old and was bred from a Shorthorn cow mated to a Friesland bull. She is black and white in colour and reveals no Shorthorn characteristics. In 1939 she gave birth to one normal calf. In March last year she gave birth to twin calves both of which died. The owner stated that they were about four weeks premature.

On the 18th February 1941 she gave birth to two heifer calves and two bull calves. The first calf was born at about 9 a.m. and the last at 5 p.m. The last calf died about half an hour after birth. They were all black and white in colour and weighed approximately 60 - 70 lb. each. At birth they were as large as the ordinary Jersey calf.

In addition to these calves an oval body about the size of a cricket ball was found attached to the afterbirth. The owner estimated that it weighed between half and one lb. He described it as resembling the scrotum (containing testicles) of a young bull. This body was encased in distinct skin which was covered with red and white hairs. It is interesting to notice that the colours were red and white in contradistinction to the calves which were black and white. The above body was found with the afterbirth to which it was attached by means of a cord about nine inches long. This body possibly represented an acardiac amorphous foetus and thus the cow conceived quintuplets.

The cow was in good health and in fair condition. There was partial retention of the afterbirth which however soon cleared up on treatment.

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Report on a Case of Acetonaemia in a Cow at Onderstepoort

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Onderstepoort

Although acetonaemia is a fairly common disease of dairy cows in Europe and America I have been prompted to report this case in some detail because the disease is either uncommon in South Africa or is usually not recognised. It is mainly a disease of well-fed dairy animals which do not have much exercise. It is described as one of the group of metabolic diseases, is often confused with milk fever, and frequently unsuccessfully treated as milk fever.

The symptoms observed in the case to be described were classical and may serve as a useful guide to colleagues encountering the disease.

Anamnesis. — A Friesland cow in exceptionally good condition, commencing the third lactation, calved normally on 27:9:40. The first symptoms of illness were noticed during the days between 13:10:40 and 18:10:40, that is, approximately 14 days after calving.

The cow had been kept in a small paddock, about a quarter of an acre in size, since her first pregnancy. There was a small amount of grazing consisting mainly of garden weeds and grass. The only plant suspected of being toxic which could be found was *Erigeron linifolius.* *

The animal had been dried off about three months before calving, from 1:7:40. The ration during the dry period had consisted of lucerne and teff hay *ad lib.* A green feed of fresh lucerne and occasionally oats was given in the evening with a small dishful of a concentrate mixture consisting of: yellow crushed mealies 400 lb., wheaten bran 200 lb., peanut-cake 200 lb., bonemeal 60 lb. and salt 20 lb. After having had the evening feed in the stable the cow was taken back to the small paddock which adjoined the stable. Exercise was, therefore, restricted. During the last ten days before calving the concentrate mixture was stopped and a smaller amount of pure bran was fed.

Pure bran was continued for about a week after calving and thereafter the usual concentrate ration gradually introduced, but this was not eaten with the usual relish, and the full ration was never consumed. The hay ration and green feed were eaten well and in normal quantities. During the period 13:10:40 to 18:10:40 the appetite became capricious and concentrates were completely refused, whereas the weeds and green growth

* This plant was identified by the Botanical Section of the Department of Agriculture in Pretoria

in the small paddock were greedily consumed. The plant *Erigeron linifolius,* which had been disregarded before, was now found, by the owner, to have been cropped short over the whole paddock. The milk yield had meanwhile been decreasing steadily and the animal was noticed to have lost condition.

On 20:10:40 nervous symptoms suddenly appeared. These consisted of torticollis and circling towards the left side. The symptoms were somewhat reminiscent of cotyledon poisoning. Rumination, which had been normal, now stopped. The temperature was normal, ruminal movements decreased, and atony of the rumen was diagnosed by the owner. Faeces decreased in quantity and became hard.

Vegetable poisoning was suspected and a bottle of raw linseed oil and 2 oz. turpentine was administered. On the 21st, ruminal atony and constipation were considered to be the most prominent symptoms requiring relief, and during that evening ¼ grain physostigmine was injected subcutaneously at half-hourly intervals until defecation took place. The faeces were dry.

During the 22nd the appetite improved somewhat and there was general improvement. Concentrates were taken in small amounts, but at times the animal seemed to be somewhat unsteady on its legs.

There was no change in the condition of the animal until the 24th and during the forenoon the owner observed nothing which could make him suspect that improvement was not being maintained. When, however, the owner again saw the animal during the late afternoon marked nervous symptoms had developed. They were — widely dilated and staring eyes, a degree of coma and stupidity of expression, slight torticollis and circling to the right, paresis and motor inco-ordination. It was found difficult to drive the animal out through the paddock gate and into the stable, because of the nervous symptoms described. The pulse was slow but remarkably full and strong.

As the owner was going out for the evening he decided to give an intravenous injection of calcium gluconate and I was asked to do the injection. It was at this stage that I saw the animal for the first time and made a tentative diagnosis of acetonaemia. This was confirmed by analysis of the blood and urine on the following day. The clinical diagnosis was based on the symptoms and history described and on the presence of a smell of acetone or chloroform in the breath. The last symptom, provided it can be clearly and unmistakably recognised, is regarded as pathognomonic of the disease. Clinical observations should be confirmed by the urinary test described by Udall (1933).

The treatment immediately adopted was that which is most commonly recommended (Udall), viz. the intravenous administration of large quantities of glucose and the administration *per os* of a saline purgative and sodium bicarbonate. By 25:10:40 the general condition and appetite had

greatly improved but acetonaemia, acetonuria, and the odour of chloroform in the breath were still marked. The treatment described was repeated.

The clinical condition of the animal continued to improve gradually during succeeding days but this improvement was slow and the results of blood analyses shown in the table indicated that there was only slight improvement in the amount of acetone bodies present. After the 25th the administration of glucose was not repeated, but sodium bicarbonate was given in the feed.

Dr. J. W. Groenewald, of this institute, was consulted about the case and he brought an article by Carlström, Myrbäck and Larsson (1939) to our notice. These authors reported the successful treatment of acetonaemia with vitamin B_1. They used "Aneurine." They advanced the theory that acetonaemia and a number of other metabolic diseases are associated with and may be caused by a vitamin B_1 deficiency, and they recorded critical observations in support of their claims. This vitamin, according to these workers, is synthesized in the digestive tract of ruminants, and digestive disturbances may therefore cause acetonaemia as a secondary complication.

As a result of a recommendation made by Dr. Groenewald, two cc. of Betaxan, another vitamin B_1 preparation, was injected intravenously on 29:10:40. The rapid drop in the acetone bodies in the blood is indicated by the blood analyses made on 31:10:40. This improvement in the blood was accompanied by an even more marked clinical improvement and thereafter the cow made an uninterrupted recovery. No further analyses were undertaken.

Blood Analyses in Acetonaemia

Blood constituents.	Dates.		
	25:10:40.	28:10:40.	31:10:40.
Acetone and aceto-acetic acid in mgm. per 100 cc.	35.30	38.20	11.12
B-hydroxy-butyric acid (mgm. %)	21.00	10.15	16.92
Total acetone bodies	56.30	48.35	28.04
Phosphorus (mgm. %)	3.6	3.7	3.8
Calcium (mgm. %)	8.1	8.3	8.3

The normal figures for total acetone bodies are approximately 1-2 mgm. per cent.

Discussion

The history of this case was classical, viz. the appearance of a poor appetite affecting especially the inclination for concentrates, followed by the appearance of definite symptoms approximately two to three weeks after calving. The whole syndrome, including the temporary improvements and subsequent recurrence of symptoms, was typical of acetonaemia.

The possibility that the plant *Erigeron linifolius,* which is said to contain tannic acid and exercise a remarkably astringent effect when consumed, caused the primary digestive disturbance which was followed by the development of acetonaemia has been suggested. This theory would apparently be strengthened by the suggestions of Carlström, Myrbäch and Larsson; but opposed to this is a common observation that acetonaemia is associated with a capricious appetite, and the fact that the plant under consideration had apparently not been grazed by this cow, nor its mate in the same paddock, before, indicates that the abnormal appetite present after the first symptoms of the disease had developed may have been the primary condition. The ingestion of this plant would then have been merely incidental to the abnormal condition of the cow and not the primary cause of the condition.

The similarity of acetonaemia to milk fever is striking and is the commonest cause of an incorrect diagnosis in cases of acetonaemia. This similarity led the owner to suspect milk fever and prompted his decision to administer calcium gluconate. The calcium and phosphorus content of the blood was therefore determined and the values obtained are reproduced in the table. The phosphorus figures must be considered rather low [Groenewald (1935)] but if this observation is of any significance in this disease it is not yet understood.

Acknowledgements

Our thanks are due to Dr. H. Graf and Dr. J. G. Louw for undertaking the blood analyses and to Mr. J. H. R. Bisschop for obtaining and administering the Betaxan.

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Examination of the Trypanocidal Value of Anthiomaline

H. E. HORNBY
From the Tsetse Research Laboratory, Shinyanga, Tanganyika Territory

Since 1906, when Nicolle and Mesnil first proposed the use of antimony salts for trypanosomiasis, there has been steady development of antimony therapy in tropical medicine. Of the many preparations that have been synthesized in the course of this development, antimony pyrocatechin disulphonate of sodium — known as Antimosan and also as Fouadin — has proved of most value in the treatment of animal trypanosomiases. But this drug is likewise valuable in the treatment of human bilharziasis and lymphogranuloma inguinale. Hence, when looking through the literature of antimony therapy and noting that antimony thiomalate of lithium (Anthiomaline) is recommended for the treatment of bilharziasis and lymphogranuloma inguinale I wondered whether it would also act against animal trypanosomiases. Through the courtesy of the manufacturers, Messrs. May and Baker, Ltd., I obtained a quantity for free trial, and I tender to them my thanks.

In veterinary medicine one cannot ignore monetary cost. The drug most widely used against animal trypanosomiases is tartar emetic, because in spite of its manifold disadvantages it is very cheap indeed. Antimosan has the same trypanocidal action as tartar emetic, but it is less toxic, and so much less irritant that it can be given subcutaneously; not merely as a single injection, but as a series of injections without much regard being paid to whether the same site is used more than once. For any drug to be a satisfactory substitute for antimosan it must have the same or higher trypanocidal value, and be bland enough for subcutaneous injection.

The form in which I received Anthiomaline was a six per cent. slightly pinkish solution of lithium antimony thiomalate in 20 cc. ampules. This contains 10 mg. antimony per cubic centimetre. As one gm. of tartar emetic (the usual dose for an African native ox) contains 36·5 per cent. antimony, I worked on the basis that 35 cc. of Anthiomaline would correspond to 1 gm. of tartar emetic. I reckoned, therefore, since I knew of no veterinary literature on the drug, that seven cc. per cwt. live weight might be considered a normal dose for an ox and 10 cc. per cwt. for smaller species. As a test, a young goat was given twice this amount, 10cc., subcutaneously. No appreciable local or systemic reaction was detected.

A number of experiments were devised to determine the toxicity and the irritancy of the drug, and its efficacy in *T. vivax, T. congolense,* and *T. brucei* infections. Cattle, sheep, goats and a dog were used in these experiments and the drug was given subcutaneously and intramuscularly. The details are shown in the table.

No. of experiment	Species of animal	Infected with *Trypanosoma*	Treatment		Reaction.		Efficacy of treatment
			Dose of Anthiomaline, 6%	Method of administration	Local	Systemic	
1.	bovine	*vivax* (old standing)	30 cc.	i.m.	very slight	nil	Smears negative for 20 days.
2.	bovine	*congolense* (previous infection with other tryps)	40 cc.	i.m.	slight	nil	Smears negative for 27 days.
3.	bovine	*congolense*	40 cc.	s.c.	nil	nil	Smears positive on 10th day.
	bovine	*congolense*	40 cc.	s.c.	nil	nil	,, ,, ,, ,,
	bovine	*congolense* (all had previous treatments and relapses)	40 cc.	s.c.	nil	nil	,, ,, ,, ,,
4.	sheep	*congolense*	5 cc. on 3 successive days	s.c.	nil	nil	Smears positive on 23rd day.
	sheep	*congolense*		s.c.	nil	nil	,, ,, ,, ,,
	sheep	*congolense*		s.c.	nil	nil	Smears positive on 28th day.
5.	bovine	*brucei*	40 cc.	s.c.	nil	nil	Smear positive on 21st day.
6.	bovine	*congolense*	35-40 cc. on 1st, 12th & 13th day	s.c.	severe	—	Examined for 2 months and no parasites found.
	bovine	*congolense*		s.c.	nil	—	Smear positive on 18th day.
	bovine	*congolense*	35-40 cc. on 1st and 2nd day	s.c.	severe (swellings lasted a month)	—	Smear positive on 18th day.
7.	dog	mixed infection	5 cc.	s.c.	severe	severe	Other treatment interfered with interpretation of results.
8.	goat	*congolense*	10 cc. (5 cc. in front of each shoulder)	s.c.	nil	nil	Smear positive on 13th day.
9.	bovine	*vivax*	40 cc.	s.c.	very severe	—	Intention had been to give weekly treatments, but this was abandoned owing to severity of reactions.
	bovine	*congolense*	40 cc.	s.c.	nil	—	
	bovine	mixed	40 cc.	s.c.	very severe	—	
	bovine	mixed	40 cc.	s.c.	very severe (evolution of swellings took some weeks)	—	

i.m. = intramuscular. s.c. = subcutaneous.

Discussion

In assessing the trypanocidal action of a drug, one must take into consideration the case histories of the animals treated. The cattle on which anthiomaline was tested were chronic cases that had relapsed after treatment with other drugs. Had cure of these animals been effected by small series of injections of anthiomaline, then this drug would have exhibited trypanocidal action markedly superior to that of tartar emetic or antimosan. As it was, the effects on the trypanosomes were what I should have expected had I used either of these other drugs, and therefore I formed the opinion that the trypanocidal action of anthiomaline was probably the same as that of the other two antimony preparations. In irritancy, on the other hand, it lies between these two, though nearer to antimosan. Single injections can be given subcutaneously to goats, sheep and cattle without much danger of causing local swelling, but such injections cannot be repeated without grave risk of causing severe reactions. For this reason, anthiomaline cannot be recommended as a substitute for antimosan in the treatment of animal trypanosomiases.

Conclusion

Anthiomaline has approximately the same trypanocidal action as tartar emetic or antimosan. In irritancy it lies between the two, though nearer to antimosan. Its toxicity was not ascertained, but there was no evidence that it was more toxic than the other two drugs.

BOOK REVIEW

The Principles and Practice of Feeding Farm Animals. E. T. Halnan and F. H. Garner.

This book * is an exceedingly useful work of reference and well merits its place in the bookshelf.

The first four chapters are devoted to theoretical considerations and comprize 84 pages of particularly valuable information. The latest advances in nutritional research and development have received adequate consideration, and in this respect the inclusion of the biological values and of the fatty acid and vitamin content of feeds is of special interest. The illustrations in this section are well done and well reproduced. At the end of the book are nine pages of tables giving: the composition and nutritive value of feeding stuffs; the mineral content of feeding stuffs; a ration ready-reckoner giving the nutrient amounts for various quantities of different feeds; ready-reckoners for the determination of prices; and the weights of food by measure.

The second half of the book comprises 246 pages and deals with a variety of practical feeding considerations. Chapter V deals with the ordinary feeds

of the British Isles; some of these are not usually found in South Africa. In Chapter VI, the uses of grassland and its products are discussed, but this refers especially to intensive artificial pasturage and only applies to a limited extent in South Africa. The two following chapters are very useful and deal with the preparation of feeding stuffs for farm animals, and the principles and practice of feeding farm animals. Thereafter chapters are devoted to the feeding of dairy cows; the fattening of cattle; sheep feeding; pig feeding; the feeding of horses; and poultry feeding. The last chapter deals with the feeding of farm animals during war-time and is thus of great importance.

These chapters are all excellent. They are, however, written for the European and, primarily, the British farmer. The information given, therefore, has often no direct applicability to South African husbandry. Some modifications are necessary to make this book as useful to our farmers as to those for whom it is, primarily, intended.

J.W.G.

* "The Principles and Practice of Feeding Farm Animals", by E. T. Halnan, M.A., School of Agriculture, Cambridge, and F. H. Garner, M.A., Cambridge University lecturer in agriculture. pp. x + 359, eight diagrams and 23 illustrations. London: Longmans, Green & Co. 1940. Price 15/-.

THE ASSOCIATION

Secretary's Report for the Year Ending 31st March, 1941.

Membership.

The membership on 31st March, 1941, was 179, an increase of three over the previous year. Seven new members were elected at the last General Meeting. The deaths of four members (J. H. L. Cloete, A. E. Lund, J. L. Mainprize and T. H. Sandrock) are recorded with sincere regret.

Council.

The policy of curtailing meetings as far as possible during the war period was continued during the year under review and, accordingly, only two meetings were held. The vacancy on Council which was created by the death of Dr. Cloete was filled by the election of Dr. P. S. Snyman.

General Meeting.

The 35th General Meeting was held on 13th November, 1940. Considering that this too was a war period meeting, the attendance must be regarded as very satisfactory and it indicates that members are not allowing their interest in the Association to be damped by the prevailing unsettled conditions.

Abattoir Control.

A question which has been receiving serious attention from Council as well as from the Natal branch and from individual members, is the insignificant part which is still being played by members of the veterinary profession in abattoir control and meat inspection in South Africa. This matter recently received serious consideration from a Departmental Committee on which the veterinary profession was represented by Dr. Fourie. The report of this Committee is eagerly awaited, as it is felt that this will go a long way towards remedying the present unsatisfactory position. Further action by the Association is suspended pending the publication of this report.

Constitution.

A handbook containing the Constitution, Act 16 of 1933, and Government Notice 925 of 1934 published under this Act, was printed and issued to members during the year.

Finance.

A new and simplified system of bookkeeping was introduced on the recommendation of our auditor. Consequently the Group Endowment Insurance Fund is no longer run as a separate banking account, but as can be seen from the balance sheet, a profit of £20.14.3. was derived from this fund during the year.

The balance sheet also shows a credit balance on 31:3:41 of £1,831.2.7. as against £1,458.10.11. the previous year. The increase of £372.11.8. is due mainly to interest on Union Loan Certificates which matured during the year and to £75 profit transferred from the book fund.

The benevolent fund has increased from £459.2.9. to £520.5.4. During the year Council decided to pay a pension of £2 per month to the widow of a late member.

In view of the all round drop in the rates of interest paid on our investments, our revenue from this source for the next year is bound to show a marked decline.

The report of the Finance Committee with the relevant statements is appended.

S. W. J. v. RENSBURG,
Hon. Sec.-Treas. S.A.V.M.A.

Report of Finance Committee 1940-41

Members of Committee: H. H. Curson, B. S. Parkin, A. D. Thomas and J. J. van der Wath.

The following statements are submitted as reflecting the financial position of the Association at the close of the financial year on 31st March, 1941.

A. CASH STATEMENT OF INCOME AND EXPENDITURE 1940 - 41.

RECEIPTS.				EXPENDITURE.			
Credit Balance on 1/4/40	£129	18	6	Printing of Journal	£213	7	0
Membership subscriptions....	334	13	6	Journal postage and stationery	8	13	6
Book Fund	568	11	3	Book Fund	494	18	0
Insurance Premiums	928	17	3	Insurance premiums	905	18	11
Journal subscriptions	9	5	6	Printing of Constitution	45	15	0
Advertisements	49	19	6	Union Loan Certificates	722	18	6
Sale of reprints	6	0	0	United Building Society	75	0	0
Union Loan Certificates matured	684	16	0	Government securities	398	0	0
Interest	218	4	10	Student advances	26	3	6
United Building Society	200	0	0	Benevolent Fund	59	2	7
Premiums overpaid	8	10	2	Book Fund Prize	10	0	0
				Refund Insurance Premium	8	10	2
				Wreaths	5	13	5
				Typing and clerical assistance	43	12	7
				Auditing	5	5	0
				Bank charges	8	3	3
				Stationery	5	16	6
				Telephone, telegrams and postage	10	14	9
				Sundry expenses	3	0	6
				Credit Balance on 31/3/41....	88	3	4
	£3,138	16	6		£3,138	16	6

B. Balance Sheet: 1940 - 41.

ASSETS.				LIABILITIES.			
Union Loan Certificates	£788	10	6	Subscriptions paid in advance	£4	12	6
United Building Society	375	0	0	Natal Branch	9	12	0
Government Securities	398	0	0	Group Endowment Fund	20	14	3
Arrear subscriptions	204	4	0	Benevolent Fund	14	0	0
Student Advance	26	3	6	31/3/41 Credit Balance	1,831	2	7
Cash in Bank 31/3/41	47	0	9				
Cash in hand 31/3/41	41	2	7				
	£1,880	1	4		£1,880	1	4

C. Benevolent Fund.

Credit Balance on 1/4/40	£459	2	9	To Widow "D"	£12	0	0
From General Account	36	0	0	Credit Balance on 31/3/41	520	5	4
From Insurance Account	19	19	1				
Interest	17	3	6				
	£532	5	4		£532	5	4

S. W. J. van Rensburg,
HON. SEC.-TREAS. S.A.V.M.A.

Jl. S.A.V.M.A.
XII(4) : 97 - 102
1941

Pregnancy Disease, or "Domsiekte," in Ewes

R. CLARK and J. W. GROENEWALD
Onderstepoort

Introduction

Pregnancy disease in ewes has been described from practically all over the world and it has now been definitely established that the disease known in South Africa as 'domsiekte' is the same condition. It usually occurs in pregnant ewes before lambing and especially in those carrying twins. Older ewes are more susceptible. The disease is characterised by nervous symptoms which may appear as a hypersensitivity, shown by a wild stare in the eyes, frequent micturition, and spasms of the body. Sometimes the sheep turns round in circles, pivoting on the hind feet, and it is often blind in one or both eyes. Another form, which frequently follows that just described, is one of hyposensitivity. The animal becomes dull, listless, and stupid, thus giving rise to the appropriate Afrikaans name 'domsiekte' meaning 'stupid sickness.' The final stage is often one of coma, the animal lying with the head to the flank, like a cow with milk fever, or flat on the belly with the hind legs straddled out. The corneal reflex is often completely absent.

The post mortem in a typical case shows a severe fatty infiltration of the liver and fatty changes in the renal cortex, although in peracute cases there may be very little change present.

Aetiology

Numerous and varied causes are suggested in the literature, such as over-fatness, lack of exercise, and mineral deficiency. It has been shown at Onderstepoort, however, that the disease can be produced by suddenly reducing the ration of ewes when they are about four months pregnant. Full reports of these experiments will shortly appear in the Onderstepoort Journal, and it is not intended to give details of them here.

The mechanism of the production of the disease under these conditions would presumably be as follows. In the absence of sufficient carbohydrate, the glycogen reserves of the body become seriously depleted, as evinced by the hypoglycæmia. Consequently the oxidation of fat is incomplete, a fatty infiltration of the liver occurs and ketone bodies appear in the blood.

The theory that the disease is due to a mineral deficiency can be discarded. Chemical analyses of the blood in 'domsiekte' show normal figures for calcium and phosphorus.

Pregnancy, especially from the fourth month, when the most rapid

growth of the foetus occurs, would greatly increase the carbohydrate drain which would, of course, be even greater in twin pregnancies. This is probably why the disease is usually confined to pregnant sheep towards the end of gestation, but it has been shown that non-pregnant ewes, and even wethers, will show the condition when starved. The period of starvation required is, however, very much longer, as pregnant sheep show symptoms in from three to ten days after the cut in the ration, whereas non-pregnant sheep take from 24 to 40 days. There is, however, another very important peculiarity of pregnant sheep which must have a very significant bearing on the ætiology of 'domsiekte.' It has been noted that if a heavily pregnant ewe is starved for a short period, even 48 hours, she will often refuse food when it is again offered. The sheep then voluntarily starves itself into the disease. This is not the case in non-pregnant ewes or wethers. This is very important in that even a very temporary upset in the feeding of pregnant ewes, at the critical period of gestation, may lead to serious results, whereas it would not affect the non-pregnant stock.

It must be borne in mind that the disease is so closely associated with pregnancy, as evinced by the names 'pregnancy disease,' 'twin lambing disease,' etc., that one is loth to diagnose pregnancy disease in a wether or non-pregnant ewe, although the typical signs may be present. That such a condition may arise was well shown in a recent experiment at Onderstepoort, where a heavy mortality occurred among the control, i.e. well-fed group. These deaths took place after a prolonged period of rain and all the sheep showed a fatty infiltration of the liver on post mortem. No other cause of death could be demonstrated. The sheep were fed in individual pens, but in the open, and had refused practically all their ration during the rainy period. This disinclination of sheep to feed during wet weather will be discussed later.

If the hypothesis that 'domsiekte' is caused by an interruption in the diet of the animal is accepted, this must be correlated with the occurrence of the disease under farming conditions. A starvation may occur in many ways, as follows.

i. Lack of feed. The simplest cause would be a lack of feed at the critical period of gestation. Apparently a gradual diminution of the available food, as when a flock grazes out a paddock, would not be so serious as a sudden change. Sheep appear to become adapted to a lower intake and no sudden demand is made on large fat depots. The available feed might suddenly give out owing to untimely frost or drought. Should this occur towards the end of pregnancy, 'domsiekte' might be expected. It has often been stated that 'domsiekte' appears after a 'break' in the grazing.

ii. Inclement Weather. The fact that sheep do not feed readily in rainy or inclement weather is well known. Prolonged periods of rain or bad weather at the critical time might, therefore, set up the condition in

the presence of abundant food. As pointed out above, even a very temporary interruption in the feeding of heavily pregnant ewes may lead to continued starvation and so set up the disease. This may be why numerous outbreaks have been reported as occurring after rainy spells. Overseas writers frequently mention that outbreaks have followed snow storms. This may be because the sheep would not feed during the storm or because the feed became unavailable.

iii. Any Sudden Change in Environment. Sheep are well known to be very sensitive to sudden changes and may refuse to feed in strange surroundings or when they are confronted with an unaccustomed diet. In Holland the disease is known as 'ophaalziekte' and is said to occur when the pregnant ewes are brought in to be given extra feed. This observation has led to the theory that too high a protein diet causes the disease, but it may well be that the sheep refuse to feed in the new surroundings so that their intake actually drops, despite a more abundant food supply.

iv. Disturbances in the Function of the Digestive Tract. During the winter of 1941 the authors were fortunate in being able to observe an outbreak which occurred in the Bredasdorp district of the Cape and which showed many peculiar aspects. The cases occurred on a land of young lucerne and were all peracute, many sheep being in a state of coma within a few hours of having appeared normal. At autopsy, in all these cases, the rumen was found to contain a fairly large amount of rather watery green ingesta, which was frothy and smelled putrid. The intestinal tract distal to the rumen was entirely empty, except for a few hard, mucus-covered balls in the cæcum. Fatty infiltration of the liver, which is usually looked upon as the most striking and constant post-mortem finding in 'domsiekte,' was not marked in any of these sheep. In the very peracute cases the livers were normal.

The cause of the 'domsiekte' was assumed to be a physiological starvation following on an atony of the rumen. The cause of this atony could not be established. Prussic acid was not found on analysis of the ruminal contents. It is possible that the lack of roughage in the exceedingly young lucerne shoots may have suppressed ruminal movement. It is also quite conceivable that the pressure of the foetus, especially in older ewes where the uterine attachments may be slack, might predispose to ruminal atony. This might also be a factor explaining the frequency of the condition in ewes with twin lambs, where the pressure would naturally be much greater. In any case it is quite understandable that any impairment of the function of the digestive tract, leading to the cessation of absorption, will have the same effect as an actual starvation. Any digestive disturbance in late pregnancy may, therefore, lead to 'domsiekte.'

v. Poisonous Plants. Certain plants such as *Othonna pallens* (sprinkaanbos), *Lippia* and *Lantana* spp., and *Tribulis terrestris* (in the

wilted state) cause paresis in the fore-stomachs and intestines of sheep.* Their presence may, therefore, set up the chain of events leading to 'domsiekte.' Prussic acid in small doses also has a paralysing effect on the alimentary tract.

Prevention

If it is accepted that 'domsiekte' is primarily caused by dietetic factors, its prevention will be mainly a matter of grazing and flock management. It must be remembered that, under ordinary circumstances, the disease is confined to older pregnant ewes in the last two months of gestation. The number of sheep that should be given preferential treatment and the period over which this will have to be done are, therefore, limited. With careful planning beforehand, suitable arrangements should be quite practicable. The basis of prevention should be to ensure that there is no loss of body weight in the 'susceptible' ewes over the critical period. Suitable camps should, therefore, be reserved for them and any sudden change in environment or feed should be avoided.

In order to guard against a spell of bad weather at the dangerous time, the selected flock could previously be made accustomed to feeding under cover. Should the need arise they could then be brought in and fed without any setback. This would apply especially to stud ewes.

Factors tending to cause atony or impaction of the rumen must be avoided. These would include — too succulent grazing, insufficient roughage, excessively dry grazing, and lack of water. Where the presence of prussic acid in the grazing is suspected, licks containing sulphur may be given.

The feeding of molasses, either with a lick or in the drinking water, is widely advocated as a prophylactic. It has been shown at Onderstepoort that the daily dosing with molasses prevents the development of the disease. Molasses would act as a valuable carbohydrate supplement to the diet as well as tending to combat intestinal stasis.

Treatment

No specific treatment can be laid down, but the following method appears rational and has given good results in a limited number of cases.

i. Glucose. As a severe hypoglycæmia is one of the most outstanding symptoms, at least in the early stages, the administration of glucose is clearly indicated. The fact that numerous authors report no beneficial results with glucose need not deter us from its use, but glucose alone will not effect a cure. It is best given intravenously as a 10 to 20 per cent. solution, 10 gm. being given at a time to the average-sized sheep. This can be repeated at intervals of a few hours if necessary.

* This information was kindly communicated by Dr. D. G. Steyn, Section of Toxicology, Onderstepoort.

ii. Vitamin B1 (Thiamin). As 'domsiekte' appears to be primarily a disturbance of the carbohydrate metabolism, the role of Vitamin B1 must be considered. It is generally stated that ruminants do not require this vitamin in the diet as it is synthesized in the rumen. This is doubtless the case under normal conditions, but where there is a stasis of the ruminal contents the vitamin may well not be absorbed. A further indication for the use of vitamin B1 is its successful employment in the treatment of acetonæmia of cows, a disease that appears to resemble 'domsiekte' in many respects. The authors at one time suspected that the nervous symptoms of 'domsiekte' might be due to a simple B1 deficiency. Injection of this vitamin into a sheep with coma, however, had no effect. Nevertheless, it is felt that the indications for its use are so strong that it has been given at the same time as the glucose, three thousand international units being injected intramuscularly daily.

iii. Stimulation of the rumen. As ruminal atony appears to occur in practically every case of 'domsiekte,' no true recovery can be expected unless this condition is relieved. Lentin (1 mg. subcut.) has been successfully used for this purpose, but any reliable ruminal tonic could be employed.

General Principles of Treatment

If the sheep can be induced to feed again, a cure is practically certain. This can only be done by alleviating the nervous symptoms and restoring the ruminal movements. Glucose and vitamin B1 appear to help the nervous condition, but it is often difficult to get the animal to resume feeding. Under these circumstances a relapse will occur unless the animal is force fed. Contrary to expectations it has been found that a highly proteinaceous food is the best for this purpose. Meat meal can easily be dosed in small quantities and has given good results. Molasses can also be dosed safely, even while the animal is in coma.

A complication that has been encountered is that at an early stage of the disease the foetus dies, but is frequently not immediately expelled, probably due to weakness of the ewe. The sheep may recover from the symptoms of 'domsiekte,' but remain listless and ill. She may abort later or die, revealing the presence of a disintegrating foetus at autopsy. Where early abortion takes place, the sheep often recovers spontaneously but may contract metritis later. The frequency of abortion in the early stages of 'domsiekte' should be borne in mind when investigating an outbreak of abortion in ewes.

Summary

(1) Since typical 'domsiekte' can be produced by a sudden cut in the ration of pregnant ewes, it is assumed that a deficient calorific intake, or absorption, is the primary cause of the disease.

(2) This may occur through a lack of sufficient intake or disturbances in the alimentary system.

(3) Heavily pregnant sheep are peculiar in that a temporary cessation of feeding often leads to a prolonged voluntary starvation, thus setting up the disease.

(4) Prevention is a matter of flock and pasture management, which should be designed to prevent any loss of weight during the last two months of pregnancy.

(5) Any sudden change in the diet or management of heavily pregnant ewes should be avoided, as this may lead to an interruption in their feeding.

(5) Treatment with vitamin B_1, glucose, and ruminal tonics is suggested.

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The Immunisation of Calves Against Heartwater

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Introduction

It is well known that young animals possess a high degree of resistance to certain protozoal infections. For instance the course of diseases such as anaplasmosis, babesiosis and piroplasmosis in young animals is usually so mild that objective symptoms can be detected only after systematic clinical examination. This resistance bears no relationship to the susceptibility or immunity of the dams and must not be confused with the passive immunity transmitted through the colostral milk to the new-born progeny of dams immune to certain bacterial, virus, and toxic infections.

Towards the end of 1936 it was shown experimentally that lambs and calves were resistant to heartwater (*Rickettsia ruminantium* infection). The application of this finding to the development of a method of artificial immunization forms the basis of this preliminary report.

Laboratory Experiments

For this work the "Mara" strain of heartwater was maintained by serial passage in sheep, subinoculation being carried out at what was judged to be the height of the febrile reaction. No difficulty was experienced in maintaining the strains, but it is essential to include at least two sheep in each passage because it has been found that where a 10 cc. infecting dose of blood is given intravenously, about one to two per cent. of sheep fail to react. This failure to react is probably due to the low infectivity of the blood since in every case the sheep reacted normally when re-injected.

For the first experiment five merino lambs aged from one to seven days, the progeny of susceptible ewes, were selected. The infecting dose decided upon was three cc. of blood intravenously and five cc. subcutaneously. It will be seen from Table 1 that three of the lambs showed no deviation from normal health while two passed through mild febrile reactions from which they made uninterrupted recoveries. At the time it was assumed that the infectivity of the blood used was particularly low or that the dose used was too small. However, the experiment was carried to its logical conclusion by subjecting the lambs to an immunity test. The interval between the infecting injection and the immunity test in one case was 36 days, in the remaining four cases 54 days. None of the lambs reacted, whereas a control aged 284 days reacted and died.

It is now realized that this experiment was hardly conclusive because the oldest lamb in the experiment was only 61 days old at the time of the immunity test and no data were available as to the susceptibility of lambs 284 days old, the age of the control used.

However, the result prompted a similar experiment in calves.

TABLE 1.

The Infection of Lambs with Heartwater

The artificial infection of merino lambs with heartwater				The immunity test		
Laboratory number of lamb	Age in days	Dose of blood	Result	Interval in days	Dose of blood	Result
53749	7	3 cc. i.v. 5 cc. s.c.	Very mild febrile reaction	54	10 cc. i.v.	Immune
53480	7	3 cc. i.v. 5 cc. s.c.	No febrile reaction	54	10 cc. i.v.	Immune
53482	7	3 cc. i.v. 5 cc. s.c.	Very mild febrile reaction	54	10 cc. i.v.	Immune
53483	7	3 cc. i.v. 5 cc. s.c.	No febrile reaction	54	10 cc. i.v.	Immune
47314	1	3 cc. i.v. 5 cc. s.c.	No febrile reaction	36	10 cc. i.v.	Immune
45329	284	10 cc. i.v.	Reacted and died from heartwater	—	—	—

i.v. = intravenously, s.c. = subcutaneously.

Details of the observations on the 37 calves included in this experiment are shown in Table 2. The calves were the progeny of nondescript stock running on a farm where heartwater had been controlled by systematic dipping over a number of years, but no definite opinion can be expressed as to the susceptibility or immunity of the cows. The ages of the calves varied from 7 to 97 days. It is certain that the younger animals had not been exposed to infection by tick infestation at the time of their selection. It is also believed that the older animals were fully susceptible, and this belief is supported by the fact that the oldest calf reacted and died of heartwater as a result of the artificial infection. The immunizing or infecting dose consisted of 10 or 25 cc. of sheep blood tapped from sheep at the height of a heartwater reaction. The infectivity of the blood was controlled in sheep; of the 15 control sheep all reacted, 13 died and 2 recovered.

Consideration of Table 2 shows that heartwater reactions were observed in only 10 out of the 34 experimental calves; of these 8 died and 2 recovered. The youngest calf which died was 19 days old at the time of the injection.

Of 19 calves younger than 4 weeks only 2 died (10·5%), whereas of 15 calves between the ages of 4 and 14 weeks 6 died (40%). In addition it must be pointed out that of the 26 survivors 24 showed no febrile reaction whatever, one aged 15 days showed a mild reaction, and one aged 73 days a reaction which must be considered more severe, but was nevertheless unaccompanied by any clinical symptoms other than those attributable to hyperthermia.

Some of the calves were given an immunity test approximately four weeks after the first injection. None reacted to the 20 or 25 cc. of infective sheep blood given intravenously, but it was realized that the animals should be kept for at least a year before applying an immunity test if the results were to have any significance. The calves were therefore turned out to graze with the original herd. During this period they may have been exposed to natural infection, but this is doubtful because no case of heartwater was encountered in the entire herd. A number of animals were used for other purposes, but eventually 10 were returned to the laboratory for an immunity test. In addition three animals of approximately the same age were selected to serve as controls for the test; these animals had run with the herd from birth and had received no previous treatment whatever. Of the immunized animals none reacted; of the three controls one reacted and died, one reacted severely but recovered, and one failed to react.

From these two experiments it is justifiable to conclude that lambs up to the age of seven days and calves up to the age of about four weeks possess a high degree of resistance to heartwater. This resistance gradually decreases until full susceptibility is reached at an unknown, but probably variable, age. If infected during the resistant period a durable immunity is produced no matter whether a clinical reaction is produced or not.

Unfortunately the number of animals included in the experiments was far too small to permit of any statistical survey of the risks involved and the degree of immunity produced. Sheep could have been made available for further tests, but it is appreciated that results obtained with sheep need not be applicable to cattle. For these reasons an endeavour was made to continue the work in the field, where a larger number of calves might be obtained.

Field Experiments.

A. Mr. B. van der Vijver, Government Veterinary Officer, Pretoria, made arrangements with a dairy farmer in the Pretoria district to use his grade Friesland calves for experimental purposes. This dairyman had found that it was not economical to rear the progeny of his cows to maturity under the conditions prevailing in his dairy close to a large town. Consequently it was his practice to send all his calves to a farm in the Northern Transvaal, but there his mortality from heartwater was practically 100 per cent. Altogether five calves less than four weeks of age and three calves approximately

TABLE 2.

The Artificial Infection of Young Calves with Virulent Heartwater Blood.

The artificial infection of the nondescript calves with heartwater				Immunity test					
Laboratory number of calf	Age in days at time of infection	Dose of blood intrav. in cc.	Result	Interval in days between artificial infection and exposure to natural infection	Period in days at grass	Remarks	Interval in days between artificial infection and immunity test	Dose of blood intrav. in cc.	Remarks
7409	7	10	No reaction observed	—	—	—	38	25	Immune
7419	7	25	„ „ „	300	540	No reaction observed	—	—	—
7734	11	10	„ „ „	28	820	„ „ „	850	25	Immune
7677	12	10	„ „ „	—	—	—	26	20	Immune
7575	13	10	„ „ „	28	820	No reaction observed	1270	25	Immune
7733	14	10	„ „ „	—	—	—	26	20	Immune
7673	15	10	„ „ „	—	—	—	26	20	Immune
7576	15	10	„ „ „	28	820	No reaction observed	1270	25	Immune
7741	15	10	Mild febrile heartwater reaction	21	480	„ „ „	—	—	—
7417	16	25	No reaction observed	250	540	„ „ „	—	—	—
7418	16	25	„ „ „	300	690	„ „ „	1476	25	Immune
7569	19	10	Died from heartwater 22 days later	—	—	—	—	—	—
7570	19	10	No reaction observed	28	270	No reaction observed	—	—	—
7568	20	10	„ „ „	28	365	„ „ „	—	—	—
7577	22	10	„ „ „	28	730	„ „ „	1270	25	Immune
7566	26	10	Died from heartwater 25 days later	—	—	—	—	—	—
7567	26	10	No reaction observed	28	850	No reaction observed	—	—	—
7565	27	10	„ „ „	28	630	„ „ „	—	—	—
7564	27	10	„ „ „	28	630	„ „ „	—	—	—
7563	33	10	„ „ „	28	1020	„ „ „	—	—	—
7415	42	25	Died from heartwater 21 days later	—	—	—	—	—	—

TABLE 2 — (continued)

The Artificial Infection of Young Calves with Virulent Heartwater Blood

The artificial infection of the nondescript calves with heartwater				Immunity test					
Laboratory number of calf	Age in days at time of infection	Dose of blood intrav. in cc.	Result	Interval in days between artificial infection and exposure to natural infection	Period in days at grass	Remarks	Interval in days between artificial infection and immunity test	Dose of blood intrav. in cc.	Remarks
7553	43	10	Died from heartwater 17 days later	—	—	—	—	—	—
7559	44	10	No reaction observed	28	60	No reaction observed	—	—	—
7416	45	25	,, ,, ,,	—	—	—	—	—	—
7557	45	10	,, ,, ,,	28	900	No reaction observed	1270	25 cc.	Immune
7549	49	10	,, ,, ,,	28	900	,, ,, ,,	1270	25 cc.	Immune
7546	50	10	Died from heartwater 22 days later	—	—	—	—	—	—
7547	50	10	Died from heartwater 20 days later	—	—	—	—	—	—
7545	50	10	No reaction observed	28	1350	No reaction observed	1270	25	Immune
7550	53	10	,, ,, ,,	28	365	,, ,, ,,	—	—	—
7384	60	10	,, ,, ,,	38	1445	,, ,, ,,	1st test after 38	25	Immune
							2nd ,, ,, 1500	25	Immune
7392	65	25	Died from heartwater 19 days later	—	—	—	—	—	—
7380	73	10	Reacted and recovered from heartwater	38	1350	No reaction observed	1st test after 38	25	Immune
							2nd ,, ,, 1500	25	Immune
7381	97	25	Died from heartwater 15 days later	—	—	—	—	—	—
8538*	—	—	—	—	585	No reaction observed	—	25	Immune
8559*	—	—	—	—	566	No reaction observed	—	25	Died from heartwater 21 days later
8594*	—	—	—	—	574	No reaction observed	—	25	Reacted to heartwater & recovered

* Control animals.

six weeks old were given 10 cc. of infective sheep blood intravenously. All three of the older calves reacted very severely and two died; the younger calves showed no reaction whatever. The six survivors were sent to the bushveld farm for exposure to natural infection as an immunity test. They were alive two years later.

B. With this additional information available, arrangements were concluded with a ranching company operating two estates in the Northern Transvaal to use their stock for experimental purposes. At this stage it is no less than a duty to record our appreciation of and our thanks for the work that has been carried out. Without this ready co-operation and willingness to carry out all suggestions and instructions immediately, it would not have been possible to have collected the present valuable data.

Again infective sheep blood was used for the immunizing injection so as to obviate the possibility of transmitting cattle diseases such as anaplasmosis or piroplasmosis. With the railway facilities available it was possible to deliver the blood within twelve hours of bleeding and it was stipulated that all injections had to be completed within the next six hours. To control the infectivity of the blood a sample was retained in the laboratory for injection into sheep twenty-four hours after bleeding, i.e. six hours after the last injection had been carried out on the ranch. All these control sheep reacted, so that it is reasonable to conclude that the calves which received a ten cc. dose of blood at an age of not more than four weeks were given an infective dose. In spite of this and previous observations that *Rickettsia ruminantium* may remain viable for periods up to twenty-four hours outside the animal body, a note of warning is sounded that under ordinary field conditions the time of survival may be considerably decreased. This warning is sounded because the infective titre of the blood used was markedly increased by giving the donor an injection of neosalvarsan (0·45 gm.) or the antimony arsenic compound Std 386 B* (1·0 gm.) 24 to 48 hours before bleeding, in accordance with observations made by Neitz (1940) that arsenic has a marked stimulating effect upon multiplication of the *Rickettsiæ*.

Immunization of grade Aberdeen Angus calves was commenced in June, 1939. During the year 187 calves on the two estates were treated: six (3·2 per cent.) died from heartwater as a direct result of the injection. The surviving 181 calves were exposed to natural infection on the farm, the experience being that the greatest mortality occurred when the animals had reached an age of from fifteen months to two years. In animals of this age group, a mortality of from 20 – 60 per cent., depending upon climatic conditions and the season, could be expected. At the end of two years fourteen (7·7 per cent.) had died from heartwater. In this group of animals

* Supplied by Bayer Products Ltd., London.

therefore, over a period of two years, the mortality as a result of immunization and natural infection was 20 out of 187 (10·7 per cent.).

The reduction in mortality was considered to be sufficiently encouraging to justify persevering with the method. In addition data were being accumulated on the value of uleron and the water soluble uleron sodium in the treatment of animals reacting to the immunizing injection and to subsequent natural infection [Neitz (1939)]. Moreover, the introduction of a system whereby blood smears were obtained from every experimental animal showing any deviation from normal health, and jugular vein intima preparations together with brain specimens from every animal that died, threw considerable light on the incidence of other infections such as anaplasmosis and piroplasmosis.

In 1940 a total of 590 calves were treated. Of these 22 (3·7 per cent.) died from heartwater as a result of the injection. The surviving 568 calves were exposed to natural infection for a period of about fifteen months; the mortality from heartwater was 41 (7·2 per cent.). The total loss in this group up to the middle of 1941 was 63 calves (10·7 per cent.), exactly the same figure as in the previous year.

During the first five months of 1941, 549 calves were immunized. As the result of the injection five (0·9 per cent.) died. The remaining 545 calves have been exposed to natural infection for a period of two to five months; nineteen (3·5 per cent.) have died, but it must be expected that this figure will be increased somewhat during the coming season when the animals reach the critical age of approximately eighteen months.

It is realized that exposure to natural infection by tick infestation constitutes a crude and unsatisfactory immunity test on a farm where regular dipping is enforced, even though the incidence of heartwater amongst susceptible stock may be high. Consequently six animals of the 1939 group and six from the 1940 group were selected at random for an artificial immunity test. This was done when the animals were approximately eighteen months old. The dose of infective sheep blood was again ten cc. Ten were solidly immune; two died of heartwater; in one the disease was complicated with redwater, a condition of the experiment being that no treatment with uleron should be applied.

While these experiments were in progress a request was made to immunize pure-bred calves, particularly bulls, that were being bred for stud purposes. As practically nothing was known of the relative susceptibility of grade and pure-bred calves, a preliminary experiment on nine, consisting of one Aberdeen Angus and eight Herefords, the progeny of fully susceptible pedigreed stock, was planned. That this caution was justified is shown by the results, which were disastrous. The Aberdeen Angus showed no clinical reactions, but three of the Herefords died, though it must be stated that treatment with uleron was not undertaken.

Further immunization was postponed pending the results of current experiments at Onderstepoort on the effect of administration of a single dose of uleron during the incubation period of the disease. Full details of this work will form the subject of a separate publication [Neitz (to be published in the Onderstepoort Journal)]. The results were so satisfactory that the method was applied to 24 Aberdeen Angus and 54 Hereford calves. The only treatment was that fifteen cc. of a ten per cent. solution of uleron sodium was injected eight days after the infective dose of blood. Of the 24 Angus calves only one showed clinical symptoms of heartwater and died; of the Herefords five reacted clinically and three died. The survivors were far too valuable to submit to an artificial immunity test and they continued to run with the pure-bred herd, where they are doing well.

Discussion

Up to the time of writing this preliminary report 1,349 grade Aberdeen Angus calves under the age of four weeks have been inoculated with virulent heartwater blood; 33 (2·5 per cent.) died from heartwater as a direct result of the injection; 74 (5·5 per cent.) on subsequent exposure to natural infection. The total mortality was eight per cent.

In the later experiments the mortality from the injection was reduced to less than one per cent. This must be attributed solely to more careful handling of the calves. Reactions, when they do occur, are usually noticed between the fourteenth and twenty-fourth day. All the calves were inspected twice daily and particular attention was paid to them during this critical period. At the first sign of ill-health blood smears were taken and submitted for examination and the calf received uleron. Frequently redwater (*Piroplasma bigeminum* or *Babesia bovis*) or anaplasmosis was found as a complication and the requisite treatment with Gonacrine, Pirevan, or Mercurochrome was prescribed. The value of this procedure was early apparent and now the work is proceeding with few misgivings. During the next critical period, i.e., when the animals reach an age of about eighteen months and are being exposed to the risk of natural infection, they should again be watched, because it has been shown conclusively that the timely administration of uleron is attended by excellent results.

The failure of a small percentage of animals to develop an adequate immunity is an observation which is receiving further attention. Obviously it may be associated with the injection of a subinfective dose of infective blood due to loss of infectivity of the *Rickettsias* on storage outside the animal body. In our opinion some factor in addition to this obvious explanation is involved.

The method of administering uleron during the period of incubation of the disease set up by artificial infection in the treatment of hyper-susceptible pure-bred calves is interesting. The possibility of its application to the immunization of adult cattle is receiving close attention.

In conclusion it must be emphasized that this is a preliminary report. The method is not a practical one and could not be introduced into general practice because of the great difficulty of ensuring that viable *Rickettsias* are injected into the resistant calves. Meantime valuable data on immunity and immunity production are being accumulated. If in the future a method of preserving *Rickettsia ruminantium* is elaborated, then at least part of the groundwork of an effective method of immunization against heartwater will have been completed.

Summary

(1) Calves up to the age of four weeks and lambs at least up to the age of seven days possess a marked degree of resistance to heartwater.

(2) This resistance is independent of the susceptibility or immunity of the dams and must be differentiated from passive transmitted immunity.

(3) Pure-bred Aberdeen Angus and Hereford calves are less resistant than grade calves.

(4) Mortality from heartwater in grade calves receiving an injection of infective heartwater blood was only 2·5 per cent. By careful treatment, judicious use of uleron and an appreciation of concurrent infections, this mortality was reduced to less than one per cent.

(5) Treated calves develop a durable immunity, though this immunity may be broken down in about seven per cent. of cases.

(6) Pure-bred calves may be immunized by administering uleron during the period of incubation of the artificially produced disease.

(7) At present this method of immunization is not suitable for application to the field on a large scale.

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Seasonal Distribution of Calving in Native Herds in Nyasaland

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The Mzimba district in the northern part of Nyasaland, by nature of its varying soil fertility, has come to be regarded as an area more suited for native cattle farming than for the production of economic crops. The following notes have been collected from work carried out on the Veterinary Stock Farms established to foster the growing native dairy industry.

The vegetation, soils, and native agricultural practice have already been described by Hornby (1938) and Wilson (1941). Various Mission publications have described the original Tumbuka inhabitants and the subsequent Angoni invasion in 1850, since when the tribes have intermixed freely.

Native Cattle Types

The district at present supports 60,000 head of cattle and approximately 10,000 sheep and goats without, as yet, showing any signs of general overstocking. The original cattle owned by the Tumbuka tribe were undoubtedly short-horned Zebu, but, while this breed has always remained predominant, it is no longer pure. Admixture of blood has occurred through migratory wanderings of native tribes with consequent mixing of herds. The most certain and definite record was that due to the Angoni invasion mentioned above. These people, fleeing northwards from Zululand in the early half of the 19th century, possessed during their wanderings through Southern Rhodesia herds of dwarf Makalanga cattle [Nobbs (1927), Bisschop and Curson (1933)] and the large Mangwato cattle [Nobbs (1927)].

While crossing the Zambesi River in November 1835, according to native tradition, the Angoni lost all their cattle [Young (1923), Lane-Poole (1934)]. Settling in Mzimba, however, raiding parties penetrated as far north as Lake Victoria and brought back Ankole (Sanga) cattle. These Ankole herds, being still concentrated around villages of chiefs, suffered heavily during the rinderpest epidemic in July 1892. Sufficient survived, however, to impose a lasting mark on the Mzimba cattle, as shown in increased horn and skeletal development and loss of the characteristic Zebu hump. Other characteristics of the Ankole cross are their poor milking qualities, with low butter-fat content, and poor resistance to tsetse-fly infection. In spite of these defects, the natives have continued to encourage the breed and Mzimba has long been known as the home of big cattle.

Previous writers e.g. Curson (1936) have credited Nyasaland with pure short-horned Zebu herds. Curson and Thornton (1936) have added a footnote to the effect that some mixture with Sanga cattle may have occurred. Pure short-horned Zebu are now found only in Karonga district on the north-western shores of Lake Nyasa, where, owing to the surrounding Misuku and Nyika highlands, native migrations have been limited.

Seasonal Calving Period

The native system of animal husbandry is the simplest imaginable, the cattle being kraaled at night in open *bomas* and grazed through the day on communal lines over areas of bush, deserted gardens, or in low-lying, damp, uncultivated land. They invade the cultivated lands after the harvest to eat crop residues. The work of herding and milking is left entirely to the young boys of the tribe.

Analysis of census figures shows the percentage distribution in herds to be 49 per cent. cows, 6 per cent. mature bulls, 1 per cent. oxen and 44 per cent. immature beasts under three years. The preponderance of the ratio of adults to immature animals of 56 per cent. to 44 per cent. is explained by the prevalence of East Coast fever. As herding is usually concentrated over a limited area, the percentage of bulls is ample to ensure that all cows in "heat" are detected even though the "heat" period may be of short duration [Anderson (1936)].

Under these uncontrolled conditions, with cattle perfectly acclimatized, any seasonal periodicity of calving will be due more to seasonal fluctuations in the sexual activity of females, than to any rise or fall in the fertility of bulls. In a small preliminary experiment in 1938 the following results were recorded in the Zombwe Farm herd:—

TABLE 1

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
No. of cows in herd .	13	13	14	14	15	15	15	15	16	16	16	16
No. of calves born . . .	1	—	—	—	1	6	—	—	4	1	1	—
% Calving .	8	—	—	—	7	42	—	—	25	6	6	—

Thus 79 per cent. of calves were born during May to September, while over 90 per cent. of cows calved.

In 1939 two large areas were kept under observation near two native dairies, to which surrounding native farms were selling milk.

The results are given in Tables 2 and 3.

TABLE 2

ENLANGENI AREA, 1939

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Total no. of cows in herds	718	676	676	662	659	679	681	693	670	681	700	726	
No. of calves born . . .	15	2	5	33	82	84	109	93	41	39	29	17	549
% distribution of calving . .	2	0.3	0.7	5.0	12.4	12.3	16.0	13.4	6.0	5.7	4.1	2.3	80.2
Rainfall in inches . . .	9.0	4.1	4.5	5.8	0.25	—	—	0.25	—	—	1.0	2.0	26.9

i.e., 67 per cent. of calves during the months May – August, while 80 per cent. of cows calved during the whole year.

TABLE 3

EDUNDU AREA 1939

	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
No. of cows in herds . .	651	695	692	635	655	677	670	690	691	685	685	629	
Total no. of calves born	8	13	15	57	101	140	96	119	40	25	22	10	646
% distribution of calves . .	1.2	1.8	2.1	9.0	15.2	20.6	14.3	17.2	5.5	3.7	3.2	1.6	95.4

In this area 71 per cent. of calves were born from May – August while 95 per cent. of cows calved during the year.

From a study of the tables it is clear that there is a particular period i.e. May to August, when about 70 per cent. of calves are born. At Elangeni the peak month was July (16 per cent.), while at Edundu the maximum number of calvings was in June (20 per cent.) with a secondary rise in August (17 per cent.). The minimum number of calvings was in February. Proof that these facts correspond with experience is provided each year by the scarcity of milk during February to April, and its relative abundance in the second half of the year. Milk for clarified butter production reaches its peak about October each year.

The rainfall figures for the area are given in Table 2. Owing to technical difficulties it was impossible to take daily thermometer readings. Briefly it may be stated that during the spring and summer months from October to April shade temperatures average 80°F, with maximum readings during November to December of 92°F. During the dry winter months, nights are cold with occasional ground frosts and maximum day temperatures average 60°F.

On an isothermal map the areas covered by the experiment lie between isothermal lines 60°F, allowing correction for an altitude of 3,000 ft. above sea level.

Discussion

The fact that the duration and intensity of oestrus shows a seasonal variation is well known. Marshall and Hammond (1933) point out that the period of rut in wild animals is probably regulated by temperature and food supply and that while such variations may be reduced under domestication, they are not altogether repressed.

In temperate climates the average duration of oestrus in cattle is longest in summer and shortest in winter months — there may be a difference of 10 hours in the average duration — and cows are usually served in summer to calve in spring.

Discussing imported breeds in the hotter regions of South Africa, Bonsma (1940) draws attention to the deleterious effects overheating of the vagina may have on sperm survival. Hot temperatures have also an effect on bulls of exotic breeds as the thin scrotal skin affords less protection from overheating than does the thick puckered skin of the scrotum of indigenous breeds, and so may repress sperm production. Further, living in a mild state of fever as they do during hot days, all activity is depressed and the bull may fail to seek cows on "heat." This is reflected in the fact that, when bulls run freely with cows, conception is highest in the cold winter months and few cows calve during June to August.

The fact that in Nyasaland conception is highest during the hotter months of the year, October – December, is yet another proof of the complete harmony existing between indigenous cattle and their environment. This also corresponds [Murray (1940)] with statistics given for European-owned herds in Southern Rhodesia.

Further, that in Nyasaland the majority of calves are born during the coldest and driest months of the year is not without significance. Until they are able to follow the herds, calves remain tethered in the villages and it may be days, or even weeks, before they are driven to water, and months before they get green succulent feeding. They are offered little, if any, protection from the weather.

These disadvantages are, however, offset by other more favourable factors. Records show that with the low-yielding native cow under village conditions the abundance, or otherwise, of green feed has little effect on milk yield, the yield being governed primarily by the stage of lactation. So milk shortage will not be any more acute for calves born in the dry winter than for summer calves. On the other hand, kraal conditions will be dry compared with the mud associated with the summer rains of the December – April period. Further during these dry months *Rhipicephalus* ticks definitely

decrease in number and so the liability of the calf to a mass attack of ticks carrying East Coast fever is reduced. It is also safe to assume that liability to gross worm infestation also decreases with aridity of pastures. The one exception to this is *Ascaris vitulorum,* infection being either *in utero* or by dust in kraals and on udders.

Taking all factors into consideration any effort to decrease calf mortality should be directed towards provision of better housing, sanitation, and management and supplementary feeding where possible and not to changing the calving period.

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Vaccination against Contagious Abortion in Calfhood: a Review

E. M. ROBINSON
Onderstepoort

In recent years the method of vaccination against contagious abortion in calfhood has received a good deal of attention, chiefly because it offers a way of combating the disease where isolation is impossible, and because it appears to be free from danger. Before reviewing the present knowledge of calfhood vaccination, I shall refer briefly to the method, introduced by Stockman in 1913, of vaccinating non-pregnant heifers or cows. This has been used in South Africa for the last 20 years, but the number of animals inoculated yearly has rarely exceeded 200,000. Opinions as to the value of this method vary and it has never been regarded as a way of eradication, but merely as a way of reducing losses from abortion. In some countries, such as Norway and Germany, this scheme has been abandoned in favour of eradication by testing and isolation. Stockman originally used virulent cultures of *Br. abortus* in the vaccine so that there was always a danger of producing latent infections. In fact, some authors considered that any immunity produced was the result of such a latent infection. Since Stockman's publication other workers have claimed good results from the use of attentuated, and even avirulent, cultures in this type of vaccine.

There is much diversity of opinion about the value of adult vaccination, for the conditions under which the method has been used varied considerably and controls were rarely kept. It will obviously make a big difference whether inoculation is carried out in the early or in the late stages of an outbreak or when the disease has existed for a considerable time.

Contagious abortion is very widespread in South Africa, particularly under ranching conditions, and although the isolation policy has been recommended, where possible, it is impracticable in many parts of the country. There is an urgent need for a more practical method of attacking the disease. We are therefore testing, in South Africa, the possibilities of vaccinating calves — a method introduced experimentally in the United States.

The strain of *Br. abortus* used is one known as Strain 19 that has been attentuated by prolonged subculturing. It will not usually cause abortion in pregnant animals, and does not appear to locate itself permanently in the body. Lesions are rarely produced in guinea pigs. The cultures, when plated, may show both smooth and rough colonies and smooth colonies must be subcultured from time to time so that the strain should remain predominantly smooth.

The most satisfactory age for inoculation seems to be between four and eight months. The animals develop positive agglutination reactions and some may show very high titres. Reactions at 1 : 6,000 or over may be present two to four weeks after inoculation, but within four to twelve months the animals are almost always negative again. There is no evidence that the organisms can produce a latent infection. In older calves the reactions may persist for two to four years, so that in these animals a latent infection may possibly be set up. However, such an infection with Strain 19 does not appear to be harmful, although it would interfere with any scheme of testing and isolation that one might wish to carry out at the same time. This combination of calfhood vaccination with testing and isolation will be discussed again later.

Buck (1930) carried out experiments using three different strains isolated eight years previously for one lot of calves, a freshly isolated strain for another lot, and Strain 19 for the third lot. Marked local reactions, and in some cases systemic reactions, occurred. All the heifers, when pregnant, were twice dosed with material from an infected foetus. Those vaccinated as calves, eleven in number, calved normally, while three of five controls aborted. At the second pregnancy the eleven inoculated animals and five fresh controls were infected *per os*. One of the eleven and three of the controls aborted. This was very satisfactory and other investigators who carried out similar experiments got equally promising results. Cotton, Buck and Smith (1934) used Strain 19 to vaccinate seventy calves, four to eight months old. Eight became infected when exposed to a heavy infection, but only one aborted. Of 73 controls, 57 became infected and 55 aborted. Thomsen (1939), in Denmark, inoculated calves on six farms, where the abortion rate was 25 to 50 per cent., with Strain 19. The animals were four to six months old and two-thirds were inoculated and one-third kept as controls. The injections were done subcutaneously and the temperatures sometimes rose to 107°F, but there were no abscesses.

Three per cent. of the inoculated and twenty-five per cent. of the controls became infected, and the percentage of abortions in the inoculated animals was lower than usual.

These promising results encouraged the United States Bureau of Animal Industry to carry out extensive field trials of calfhood vaccination. In 1936 a five-year programme was started in 260 herds in 24 states. By 1940 the results of work on 17,000 calves were available. Of these, 8,182 had had three pregnancies and 7,872 (96%) calved normally, but reacted to the agglutination test; 947 (12%) calved normally, but gave suspicious reactions. Abortions occurred in 310 (3·8%) of which 182 were negative, 99 positive, and 29 suspicious reactors. On the basis of the agglutination test only 128 (1·6%) of the abortions in this group of 8,182 animals could be attributed to brucella infection. Another group of only 44 animals had four parturitions and 41 calved normally while three aborted. One gave a positive reaction,

In 1,346 that calved normally and showed a positive or suspicious reaction, 500 were negative six months later. In one group of 97 animals that had a normal first calving, but showed a positive or suspicious reaction, a re-test two-and-a-half years later showed that 75 per cent. were negative.

In these field tests it was not possible to do all the investigations that should have been done, and there is little doubt that some animals that reacted at the second, third, and fourth pregnancies would probably have been found positive at the first, had they been tested. However, the number of these animals is insignificant. No controls could be kept, as the experiments were done on private herds; but no herd with an abortion rate of less than 15 per cent. per annum was used. In the herds there were 5,531 (29·1%) positive and 1,593 suspicious reactors. In July 1940, 24 per cent. of the original positive and 21 per cent. of the suspicious reactors were still in the herds and some had aborted every year during the experiment. Thus there was a heavy natural infection. Seven per cent. of the original reactors became negative during the three-and-a-half years of the experiment.

These interim results were published by Mohler, Wright and O'Rear (1940) who then formulated a plan for an official policy involving the recognition of calfhood vaccination. The main points were as follows.—

(1) All animals over six months old in a herd under co-operative supervision must, if vaccination is adopted, be tested before vaccination and once a year thereafter.

(2) A proper record of the herd must be kept to facilitate control of movements.

(3) Only heifers four to eight months, and if possible not more than six months old, shall be inoculated.

(4) The age, date of vaccination, and identity of the animals must be properly recorded.

(5) No reactor in a vaccinated herd may be sold, except for slaughter, without special permission.

(6) A vaccinated herd may be certified free from contagious abortion for a period of one year when all animals over two years old have been negative in two successive official tests, properly spaced, and when non-vaccinated heifers under two years have also proved negative, and when vaccinated heifers under two years either show a satisfactory decline in agglutination titre, or are removed for slaughter.

Rabstein and Welsh (1941) in an article on field experiments with calfhood vaccination give the results of tests on calves and heifers vaccinated at different ages. They had three groups, (1) three to eight months, (2) nine to twelve months, (3) thirteen to twenty-one months. In group (1) 79 per cent. were negative within six months, 91 per cent. in nine months, and 95 per cent. in eighteen months. Only one per cent. remained positive. In

group (2) 50 per cent. were negative in nine months, 71 per cent. in eighteen months, and six per cent. remained positive. In group (3) 10 per cent. were negative in six months, 20 per cent. in nine months, and 40 per cent. in eighteen months. Twenty per cent. remained positive. Some calves did not react at all to vaccination even after several doses at intervals. Some observations were made on the elimination of *Br. abortus* from the udders of vaccinated animals. Samples from the quarters of 77 heifers were tested and *Brucella* was isolated from two, but did not appear to be Strain 19. No infection could be demonstrated in the milk of ten cows vaccinated as adults.

In conclusion a recent article by Haring and Traum (1941) may be quoted. It is in the form of a questionaire and an attempt is made to answer various points. To the question about the degree of protection conferred by Strain 19, the answer given is that there may be 100 per cent. protection in experiments where the immunity is tested by conjunctival infection, but that the immunity may break down if the infection is a mass one. Only field tests with exposure to natural infection can be considered satisfactory. It is difficult to control such tests adequately, but in herds where calves were vaccinated there was a marked decline in the incidence of the disease in the vaccinated animals, although they were exposed to infection. A case is quoted where a heifer showed the vaccine organisms in the udder secretions at three tests during pregnancy. The age at which vaccination is carried out has little effect on the results of parturitions. Ninety per cent. of adult vaccinated cows calved normally, and the average of normal calvings for animals vaccinated at all ages was 94 per cent. However, the vaccination of pregnant animals is not without danger, as in one case one out of 28 animals aborted.

The duration of the resistance conferred is uncertain. In four consecutive pregnancies in vaccinated animals the percentage of normal calvings did not vary appreciably. As yet, little information is available on the question of re-vaccination.

Another question is whether systematic vaccination of calves is likely to result in an eradication of the disease. It may be of great help in those problem herds in which cases of *Brucella* infection crop up in spite of regular testing and isolating of reactors. Calfhood vaccination when combined with the test and slaughter method should materially hasten eradication. Haring (1938) quotes a case in California where calfhood vaccination reduced the percentage of infection from 41 to 0 in five years.

Calves should be tested when vaccinated and twice subsequently at three to six weeks intervals, to see what reaction occurs. An agglutination at 1 : 200 or higher should be found, although it is not an indication of the degree of immunity. It is inadvisable to expose vaccinated calves to heavy infection during the last three months of their subsequent pregnancies, as a

mass infection may break down their immunity. It is quite likely that exposure to infection before pregnancy would increase immunity.

The length of time vaccinated calves continue to react depends on several factors. Older heifers react longer than younger heifers and calves, and some remain positive for years. Ninety-five per cent. of vaccinated calves become negative before their first pregnancy, but some show low titres — around 1 : 50 — for one or two years. If a vaccinated animal becomes infected naturally it may show a second rise in titre, but as a rule this is not high. Continuous reactors need not be removed from the herd. It is doubtful whether vaccinated animals can infect susceptible animals with which they come in contact, for vaccinated calves introduced into clean herds have not set up an infection.

There is no justification for not vaccinating adult animals with Strain 19 in herds where the isolation policy is not being carried out. Such a scheme has had good results in California. However, Haring (1938) reports a marked drop in milk yield in vaccinated lactating cows. Recovery to full production sometimes took as long as two weeks.

Calfhood vaccination must still be considered an experimental procedure, for we do not know enough about the antigenic and pathogenic characters of *Brucella* strains subcultured for long periods. Dissociation is continuous and some unexpected and unexplained results may be due to the production of variants. There is some evidence that the resistance of vaccinated animals decreases after two or three pregnancies. The advisability of re-vaccination may therefore have to be considered.

Because of the promising results obtained in the United States, the method is being tested out in South Africa under various conditions. Calfhood vaccination should not be considered where it is possible to undertake eradication by isolation, but it may be of help where isolation methods are unlikely to be successful until the incidence of infection has been materially reduced. Under ranching conditions such as in the bushveld of the Transvaal, where regular testing and isolation are impossible, calfhood vaccination may be a means of greatly reducing infection, even if it cannot eradicate it completely.

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Grahamella (Brumpt 1911) of the Rat: Its Occurrence in Natal

A. S. CANHAM

Allerton Laboratory, Pietermaritzburg

While examining blood smears made from a rat (*Rattus rattus*) that was caught in one of the stables at Allerton Laboratory, Pietermaritzburg, Natal, I saw small bodies in a number of the erythrocytes. The smear was stained with Giemsa. Mr. Neitz, of Onderstepoort, identified the organisms as *Grahamella* and stated that this was the first time they had been seen in rats in South Africa.

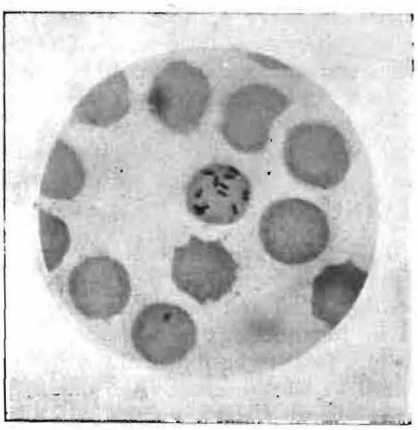
The *Grahamella* was first described by Graham-Smith (1905) in the red cells of English moles, but, judging from the coloured plate accompanying this article, it is rather difficult to agree that these organisms are similar to those now known as *Grahamella*. Figure 5 of the photographic reproductions show the closest resemblance to the organisms now being recorded. Brumpt (1911) regarded them as a parasite and founded the new genus *Grahamella*. Macfie (1914) has a good coloured plate of *Grahamella* in his work on blood parasites collected in Nigeria. Kikuth (1934) in comparing the *Bartonella* with the *Grahamella* gives a description of the latter which appears to coincide with that given in this paper. Laveran and Marullaz (1914) expressed the opinion that the bodies seen by Graham-Smith in the red cells of the English mole were changes in the red cells analogous to the basophilic degeneration seen in anæmic conditions. Wenyon (1926) in his book "Protozoology" does not appear satisfied that these bodies are parasitic. He states.— 'It was possible to trace gradations from the more irregular granules which everyone admits to be an indication of basophilic degeneration to the more uniformly shaped rods of the *Grahamella* type.' In the blood of cattle recovering from anaplasmosis punctate basophilia is frequent and one sees many other anæmic changes such as poikilocytosis, anisocytosis, and the presence of immature cells. The blood of the rat from which the smears showing the *Grahamella* were made, showed no marked anæmic changes. An occasional cell showed a slight diffuse basophile staining but this appears common in most smears made from rats. Topley

and Wilson (1936) state that though these bodies are probably living micro-organisms the evidence in favour of this is not yet conclusive.

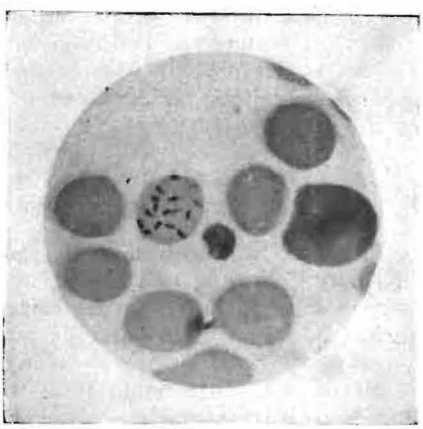
The organisms appear to resemble bacteria, some being straight rods and others somewhat curved. Some show a constriction at the middle, while others appear to stain more deeply at one end than at the other. There is even a suggestion of a 'beaded' staining in some of the organisms.

The rat from which the smear was obtained appeared perfectly healthy.

In the two photographs the points mentioned can be seen.



Grahamella of the rat.
Rattus rattus × 1500.



Grahamella of the rat.
Rattus rattus × 1500.

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OBITUARIES.

WILLIAM McKIE.

William McKie was born at Inch Parish, Stranraer, Scotland, in 1864. He qualified at Glasgow Veterinary College in 1887 and then practised at Carlisle. He came to South Africa during the Anglo-Boer War and thereafter farmed until his children had to go to school, whereupon he started private practice in Bloemfontein. At the outbreak of the Great War he joined the South African Veterinary Corps with the rank of captain. McKie saw service in the South West and in the East African campaigns and was also employed on the transport *S.S. Hymethus* which plied between Durban and East Africa. In 1920 he was released from the army and joined the civil veterinary department in South West Africa. In 1926 he retired and went farming. McKie was essentially a farmer and had large interests at Blenheim, a farm in the Orange Free State. He died of heart failure on the eighteenth of March, 1939, while superintending work on the farm. To his son and daughter we offer our sympathy.

H.H.C.

JOHN KIRBY PILKINGTON.

Pilkington was born near Liverpool in 1857. He qualified at the Liverpool Veterinary College in 1881 and practised in that town for twenty years. During the second Anglo-Boer War he came to South Africa and served as a C.V.S. attached to the A.V.D. Later he practised in Johannesburg and took a keen interest in sport and in the volunteer movement. In 1914 he joined the S.A.V.C. and served in German South West Africa until he was released from duty and returned to private practice. In 1921 he was awarded the Colonial Auxiliary Forces Decoration in addition to other medals.

He was a foundation member of the Johannesburg and Germiston Bowling Clubs and a past president of the former. In 1926 he visited England as a member of the Springbok bowling team.

He died on the fourth of August, 1941, and was buried at the Brixton Cemetery. He is survived by his widow and three sons of his first marriage.

H.H.C.

JOSEPH BUCK

Joseph Buck was born in 1872 at Ragdale Hall, Leicestershire. On leaving school he was apprenticed to a chemist at Cannock, but, preferring the out-door life, he decided to study veterinary science. He qualified at the New Edinburgh Veterinary College in 1895, and came to South Africa during the second Anglo-Boer War as a C.V.S. attached to the A.V.D. Buck displayed energy and initiative in his military duties and was mentioned in despatches.

After the war Buck settled in Kimberley as a private practitioner. He was frequently employed by the De Beers Company, the Kimberley Municipality, and the Southern Rhodesian Government. In 1923 he received an appointment in the Union Veterinary Service, which he relinquished on reaching the age limit in 1932. Thereafter he returned to private practice which he carried on until his death in 1941. To his widow, his two daughters, and his son (on active service with the Kimberley Regiment) we extend our sympathy in their sad loss.

H.H.C.

THE ASSOCIATION.

Council Meeting held at Polley's Hotel, Pretoria, on 4th November, 1941, at 8 p.m.

Present. — S. T. Amos (President), R. A. Alexander, J. L. Dickson, P. J. du Toit, A. C. Kirkpatrick, P. S. Snyman, D. G. Steyn, M .Sterne and H. H. Curson representing N. F. Viljoen, who submitted an apology for absence, and S. W. J. van Rensburg (Hon. Sec.-Treas.). The President welcomed Dr. Snyman to Council.

(1) *Minutes of Meeting* held on 16th April, 1941, were taken as read and were confirmed.

(2) *Arising from the Minutes.* —

(a) *Lecturer in Anatomy.* — Dr. du Toit informed the meeting of the latest developments in connection with this appointment. After full discussion, Dr. Alexander proposed and Dr. Sterne seconded that "A deputation from this Council lays before the Secretary for Agriculture the objection of Council to interference by Pretoria University with veterinary education, with special reference to the vacancy of the above post." This was carried by 5 votes to 2, Mr. Amos, Dr. Steyn and Dr. Alexander to form this deputation.

(b) *Representation on Meat and Dairy Control Boards.* — Letter read from the Secretary for Agriculture intimating that the Department could not recommend representation on these two Boards being granted to the Veterinary profession, since the Boards which are already unwieldy, are concerned with non-veterinary aspects such as marketing and price control, while questions like the health and breeding of livestock are largely controlled by the Department, of which the Veterinary Division is an integral part.

This reply was not considered satisfactory, and finally Dr. du Toit was asked to press for fuller recognition to be given to the Division of Veterinary Services.

(3) *New Members.* — Maj. A. B. Bowhay and Mr. W. C. Viljoen were proposed and their acceptance by the General Meeting was recommended.

(4) *Arrears.* — Decided to recommend to the General Meeting that the name of Mr. P. D. Huston be deleted from the list of Members.

(5) *Student Loan.* — An application from student A for increasing the loan advanced to him, was considered. Decided that the grant of an extra £50 p.a. for the next two years be recommended to Finance Committee.

(6) *Mallein and Tuberculin Tests.* — A letter from the Director of Veterinary Services, giving a list of private and municipal veterinarians approved to carry out these tests, was read. Decided that the Secretary should write to all those not included in the list, advising them to apply for such approval without delay.

(7) Dr. Sterne proposed and Dr. Curson seconded.—"Since the S.A.V.M.A. represents and is representative of the Veterinary profession in South Africa, and since the members of the Association are well aware of the needs of the public, it is felt that the interests of both the profession and the public would be well served if the Association were represented on the bodies controlling veterinary education in South Africa."

The general feeling of the meeting was that the present time was inopportune for considering this matter, and it was agreed to let it stand over for the duration of the war.

(8) *General.* —

(a) *Auditor's Report.* — This was read by the Secretary and it was agreed that Finance Committee be asked to give careful consideration to the various points raised. A suggestion by Dr. du Toit for an honorarium for the Secretary was also referred to Finance Committee.

(b) *Students on Active Service.* — Dr. Alexander brought to notice the position of students who qualify and then enlist for active service, thereby incurring the risk of finding themselves without an opportunity for getting employment after the war. Decided that Dr. Alexander, as Officer in Charge of the Unit concerned, should approach the Minister of Agriculture for a ruling on this matter.

(c) *General Meeting.* — In reply to an enquiry by Dr .Alexander, the Secretary stated that it was at first intended merely to have a business meeting. Members were therefore not invited to offer papers. The few papers which were eventually included, were suggested by the Director of Veterinary Services and were approved of by the President.

(d) *Cost of Blocks.* — Dr. Curson raised the question of payment for blocks required in connection with the publication of the history of the S.A.V.C. This was referred to Finance Committee.

(e) *Standing Committees.* — The following were elected for 1941-42:

Editorial. — P. J. du Toit, A. D. Thomas, C. Jackson, H. P. Steyn, J. H. Mason and M. Sterne.

Finance. — H. H. Curson, R. A. Alexander, A. D. Thomas and B. S. Parkin.

Library. — E. M. Robinson, D. G. Steyn, C. Jackson, G. de Kock and A. D. Thomas.

General Purposes. — R. A. Alexander, C. J. van Heerden, A. C. Kirkpatrick, P. S. Snyman and P. J. J. Fourie.

The meeting closed at 11.15 p.m., with a hearty vote of thanks to the President.

S. W. J. van Rensburg,
HON. SEC.-TREAS., S.A.V.M.A.

ANNUAL GENERAL MEETING.

36th General Meeting held at Onderstepoort, 5th November, 1941.

Present. — S. T. Amos (President), P. J. du Toit, J. Zwarenstein, J. Nicol, E. M. Robinson, J. Spruell, A. C. Kirkpatrick, R. Alexander, (Mrs.) J. A. Robinson, W. G. Barnard, H. H. Curson, H. O. Mönnig, P. S. Snyman, D. G. Steyn, M. Sterne, R. Clark, J. W. A. Brookes, J. L. Dickson, C. F. B. Hofmeyr, D. Coles, J. Quin, P. J. J. Fourie, P. J. Meara, C. J. van Heerden, J. R. Scheuber, M. W. Henning, J. H. R. Bisschop, J. J. G. Keppel, R. du Toit, D. T. Mitchell, A. S. Canham, J. G. Bekker, V. Cooper, W. O. Neitz, M. M. Neser, A. M. Diesel, N. C. Starke, W. G. van Aswegen, J. G. van der Wath, H. P. A. de Boom, D. Haig, S. J. van der Walt, K. Schulz, and S. W. J. van Rensburg (Hon. Sec.-Treas.).

Apologies for absence. — Dr. J. Quinlan, Dr. N. F. Viljoen and Mr. S. B. Woollatt.

(1) *Minutes of Meeting* held on 13th November, 1940, were taken as read and were confirmed.

(2) *New Members.* — The following were unanimously elected: G. P. Bishop, A. B. Bowhay, G. Faull, F. J. D. Hempstead, and W. C. Viljoen.

(3) *Election of Council.* — The ballot for four members of Council resulted in the re-election of the four retiring members, — (R. A. Alexander, J. L. Dickson, P. J. du Toit and A. C. Kirkpatrick.) Council for 1941-42 was therefore announced as follows:

President. — S. T. Amos.
Vice-President. — C. J. van Heerden.
Hon. Sec.-Treas. — S. W. J. van Rensburg.
Members. — R. A. Alexander, J. L. Dickson, P. J. du Toit, A. C. Kirkpatrick, P. S. Snyman, D. G. Steyn, A. D. Thomas, N. F. Viljoen.

(4) *Presidential Address.* — The President stated that in view of the unsettled conditions he did not come with a prepared address, and therefore merely wished to make a few remarks. He expressed appreciation of the services rendered to the profession by the Vice-President and also by the Director and Deputy Director of Veterinary Services. Attention was drawn to one discordant note, namely the presence in the veterinary profession of individuals who do not live up to the standard of etiquette required of professional men. Yet none of these have up to the present time been summoned to appear before the Veterinary Board. The President appealed to every member to bring to the notice of the Board any cases of flagrant breach of the rules of etiquette or of improper conduct, in order that such individuals may be made to understand that they have to conform with the rules.

(5) *Arrear Subscriptions.* — On the recommendation of Council it was decided that the name of Mr. P. D. Huston be deleted from the list of members.

(6) *Resignation.* — A letter tendering his resignation was submitted by Mr. E. T. Perossi. Several members expressed regret that circumstances should have forced Mr. Perossi to resign, and it was decided to refer this matter to Council to investigate the possibility of allowing him to continue membership without paying subscriptions.

(7 and 8) *Reports.* — The reports of the Secretary and of Finance Committee were submitted and approved.

(9) *General.* —

(a) *National Nutritional Council*: Dr. Quin pointed out the desirability of the Veterinary profession being represented on this Council and it was decided that this be referred to Council for their further consideration.

(b) *Students' Loan Fund*: The President suggested that Finance Committee should consider the advisability of starting such a fund.

This concluded the business and the meeting immediately went over to a consideration of the scientific section, when the following programme was submitted:

Globidium Infection in Cattle. — Drs. P. J. du Toit and G. de Kock, and Mr. C. F. B. Hofmeyr.

Recent Investigations on Bloating in Ruminants. — Dr. J. Quin.

Malignant Catarrh in Bovines. — Drs. G. de Kock and P. S. Snyman.

The Blowfly Problem. — Dr. H. O. Mönnig.

Visit to Kaalplaats.

S. W. J. van Rensburg,
HON. SEC.-TREAS., S.A.V.M.A.

VOLUME XII. — AUTHOR INDEX

VOLUME XII. — SUBJECT INDEX