

The Immunity in Heartwater.

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INTRODUCTION.

FIELD observations as well as immunity tests under laboratory conditions have shown that animals are capable of developing an immunity against heartwater after recovery. The nature of the immunity however is obscure. *Rickettsia ruminantium* parasitizes the endothelial cells of the blood vessels, and its presence in the peripheral blood can be demonstrated by sub-inoculation of blood into susceptible animals during the reaction and for a limited period after recovery. Alexander (1931) found that in some cases no "virus" could be demonstrated by the intra-jugular sub-inoculation of 10 c.c. blood 8 days after the height of the fever reaction, while in others transmission was successful 35 days after the reaction. In an experiment to be described later, it was possible to detect the presence of heartwater "virus" in a recovered sheep 60 days after recovery. From the data it must be concluded that a preimmunity may exist for a period of two months after recovery, and that the nature of the immunity after this period is obscure and needs further investigation.

In the case of *Rickettsia bovis* which parasitizes the leucocytes de Kock, van Heerden, du Toit and Neitz (1937) showed that a preimmunity develops, and that splenectomy results in a relapse.

A factor to be considered in these studies is the resistance of the various species of animals. As far as is known all the ruminants are susceptible to heartwater, but their susceptibility varies. The artificial infection of two species of antelopes viz. the blesbuck (*Damaliscus albifrons*) and the black wildebeest (*Conochaetes gnu*) did not result in clinical symptoms of heartwater, although the heartwater "virus" was demonstrable by sub-inoculation of blood into susceptible sheep. (Neitz 1933, 1935 and 1937.) Alexander (1931) states that indigenous sheep and blackhead-persians possess a higher degree of resistance than imported breeds. In the former breeds the mortality is approximately 6 per cent., whereas in the merino sheep up to 80 per cent. may die. Similar observations have been made with indigenous and imported breeds of goats. Insufficient information has been collected about the resistance of cattle breeds, but from field observations it would appear that one can expect the mortality to be in the neighbourhood of 60 per cent.

A third factor to be considered in these investigations is the possibility of the existence of heterologous strains. Spreull (1904), Theiler (1909) and Alexander (1931) conclude from their experiments that there are immunologically different strains. However du Toit (1924)* and the writer in these experiments have failed to confirm this statement. The observations by the former workers are summarized in Table 1 and those of the latter workers in Table 2. The immunity tests (Table 1) in the cattle, sheep and goats were applied from 3 weeks to 2 years after the recovery of the animals. In some cases the animals received virulent blood, and in other instances they were exposed to natural infection. No satisfactory explanation can be given why the earlier workers encountered immunologically different strains. A possible explanation may be that since the presence of *Eperythrozoon ovis* (Neitz, Alexander and du Toit, 1934) and that of a new "virus" which will be referred to as "virus A" in these studies was not known, incorrect conclusions may have been drawn from the febrile reactions set up by these two diseases in sheep. It will be noticed from Table 1 that no breakdown is recorded in cattle where the *Eperythrozoon* and the "virus A" disease do not play an important rôle, whereas in sheep and goats a large number reacted when the immunity test was applied. This argument is brought forward to show how misleading a febrile reaction may be, particularly in diseases like heartwater and "virus A" disease where the temperature may be the only symptom. In such doubtful cases therefore sub-inoculations and cross immunity tests have to be resorted to before a correct interpretation of the reaction can be given.

OBSERVATIONS AT ONDERSTEPOORT.

For these experiments the highly susceptible merino sheep was used. The strains were passed by sub-inoculating blood at the height of the reaction into susceptible sheep. These animals incidentally served as controls to the various experiments. No difficulty was experienced in maintaining the infection in this way, although occasionally one out of two injected sheep failed to react, in spite of the fact that such an animal was subsequently found to be fully susceptible on receiving a second infective dose. Similar observations have been recorded previously by Alexander (1931) in sheep, and by Neitz (1937) in the sub-inoculations made from infected blesbuck into sheep. The inability to transmit heartwater at times may be due to a very low concentration of the heartwater "virus" in the blood. In any of the sheep used in these experiments in which no reaction, or a doubtful one was observed, a second infective dose was given, in order to definitely determine whether the animal was susceptible or not. The mortality from the various strains was as high as 80 per cent., and consequently a relatively small number of sheep were available for these studies. Blood smears which were examined from time to time from the passage sheep during the heartwater reaction frequently showed the presence of *Eperythrozoon ovis*. These recovered animals developed a premonition to *Ep. ovis* infection, and did not react again to this disease when the

* Quoted by Alexander (1931).

TABLE 1.
Summary of the Immunity Tests by the Earlier Workers.

| ANIMAL THAT RECOVERED FROM HEARTWATER. | | Interval after Recovery. | IMMUNITY TEST. | | | | Year. |
|--|--|--------------------------------------|--|--|---|---------------|-------|
| Number. | Heartwater Strain. | | Heartwater Strain. | Reaction. | Result. | Author. | |
| 5 Cattle. | Not stated..... | Various intervals up to 17 months | Homologous strain injected in doses of 1000-3000 c.c. i.v. | No reaction.. | Immune..... | Theiler..... | 1909 |
| 8 Cattle. | Not stated..... | 2 years..... | Homologous strain and subsequently exposed to natural infection together with controls | No. reaction. Controls contracted heartwater | Immune..... | Theiler..... | 1909 |
| 3 Goats. | Not stated..... | ? | Probably heterologous strain... | 1 No reaction, 2 reacted | Immune, 1 died | Spreull..... | 1904 |
| 3 Sheep. | Not stated..... | ? | Probably heterologous strain... | 2 No reaction, 1 Reacted... | Immune..... Died. | Spreull..... | 1904 |
| 8 Sheep. | Sjambokkraal and later hyperimmunized with the same strain | ? | Komatipoort..... | 8 Reacted... | 7 Recovered, 1 Died | Theiler..... | 1909 |
| 8 Sheep. | Not stated..... | At various intervals up to 18 months | Homologous strain injected in doses of 50-400 c.c. i.v. | No reaction.. | Immune..... | Theiler..... | 1909 |
| 34 Sheep. | Heartwater strain..... | 22-205 days.... | Homologous strain..... | 27 No reaction 7 Reacted... | Immune..... 5 Recovered 2 Died..... | Alexander... | 1929 |
| 37 Sheep. | Heartwater strain..... | 30-173 days.... | Heterologous strain..... | 24 No reaction 13 Reacted.. | Immune..... 8 Recovered 5 Died..... | Alexander.... | 1929 |

heartwater immunity test was applied. On the other hand it was noticed that those sheep which contracted and recovered from a natural infection of heartwater were susceptible to *Ep. ovis*. This complicating factor can be excluded by treating the donors infected with heartwater and *Ep. ovis* with the antimony-arsenic compound Std. 386 B. 24 hours before sub-inoculating blood (Neitz 1937).

In the course of these experiments a hitherto undescribed "virus A" distinct from heartwater, blue-tongue, and tick-borne fever was isolated. This determination was important, because wrong interpretations would have been given to the reactions, if the existence of this "virus A" had not been recognized. The incubation period that follows the injection of "virus A" is as a rule longer (12-21 days) but may be as short as that of heartwater. The duration and the type of the febrile reaction are very similar to those of heartwater, and its identity can in most cases only be recognized by carrying out sub-inoculations and cross-immunity tests.

The nature of "virus A" is obscure. It can not be transmitted by the ticks *Amblyomma hebraeum*, the vector of heartwater, and *Rhipicephalus appendiculatus*. It does not pass through a Berkefeld or a Seitz filter, and does not live longer than 24 hours at room temperature in citrated blood. The mortality is extremely low and up to the present approximately 2 per cent. of the infected sheep have died. Clinically the only symptoms seen are inappetence and general weakness. At post-mortem the lesions resemble very closely those of heartwater, and the only way to differentiate these two diseases is the microscopic examination of the intima smears, and of sections of the hippocampus for the presence of *Rickettsia ruminantium*. A very important difference between "virus A" disease and heartwater is the fact that the former can be transmitted to horses, in which a mild reaction is produced after an incubation period of 18-21 days.

In the tables mentioned in Appendix I it will be noticed that "virus A" produced febrile reactions in heartwater immune and susceptible sheep on several occasions. This complicated the interpretations of the reactions and necessitated further sub-inoculations in order to ascertain the nature of the febrile reactions.

The experiments are discussed under two headings:—

A To determine the duration of the immunity in heartwater and to ascertain whether immunologically different strains exist.

B. To determine how this immunity is maintained.

A. TO DETERMINE THE DURATION OF THE IMMUNITY IN HEARTWATER AND TO ASCERTAIN WHETHER IMMUNOLOGICALLY DIFFERENT STRAINS EXIST.

I. EXPERIMENTAL SHEEP.

The details of the experiments on which this discussion is based are given in Appendix I. A large number of sheep was used and the observations on the various groups of sheep are presented in

tabular form at the end of each experiment. Another table summarizing the results of all the experiments described in Appendix 1 is given at the end of this discussion. In addition the same information is given on a chart on which the observations are demonstrated graphically. It will be seen that an insufficient number of sheep were available for each group. Nevertheless the data gives one some idea about the duration of the immunity.

For these experiments merino sheep which had recovered from an artificial infection of heartwater were used. These sheep together with a large number of others which were fully susceptible to heartwater were kept in a camp comparatively free of ticks. During the period in which they were kept in the camp no reactions and no deaths from heartwater were observed. It was therefore concluded that a natural infection of heartwater did not take place. In order to confirm this observation 13 of the susceptible sheep which are mentioned in Appendix 1 Table 8 (a) were used as controls to these experiments. Besides these 13 controls at least 2 susceptible sheep were included in each group of recovered heartwater sheep which were being tested for their immunity. Wherever possible the endothelial cells of the jugular veins of the control sheep that died were examined for the presence of *Rickettsia ruminantium* in order to be sure that the reactions observed were due to heartwater and not possibly due to "virus A" disease. It is of interest to mention that on several occasions it was possible to demonstrate the presence of *R. ruminantium* in the endothelial cells of the jugular veins of sheep which had died early in the evening, the next morning approximately 12 hours after death. In most of the sheep the decomposition changes were far advanced at the time when the post-mortem examination was carried out. In some instances where a sheep died late in the afternoon, and where it was not possible to carry out a post-mortem examination the same day, the jugular veins were removed and placed in a refrigerator and examined the next morning for the presence of parasites. This procedure proved very satisfactory. In one instance small portions of the jugular vein which were kept in a refrigerator were examined daily for a period of 10 days after the death of the animals. *Rickettsia* colonies which showed slight morphological changes could be demonstrated throughout this period. Whether they were still viable or not can not be stated as no biological test was made.

2. ORIGIN OF THE HEARTWATER STRAINS.

All the ten strains mentioned below were obtained from different localities in the Transvaal. An opportunity did not present itself to study the nature of the heartwater strains which are known to occur in Natal and in the Cape Province.

- (1) The strains "C.853", "C.860" and "C.1024" were isolated from three naturally infected cattle which were exposed at Onderstepoort.
- (2) The strain "S.4377" was isolated from a naturally infected sheep that contracted the disease at Onderstepoort.

- (3) The "Harding" strain was isolated from a naturally infected ox in the vicinity of Pretoria.
- (4) The "Krugersdorp" strain was isolated at Krugersdorp from naturally infected sheep which contracted the disease while they were on their way from the Lowveld in the Eastern Transvaal. Krugersdorp lies on the Highveld of the Transvaal, and heartwater may make its appearance there in animals that have recently been introduced from areas where heartwater is known to exist. The climatic conditions of the Highveld are such that the bont-tick (*Amblyomma hebraeum*) does not thrive there.
- (5) The "Mara" strain was isolated from naturally infected sheep that were exposed on the Government Experimental Station at Mara near Louis Trichardt in the Zoutpansberg district.
- (6) The "Northam" strain was isolated from naturally infected sheep which were exposed on the Government Experimental Station at Northam in the Rustenburg district.
- (7) The "Strydom" strain of heartwater was isolated from naturally infected cattle on a farm in the vicinity of Warmbad in the Waterberg district.
- (8) The "Zoutpansberg" strain of heartwater was isolated from naturally infected cattle in the Zoutpansberg district.

3. SUMMARY OF THE IMMUNITY AND CROSS IMMUNITY TESTS.

The observations made in experiments 1-9 (*b*) which are mentioned in Appendix 1 have been summarized in Table 2 of the text. It will be seen that a solid immunity was present in 121 sheep for periods up to 58 months after recovery in all of them with the exception of 10 sheep in which febrile reactions due to the heartwater test injection were noticed 7 to 34 months after recovery. No clinical symptoms other than febrile reactions were noticed. Of the 8 sheep which had recovered from the "Mara" strain febrile reactions were seen after 7 months (1 sheep), after 12 months (1 sheep), after 15 months (1 sheep), after 16 months (1 sheep); after 25 months (1 sheep), after 30 months (2 sheep) and after 34 months (1 sheep), on testing their immunity against the "Mara" heartwater strain. In one sheep which had recovered from the "S.4377" strain of heartwater a mild reaction was noticed on testing the immunity against the "Mara" strain 20 months after the primary reaction. Another sheep which recovered from both "Strydom" and "Mara" strains reacted on testing the immunity after 10 months with the "Strydom" strain of heartwater. The mild reactions indicated that there was still a partial immunity present. The cause of death in one sheep which was exposed at "Mara" six months after recovering from the "S.4377" strain could not be definitely determined. The other febrile reactions in the tested sheep were due to *Ep. ovis* in 5 cases and due to "virus A" in 5 cases.

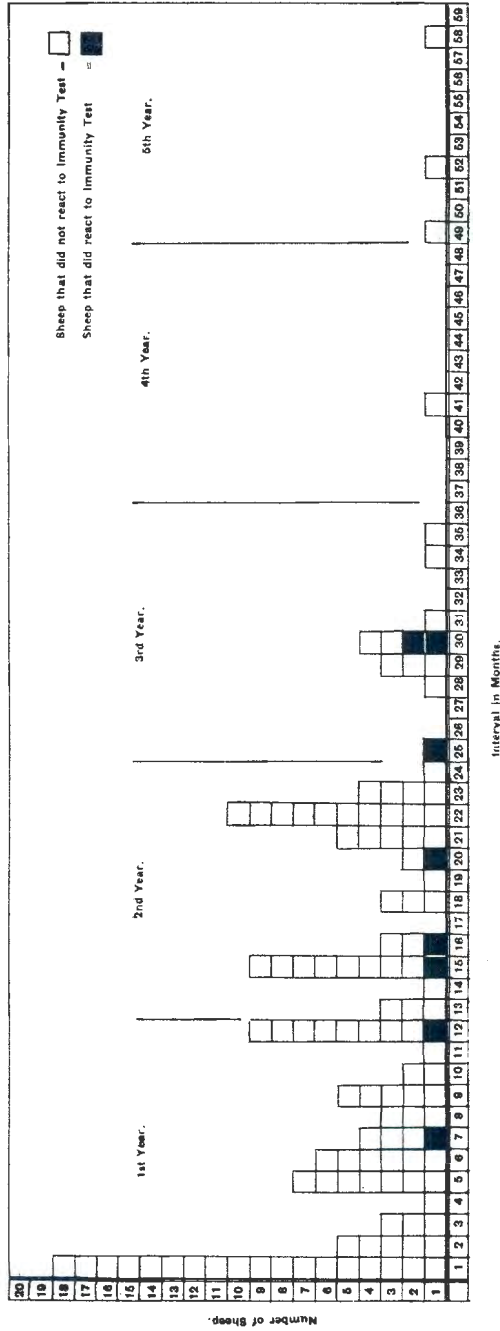
TABLE 2.
Summary of Tables 1-9.

| SHEEP THAT RECOVERED FROM HEARTWATER. | | IMMUNITY TEST. | | | | |
|---------------------------------------|----------------------|--|----------------------|---|---------------------------|-------------------------|
| Number. | Strain. | Approximate interval in months between the recovery and the Immunity Test. | Strain. | | | |
| | | | Reaction. | | | |
| | | | Result. | | | |
| | | | Table of Appendix I. | | | |
| 1 | "Harding" | 1 | "Harding" | No reaction. | Immune. | 1 |
| 3 | "Harding" | 2 and 12 | "Zoutpansberg" | No reaction. | Immune. | 1 |
| 1 | "Harding" | 5 | "C. 860" | No reaction. | Immune. | 1 |
| 1 | "Harding" | 5 | "C. 1024" | No reaction. | Immune. | 1 |
| 1 | "Zoutpansberg" | 1 | "Zoutpansberg" | No reaction. | Immune. | 1 |
| 2 | "Zoutpansberg" | 1 | "Harding" | No reaction. | Immune. | 1 |
| 2 | "Zoutpansberg" | 6 | "C. 860" | No reaction. | Immune. | 1 |
| 2 | "Zoutpansberg" | 5 and 6 | "C. 1024" | No reaction. | Immune. | 1 |
| 2 | "Krugersdorp" | 1 | "C. 860" | No reaction. | Immune. | 1 |
| 2 | "Krugersdorp" | 1 | "C. 1024" | No reaction. | Immune. | 1 |
| 1 | "C. 853" | 1 | "C. 1024" | No reaction. | Immune. | 1 |
| 1 (goat) | "C. 853" | 1 | "C. 1024" | No reaction. | Immune. | 1 |
| 1 | "C. 853" | 2 | "C. 860" | No reaction. | Immune. | 1 |
| 1 (goat) | "C. 853" | 1 | "C. 860" | No reaction. | Immune. | 1 |
| 1 | "C. 853" | 1 | "Krugersdorp" | No reaction. | Immune. | 1 |
| 1 (goat) | "C. 860" | 1 | "C. 1024" | No reaction. | Immune. | 1 |
| 2 | "C. 1024" | 1 and 2 | "Krugersdorp" | No reaction. | Immune. | 1 |
| 9 | "Mara" | 21-23 | "Mara" | No reaction. | Immune. | 2 (a) |
| 10 | "S. 4377" | 2-8 | "Mara" | 8 No reaction. 1 Reacted. 1 Abscess in lungs. | Immune. Died. Died. | 2 (b) 2 (b) 2 (b) |
| 8 | "Mara" and "S. 4377" | 22 | "Mara" | No reaction. | Immune. | 2 (b) |
| 5 | "Northam" | 12 | "Mara" | No reaction. | Immune. | 3 |
| 2 | "Mara" | 2 | "Northam" | No reaction. | Immune. | 4 |

TABLE 2 (continued).

| SHEEP THAT RECOVERED FROM HEARTWATER. | | IMMUNITY TEST. | | | | |
|---------------------------------------|----------------------|--|-----------|---|----------------------------------|----------------------|
| Number. | Strain. | Approximate interval in months between the recovery and the Immunity Test. | Strain. | Reaction. | Result. | Table of Appendix I. |
| 2 | "Mara" and "Northam" | 12 | "Mara" | 2 No reaction to heartwater. 1 Reacted to "Virus A." | Immune. Immune. | 4 4 |
| 2 | "Mara" | 18 | "Mara" | No reaction. | Immune. | 5 |
| 2 | "Mara" | 23 | "Mara" | 1 No reaction to heartwater. 1 Reacted to "Virus A." | Immune. Immune. | 5 5 |
| 3 | "Mara" | 12-16 | "Mara" | No reaction. | Immune. | 5 |
| 1 | "Mara" | 25 | "Mara" | Reacted to heartwater. | Recovered. | 5 |
| 2 | "S. 4377" | 20 | "Mara" | 1 No reaction. 1 Reacted to heartwater. | Immune. Recovered. | 6 6 |
| 6 | "S. 4377" | 7-11 | "Mara" | No reaction. | Immune. | 7 |
| 6 | "S. 4377" and "Mara" | 15 | "Mara" | No reaction. 1 Reacted to "Virus A." 1 Reacted to heartwater. | Immune. Immune. Recovered. | 7 7 7 |
| 5 | "Mara" | 3-6 | "Mara" | No reaction. | Immune. | 8 |
| 1 | "Mara" | 7 | "Mara" | Reacted to heartwater. | Recovered. | 8 |
| 3 | "Mara" | 8-9 | "Mara" | No reaction. | Immune. | 8 |
| 1 | "Mara" | 12 | "Mara" | Reacted to heartwater. | Recovered. | 8 |
| 7 | "Mara" | 13-15 | "Mara" | No reaction. | Immune. | 8 |
| 1 | "Mara" | 16 | "Mara" | Reacted to heartwater. | Recovered. | 8 |
| 2 | "Mara" | 18-19 | "Mara" | No reaction. | Immune. | 8 |
| 4 | "Mara" | 28-29 | "Mara" | No reaction. | Immune. | 8 |
| 4 | "Mara" | 30 | "Mara" | 2 No reaction. 2 Reacted to heartwater. | Immune. Recovered. | 8 8 |
| 3 | "Mara" | 31-35 | "Mara" | No reaction. | Immune. | 8 |
| 1 | "Mara" | 41 | "Mara" | No reaction. | Immune. | 8 |
| 2 | "Strydom" and "Mara" | 9 | "Strydom" | No reaction. | Immune. | 9 |
| 1 | "Strydom" and "Mara" | 10 | "Strydom" | Reacted to heartwater. | Recovered. | 9 |
| 1 | "Mara" | 28 | "Mara" | No reaction. | Immune. | 9 |
| 1 | "Mara" | 34 | "Mara" | Reacted to heartwater. | Recovered. | 9 |
| 1 | "Mara" | 35 | "Mara" | No reaction. | Immune. | 9 |
| 1 | "Mara" and "Northam" | 49 | "Mara" | No reaction. | Immune. | 9 |
| 1 | "Mara" and "Northam" | 52 | "Mara" | No reaction. | Immune. | 9 |
| 1 | "Mara" | 58 | "Mara" | No reaction. | Immune. | 9 |

CHART INDICATING DURATION OF IMMUNITY TO HEARTWATER.



4. DISCUSSION.

The results of the experiments indicate that once an animal has recovered from heartwater a solid immunity lasting for periods up to 58 months may be expected. In a few individual animals however only a partial immunity may be present after a period of 7 months. These observations conform with those made under field conditions. The fact that it was possible to demonstrate the presence of heartwater "virus" by sub-inoculating blood into susceptible sheep from partially immune sheep, which were reacting to the heartwater immunity test has brought forward a very important practical point in connection with the control of heartwater in the field. Should partially immune animals become reinfected with heartwater, they can act as excellent reservoirs for infecting ticks. The disease can therefore be maintained in the absence of fully susceptible animals. Mortality due to heartwater can therefore be expected on apparently heartwater free farms when susceptible stock is introduced.

In these experiments no immunological difference could be detected between the various strains employed. Reactions sometimes followed by death, have been noticed in cattle and sheep reared on heartwater veld. Such reactions should not be ascribed to a reinfection with an immunologically different strain but to a partial or a complete loss of immunity.

B. TO DETERMINE HOW THE IMMUNITY IS MAINTAINED IN HEARTWATER.

1. SUMMARY OF THE EXPERIMENTS DESCRIBED IN APPENDIX II.

In experiments 10 and 11 mentioned below, attempts were made to ascertain how the immunity is maintained in heartwater. In case of the bacterial diseases most of the essential aspects of immunity are known, because many methods are available for the *in vitro* and *in vivo* studies of the bacteria and their products. In case of heartwater, only *in vivo* investigations can be carried out. The cultivation of *R. ruminantium* on artificial media and the transmission of this disease to small laboratory animals have not been successful up to the present (Mason and Alexander 1938).

The blood sub-inoculations recorded by Alexander (1931) have shown that the virus may still be present 35 days after a heartwater reaction. Donatien and Lestoquard (1937) state that they were able to demonstrate the presence of heartwater "virus" in sheep 105 days after recovery. Two sheep that received large quantities of blood from a sheep 105 days after recovering from heartwater failed to react to heartwater, but a third sheep which was injected with an emulsion prepared from the endothelial cells of the blood vessels reacted to heartwater 23 days later. This animal died and at post-mortem the characteristic exudations which are seen in heartwater were observed. No attempts were made by them to confirm their diagnosis by sub-inoculation of blood into susceptible sheep or by demonstrating the presence of *R. ruminantium*.

In the experiments described in detail in Appendix II splenectomy of heartwater recovered sheep and the sub-inoculation of blood and organ emulsions were carried out in order to ascertain whether *R. ruminantium* can be demonstrated in the recovered heartwater sheep.

The results of experiment 10 can be summarized as follows:—The splenectomy of 5 recovered heartwater sheep, resulted in a relapse to either *Eperythrozoon ovis*, *Anaplasma ovis* or *Theileria ovis*, but not to heartwater. The rôle, if any, which is played by the spleen in maintaining the immunity in heartwater appears to be totally different to that observed in the protozoal diseases. Blood was sub-inoculated from two of the splenectomized sheep at varying intervals up to 45 days after the operation. Of the 20 sub-inoculated sheep only one reacted to heartwater, while the others reacted to *Anaplasma ovis* or *Eperythrozoon ovis*. The sheep which reacted to heartwater, had received blood from the splenectomized sheep, which had recovered from heartwater 60 days previously. The concentration of the heartwater “virus” of the splenectomized sheep must have been very low, because another sheep injected at the same time failed to react. The presence of the heartwater “virus” apparently did not stand in any relation to the splenectomy and in all probability would also have been demonstrable at that time had the spleen not been removed.

In the experiment 11 in which blood, endothelial cell scrapings of the jugular vein and emulsions prepared from the organs, were injected into 24 susceptible sheep no heartwater reactions were observed. On the contrary in three of the sub-inoculated sheep reactions due to “virus A” resulted.

2. DISCUSSION.

Of the 44 sheep which received either blood organ emulsions or endothelial scrapings prepared from the jugular veins, only one sheep reacted to heartwater. This sheep received blood from a splenectomized sheep which had recovered from heartwater 60 days previously. In three of the heartwater recovered sheep the presence of “virus A” could be demonstrated by sub-inoculating blood into susceptible sheep. From the above results it is impossible to explain how the immunity is maintained in heartwater. If the immunity is a premunition one would have expected that several more sheep should have reacted in these tests, particularly those that were injected with endothelial cells or organ emulsions. All that is known at the present moment is that *R. ruminantium* may be present in sheep 31, 60 and 105 days after recovery. Whether this parasite is still present after this period in sheep that were found to be solidly immune after several years is not known. Another aspect of the immunity that can not be explained is that in several of the heartwater recovered sheep only a partial immunity was observed. If the immunity is due to a labile infection the question arises, why is it partial in some animals?

Before final conclusions can be drawn it is suggested that further experiments of this nature be carried out. It should however be

remembered that recovered heartwater sheep may harbour latent infections of other diseases, and that a febrile reaction that may occur in a subinoculated sheep need not necessarily be that of heartwater.

GENERAL CONCLUSIONS.

1. Studies were undertaken in order to ascertain whether immunological different strains of heartwater referred to by Spreull, Theiler and Alexander exist. For this purpose cross-immunity experiments with 10 strains obtained from different localities in the Transvaal were utilized. No difference was detected.

2. During the investigations "virus A" disease which produces a febrile reaction in sheep very similar to that of heartwater was observed on several occasions. The origin of the virus is not clear, but it would appear that a small number of the passage sheep harboured this infection. The mortality from this disease is very low viz. 2 per cent., and the lesions at post-mortem resemble very closely those of heartwater. The possibility exists that this disease may have been responsible for some of the febrile reactions and even deaths, which were noticed by the earlier workers. This suggestion is made because in the limited number of animals used in these experiments the presence of "virus A" was encountered no less than 8 times.

3. The susceptibility of the horse to "virus A" and the resistance of the horse to heartwater can be employed as a method to differentiate the two diseases, and in case of a mixed infection as a method to obtain a pure strain of "virus A".

4. The duration of the immunity was studied in 121 sheep for a period up to 58 months after the recovery from a heartwater reaction. A solid immunity lasting for at least 6 months was observed. In the majority of cases the immunity is complete after this period, but in a very few only a partial immunity may be present at 7, 10, 12, 15, 16, 20, 25, 30 and 34 months after recovery.

5. It is suggested that partially immune animals reacting to heartwater can play an important rôle in maintaining heartwater infection in the bont-tick in the absence of fully susceptible animals.

6. Splenectomy of heartwater recovered sheep does not result in a relapse. In one of the splenectomized sheep the presence of heartwater in the circulating blood could be demonstrated 60 days after recovering from the heartwater reaction.

7. Experiments to demonstrate the presence of *Rickettsia ruminantium* for a period longer than 60 days by the subinoculation of blood and organ emulsions from recovered heartwater sheep were entirely negative.

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APPENDIX I.

A. TO DETERMINE THE DURATION IN THE IMMUNITY OF HEARTWATER AND TO ASCERTAIN WHETHER IMMUNOLOGICALLY DIFFERENT STRAINS EXIST.

Experiment 1 (S.1888, S.2142, S.2143, S.2149 and S.2150).

Object.—To test the immunity of sheep and goats with the same strain or a strain other than that from which the animals had recovered.

Method. 22 sheep and 2 goats which had recovered from "Harding", "Zoutpansberg", "Krugersdorp", "C.853", "C.860" and "C.1024" strains of heartwater were injected with blood from sheep reacting to the strains indicated in Table 1. The sheep used for maintaining the various strains acted as controls.

Result.—All the animals listed in Table 1 were found to be solidly immune for a period of 27 to 363 days after receiving the infective dose of heartwater blood. The control sheep all reacted to heartwater; most of them died.

Conclusion. No immunological difference could be detected between the six strains employed in these experiments.

Experiment 2 (a) (S.5507).

Object. To compare the resistance of sheep susceptible and of sheep immune to heartwater to a natural infection of heartwater.

Method.—23 susceptible sheep and 10 sheep which had recovered from an artificial infection of the heartwater strain "S.4377" were exposed at Mara in the Northern Transvaal, a locality which is known to be a very bad heartwater area.

Result.—It will be noticed from Table 2 (a) that of the 23 susceptible sheep 12 died from heartwater from the 20th to the 35th day after exposure. 2 showed clinical symptoms and recovered and in 9 no symptoms were observed.

TABLE 1.

Experiment 1 (S.1888, S.2142, S.2143, S.2149 and S.2150).—To test the immunity of sheep and goats that recovered from heartwater against different strains of heartwater.

| D.O.B. Number Sheep. | Date of Injection. | Heartwater Strain. | Result. | Interval in days between date of Injection and Immunity Test. | IMMUNITY TEST. | | | Result. |
|----------------------|--------------------|--------------------|---------------------------------|---|--------------------|--------------------|--------------------|--------------|
| | | | | | Heartwater Strain. | Date of Injection. | Dose of Blood i.v. | |
| 6276..... | 13/10/23 | "Harding"..... | Recovered from heartwater..... | 363 | "Zoutpansberg" | 11/11/24 | 10 c.c. | No reaction. |
| 8624..... | 22/9/24 | "Harding"..... | Recovered from heartwater..... | 50 | "Zoutpansberg" | 11/11/24 | 10 c.c. | No reaction. |
| 9161..... | 8/9/24 | "Harding"..... | Recovered from heartwater..... | 65 | "Zoutpansberg" | 13/11/24 | 10 c.c. | No reaction. |
| 8930..... | 13/10/24 | "Harding"..... | Recovered from heartwater..... | 29 | "Harding" | 12/11/24 | 10 c.c. | No reaction. |
| 8775..... | 9/12/24 | "Harding"..... | Recovered from heartwater..... | 138 | "C. 1024"..... | 16/5/25 | 5 c.c. | No reaction. |
| 7795..... | 4/10/24 | "Zoutpansberg" | Recovered from heartwater..... | 38 | "Zoutpansberg" | 11/11/24 | 10 c.c. | No reaction. |
| 7710..... | 4/10/24 | "Zoutpansberg" | Recovered from heartwater..... | 38 | "Harding"..... | 12/11/24 | 10 c.c. | No reaction. |
| 8864..... | 13/10/24 | "Zoutpansberg" | Recovered from heartwater..... | 29 | "Harding" | 12/11/24 | 10 c.c. | No reaction. |
| 8713..... | 20/10/24 | "Zoutpansberg" | Recovered from heartwater..... | 148 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 8864..... | 13/10/24 | "Zoutpansberg" | Recovered from heartwater..... | 185 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 7795..... | 4/10/24 | "Zoutpansberg" | No reaction after 2nd injection | 190 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 8655..... | 11/11/24 | "Zoutpansberg" | No reaction after 2nd injection | 168 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 9042..... | 31/12/24 | "Zoutpansberg" | No reaction..... | 181 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 10941..... | 3/4/25 | "Krugersdorp" | Recovered from heartwater..... | 43 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 11369..... | 8/4/25 | "Krugersdorp" | Recovered from heartwater..... | 43 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 11094..... | 8/4/25 | "Krugersdorp" | Recovered from heartwater..... | 47 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 10684..... | 23/4/25 | "Krugersdorp" | Recovered from heartwater..... | 27 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 10529..... | 17/4/25 | "C. 853" | Recovered from heartwater..... | 29 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 10294 (goat)..... | 31/3/25 | "C. 853" | Recovered from heartwater..... | 46 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 11112..... | 31/3/25 | "C. 853" | Recovered from heartwater..... | 51 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 9874 (goat)..... | 17/4/25 | "C. 853" | Recovered from heartwater..... | 34 | "C. 860" | 20/5/25 | 5 c.c. | No reaction. |
| 10629..... | 17/4/25 | "C. 853" | Recovered from heartwater..... | 34 | "Krugersdorp" | 20/5/25 | 5 c.c. | No reaction. |
| 10303 (goat)..... | 2/4/25 | "C. 860" | Recovered from heartwater..... | 44 | "C. 1024" | 16/5/25 | 5 c.c. | No reaction. |
| 6551..... | 26/3/25 | "C. 1024" | Recovered from heartwater..... | 55 | "Krugersdorp" | 20/5/25 | 5 c.c. | No reaction. |
| 10815..... | 17/4/25 | "C. 1024" | Recovered from heartwater..... | 33 | "Krugersdorp" | 20/5/25 | 5 c.c. | No reaction. |

Of the 10 heartwater recovered sheep mentioned in Table 2 (b), 8 showed no clinical symptoms, one died and at the post mortem examination showed lesions resembling those of heartwater, and another died showing multiple, localized abscesses in the lungs.

Conclusion.—The mortality in the susceptible sheep was 52 per cent. Although no clinical symptoms of heartwater were observed in 9 of the susceptible sheep exposed at Mara, it is assumed that these sheep reacted mildly to a natural infection of heartwater. It seems hardly possible that these sheep could have escaped from a natural infection if one considers the presence of the large number of bont ticks that occurred on the veld at the time. The duration of immunity of 8 of these sheep and of one which showed clinical symptoms of heartwater are discussed in the following experiment 2 (b). In 8 of the immune sheep the immunity was solid for a period varying from 70 to 257 days after recovering from an artificial infection of heartwater. The cause of death could not be determined in one of the sheep on account of the lack of facilities at the time in the field to carry out the necessary examination.

Experiment 2 (b)

Object.—To ascertain whether 9 of the sheep that survived from a natural infection at Mara are immune to heartwater for a period of approximately 21-23 months after exposure.

Method.—The 9 sheep mentioned in Table 2 (a) together with 2 susceptible sheep were injected intravenously with blood of the "Mara" strain of heartwater.

Result.—All the 9 sheep were found to be immune. Three of them however reacted to *Eperythrozoon ovis*, and one of them died from this infection 33 days after receiving the test dose. The 2 susceptible control sheep both reacted and died of heartwater.

Conclusions.—The immunity was solid against the homologous heartwater strain for a period of 21-23 months after exposure at Mara. *Ep. ovis* produced febrile reactions in three of these animals.

Experiment 2 (c).

Object.—To test the immunity of the 8 sheep, which had recovered from the "S.4377" strain of heartwater, and which had also been exposed at Mara, approximately 22 months later, against the "Mara" strain of heartwater.

Method.—These sheep together with 2 susceptible ones were injected intravenously with blood of the "Mara" strain of heartwater.

Result.—The 8 sheep referred to in Table 2 (b) did not react whereas both control sheep reacted and died from heartwater.

Conclusion.—The immunity was solid against the homologous strain of heartwater for a period of 22 months after exposure at Mara, and for a period of 25 to 31 months after recovering from an artificial infection with the heartwater strain "S.4377".

Experiment 3 (S.5688 and S.5690).

Object.—To test the immunity of 2 sheep which had recovered from a natural, and 3 sheep which had recovered from an artificial infection of the "Northam" strain of heartwater approximately 12 months after recovery.

Method.—The 5 sheep together with 2 susceptible ones were injected intravenously with blood of sheep reacting to the "Mara" strain of heartwater. The two sheep that recovered from the natural infection at Northam in the Rustenburg district reacted to *Eperythrozoon ovis*. The two control sheep both reacted and died.

Conclusions.—The 5 sheep were found to be solidly immune approximately 12 months after recovering from heartwater. No immunological difference could be found between the "Northam" and the "Mara" strains of heartwater.

TABLE 2 (a).
Experiments 2 (a) and 2 (b) (S.5507).—Sheep susceptible to heartwater exposed at Mara.

| D.O.B. Number of Sheep. | Period of Exposure at Mara. | Result. | Interval in Days between First Day of Exposure at Mara and the Immunity Test. | IMMUNITY TEST. | | | | Result. |
|-------------------------|-----------------------------|--|---|--------------------|--------------------|--------------------|--|---------|
| | | | | Heartwater Strain. | Date of Injection. | Dose of Blood i.v. | | |
| 29365 | 4/10/34- 15/ 2/35 | No reaction observed..... | 690 | " Mara " .. | 5/ 9/36 | 10 c.c. | No reaction. | |
| 32798 | 4/10/34 15/ 2/35 | No reaction observed..... | 690 | " Mara " .. | 5/ 9/36 | 10 c.c. | No reaction. | |
| 37362 | 4/10/34- 15/ 2/35 | No reaction observed..... | 626 | " Mara " .. | 23/ 6/36 | 10 c.c. | No reaction. | |
| 39347 | 4/10/34- 15/ 2/35 | No reaction observed..... | 626 | " Mara " .. | 23/ 6/36 | 10 c.c. | Showed a febrile reaction due to <i>Ep. ovis</i> . | |
| 39414 | 4/10/34- 15/ 2/35 | No reaction observed..... | 626 | " Mara " .. | 23/ 6/36 | 10 c.c. | No reaction. | |
| 39985 | 4/10/34- 15/ 2/35 | No reaction observed..... | 626 | " Mara " .. | 23/ 6/36 | 10 c.c. | No reaction. | |
| 40027 | 4/10/34- 15/ 2/35 | No reaction observed..... | 626 | " Mara " .. | 23/ 6/36 | 10 c.c. | Showed a febrile reaction due to <i>Ep. ovis</i> . | |
| 40126 | 4/10/34- 15/ 2/35 | Showed clinical symptoms of heartwater, 27/10/34 and recovered | 676 | " Mara " .. | 12/ 8/36 | 10 c.c. | No reaction. | |
| 40375 | 4/10/34- 15/ 2/35 | No reaction observed..... | 676 | " Mara " .. | 12/ 8/36 | 10 c.c. | No reaction. | |
| 38138 | 4/10/34- 15/ 2/35 | No reaction observed..... | — | — | — | — | — | |
| 39462 | 4/10/34- 15/ 2/35 | Showed clinical symptoms of heartwater, 27/10/34 and recovered | — | — | — | — | — | |
| 37073 | 4/10/34 | Died from heartwater 21 days after exposure | — | — | — | — | — | |
| 37889 | 4/10/34 | Died from heartwater 26 days after exposure | — | — | — | — | — | |
| 37911 | 4/10/34 | Died from heartwater 20 days after exposure | — | — | — | — | — | |
| 38190 | 4/10/34 | Died from heartwater 20 days after exposure | — | — | — | — | — | |
| 38316 | 4/10/34 | Died from heartwater 28 days after exposure | — | — | — | — | — | |
| 38576 | 4/10/34 | Died from heartwater 26 days after exposure | — | — | — | — | — | |
| 39640 | 4/10/34 | Died from heartwater 28 days after exposure | — | — | — | — | — | |
| 40036 | 4/10/34 | Died from heartwater 35 days after exposure | — | — | — | — | — | |
| 40130 | 4/10/34 | Died from heartwater 35 days after exposure | — | — | — | — | — | |
| 40159 | 4/10/34 | Died from heartwater 28 days after exposure | — | — | — | — | — | |
| 40162 | 4/10/34 | Died from heartwater 18 days after exposure | — | — | — | — | — | |
| 40174 | 4/10/34 | Died from heartwater 21 days after exposure | — | — | — | — | — | |

TABLE 2 (b).
Experiment 2 (c) (S.5507).—*Sheep immune to "S.4377" strain of heartwater exposed at Mara.*

| D.O.B. Number of Sheep. | Date of Injec-tion. | Heart-water Strain. | Result. | Interval in Days between Injec-tion and First Day of Expo-sure. | Period of Exposure at Mara. | Result. | IMMUNITY TEST. | | | | | |
|-------------------------|---------------------|---------------------|---------------------------|---|-----------------------------|--|---|---|---------------------|--------------------|---------------------|--------------|
| | | | | | | | Interval in Days between Artificial Infection and Immu-nity Test. | Interval in Days between First Day of Expo-sure and Immu-nity Test. | Date of Injec-tion. | Dose of Blood i.v. | Heart-water Strain. | Result. |
| 31788 | 15/7/34 | "S. 4377" | Recovered from heartwater | 81 | 4/10/34— 15/ 2/35 | No reaction observed | 757 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 37767 | 26/7/34 | "S. 4377" | Recovered from heartwater | 70 | 4/10/34— 15/ 2/35 | No reaction observed | 747 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 37828 | 4/6/34 | "S. 4377" | Recovered from heartwater | 122 | 4/10/34— 15/ 2/35 | No reaction observed | 799 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 38195 | 5/3/34 | "S. 4377" | Recovered from heartwater | 213 | 4/10/34— 15/ 2/35 | No reaction observed | 890 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 39531 | 13/4/34 | "S. 4377" | Recovered from heartwater | 174 | 4/10/34— 15/ 2/35 | No reaction observed | 851 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 37096 | 20/1/34 | "S. 4377" | Recovered from heartwater | 257 | 4/10/34— 15/ 2/35 | No reaction observed | 934 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 38013 | 15/6/34 | "S. 4377" | Recovered from heartwater | 111 | 4/10/34— 15/ 2/35 | No reaction observed | 788 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 38871 | 24/4/34 | "S. 4377" | Recovered from heartwater | 164 | 4/10/34— 15/ 2/35 | No reaction observed | 841 | 677 | 12/8/36 | 10 c.c. | "Mara" | No reaction. |
| 38921 | 4/4/34 | "S. 4377" | Recovered from heartwater | 183 | 4/10/34 | Died 15/11/34 Cause of death was not deter-mined | — | — | — | — | — | — |
| 38932 | 24/3/34 | "S. 4377" | Recovered from heartwater | 194 | 4/10/34 | Died 16/12/34 from mul-tiple local-ized absces-ses in the lung | — | — | — | — | — | — |

TABLE 3.

Experiment 3 (S. 5688 and S. 5690).—Immunity test in sheep that recovered from "Northam" heartwater reaction.

| D.O.B. Number of Sheep. | Date of Infection. | Heartwater Strain. | Reaction. | Interval between Heart-water Infection and Immunity Test. | IMMUNITY TEST. | | | Result. |
|-------------------------|-----------------------|--------------------|--|---|---------------------|--------------------|--------------------|--|
| | | | | | Heart-water Strain. | Date of Injection. | Dose of Blood i.v. | |
| 41839 | 11 and 14/6/35, . . . | "Northam" | Recovered from heartwater | 376 days | "Mara" | 23/6/35 | 10 c.c. | No reaction. |
| 43749 | 15/7/35, | "Northam" | Recovered from heartwater | 342 days | "Mara" | 23/6/35 | 10 c.c. | No reaction. |
| 41835 | 25/6/35, | "Northam" | Recovered from heartwater | 363 days | "Mara" | 23/6/35 | 10 c.c. | No reaction. |
| 42789 | Exposed at Northam | "Northam" | Reacted to a natural infection 23/6/35 and recovered | ± 365 days | "Mara" | 23/6/35 | 10 c.c. | Showed a febrile reaction due to <i>Ep. ovis</i> . |
| 43153 | Exposed at Northam | "Northam" | Reacted to a natural infection 23/6/35 and recovered | ± 365 days | "Mara" | 23/6/35 | 10 c.c. | Showed a febrile reaction due to <i>Ep. ovis</i> . |

Experiment 4 (a) (S.5691).

Object.—To test the immunity of 2 sheep which had recovered from an artificial infection of the “Mara” strain against the “Northam” strain of heartwater approximately 2 months after recovery.

Method.—The two recovered sheep and 2 susceptible ones were injected intravenously with blood of a sheep reacting to the “Northam” strain of heartwater.

Result.—The two sheep mentioned in Table 4 were found to be immune, whereas both the controls reacted and died.

Conclusion.—There is no immunological difference between the “Northam” and “Mara” strains of heartwater.

Experiment 4 (b) (S.5691).

Object.—To test the immunity of the two sheep mentioned in experiment 4 (a) and Table 4 for a second time with the “Mara” strain of heartwater approximately 12 months after the first immunity test

Method.—Blood from a sheep reacting to heartwater was injected intravenously into the above-mentioned and into two susceptible sheep.

Result.—One sheep showed no reaction, whereas the other 41021 developed a febrile reaction on the 15th day. Subsequent subinoculation experiments showed that this reaction was caused by “virus A”. The control sheep reacted and died of heartwater.

Conclusion.—The immunity was solid for a period of 14 months after the recovery from the “Mara” strain of heartwater, and for a period of 12 months after the first immunity test with “Northam”. The febrile reaction observed in one of the sheep was caused by “virus A”.

Experiment 5 (S.5507, S.5527, S.5623, S.5627, S.5961 and S.6096).

Object.—To ascertain the duration of immunity in sheep which had recovered from an artificial infection of the “Mara” strain of heartwater.

Method.—The eight sheep mentioned in Table 5, together with two susceptible ones, were injected intravenously with the “Mara” strain of heartwater.

Result.—It will be noticed that in six of the sheep no febrile reactions were noticed. One sheep 41016 showed a febrile reaction which may have been due to heartwater. This observation, however, was not confirmed by subinoculation into susceptible sheep. Sheep 47051 showed a febrile reaction due to “virus A” which disease was first noted in experiment 4 (b). It was not possible to demonstrate the presence of heartwater “virus” in sheep 39185, in which no reaction was observed.

Both the control sheep reacted to heartwater and died.

Conclusion.—The immunity against the homologous strain of heartwater was solid for a period of 12 to 23 months in seven of the tested sheep. In one sheep whose immunity was tested after 25 months a febrile reaction, probably due to heartwater, was noticed. The mild febrile reaction noticed in another of the sheep was due to “virus A”. In an immune sheep circulating virus could not be demonstrated by subinoculation of blood.

TABLE 4.
Experiment 4 (a) and 4 (b) (S. 5691).—Immunity test in sheep that recovered from "Mara" strain of heartwater.

| D. O. B. Number of Sheep. | Date of Injection. | Heart-water Strain. | Result. | FIRST IMMUNITY TEST. | | | | SECOND IMMUNITY TEST. | | | | | |
|---------------------------|--------------------|---------------------|---------------------------|---|---------------------|--------------------|---------------------|-----------------------|--|---------------------|--------------------|---------------------|--|
| | | | | Interval in Days between Injection and First Immunity Test. | Heart-water Strain. | Date of Injection. | Dose of Blood i. v. | Result. | Interval in Days between First and Second Immunity Test. | Heart-water Strain. | Date of Injection. | Dose of Blood i. v. | Result. |
| 39435 | 15/5/35 | "Mara" | Recovered from heartwater | 54 | "Northam" | 8/7/35 | 10 c.c. | No reaction | 350 | "Mara" | 22/8/35 | 10 c.c. | No reaction. |
| 41021 | 23/4/35 | "Mara" | Recovered from heartwater | 67 | "Northam" | 22/8/35 | 10 c.c. | No reaction | 359 | "Mara" | 22/8/35 | 10 c.c. | On the 15th day after injection sheep showed a febrile reaction which lasted for 14 days. The highest temperature recorded was 106.4° F. On subinoculating blood on the 4th day of reaction into sheep 45340 and 45750 it was found that the febrile reaction in sheep 41021 was due to "virus A." |

Experiment 6 (S.4377 and S.6095).

Object. To ascertain whether sheep which had recovered from an artificial infection of the heartwater strain "S.4377" are immune to the "Mara" strain approximately 20 months after recovery.

Method.—Virulent "Mara" heartwater blood was injected intravenously into two recovered and two susceptible sheep.

Result. The details of the results will be found in Table 6. One of the sheep was found to be solidly immune. In this animal circulating heartwater could not be demonstrated by the subinoculation of blood into susceptible sheep. The febrile reaction noted in the second sheep was found to be due to heartwater. This sheep, however, did not show any clinical symptoms.

Conclusion. The immunity in one of the sheep was complete for a period of 20 months, and in the other a mild febrile reaction, due to heartwater, was observed. It would thus appear that although the sheep reacted a sufficient resistance or partial immunity was still present to prevent a severe attack.

Experiment 7 (a) (S.4377, S.5722 and S.6045).

Object. To note the nature of immunity in sheep which had recovered from an artificial infection of heartwater strain "S.4377" by testing them with the "Mara" strain approximately seven to 11 months after recovery.

Method.—Virulent heartwater blood was injected into the six recovered and into two susceptible sheep.

Result. It will be noticed from Table 7 that all the sheep were immune. Both controls reacted and died.

Conclusion. The sheep were found to be fully immune for a period of seven to 11 months after recovery. No immunological difference could be detected between the "Mara" and the "S.4377" heartwater strains.

Experiment 7 (b).

Object. To test the immunity of the six sheep mentioned in experiment 7 (a) and Table 7 for a second time against the "Mara" strain of heartwater approximately 15 months after the first immunity test.

Method.—Virulent heartwater blood was injected intravenously into the six sheep and also into two susceptible ones.

Result. In five of the sheep no reactions were noted. In one of the animals a reaction due to "virus A" was noticed and in another a febrile reaction due to heartwater was seen.

Conclusion.—The immunity was solid for a period of 20 to 25 months after the recovery from the "S.4377" strain and for a period of 15 months after the first immunity test with the "Mara" strain of heartwater. In one sheep a mild febrile reaction due to heartwater was noticed 24 months after recovery from the primary reaction and 15 months after the first immunity test.

Experiment 8.

Object. To test the immunity of 32 sheep which had recovered from an artificial infection of the "Mara" strain of heartwater against the same strain three to 41 months after recovery.

Method.—Virulent "Mara" heartwater blood was inoculated intravenously in doses of 10 c.c. into the 32 heartwater recovered sheep mentioned in Table 8 and into 11 susceptible sheep mentioned in Table 8 (a).

Result.—All the susceptible sheep reacted to heartwater, and eight died. In the smears prepared from the intima of the jugular veins of the latter *Rickettsia ruminantium* could be demonstrated. Of the 32 recovered heartwater sheep, 27 showed no reaction and five showed febrile reactions, when tested after periods of seven, nine, 16 and 30 months. None of these sheep died.

Conclusions.—The immunity in the majority of sheep was solid for periods up to 41 months after recovery. In only five of the sheep febrile reactions due to heartwater were noticed when tested at intervals from seven to 30 months after the primary reaction. The control sheep were found to be fully susceptible, indicating that the camp in which the sheep were allowed to run was free of infected heartwater ticks.

Experiment 9 (a).

Object.—To test the immunity of 9 sheep mentioned in Table 9 which had recovered from an artificial infection of the "Strydom" or "Mara" strain of heartwater 1 to 25 months after recovery from the "Mara" or the "Northam" strain of heartwater.

Method.—(1) The 3 "Strydom" heartwater recovered sheep each received 10 c.c. virulent "Mara" heartwater blood intravenously.

(2) The 4 "Mara" heartwater recovered sheep each received 10 c.c. virulent "Mara" heartwater blood intravenously.

(3) The 2 "Mara" heartwater recovered sheep each received 10 c.c. virulent "Northam" heartwater blood intravenously.

Result.—It will be seen from Table 9 that all the sheep were found to be solidly immune to the strains of heartwater used. Febrile reactions due to "virus A" disease were observed in two of the sheep which recovered from the "Strydom" strain of heartwater.

Conclusions.—The immunity was complete for periods varying from 1 to 25 months after recovery from heartwater. No immunological differences could be detected between the "Mara", "Strydom" and "Northam" strains of heartwater.

Experiment 9 (b).

Object.—To test the immunity of the 9 sheep mentioned in experiment 9 for periods varying from 9 to 58 months after the recovery from the infection.

Method.—(1) The 3 sheep that recovered from the "Strydom" strain of heartwater and 2 susceptible sheep mentioned in Table 8 (a), each received 10 c.c. virulent "Strydom" heartwater blood intravenously.

(2) The 6 sheep that recovered from the "Mara" strain of heartwater each received 10 c.c. virulent "Mara" heartwater blood intravenously. The 10 susceptible sheep which were inoculated with virulent "Mara" heartwater blood in experiment 8 also served as controls in this experiment.

Result.—(1) It will be seen from Table 9 that 2 of the "Strydom" heartwater recovered sheep were solidly immune after an interval of 9 months. The third animal which was tested after 10 months showed a febrile reaction and recovered. Both the susceptible sheep injected with the "Strydom" strain of heartwater reacted. One of them died, and in the smears prepared from the intima of the jugular vein *Rickettsia ruminantium* could be demonstrated.

(2) Five of the "Mara" recovered sheep were found to be solidly immune when tested at intervals of 28 to 58 months after the primary infection. In the remaining sheep a mild febrile reaction due to heartwater was observed when tested after 34 months.

Conclusions.—Out of the 9 sheep 7 were found to be solidly immune for periods varying from 9 to 58 months. Febrile reactions were noticed in 2 sheep at 10 and 34 months after the primary reaction.

Experiment 5 (S. 5507, S. 5527, S. 5623, S. 5627, S. 5961 and S. 6086).—Immunity test in sheep that recovered from "Mara" strain of heartwater.

| D.O.B. Number of Sheep. | Date of Injection. | Heart-water Strain. | Result. | Interval in Days between Artificial Infection and Immunity Test. | Heart-water Strain. | Date of Injection. | Dose of Blood i.v. | Immunity Test. | Result. |
|-------------------------|----------------------|---------------------|--|--|---------------------|--------------------|--------------------|---|---------|
| 40962 | 27/10/34 14/12/34 | "Mara" "Mara" | Recovered from heartwater No reaction after second injection..... | 710 | "Mara" | 4/12/36 | 10 c.c. | No reaction. | |
| 40184 | 20/ 2/35 15/ 6/35 | "Mara" "Mara" | Recovered from heartwater No reaction after second injection..... | 537 | "Mara" | 4/12/36 | 10 c.c. | No reaction. | |
| 41016 | 27/10/34 | "Mara" | Recovered from heartwater | 768 | "Mara" | 4/12/36 | 10 c.c. | On the 7th day after injection sheep showed a febrile reaction which lasted for 5 days. The highest temperature recorded was 107 F. This febrile reaction in all probability was due to heartwater. | |
| 39185 | 11/ 4/35 15/ 5/35 | "Mara" "Mara" | Recovered from heartwater No reaction after second injection..... | 568 | "Mara" | 4/12/36 | 10 c.c. | No reaction. On the 12th day after injection 10 c.c. blood of this sheep was injected into 2 susceptible heartwater sheep 46420 and 43391 in order to ascertain whether any heartwater "virus" was circulating in the blood stream. No reactions however were noticed. | |
| 47051 | 22/12/34 | "Mara" | Recovered from heartwater | 762 | "Mara" | 4/12/36 | 10 c.c. | On the 9th day after injection sheep showed a mild febrile reaction which lasted for 4 days. The highest temperature recorded was 104 F. On the 4th day of the reaction 10 c.c. blood was injected into sheep 46121 and 46691. Both showed a febrile reaction on the 16th day due to "virus A." On testing the immunity of these two sheep against heartwater they both reacted and died from heartwater. | |
| 41587 | 15/ 6/35 | "Mara" | Recovered from heartwater | 373 | "Mara" | 23/ 6/36 | 10 c.c. | No reaction. | |
| 41897 | 14/ 1/35 | "Mara" | Recovered from heartwater | 525 | "Mara" | 23/ 6/36 | 10 c.c. | No reaction. | |
| 41524 | 22/11/35 | "Mara" | Recovered from heartwater | 578 | "Mara" | 23/ 6/36 | 10 c.c. | No reaction. | |

IMMUNITY IN HEARTWATER.

TABLE 6.
Experiment 6 (S.4377 and S.6095).—To test the immunity of sheep that recovered from "S.4377" strain against "Mara" strain.

| D.O.B. Number of Sheep. | Date of Injection. | Heart-water Strain. | Reaction. | Interval in Days between Infection and Immunity Test. | IMMUNITY TEST. | | | |
|-------------------------|--------------------|---------------------|---------------------------|---|---------------------|--------------------|--------------------|--|
| | | | | | Heart-water Strain. | Date of Injection. | Dose of Blood I.V. | |
| 39295 | 2/4/35 | "S. 4377" | Recovered from heartwater | 610 | "Mara" | 3/12/36 | 10 c.c. | No reaction. On the 12th day after injection, blood of sheep 39295 was injected into two susceptible heartwater sheep in order to ascertain whether heartwater virus was circulating in the blood, although no febrile reaction was observed. Both s.b.inoculated sheep 46108 and 47029 did not react. |
| 39158 | 2/4/35 | "S. 4377" | Recovered from heartwater | 610 | "Mara" | 3/12/36 | 10 c.c. | On the 8th day after injection sheep showed a febrile reaction which lasted for 6 days. Highest temperature recorded 105° F. On the 5th day of the reaction blood was injected into sheep 40063 which reacted to heartwater and died, and into another susceptible heartwater sheep 46715 which did not react; subsequently on testing the immunity with known virulent heartwater virus sheep 46715 reacted and recovered. Sheep 39158 therefore showed a febrile reaction due to heartwater. |

IMMUNITY IN HEARTWATER.

Experiment 7 (a) and 7 (b) (S.4377, S.5722 and S.6045

| D.O.B. Number of Sheep. | Date of Injection. | Heartwater Strain. | Result. |
|----------------------------------|-----------------------|-----------------------|--------------------------------|
| 40936 | 28/ 8/34 | " S. 4377 "..... | Recovered from heartwater..... |
| 41107 | 19/10/34 | " S. 4377 "..... | Recovered from heartwater..... |
| 41552 | 14/12/34 | " S. 4377 "..... | Recovered from heartwater..... |
| 41557 | 3/12/34 | " S. 4377 "..... | Recovered from heartwater..... |
| 39205 | 10/ 9/34 | " S. 4377 "..... | Recovered from heartwater..... |
| 39853 | 28/ 9/34 | " S. 4377 "..... | Recovered from heartwater..... |

p269-270b →

269-270a

TABLE 7.

ment 7 (a) and 7 (b) (S.4377, S.5722 and S.6045).—To test the immunity in sheep that recovered from “S.4377” str

| FIRST IMMUNITY TEST. | | | | |
|--|--------------------|--------------------|--------------------|-----------------|
| Interval in Days between Artificial Infection and Immunity Test. | Heartwater Strain. | Date of Injection. | Dose of Blood i.v. | Result. |
| 329 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |
| 236 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |
| 211 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |
| 222 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |
| 306 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |
| 288 | “Mara”..... | 13/ 7/35 | 10 c.c. | No reaction.... |

← 269-270a

p269-270c →

269-270b

BLE 7.

sheep that recovered from "S.4377" strain against "Mara" heartwater strain.

| SECOND IMMUNITY TEST. | | | | | |
|--|---|--------------------|--------------------|--------------------|---|
| Interval in Days between Artificial Infection and 2nd Immunity Test. | Interval in Days between 1st and 2nd Immunity Test. | Heartwater Strain. | Date of Injection. | Dose of Blood i.v. | Result. |
| 773 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | No reaction. |
| 680 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | No reaction. |
| 655 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | No reaction. |
| 666 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | No reaction. |
| 750 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | On the 20th day after the injection sheep showed a febrile reaction which lasted 5 days. On further tests it was found that this reaction was produced by "virus A." |
| 732 | 444 | "Mara"..... | 30/9/36 | 10 c.c. | On the 8th day after injection sheep showed a febrile reaction which lasted for 12 days. The highest temperature recorded was 105.2° F. On the 10th day of the reaction blood was injected into 2 susceptible sheep 46439 and 45823. Both these animals developed heartwater and died. The febrile reaction in the sheep therefore was due to heartwater. |

← 269-270b

269-270c

0-270

Experiment 8.—To test the duration of

| D.O.B. Number of Sheep. | Injected with. | Expt. S. | Date of Injection. | Incub. Period in Days. | Duration of Disease in Days. |
|-------------------------------|----------------------|----------|--------------------------|------------------------------|--|
| 51424 | Mara HW. strain..... | 6103 | 19/ 4/39 | 12 | 17 |
| 50059 | Mara HW. strain..... | 6599 | 13/ 2/39 | 10 | 12 |
| 51449 | Mara HW. strain..... | 6571 | 13/ 2/39 | 17 | 14 |
| 52748 | Mara HW. strain..... | 6607 | 21/ 1/39 | 10 | 15 |
| 52795 | Mara HW. strain..... | 6607 | 21/ 1/39 | 10 | 14 |
| 52632 | Mara HW. strain..... | 6571 | 9/ 1/39 | 11 | 8 |
| 51386 | Mara HW. strain..... | 6559 | 21/11/38 | 7 | 15 |
| 52126 | Mara HW. strain..... | 6531 | 10/11/38 | 7 | 12 |
| 51683 | Mara HW. strain..... | 6531 | 31/10/38 | 7 | 14 |
| 41498 | Mara HW. strain..... | 5527 | 28/ 7/38 | 11 | 10 |
| 49384 | Mara HW. strain..... | 5527 | 1/ 6/38 | 8 | 17 |
| 49979 | Mara HW. strain..... | 5527 | 27/ 6/38 | 8 | 7 |
| 49929 | Mara HW. strain..... | 5527 | 14/ 6/38 | 10 | 12 |
| 50384 | Mara HW. strain..... | 5527 | 21/ 5/38 | 6 | 20 |
| 49681 | Mara HW. strain..... | 5527 | 11/ 5/38 | 6 | 22 |
| 49386 | Mara HW. strain..... | 5527 | 19/ 4/38 | 9 | 11 |
| 50319 | Mara HW. strain..... | 6400 | 19/ 4/38 | 8 | 10 |
| 50306 | Mara HW. strain..... | 5527 | 26/ 3/38 | 10 | 17 |
| 50360 | Mara HW. strain..... | 5527 | 9/ 2/38 | 9 | 20 |
| 49876 | Mara HW. strain..... | 5527 | 12/ 1/38 | 6 | 21 |
| 46124 | Mara HW. strain..... | 6031 | 15/ 4/37 | 9 | 14 |
| 42531 | Mara HW. strain..... | 6031 | 1/ 3/37 | 10 | 13 |
| 43482 | Mara HW. strain..... | 6031 | 1/ 3/37 | 12 | 11 |
| 45747 | Mara HW. strain..... | 5527 | 19/ 2/37 | 8 | 19 |
| 46369 | Mara HW. strain..... | 5527 | 8/ 2/37 | 10 | 21 |
| 43391 | Mara HW. strain..... | 6096 | 8/ 2/37 | 8 | 15 |
| 46715 | Mara HW. strain..... | 6095 | 8/ 2/37 | 10 | 12 |
| 47029 | Mara HW. strain..... | 6095 | 8/ 2/37 | 9 | 8 |
| 43755 | Mara HW. strain..... | 6104 | 7/ 1/37 | 11 | 14 |
| 47078 | Mara HW. strain..... | 5527 | 30/ 9/36 | 9 | 24 |
| 46230 | Mara HW. strain..... | 6011 | 10/ 9/36 | 7 | 15 |
| 44586 | Mara HW. strain..... | 5623 | 9/ 3/36 | 7 | 12 |

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TABLE 8.

Experiment 8.—To test the duration of immunity in sheep that recovered from the "Mara" strain of heartwater.

| Result. | Interval between Infection and Immunity Test. | | Injected with. | Date. | IMMUNITY |
|----------------|---|-------------------------------|----------------------|----------|----------|
| | Number of Days. | Approximate Number of Months. | | | |
| Recovered..... | 104 | 3 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 168 | 5 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 168 | 5 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 191 | 6 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 191 | 6 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 203 | 7 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 252 | 8 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 263 | 9 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 274 | 9 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 369 | 12 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 395 | 13 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 401 | 13 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 412 | 13 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 437 | 14 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 447 | 15 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 468 | 15 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 468 | 15 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 492 | 16 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 538 | 18 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 565 | 19 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 837 | 28 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 882 | 29 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 882 | 29 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 893 | 29 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 903 | 30 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 903 | 30 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 903 | 30 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 903 | 30 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 935 | 31 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 1,035 | 34 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 1,054 | 35 | Mara HW. strain..... | 31/ 7/39 | |
| Recovered..... | 1,239 | 41 | Mara HW. strain..... | 31/ 7/39 | |

← 271-272a

271-272c →

271-272b

from the "Mara" strain of heartwater.

IMMUNITY TEST.

| Date. | Incub. Period in Days | Duration of Disease in Days. | Remarks. |
|----------|-----------------------|------------------------------|---|
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | 11 | 11 | Febrile reaction 108° F. No clinical symptoms—Recovered. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | 10 | 10 | Febrile reaction 107° F. No clinical symptoms.—Recovered. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | 13 | 8 | Febrile reaction 107° F. No clinical symptoms—Recovered. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | 17 | 8 | Febrile reaction 107.4° F. No clinical symptoms.—Recovered. |
| 31/ 7/39 | 11 | 3 | Febrile reaction 105° F. No clinical symptoms. Recovered. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |
| 31/ 7/39 | — | — | No reaction. |

← 271-272b

TABLE 8 (a).

The infection with heartwater of the 13 control sheep which were kept together with the heartwater recovered sheep in a camp.

| D.O.B. Number of Sheep. | Injected with Heartwater Strain. | Expt. S. | Date of Injection. | Incubation Period in Days. | Duration of Disease. | Remarks. |
|-------------------------|----------------------------------|----------|--------------------|----------------------------|----------------------|--|
| 40955 | "Mara" | 5263 | 31/7/39 | 11 | 6 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 42993 | "Mara" | 5263 | 31/7/39 | 8 | 8 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 43126 | "Mara" | 5263 | 31/7/39 | 8 | 7 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 45956 | "Mara" | 6031 | 31/7/39 | 8 | 7 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 40306 | "Mara" | 6730 | 31/7/39 | 9 | 10 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 47071 | "Mara" | 6730 | 31/7/39 | 10 | 8 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 48769 | "Mara" | 6730 | 31/7/39 | 11 | 7 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 45928 | "Mara" | 6730 | 31/7/39 | 10 | 5 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 49050 | "Mara" | 6730 | 31/7/39 | 11 | 4 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |
| 55452 | "Mara" | 6711 | 31/7/39 | 7 | 15 | Recovered. |
| 54526 | "Mara" | 6711 | 31/7/39 | 8 | 15 | Recovered. |
| 54913 | "Strydom" | 6710 | 31/7/39 | 8 | 12 | Recovered. |
| 55536 | "Strydom" | 6710 | 31/7/39 | 9 | 7 | Died. <i>Rickettsia ruminantium</i> found in the intima smears |

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Experiments 9 (a) and 9 (b).—To test the

| D.O.B. Number of Sheep. | Injected with. | Expt. S. | Date of Injection. | Incub. Period in Days. | Duration of Disease in Days. | Result. |
|----------------------------------|----------------------------|-------------|-----------------------|---------------------------------|---------------------------------------|---------------------|
| 51473 | Strydom HW. strain | 6547 | 20/10/38 | 8 | 10 | Recovered |
| 51631 | Strydom HW. strain | 6547 | 20/10/38 | 10 | 9 | Recovered |
| 52794 | Strydom HW. strain | 6547 | 8/10/38 | 10 | 8 | Recovered |
| 46098 | Mara HW. strain | 6031 | 3/ 3/37 | 13 | 15 | Recovered |
| 45974 | Mara HW. strain | 6020 | 16/10/36 | 13 | 15 | Recovered |
| 43659 | Mara HW. strain | 6020 | 18/ 9/36 | 6 | 9 | Recovered |
| 43749 | Mara HW. strain | 5688 | 23/ 6/35 | 8 | 13 | Recovered |
| 41021 | Mara HW. strain | 5688 | 23/ 4/35 | 8 | 12 | Recovered |
| 41016 | Mara HW. strain | 5507 | 27/10/34 | 13 | 14 | Recovered |

275-276b 

275-276a

TABLE 9.

(a) and 9 (b).—To test the duration of immunity in sheep that recovered from the "Mara" and "Strydom" strain of heartwater.

| Interval in Days between Injection and 1st Immunity Test. | FIRST IMMUNITY TEST. | | | Interval between Injection and 2nd Immunity Test. | |
|---|------------------------|--------------------|--------------------------|---|-------------------------------|
| | Injected with. | Date of Injection. | Result. | Number of Days. | Approximate Number of Months. |
| 32 | Mara HW. strain..... | 21/11/38 | Reacted to virus "A".... | 284 | 9 |
| 32 | Mara HW. strain..... | 21/11/38 | Reacted to virus "A".... | 284 | 9 |
| 44 | Mara HW. strain..... | 21/11/38 | No reaction..... | 296 | 10 |
| 42 | Mara HW. strain..... | 15/ 4/37 | No reaction..... | 837 | 28 |
| 36 | Mara HW. strain..... | 21/11/36 | No reaction..... | 1,018 | 34 |
| 64 | Mara HW. strain..... | 21/11/36 | No reaction..... | 1,046 | 35 |
| 23 | Northam HW. strain.... | 15/ 7/35 | No reaction..... | 1,475 | 49 |
| 68 | Northam HW. strain.... | 29/ 6/35 | No reaction..... | 1,560 | 52 |
| 769 | Mara HW. strain..... | 4/12/36 | No reaction..... | 1,738 | 58 |

← 275-276a

275-276c →

275-276b

and "Strydom" strain of heartwater.

| SECOND IMMUNITY TEST. | | | | |
|-------------------------|--------------------|----------------|----------------------|--|
| Injected with. | Date of Injection. | Incub. Period. | Duration of Disease. | Result. |
| Strydom HW. strain..... | 31/7/39 | — | — | No reaction. |
| Strydom HW. strain..... | 31/7/39 | — | — | No reaction. |
| Strydom HW. strain..... | 31/7/39 | 16 | 3 | Febrile reaction 106° F. No clinical symptoms. |
| Mara HW. strain..... | 31/7/39 | — | — | No reaction. |
| Mara HW. strain..... | 31/7/39 | 11 | 3 | Febrile reaction 105·6. No clinical symptoms. |
| Mara HW. strain..... | 31/7/39 | — | — | No reaction. |
| Mara HW. strain..... | 31/7/39 | — | — | No reaction. |
| Mara HW. strain..... | 31/7/39 | — | — | No reaction. |

APPENDIX II.

B. TO DETERMINE HOW THE IMMUNITY IS MAINTAINED IN HEARTWATER.

Experiment 10 (S.6123).

Object.—To ascertain whether sheep that have recovered from heartwater will show a relapse to this disease after splenectomy.

Method. 1. Five sheep which are mentioned in the appended Table 10 (a) were splenectomized by Dr. Quiñan, of this Institute. Two of these animals had been infected artificially with heartwater two months previously, two approximately three months previously and one which was exposed to a natural infection 16 months ago had been subsequently reinfected with blood three and a half months before it was operated on.

2. Blood from two sheep 45972 and 46053 was injected into susceptible sheep before the operation and subsequently at varying intervals up to 45 days after the removal of the spleen.

3. The temperatures were recorded twice daily.

4. Blood smears were examined daily for the appearance of blood parasites.

Result. The details of the observations are mentioned in Tables 10 (a) to 10 (c). It will be noticed that sheep 37862 showed a relapse to *Ep. oris*, sheep 35004 and 37362 relapsed to *A. oris* and sheep 45972 and 46053 showed relapses to both *A. oris* and *Th. oris*. In none of these splenectomized animals, however, was a heartwater reaction noticed. Of the 10 sheep which were injected with blood of sheep 46053, seven reacted to *A. oris*, one to *A. oris* and *Ep. oris*, and two showed no blood parasites. No reactions to heartwater were noticed in any of these sheep. A heartwater immunity test showed all the sheep to be fully susceptible.

Of the 10 sheep which had been injected with blood from 45972, six reacted to *A. oris*, two reacted to *Ep. oris* and *A. oris*, one reacted to *Ep. oris*, and one reacted to *A. oris* and to heartwater. A heartwater immunity test gave reaction in all the sheep except in sheep 43089, which had reacted previously to heartwater after receiving blood from sheep 45972 on the ninth day after splenectomy.

Conclusion. The splenectomy of heartwater recovered sheep resulted in a relapse to *A. oris*, *Ep. oris* and *Th. oris*, but not to heartwater. The presence of circulating heartwater "virus" in sheep 45972 was demonstrated by the subinoculation of blood into sheep 43089 on the ninth day after splenectomy. The concentration of "virus" in this sheep must have been very low, because sheep 54832, which was injected at the same time as 43089, failed to react.

Experiment 11 (S.6400, S.6437 and S.6403).

Object.—To ascertain how long recovered heartwater sheep may remain carriers of *Rickettsia ruminantium*.

Method.—For this experiment three sheep which had recovered from an artificial infection with the "Mara" strain of heartwater were used as indicated in the subjoined Table 11.

(a) Sheep 48596 was destroyed approximately four and a half months after receiving the infective dose of heartwater blood. Organ emulsions in saline prepared from the brain, liver, kidney, spleen, the endothelial cells of the jugular vein and blood were injected into susceptible sheep.

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(b) Sheep 48360 was destroyed approximately four months after being infected with heartwater "virus". Blood, organ emulsions, and the endothelial cells of the jugular veins were injected into sheep.

(c) Blood of sheep 48021 was injected into several sheep 63 and 69 days after being infected with heartwater.

Result.—(a) None of the sheep injected from 48596 reacted to heartwater. The two sheep which received the injection of blood, reacted to "virus A". These sheep were later found to be susceptible to heartwater. The sheep which received the organ emulsions were found to be susceptible to "virus A". Their susceptibility to heartwater was not tested.

(b) No heartwater reactions were observed in any of the sheep injected from 48360. Four of the sheep reacted and died as the result of a heartwater immunity test.

(c) The sheep injected from sheep 48021 failed to react to heartwater. In one of them, however, a reaction to "virus A" was observed. This sheep and another one were subjected to a heartwater test. Both of them reacted and recovered.

Conclusion. It was not possible to demonstrate the presence of *Rickettsia ruminantium* by the subinoculation of blood, endothelial cells of the jugular vein and organ emulsions from the three recovered sheep after a period of 63 to 135 days after receiving an infective dose of heartwater. Two of the sheep were found to be carriers of the "virus A".

Table 10 (a).
The splenectomy of heartwater recovered sheep.

| D.O.B. Number of Sheep. | Date of Injection. | HEARTWATER REACTION. | | Interval in Days between Injection and Splenectomy. | Date. | Result. |
|-------------------------|---|----------------------------|---|---|------------|---|
| | | Incubation Period in Days. | Reaction. | | | |
| 35004 | 30/10/33 and 1/1/33..... | 8 | Typical heartwater reaction from 6/11/33-17/11/33 | 98 | 6/ 2/34 | Relapse to <i>A. ovis</i> . No indication of a relapse to heartwater. |
| 37802 | 30/10/33 and 1/11/33..... | 10 | Typical heartwater reaction from 9/11/33-20/11/33 | 100 | 8/ 2/34 | Relapse to <i>Ep. ovis</i> . No indication of a relapse to heartwater. |
| 37362 | Exposed to natural infection of heartwater at Mara from 3/10/34-15/2/35 Injected with heartwater blood 23/6/36 | — | No clinical symptoms seen | 595 | } 13/10/36 | Relapse to <i>A. ovis</i> . No relapse to heartwater. |
| | | | No reaction..... | 112 | | |
| 45972 | 11/11/36..... | 7 | Typical heartwater reaction from 18-29/11/36 | 62 | 12/ 1/37 | Relapse to <i>A. ovis</i> and <i>Th. ovis</i> . No relapse to heartwater. Blood subinoculated at varying intervals after splenectomy, see Table 8b. |
| 46053 | 11/11/36..... | 7 | Typical heartwater reaction from 18-29/11/36 | 62 | 12/ 1/37 | Relapse to <i>A. ovis</i> and <i>Th. ovis</i> . No relapse to heartwater. Blood subinoculated at varying intervals after splenectomy. See Table 8c. |

Table 10 (b).
Subinoculation of blood from splenectomized sheep 45972 mentioned in Table 10 (a).

| D.O.B. Number of Sheep. | Date of Injection. | Interval in days after Splenectomy. | Dose of Blood Intrav. | Incubation Period. | Result. | Heartwater Immunity Test. |
|-------------------------|--------------------|--|-----------------------|--------------------|---|---|
| 42385 43395 | 12/1/37 12/1/37 | Subinoculation before splenectomy Subinoculation before splenectomy | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> | Reacted and recovered. Reacted and died. |
| 43089 | 21/1/37 | 9 | 10 c.c. | 13 days | Reacted to heartwater and recovered. Also reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> | The immunity of this sheep was tested twice but no reaction to heartwater was observed. Reacted and recovered. |
| 45832 | 21/1/37 | 9 | 10 c.c. | — | — | — |
| 47047 47066 | 30/1/37 30/1/37 | 18 18 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> and <i>Ep. ovis</i> . | Reacted and died. Reacted and recovered. |
| 46210 46238 | 8/2/37 8/2/37 | 27 27 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> . Died from an inter-current infection 7 weeks later | Reacted and died. — |
| 46194 47040 | 26/2/37 26/2/37 | 45 45 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> and <i>Ep. ovis</i> . Reacted to <i>Ep. ovis</i> | Reacted and died. Reacted and died. |

Table 10 (c).
 Subinoculation of blood from splenectomized sheep 46053 mentioned in Table 10 (a).

| D.O.B. Number of Sheep. | Date of Injection. | Interval in days after Splenectomy | Dose of Blood Intravenously. | Incubation Period. | Result. | Heartwater Immunity Test. |
|-------------------------|--------------------|--|------------------------------|--------------------|---|---|
| 42282 43091 | 12/1/37 12/1/37 | Subinoculation before splenectomy Subinoculation before splenectomy | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> | Reacted and died. Reacted and recovered. |
| 45835 47079 | 21/1/37 21/1/37 | 9 9 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> | Reacted and died. Reacted and recovered. |
| 46259 47055 | 30/1/37 30/1/37 | 18 18 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> Reacted to <i>A. ovis</i> | Reacted and killed. Reacted and died. |
| 46430 46197 | 8/2/37 8/2/37 | 27 27 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> No reaction..... | Reacted and died. Reacted and died. |
| 46310 47008 | 26/2/37 26/2/37 | 45 45 | 10 c.c. 10 c.c. | — — | Reacted to <i>A. ovis</i> and <i>E.p. ovis</i> . No reaction..... | Reacted and died. Reacted and died. |

Table 11.
The injectivity of blood and organ emulsions of recovered heartwater sheep into susceptible sheep.

| D.O.B. Number of Sheep. | Injected from. | Date. | Dose i.v. | Organ. | Result. | Remarks. |
|-------------------------|----------------|---------|-----------------------------------|---|---|---|
| 50319 | 48596 | 1/ 2/38 | 50 c.c..... | Blood..... | Sheep did not react to heart-water. A febrile reaction was noticed on the 14th day and lasted for 8 days. This febrile reaction was due to "virus A." | Subinoculation of blood from 50319 into sheep 50279 produced a febrile reaction not due to heartwater. On testing the immunity of sheep 50319 against the "virus A." no reaction was produced, but when injected with heartwater blood sheep reacted and recovered. |
| 50342 | 48596 | 1/ 2/38 | 50 c.c..... | Blood..... | Sheep did not react to heart-water. A febrile reaction was noticed on the 14th day and lasted 12 days. This febrile reaction was due to "virus A." | Subinoculation of blood from 50342 into sheep 50291 produced a febrile reaction not due to heartwater. On testing the immunity of sheep 50342 against the "virus A." no reaction was produced, but when injected with heartwater blood it reacted and died. |
| 50289 | 48596 | 2/ 2/38 | — | Emulsion of endothelial scrapings from the jugular vein | Both sheep failed to react to heartwater | Both sheep reacted to "virus A." on testing their susceptibility. |
| 50315 | 48596 | 2/ 2/38 | — | Brain..... | Sheep did not react to heart-water. Sheep 50280 died 5 weeks later from an inter-current infection | Sheep 50331 was found to be susceptible to "virus A." |
| 50280 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Brain..... | Sheep did not react to heart-water. | On testing the immunity of these two sheep they were both found to be susceptible to "virus A." |
| 50331 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Kidney..... | Sheep did not react to heart-water | On testing the immunity of these two sheep they were both found to be susceptible to "virus A." |
| 50328 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Liver..... | Sheep did not react to heart-water | On testing the immunity of these two sheep they were both found to be susceptible to "virus A." |
| 50343 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Spleen..... | Sheep did not react to heart-water | On testing the immunity of these two sheep they were both found to be susceptible to "virus A." |
| 48044 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 50350 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 49945 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 50123 | 48596 | 2/ 2/38 | 10 c.c. of a 5 per cent. emulsion | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 40271 | 48360 | 4/11/37 | 50 c.c..... | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 41952 | 48360 | 4/11/37 | 50 c.c..... | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |

Table 11 (continued).

| D.O.B. Number of Sheep. | Injected from. | Date. | Dose i. v. | Organ. | Result. | Remarks. |
|-------------------------|----------------|---------|---------------------------------|---------------------------------------|---|--|
| 47942 | 48360 | 4/11/37 | — | Endoth. scrapings of the jugular vein | Sheep did not react to heart-water | — |
| 50038 | 48360 | 4/11/37 | — | Endoth. scrapings of the jugular vein | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 47914 | 48360 | 4/11/37 | 10 c.c. of 5 per cent. emulsion | Brain, liver, spleen, kidney | Sheep did not react to heart-water | — |
| 49116 | 48360 | 4/11/37 | 10 c.c. of 5 per cent. emulsion | Brain, liver, spleen, kidney | Sheep did not react to heart-water | — |
| 49910 | 48360 | 4/11/37 | 10 c.c. of 5 per cent. emulsion | Brain, liver, spleen, kidney | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 50253 | 48360 | 4/11/37 | 10 c.c. of 5 per cent. emulsion | Brain, liver, spleen, kidney | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 50306 | 48021 | 3/ 2/38 | 10 c.c. | Blood..... | Sheep did not react to heart-water | On testing the immunity of this sheep against heartwater it was found to be susceptible. |
| 50363 | 48021 | 3/ 2/38 | 10 c.c. | Blood..... | Sheep did not react to heart-water | — |
| 50347 | 48021 | 9/ 2/38 | 10 c.c. | Blood..... | Sheep did not react to heart-water | — |
| 50360 | 48021 | 9/ 2/38 | 10 c.c. | Blood..... | Sheep showed a febrile reaction due to "virus A." | On testing the immunity of this sheep against heartwater it was found to be susceptible. |