

## **Studies on Immunity in Heartwater.**

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### INTRODUCTION.

FROM the results of a series of experiments on the duration and nature of immunity to heartwater in sheep it was concluded (Neitz, 1939) that:—

1. since there is complete cross-immunity between ten strains of "virus" it is possible that all strains are antigenically similar; at least no plurality of virus strains has been demonstrated up to the present;
2. recovery from heartwater is followed by a temporary period of premunition, when "virus" may be detected in the peripheral circulation for periods up to sixty days;
3. the period of premunition is followed by a period of sterile immunity during which the degree of immunity declines at a rate which varies within very wide limits in different sheep;
4. reinfection during the period of partial immunity initiates a repetition of the cycle-premunition, sterile immunity, partial immunity.

These results are of considerable practical importance to the whole problem of mass immunization in the field, but were based upon meagre data for periods longer than two years after immunization, so that arrangements were made for a repetition or extension of the work.

### EXPERIMENTAL PROCEDURE.

Merino sheep which had recovered from heartwater in a variety of experiments were confined in a small camp together with a number of susceptible controls. Daily temperatures were not taken, but the animals were under regular observation and no illness was detected; there were no deaths from heartwater among the controls so that it is safe to assume that the animals were not exposed to natural reinfection while in the camp. In May 1942 all the sheep were transferred to the experimental out-station at Bestersput in the Free State where heartwater does not occur. From May 1942 until they were returned to Onderstepoort in February 1944 for immunity tests, it is certain that there was no exposure to natural heartwater. All the immunity tests were carried out in the sheep stable under the ordinary conditions of this type of experimental work.

*Chief characteristics of the strains used.*

1. The "Mara" strain was isolated from naturally infected sheep that were exposed on the government experimental station at Mara near Louis Trichardt, in the Northern Transvaal. The inoculation period in sheep is seldom less than seven days and usually about eleven days. It is a comparatively avirulent strain, as seen from the fact that during the period May 1940 to February 1944, out of 240 sheep used for maintenance of the virus by serial passage, 50 (= 24 per cent.) recovered without treatment.

2. The "Salem" strain was isolated from a naturally infected calf at Salem in the vicinity of Grahamstown in the Eastern Province. It is characterized by an incubation period somewhat longer than that of "Mara" and is rather less virulent. It was maintained for only a limited number of passages through sheep so that the figures of percentage mortality are of little significance.

3. The "Schalekamp" strain was recovered from naturally infected Merino sheep, that became infected on the farm Bankfontein close to Middelburg in the Eastern Transvaal. Its chief characteristics are an exceedingly short period of incubation (about six days), a rapid course, frequently lasting not more than 48 hours, and 100 per cent. mortality with the presence of enormous numbers of rickettsias in intima preparations of the jugular veins.

There is complete cross-immunity between these strains of "virus" providing the immunity tests are carried out within two months of defervescence of the febrile reaction.

*Immunity Tests.*

The test dose of virulent heartwater blood was 10 c.c. citrated blood given intravenously; the virulent blood was obtained from sheep at the height of the febrile reaction to the indicated strain in routine passage experiments. The temperatures of all sheep were checked daily. Two of the controls used had been running with the sheep at Onderstepoort and at Bestersput throughout the course of the experiment and were found to be fully susceptible. Full details of the results are given in the appendix while a summary of the significant features are given in Table I.

*Results.*—If the appendix is consulted in conjunction with Table I it will be noticed that three sheep were immune to the "Salem" strain and nine to the "Mara" strain when they were given an immunity test of "Mara" virus one or two months after the immunizing injection. The twelve sheep were solidly immune. For the purposes of the investigation into the duration of immunity the interval is calculated from the date of the injection for the immunity test. Incidentally this, together with the immunity tests on the two sheep after intervals of 46 and 48 months, where the "Schalekamp" strain was used, is additional evidence on the antigenic identity of all the strains examined up to the present time.

From Table 1 it is seen that there is a gradual but progressive decrease in the degree of immunity with the passage of time as shown by the increasing proportion of sheep which reacted to the immunity test. This becomes more apparent on consulting Table 2, which summarizes the combined results of Table 1 and those similar tests after shorter intervals previously reported by Neitz (1939). Apart from the one sheep which died



TABLE 1.  
*Duration of Immunity to Heartwater.*

Number of Sheep.	Interval Months.	Reaction.	Result.
1	31	—	1 Immune.
1	32	10/6.....	1 Reacted.
1	33	—.....	1 Immune.
3	34	— 7/9, 13/3.....	1 Immune, 2 Reacted.
3	35	— 11/6 11/7*.....	1 Immune, 2 Reacted.
3	36	8/8 11/7 11/6.....	3 Reacted.
5	38	— —* 15/2 13/2 10/7‡.....	2 Immune, 3 Reacted.
1	39	—.....	1 Immune.
1	41	—.....	1 Immune.
2	42	11/6 10/7.....	2 Reacted.
1	43	11/7.....	1 Reacted.
2	44	15/8* 12/4.....	2 Reacted.
1†	46	9/11*.....	1 Reacted.
1†	48	8/6.....	1 Reacted.

In the table the numerator = period of incubation, the denominator = duration of febrile reaction.

\* Strain "Schalekamp" for immunity test. All others strain "Mara".

† Indicates sheep from which subinoculations were made; the results are given in Table 3.

TABLE 2.  
*Summary of all tests on the duration of immunity.*

Interval, Months.	Number of Sheep.	Immunity Test.		
		Immune.	Reacted and Recovered.	Reacted and Died.
1-6.....	40	40	0	0
7-12.....	24	22	1	1
13-18.....	19	17	2	0
19-24.....	23	22	1	0
25-30.....	9	6	3	0
31-36.....	13	7	6	0
37-42.....	12	5	7	0
43-48.....	5	0	5	0
49-54.....	2	2	0	0
55-60.....	1	1	0	0
1-60.....	148	122	25	1

in the 7 to 12 months group, all the reactors showed only a febrile reaction of varying severity and duration, with or without some inappetence, but no clinical symptoms of heartwater whatsoever. From a clinical point of view an aetiological diagnosis could not have been possible in a single instance. No explanation can be offered for the death of the sheep in a group where the immunity should have been solid, but isolated instances of the failure of individual animals to develop an immunity have been encountered before.

A point of interest is the fact that it was possible by an *in vivo* test, to get some idea of progressive decrease in immunity. As a general rule where the period of incubation was short the subsequent reaction was greater. For instance where the temperature did not rise until the 13th or 15th day the subsequent reaction was very mild and lasted for only about 48 hours (c.f. 34 and 38 month groups) whereas where the temperature rose earlier the reaction was much more severe (c.f. later groups).

Briefly the results may be summarized by stating that, in sheep, immunity to heartwater as a result of recovery from infection is solid for at least 12 months after which there is a gradual decrease, but a very considerable degree of immunity exists for periods up to four years, at which time the immunity is still sufficient to protect against fatal infection.

In the previous report (Neitz, 1939) attention was directed to the extreme importance of determining whether reactions, characterized by only a fever and no pathognomonic clinical symptoms, were in fact specific reactions to heartwater virus and not to some other inadvertent contaminant. Consequently a series of subinoculations into heartwater immune and susceptible sheep were made as detailed in Table 3. Initially all the sheep were immune to strain "Mara" except one (59069) which had recovered from infection with "Salem" strain virus. For the immunity tests "Mara" strain was used except in two sheep (64165 and 68744) which received the "Schalekamp" virus. The test dose of virus was 10 c.c. given intravenously but for the subinoculations 20 c.c. of fresh citrated blood was used. All the non-reactors in the subinoculation tests subsequently were found to be susceptible to heartwater.

*Results.*—A number of most important results emanated from this experiment:—

1. Four sheep, which had recovered from infection between 34½ and 44 months previously, showed febrile reactions of varying degrees of severity when subjected to an immunity test and all recovered. Subinoculation of blood drawn during the febrile reaction produced heartwater in six susceptible sheep (5 died and 1 recovered) and no reaction in three sheep known to be immune to heartwater.
2. One sheep which had recovered 37½ months previously showed no reaction on immunity test. Subinoculation of blood drawn at a time when a reaction could have been anticipated, that is on the 13th day after injection, produced heartwater in a susceptible sheep and this sheep died.
3. Two sheep which had been immunized one and two months previously were solidly resistant to an immunity test but fully virulent virus was detectable in the blood on the 15th and 25th day after injection.



TABLE 3.

IMMUNITY TESTS.						SUB-INOCULATIONS.				
Number of Sheep.	Interval, Months.	P. of I., Days.	Duration, Days.	Result.	Interval, Days.	Number of Sheep.	History.	P. of I., Days.	Duration, Days.	Result.
57191	44	15	8	Reacted	18 18	69033 69050	Susceptible Susceptible	12 12	6 4	Died. Died.
59076	42	10	7	Reacted	13	69026 69034	Susceptible Immune	10 —	16 —	Recovered. No Reaction.
58773	2 38	— 10	— 7	No Reaction Reacted	— 13 13	— 69075 69118	— Susceptible Immune	— 9 —	— 13 —	— Died X. No Reaction.*
59358	1½ 37½	— —	— —	No Reaction No Reaction	— 13	— 69113	— Susceptible	— 11	— 5	— Died.
59069	1½ 34½	— 11	— 7	No Reaction Reacted	— 13 13	— 67450 67423	— Susceptible Immune	— 7 —	— 8 —	— Died. No Reaction.*
64165	8	—	—	No Reaction	0 11 13	68907 69061 68744	Susceptible Susceptible Susceptible	— 13 —	— 4 —	No Reaction. Died. No Reaction.*
67423	1 2 3 5	— — — —	— — — —	No Reaction No Reaction No Reaction No Reaction	— — — 13	— — — 68539	— — — Susceptible	— — — —	— — — —	— — — No Reaction.
69118	1½ 2	— —	— —	No Reaction No Reaction	— 13	— 67962	— Susceptible	— 15	— 6	— Died.
68744	1	—	—	No Reaction	14	69159	Susceptible	25	3	Died.

\* See subinoculation below.  
 Died X = Died with demonstration of *R. ruminantium* in incima smears = Heartwater.  
 P. of I. = Period of Incubation.

4. One sheep failed to show any reaction to immunity tests applied on three successive occasions at monthly intervals. Two months later a fourth immunity test was given; there was again no reaction and no virus was found to be circulating on the 13th day by subinoculation into a susceptible sheep.
5. Blood was subinoculated from one sheep which received an immunity test after an interval of eight months. No virus was present in the blood immediately before the immunity test or on the 13th day afterwards, but blood drawn on the 11th day produced a fatal case of heartwater in a susceptible sheep.

#### CONCLUSION.

From the results of this series of experiments it may be concluded:—

1. The febrile reactions indicating a partial break-down in immunity were due to heartwater "virus".
2. Fully virulent "virus" may be expected to circulate in the peripheral blood of immune sheep after injection at that time when it would be found in a reacting susceptible sheep, no matter whether a detectable reaction is produced or not.

#### DISCUSSION.

The previous report of gradual decrease in the degree of immunity in sheep which have recovered from an artificially induced attack of heartwater has been confirmed, but it has been shown that after a lapse of four years this immunity is sufficient to protect against fatal infection under laboratory conditions. Since it has been shown that in the majority of cases virus has disappeared from the peripheral blood by the end of the febrile reaction, though in exceptional cases it may persist for up to 60 days, the conception of immunity in heartwater as a premunition followed by a gradually diminishing sterile immunity appears to be acceptable. The most important observation, however, is the record of circulating virus following reinfection during the period of sterile immunity, even though the immunity may be sufficiently high to block out any reaction completely, thus indicating multiplication of rickettsias in an immune or partially immune animal. Apart, entirely, from the interest and theoretical importance of this observation in general, it is of immediate practical importance to the whole problem of the control of heartwater in the field. It has always been difficult to explain the continued presence of infection in a high percentage of ticks on any given farm on the assumption that larval and nymphal ticks of the genus *Amblyomma* became infected only by feeding on a reacting animal or for a short period after recovery. Still more difficult was it to explain the almost immediate incidence of heartwater amongst susceptible animals introduced on to farms when there had been no cases of heartwater for considerable lengths of time. This has led to the belief that some reservoir of infection, other than the susceptible domestic ruminants, played a major rôle in the maintenance of infection in the tick population. While such reservoirs may still await identification, it has become quite evident that the mere presence of sheep and presumably also cattle is entirely adequate for the maintenance of infection, and the rôle of any other reservoir may be quite insignificant.

The fact that the immune animal itself constitutes the reservoir of infection necessitates a modification of views on the methods of control. Elimination of the susceptible host by mass immunization will control mortality but



cannot assist in eliminating the disease. Total eradication can only be achieved by elimination of the tick, and, at least under present conditions in South Africa, this is not possible. The only solution is the development of a practical method of immunization. There is one consoling feature, namely that search for such a method need not be confined to a field dependent upon the use of an inactivated virus, because use of an active, though possibly attenuated vaccine, can have no material effect in increasing the infection in a given area.

It is necessary to point out that the observations have been made on sheep. It is essential that similar work should be carried out on cattle.

#### SUMMARY.

1. It has been confirmed that the immunity in sheep following recovery from infection comprises a short period of premunition, followed by a period of gradually decreasing sterile immunity.

2. Infection during the period of sterile immunity again initiates the cycle premunition, sterile immunity.

3. Circulating virus is detectable in the peripheral circulation of immune sheep following reinfection, whether a demonstrable reaction is produced or not.

4. Up to a period of four years after recovery—the limit of the experiment—the residual immunity is sufficient to protect against fatal infection in sheep under laboratory conditions.

5. The significance of these findings and their relation to the problem of the control of heartwater are discussed.

#### LITERATURE.

- NEITZ, W. O. (1939). The immunity in heartwater. *Onderstepoort Jnl.*, Vol. 13, No. 2, pp. 245-283.