

PART IV.

 THE INOCULATION OF *ANAPLASMA MARGINALE* (VARIETY *CENTRALE*) AS A MEANS OF PROTECTING CATTLE AGAINST ANAPLASMOSIS.

As already stated in another part of this article, after having realized that our strain of *Anaplasma marginale* (variety *centrale*) was less virulent than *Anaplasma marginale* proper, and having noticed that animals injected with variety *centrale* did not suffer at all, or at least not so severely as those injected with *marginale*, it followed, as a matter of course, to make use of the variety *centrale* injection for protective purposes. As far as South Africa is concerned, however, the problem of protecting imported animals against anaplasmosis alone does not meet all requirements, since all imported animals are also susceptible to South African redwater.

Accordingly, for practical purposes, it is necessary at the same time to inoculate such stock against both diseases. The question to be settled was to find whether it will be advisable (1) to inject anaplasmosis first and then redwater, or (2) vice versa, or (3) to inject both simultaneously.

The inoculation against redwater does not offer much difficulty, the subject having received full consideration from various investigators. *Stockman* has fully reported on the treatment with trypan blue, which he found to constitute a powerful drug for regulating the effect of the inoculation of redwater in the majority of cases, provided the injected animals are under proper observation, and particularly if the reaction is controlled by the microscope. It has been the experience that animals treated with trypan blue in the early stages of a redwater infection easily recover, and accordingly trypan blue enables us to check the reaction when it seems likely to develop into a severe attack. Animals treated in this way are undoubtedly immune, and they retain the parasites in their blood just as if they had gone through an attack of redwater without treatment. Attention must be drawn to the fact that care has to be exercised to ensure that the blood used for the transmission of *Babesia bigemina* does not contain other parasites, particularly the virulent strain of *Anaplasma marginale*.

The following two experiments were made on pure-bred Hereford heifers, all about nine months old, imported into the Transvaal by a private owner for breeding purposes, but before the animals came into the Laboratory stables they had been running in the high veld. They were examined on their arrival, and although the tick infection in the high veld is not a severe one, yet some ticks were found on a few of the animals.

EXPERIMENT NO. 7.—*To immunize thoroughbred freshly imported Hereford Heifers against redwater, and as soon as the reaction is finished against anaplasmosis.*

(a) *Blood of Heifers 926 and 932.*

NOTE.—Heifer 926 went through an attack of redwater as the result of the injection of blood of heifer 925 on the 20th January, 1910, and heifer 932 went through an attack of anaplasmosis [due to *Anaplasma marginale* (variety *centrale*)] as the result of the injection of blood of heifer 940 on the 29th May, 1911 [*vide* Experiment No. 2 (E)].

(A) *Hereford Heifer 1176.*

Treatment.—On the 3rd December, 1910, injected subcutaneously with 5 c.c. fresh blood of heifer 926.

Remarks.—A reaction followed from the 7th to 12th day, with a rise to 104·8 F. in the evening of the 8th day. *Babesia bigemina* were noted in rare numbers during the reaction.

Second Treatment.—Fourteen days after the first injection, i.e. the 17th December, 1910, heifer 1176 was injected subcutaneously with 5 c.c. blood of heifer 932 (anaplasmosis infection).

Remarks.—Some irregular temperatures were noted shortly after, but no high evening records. From the 27th day a slight reaction set in, reaching its maximum between the 52nd and 58th days, with 103° F. as the highest record on the 53rd and 54th days. Anaplasms appeared on the 27th day for the first time, and the count was as follows:—

Day.	Per cent.	Day.	Per cent.
27th.....	0·2	38th.....	0·6
28th.....	0·1	39th.....	1·5
30th.....	0·1	40th.....	0·2
32nd.....	0·3	41st.....	0·05
34th.....	0·3	45th.....	0·1
37th.....	0·7	48th.....	0·2

(B) *Hereford Heifer 1177.*

Treatment.—Injected as above.

Remarks.—A typical redwater reaction followed from the 4th to 11th days, with a temperature of 104° F. in the evening on three occasions. *Babesia bigemina* was found in fair numbers on several days during the reaction.

Second Treatment.—Injected on the 14th day with blood of heifer 932 (see heifer 1176).

Remarks.—Nothing particular happened and there was no indication of any febrile disturbance. The blood was repeatedly examined every second or third day, and anaplasms were found for the first time on the 28th day, infecting 0·1 per cent. of the red corpuscles; the figures the next few days were:—

Day.	Per cent.	Day.	Per cent.
30th.....	0·1	38th.....	1·7
32nd.....	0·1	39th.....	2·8
34th.....	0·1	40th.....	1·6
37th.....	0·7		

The lesions of a slight anisocytosis were noted to accompany the anaplasms.

(C) *Hereford Heifer 1178.*

Treatment.—Injected as above.

Remarks.—A reaction set in from the 8th day, lasting for five days, with the maximum temperature of 105° F. on the evening of the 11th day. *Babesia bigemina* were noted in rare numbers from the 9th to 12th days and again on the 18th day.

Second Treatment.—With blood of heifer 932 (see heifer 1176) on the 14th day.

Remarks.—Nothing particular happened until the 27th day after this second injection, when a distinct febrile reaction ensued, lasting for about thirty days, but on no occasion did the temperature register over 104° F. in the evening.

Anaplasms were detected for the first time on the 27th day. The figures relating to their frequency were:—

Day.	Per cent.	Day.	Per cent.
27th.....	0·1	38th.....	7·3
28th.....	0·1	39th.....	5·8
30th.....	0·6	40th.....	4·7
32nd.....	2·2	41st.....	1·4
34th.....	4·2	42nd.....	1·2
37th.....	8·6		

The day of the maximum temperature (39th) corresponded to the time when the anaplasms were very frequent. *Babesia bigemina* was also noted on one occasion.

The blood lesions began with anisocytosis, and it was not until the 56th day that some basophile erythrocytes were noticed.

EXPERIMENT No. 8.—*To immunize freshly imported Hereford Heifers against anaplasmosis and as soon as the reaction is finished against redwater.*

(a) *Blood of Heifers 932 and 926.*

NOTE.—Heifer 932 contracted anaplasmosis as a result of the injection of blood of heifer 940, and heifer 926 contracted redwater from heifer 925 [*vide* Experiment No. 7 (A)].

(A) *Hereford Heifer 1182.*

Treatment.—Injected on the 3rd December, 1910, with 5 c.c. fresh blood of heifer 932.

Remarks.—With the exception of a sudden rise to 104° F. in the evening of the 16th day, nothing particular was noticed; blood examinations gave negative results until the 23rd day; a reaction commenced on the 28th day; the morning temperatures were above normal and a few exacerbations in the evenings were noted. The anaplasma count was as follows:—

Day.	Per cent.	Day.	Per cent.
31st.....	3·9	42nd.....	0·3
32nd.....	2·4	44th.....	0·4
33rd.....	2·3	46th.....	0·1
34th.....	2·4	48th.....	0·5
35th.....	1·6	50th.....	0·5
36th.....	1·3	51st.....	0·5
37th.....	1·1	52nd.....	0·8
38th.....	0·7	53rd.....	0·5
39th.....	0·7	54th.....	0·3
40th.....	1·0	55th.....	0·1
41st.....	1·1	56th.....	0·1

The lesions of anisocytosis, polychromasia, and basophilia were noticed on the 34th day and remained for about five to six days.

Second Treatment.—Injected on the 40th day (12th January, 1911) with blood of heifer 926 (redwater infection).

Remarks.—A typical redwater reaction followed from the 9th day, with evening temperatures above 104° F., and reaching 106·4° F. on the 11th day, when *Babesia bigemina* appeared in fair numbers; the animal showed signs of distress and did not feed well. It was accordingly considered advisable to inject it with a 1 per cent. solution of trypan blue. The following day the

temperature had dropped to normal, and with the exception of a few irregular records, remained within normal limits.

(B) *Hereford Heifer* 1183.—Injected on the 3rd December, 1910, subcutaneously with 5 c.c. fresh blood of heifer 932 (anaplasmosis infection).

Remarks.—Some irregular reactions were again noted during the incubation time, but microscopical examination of the blood gave negative results.

On the 27th day a reaction started, with evening temperatures of 103° F. on several occasions, and lasting until the 45th day.

The blood examination was recommenced on the 29th day, when anaplasms were found, and two days later they infected 6 per cent. of the corpuscles. Their presence during the succeeding days was as follows:—

Day.	Per cent.	Day.	Per cent.
32nd.....	5·2	44th.....	1·1
33rd.....	6·8	46th.....	0·2
34th.....	4·3	48th.....	1·3
35th.....	2·3	50th.....	0·6
36th.....	4·9	51st.....	0·2
37th.....	3·5	52nd.....	0·5
38th.....	1·7	53rd.....	0·2
39th.....	0·7	54th.....	0·1
40th.....	1·1	55th.....	0·6
41st.....	0·8	56th.....	0·1
42nd.....	0·9		

On the 34th day anisocytosis appeared, and the following day polychromasia and basophilia were registered.

Second Treatment.—Injected on the 40th day (12th January, 1911) with 5 c.c. fresh blood of heifer 926 (redwater infection).

Remarks.—A slight reaction followed from the 8th to 13th days, with the presence of *Babesia bigemina* from the 12th to 16th days.

(C) *Hereford Heifer* 1184.

Treatment.—Injected on the 3rd December, 1910, subcutaneously with 5 c.c. fresh blood of heifer 932.

Remarks.—A slight reaction was noted during the incubation time, but the daily examination of blood proved the absence of any parasites or blood lesions.

A temperature reaction followed from the 20th to 44th days, during which time some evening rises were noted to 104° F., with an average record of 102° F. in the morning.

The blood examinations proved the presence of anaplasma in the following numbers:—

Day.	Per cent.	Day.	Per cent.
29th.....	Rare	42nd.....	0·5
31st.....	8·2	43rd.....	1·6
32nd.....	9·0	44th.....	0·4
33rd.....	10·1	46th.....	0·6
34th.....	12·3	48th.....	0·8
35th.....	9·7	50th.....	0·5
36th.....	8·7	51st.....	0·2
37th.....	7·7	52nd.....	0·6
38th.....	3·0	53rd.....	0·5
39th.....	2·0	54th.....	0·3
40th.....	2·2	55th.....	0·7
41st.....	1·0	56th.....	0·8

The lesions of anisocytosis were noted on the 36th day; polychromasia and basophilia were also registered, remaining for the next six days.

Second Treatment.—On the 40th day (12th January, 1911) this heifer was injected subcutaneously with 5 c.c. fresh blood of heifer 926 (redwater infection).

Remarks.—A slight febrile reaction followed, during which time *Babesia bigemina* was noted on five successive days (12th to 16th).

(b) *Blood of Heifers 937 and 926.*

NOTE.—Heifer 937 had been injected with blood of heifer 938 and contracted an attack of anaplasmosis due to *Anaplasma marginale* (variety *centrale*) [*vide* Experiment No. 2 (G)].

Heifer 926 was immune to redwater (*vide* Experiment No. 7).

(D) *Hereford Heifer 1179.*

Treatment.—Injected on the 3rd December, 1910, subcutaneously with 5 c.c. blood of heifer 937.

Remarks.—A slight febrile reaction ensued from the 7th to 18th days, during which time the blood was frequently examined, but with negative results.

The anaplasmosis reaction commenced about the 28th day and lasted until the 47th day. This reaction was quite distinct, but no high temperatures were noted.

The blood examination was undertaken daily from the 31st day, when anaplasms were present, infesting 0·2 per cent. of the red corpuscles. The following figures refer to their frequency during the reaction:—

Day.	Per cent.	Day.	Per cent.
32nd.....	0·1	42nd.....	8·1
33rd.....	2·5	43rd.....	5·2
34th.....	3·9	44th.....	3·6
35th.....	3·6	46th.....	1·9
36th.....	5·7	48th.....	0·6
37th.....	10·0	50th.....	0·9
38th.....	11·2	51st.....	0·4
39th.....	13·5	52nd.....	0·1
40th.....	9·8	53rd.....	0·1
41st.....	12·8		

The blood lesions only started with anisocytosis the day after the maximum infection of anaplasmosis had been reached.

They were succeeded by polychromasia and basophile cells on the two following days.

Second Treatment.—On the 40th day after the first injection (12th January, 1911) heifer 1179 was injected subcutaneously with 5 c.c. fresh blood of heifer 926 (redwater infection).

Remarks.—A definite reaction commenced nine days after this second injection of a fairly severe character, reaching 104° F. in the morning on three occasions and touching 105° F. in the evenings. *Babesia bigemina* appeared eleven days after injection, being fairly frequent in numbers the following day, and still more numerous on the 13th day. The animal showed symptoms of distress and refused to feed; it was therefore thought desirable to inject a 1 per cent. solution of trypan blue; the following day the parasites had disappeared and twenty-four hours later the temperature returned to normal.

(E) *Hereford Heifer 1180.*

Treatment.—Injected on the 3rd December, 1910, with 5 c.c. fresh blood of heifer 937.

Remarks.—A reaction set in from the 14th day, the temperature reaching 104° F. in the evening of the 19th and 20th days, and on the 19th, 21st, and 25th days *Babesia bigemina* was noted. This infection must be attributed either to natural infection by ticks or to the blood of heifer 937. As stated before, parasites might have been present in the blood of heifer 937, due to tick infestation, and the parasites escaped notice, but considering that the previous animal, which was injected in the same way and at the same time, did not show any infection, it is more likely that the appearance of *Babesia bigemina* is due to the ticks collected by the animals whilst in the high veld.

No other fever reaction developed as a result of the injection, although there were some indications of a slight curve; anaplasms were detected on the 31st day, infecting 0·1 per cent. of the corpuscles. Their presence was registered as follows :—

Day.	Per cent.	Day.	Per cent.
31st.....	0·1	41st.....	2·2
32nd.....	1·1	42nd.....	1·3
33rd.....	0·2	44th.....	1·8
34th.....	0·4	46th.....	0·7
35th.....	0·2	48th.....	0·4
36th.....	0·4	50th.....	0·3
37th.....	0·5	51st.....	0·2
38th.....	1·4	52nd.....	0·1
39th.....	1·0	53rd.....	0·1
40th.....	0·6	54th.....	0·1

Anisocytosis was noted on the date of the heaviest anaplasma infection (41st day).

Second Treatment.—On the 40th day (12th January, 1911) heifer 1180 was injected with blood of heifer 926.

Remarks.—With the exception of a slight rise in the evening of the 13th day, nothing unusual was noticed.

Babesia bigemina was noted but in very rare numbers on the same day.

(F) *Hereford Heifer* 1181.

Treatment.—Injected on the 3rd December, 1910, with 5 c.c. fresh blood of heifer 937.

Remarks.—A slight temperature disturbance was also noted in this animal from about the 21st day, when *Babesia bigemina* was noticed in the blood (for possible explanation of this phenomenon, see previous heifer—No. 1180).

The anaplasmosis reaction was also of a very mild nature, rarely exceeding 103° F. Anaplasms were noticed on the 31st day, and their frequency was recorded as follows :—

Day.	Per cent.	Day.	Per cent.
31st.....	0·1	41st.....	2·4
32nd.....	1·0	42nd.....	1·0
33rd.....	1·0	44th.....	1·7
34th.....	0·9	46th.....	1·7
35th.....	0·5	48th.....	3·6
36th.....	0·7	50th.....	2·9
37th.....	1·6	51st.....	2·3
38th.....	1·6	52nd.....	0·8
39th.....	1·0	53rd.....	2·1
40th.....	1·3	54th.....	1·2

Babesia bigemina was also noted occasionally during this reaction.

Second Treatment.—Heifer 1181 was injected on the 40th day (12th January, 1911) with 5 c.c. fresh blood of heifer 926.

Remarks.—No febrile reaction followed, but *Babesia bigemina* was noted on one occasion (24th day).

CONCLUSIONS.

Although these nine animals did not exactly take the course I anticipated, some showing an infection of *Babesia bigemina*, which cannot be accounted for with certainty, yet the experiment has demonstrated the fact that imported animals can be successfully inoculated against anaplasmosis with but little risk. It has also been shown that even a severe infection of *Babesia bigemina* can be safely controlled with the aid of trypan blue.

These animals were not tested later by the injection of *Anaplasma marginale* proper, or by the infestation of ticks, but were exposed to natural tick infection in the low veld of the Ermelo District, near the Swaziland border, and at the time of writing (31st August, 1911) have been exposed for six months without any ill effects.

EXPERIMENT NO. 9.—*To note whether animals which were inoculated in England with redwater could be exposed to natural infection shortly after their injection in South Africa with Anaplasma marginale (variety centrale).*

NOTE.—The particulars given in respect of the inoculation of five heifers, Nos. 1226, 1227, 1228, 1229, and 1230, against redwater in England are extracted from S. Stockman's notes.

(A) Sussex Heifer 1226.

First Treatment (England).—Had been injected with blood of an English heifer immune to South African redwater.

Remarks.—An attack of redwater followed, when *Babesia bigemina* were present, accompanied with basophilia.

NOTE.—This animal arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period.

(a) Blood of Heifer 932.

NOTE.—Heifer 932 contracted anaplasmosis (due to *Anaplasma marginale* (variety *centrale*) as the result of the injection of blood of heifer 940 on the 27th May, 1910 [*vide* Experiment No. 2 (E)].

Second Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1226 was injected subcutaneously with 5 c.c. fresh blood of heifer 932.

First Test.—Sixteen days later, i.e. 8th February, 1911, this heifer was infested with blue larvae collected off animals at Onderstepoort immune against anaplasmosis (Reference No. 469).

Second Test.—On the same date (8th February, 1911) this heifer was turned out to graze on land adjoining the Laboratory.

Remarks.—Irregular temperatures with high evening exacerbations followed. *Anaplasma marginale* (variety *centrale*) were detected in the blood thirty-one days after injection of blood of 932 (15th day of exposure), and increased in

numbers during the following days, but were never very frequent. The lesions of anisocytosis and rare polychromatic and basophile cells were recorded on the 40th day after injection; *Babesia bigemina* also put in an appearance on the following day.

By the 60th day after injection the temperature had returned to normal, and the engorged blue larval ticks had been collected in great numbers between the 39th and 50th days.

The heifer was kept on the pasture until the 3rd July, 1911 (145 days), when she was sent to Potchefstroom, and was still alive on the 31st August, 1911.

(B) *Sussex Heifer* 1227.

First Treatment (England).—Injected on four different occasions with blood of English heifers immune against redwater.

Remarks.—A reaction followed the first injection, but no piroplasms were detected in the blood until the 9th day after the second injection.

NOTE.—The animal arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period.

Second Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1227 was injected subcutaneously with 5 c.c. fresh blood of heifer 932.

First and Third Test.—Sixteen days later, i.e. 8th February, 1911, this heifer was infested with blue larvae the mothers of which were collected off animals at Onderstepoort immune against redwater and anaplasmosis (Reference No. 469).

Second Test.—On the same date (8th February, 1911) this heifer was turned out to graze on ground adjoining the Laboratory.

Remarks.—Irregular temperatures with high evening rises followed; from the 29th day after injection *Anaplasma marginale* (variety *centrale*) were noted in rare numbers, together with the lesions of anisocytosis.

The ticks dropped in great numbers from the 23rd to 35th days after infestation.

The heifer was kept under observation on the pasture until the 3rd July, 1911, when she was sent to Potchefstroom, and was still alive on the 31st August, 1911.

(C) *Sussex Heifer* 1228.

First Treatment (in England).—Injected on two different occasions with blood of English heifers immune against redwater.

Remarks.—A reaction followed the first injection, but *Babesia bigemina* were not detected until the 8th day after the second injection.

NOTE.—This animal arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period.

Second Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1228 was injected subcutaneously with 5 c.c. fresh blood of heifer 932.

First Test.—Sixteen days later, i.e. 8th February, 1911, this heifer was infested with blue larvae the mothers of which were collected off animals at Onderstepoort immune against redwater and anaplasmosis (Reference No. 469)

Second Test.—On the same date turned out to graze on land adjoining the Laboratory.

Remarks.—Irregular temperature reactions with evening exacerbations were noted from the date of exposure.

Anaplasms appeared for the first time about the 35th day after injection, but never in large numbers.

Babesia bigemina appeared on the 28th day after exposure and *Babesia mutans* were also detected, these latter probably being contracted from the ticks on the pasture.

The lesions of anisocytosis, accompanied with polychromatic and basophile cells, were also noted. Engorged blue larvae were collected in great numbers from the 23rd to 35th day after exposure.

The animal was kept on the pasture until the 3rd July, 1911, when she was sent to Potchefstroom, and was still alive on the 31st August, 1911.

(D) *Sussex Heifer 1229.*

First Treatment (England).—Injected three times with blood of English cattle immune against redwater.

Remarks.—A reaction followed the first injection, reaching 100° F. on the 8th day, and the following morning *Babesia bigemina* were found in very rare numbers.

NOTE.—This animal arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period.

Second Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1229 was injected subcutaneously with 5 c.c. fresh blood of heifer 932.

First Test.—Sixteen days later, i.e. 8th February, 1911, this heifer was infested with blue larvae whose mothers were collected off animals at Onderstepoort (Reference No. 469).

Second Test.—On the same date (8th February, 1911) the heifer was turned out to graze on land adjoining the Laboratory.

Remarks.—Irregular temperature reactions with evening exacerbations were noted from the date of exposure. A definite curve commenced from the 31st day after injection, when *Anaplasma marginale* (variety *centrale*) were found to be fairly frequent. The lesions of anisocytosis and basophilia were also registered during the following days. Numbers of engorged ticks were collected off the heifer between the 23rd and 35th days after exposure.

After the anaplasmosis reaction had finished, *Babesia bigemina* were noted on one occasion. The animal was kept on the pasture until the 3rd July, 1911, when she was sent to Potchefstroom, and was still alive on the 31st August, 1911.

(E) *Sussex Heifer 1230.*

First Treatment (England).—Injected on five different occasions with blood of English cattle immune to redwater.

Remarks.—No reaction followed until the 4th day after the fifth injection, when the temperature reached 103° F. in the evening, and *Babesia bigemina* remained present from the 4th to the 8th days.

NOTE.—This animal arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period.

Second Treatment.—On the 23rd January, 1911—fourteen days after arrival—heifer 1230 was injected subcutaneously with 5 c.c. fresh blood of heifer 932.

First Test.—Sixteen days later, i.e. 8th February, 1911, this heifer was infested with blue larvae collected off animals at Onderstepoort (Reference No. 469).

Second Test.—On the same date (8th February, 1911) this heifer was turned out to graze on land adjoining the Laboratory.

Remarks.—Some irregular temperatures with high evening records were noted soon after exposure; no definite reaction could be detected, but *Anaplasma marginale* (variety *centrale*) were noted in rare numbers thirty-one days after injection. The lesions of anisocytosis and polychromatic cells were also recorded. At intervals during the following days numbers of engorged ticks were collected off this heifer between the 23rd and 35th days after exposure. The animal was kept on the pasture until the 3rd July, 1911, when she was sent to Potchefstroom, and was still alive on the 31st August, 1911.

RESULTS.

The animals which were (1) inoculated in London against redwater, (2) after their arrival in Pretoria injected with *Anaplasma marginale* (variety *centrale*), (3) infested with ticks sixteen days later, and (4) on the same date turned out on to the veld, went through a mild anaplasmosis reaction, and survived any natural infection that might be conveyed either by the ticks artificially placed on or by the ticks which the animals picked up whilst grazing.

These results had to be expected, since the incubation time of *Anaplasma marginale* when inoculated is shorter than when it is transmitted by ticks (in the latter case the shortest incubation time averages fifty-five days). It had also to be expected, by reason of the previous experiments, that recovery from anaplasmosis due to *Anaplasma marginale* (variety *centrale*) would mean protection against a severe *Anaplasma marginale* infection. As a matter of fact, no secondary fever reactions occurred and no blood examinations were made.

Accordingly, for practical purposes, animals which are inoculated with *Anaplasma marginale* (variety *centrale*) can shortly afterwards be turned out on to grazing ground with safety, always provided, of course, that the tick infestation on the grazing ground is not a gross one.

EXPERIMENT NO. 10.—*To note the effect of a simultaneous injection of (1) blood of an animal immune against Babesia bigemina and (2) blood of an animal immune against anaplasmosis due to Anaplasma marginale (variety centrale).*

(a) *Blood of Heifers 926 and 932.*

NOTE.—Heifer 926 had been injected with blood of heifer 925 on the 20th January, 1910, and developed a pure attack of redwater. Her blood had frequently been used for testing purposes [*vide* Experiment No. 1 (D)]. Heifer 932 contracted anaplasmosis [due to *Anaplasma marginale* (variety *centrale*)] as the result of the injection of blood of heifer 940 [Experiment No. 2 (E)].

(A) *Sussex Heifer 931.*—An English heifer, which arrived at the Laboratory on the 13th December, 1909. She was immediately placed in a tick-free stable and temperatured twice daily. No deviations from normal were noted during the observation period (13th December, 1909, to 20th January, 1910).

She was used later on several occasions for experiments with ticks, but without any results.

Treatment.—Injected on the 28th February, 1911, subcutaneously with 50 c.c. defibrinated blood of heifer 932 and simultaneously with 15 c.c. fresh blood of heifer 926.

Remarks.—A reaction followed from the 12th to 18th days, during which *Babesia bigemina* were detected to be very frequent on the 16th day. Another reaction commenced on the 29th day, but of a very mild nature. *Anaplasma marginale* (variety *centrale*) were noted in the blood on several occasions, as well as anisocytosis, polychromatic, and basophile cells, in rare numbers.

(B) *Heifer 939.*—An English heifer, which arrived at the Laboratory on the 13th December, 1909. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during the observation period (13th December, 1909, to 7th March, 1910).

NOTE.—This animal had been infested with ticks on various occasions, but without results.

Treatment.—Injected on the 28th February, 1911, subcutaneously with 50 c.c. defibrinated blood of heifer 932 and simultaneously with 15 c.c. fresh blood of heifer 926.

Remarks.—A slight temperature reaction was noticed from the 10th to 15th days; parasites were not detected, but the lesions of anisocytosis appeared on the 18th day, accompanied with a slight polychromasia and basophilia; accordingly it must be concluded that the *Babesia bigemina* were so rare that they escaped notice.

On the 34th day anaplasms were present, and remained during the definite but slight reaction which lasted until the 46th day; anisocytosis, polychromasia, and basophilia were occasionally noted during this reaction.

(b) *Blood of Heifers 1212 and 1216.*

NOTE.—Heifer 1212 contracted an attack of anaplasmosis [due to *Anaplasma marginale* (variety *centrale*)] as a result of the injection of blood of heifer 932 [*vide* Experiment No. 2 (H)].

Heifer 1216 contracted redwater as the result of the injection of blood of heifer 926 on the 23rd January, 1911.

(C) *Sussex Heifer 1211.*—An imported English heifer, which arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. The temperature remained within physiological limits during the observation period (9th January, 1911, to 3rd March, 1911).

NOTE.—This animal had been injected on the 3rd March, 1911—fifty-three days after arrival—with 300 c.c. of a mixture in the proportion of 1 part of filtrated blood of heifer 1192 to 12 parts of normal saline solution. (Heifer 1192 at the time she was tapped for the injection of 1211 was suffering from a severe attack of anaplasmosis, the parasites infecting about 50 per cent. of the red corpuscles.) No reaction followed the injection.

Treatment.—Injected subcutaneously on the 24th April, 1911, with 5 c.c. defibrinated blood of heifer 1216 and simultaneously with 5 c.c. fresh blood of heifer 1212.

Remarks.—A sharp rise of temperature was noted on the 7th day, when *Babesia bigemina* were recorded as being fairly frequent. As the animal commenced to pass red urine, it was injected with a solution of trypan blue on the

8th day, and by the following morning the temperature had dropped, reaching normal two days later. Anisocytosis, polychromasia, and a slight basophilia were noted occasionally during the few following days.

From the 30th day a slight reaction ensued for a few days only; *Anaplasma marginale* (variety *centrale*), accompanied with polychromasia and anisocytosis, were noticed occasionally from the 50th to 65th days.

(D) *Sussex Heifer* 1215.—An imported English heifer, which arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No abnormal records were noted during this observation period (9th January, 1911, to 23rd January, 1911).

NOTE.—She had been infested with blue larval ticks on the 23rd January, 1911, and, as a result, developed spirochaetosis eighteen days later.

Treatment.—Injected subcutaneously on the 24th April, 1911, with 5 c.c. defibrinated blood of heifer 1216 simultaneously with 5 c.c. fresh blood of heifer 1212.

Remarks.—A reaction set in from the 5th day, with high temperatures, when *Babesia bigemina* were noted, becoming very frequent two days later. The heifer was injected with trypan blue on the 8th day, when the fever was at its height, and the temperature fell to normal two days later. A slight anisocytosis, with basophilia and polychromasia, were noted occasionally from the 14th to 21st days.

An irregular reaction was noted from the 35th day, when *Anaplasma marginale* (variety *centrale*) appeared in fairly large numbers; anisocytosis, basophilia, and polychromasia reappeared at intervals between the 42nd and 53rd day.

(E) *Heifer* 1219.—An imported English heifer, which arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable. No abnormal records were noted during the observation period (9th January, 1911).

NOTE.—This heifer was injected on the 20th February, 1911, with blood of a horse, as a result of which spirochaetes appeared in the blood nine days later, but without any blood lesions.

Treatment.—Injected on the 24th April, 1911, subcutaneously with 5 c.c. defibrinated blood of heifer 1216 and simultaneously with 5 c.c. fresh blood of heifer 1212.

Remarks.—A sharp rise in the temperature occurred on the 8th day, when *Babesia bigemina* were recorded as very frequent; red urine was passed on the following day.

The heifer was injected with trypan blue on the 8th day, causing the temperature to regain normal limits on the 10th day; anisocytosis and basophilia were occasionally noted between the 14th and 21st days.

From the 28th day to about the 40th day another reaction set in, during which *Anaplasma marginale* (variety *centrale*) were noted in rare numbers. They were also noticed at intervals after this reaction had concluded.

(F) *Heifer* 1214.—An English heifer, which arrived at the Laboratory on the 9th January, 1911. She was immediately placed in a tick-free stable and temperatured twice daily. No deviations from normal were recorded during the observation period (9th January, 1911, to 23rd January, 1911).

NOTE.—This heifer had been infested with brown larval ticks on the 23rd January, 1911, but with negative results.

Treatment.—Injected subcutaneously with 5 c.c. defibrinated blood of heifer 1215 and simultaneously with 5 c.c. fresh blood of heifer 1212.

Remarks.—A temperature reaction set in from the 6th day, reaching 106.6° F. in the evening of the 8th day; *Babesia bigemina* were noted at the commencement of the reaction, rapidly increasing in numbers until on the 8th day practically every corpuscle contained one or more parasites.

The animal was injected with trypan blue on the 8th day, but apparently it was too late, as the heifer died the following day of acute redwater.

RESULTS.

The simultaneous subcutaneous injection was followed in the first instance by the appearance of *Babesia bigemina*, and secondly by *Anaplasma marginale* (variety *centrale*). All the animals (except 1214) recovered from the redwater attack before anaplasmosis set in. In the experiment (No. 10) with blood of heifer 1216 the redwater reactions were rather severe. They could, however, be checked by the injection of trypan blue, and only one animal died; in this instance death could probably have been prevented if trypan blue had been injected one day earlier.

For practical purposes therefore, since the inoculation of redwater blood is not always quite safe, it will be advisable to control it, not only by the thermometer, but also by microscope, as in this way the multiplication of the *Babesia bigemina* can be checked by the injection of trypan blue.

CONCLUSIONS.

The simultaneous inoculation of redwater and anaplasmosis is both possible and practical. The animals recovered from the result of the redwater reaction before the anaplasmosis reaction set in, which latter then took its usual course.

SUMMARY OF CONCLUSIONS.

(1) The transmission of *Anaplasma marginale* is only possible with blood containing red corpuscles. Filtrated blood failed to produce the disease when inoculated, the injected animals not showing any lesions at all and proving susceptible to subsequent blood inoculation (heifer 1211).

(2) The incubation period after the injection of blood containing anaplasms varies in length and depends on the quantity of blood injected, being shorter after an injection of a large quantity of blood and after the anaplasms have passed through a number of animals.

(3) Distinction has to be made between varieties of anaplasms. In this article one variety has been distinguished as *Anaplasma marginale* and the other as *Anaplasma marginale* (variety *centrale*).

(4) The distinction is based (1) on the different position the two parasites take up within the red corpuscle; (2) on the difference in size, there being slightly smaller individuals in the *centrale* variety; (3) on the different virulency, the *centrale* variety having caused neither death nor any serious lesions; (4) on the fact that a recovery from an infection from *Anaplasma centrale* does not cause complete immunity.

(5) Anaplasmosis was transmitted in four instances by means of *Boophilus decoloratus* larvae, and once by *Rhipicephalus simus* larvae, the mothers of which ticks were collected off immune animals.

(6) The incubation times after tick infection varied from between a few days under two months to a few days over three months.

(7) It has been noticed in one case (heifer 935) that when a very heavy infestation of ticks is made, an animal may die as a result of loss of blood due to the repletion of the engorged females.

(8) The *Anaplasma centrale* infection, transmitted either by ticks or by inoculation, in no instance caused the death of any of the thirty-nine English heifers. Accordingly an inoculation with *Anaplasma marginale* (variety *centrale*) can be made use of as a practical method of inoculation against anaplasmosis.

(9) Recovery from *Anaplasma centrale* infection gives so much protection that a subsequent inoculation of *Anaplasma marginale* no longer causes death or any serious lesions.

(10) Animals which were immune to *Babesia bigemina* could easily be infected with anaplasmosis, either by means of ticks or by injection of blood.

(11) Animals which were immune to the anaplasma infection could easily be infected with *Babesia bigemina*, either by means of ticks or by blood inoculation.

(12) Injected animals can be exposed to natural infection before the *Anaplasma centrale* reaction has run its course. The anaplasmosis infection due to ticks having a long incubation time (55 to 100 days), will not develop severely in the inoculated animal, in which the disease runs with a shorter incubation time (16 to 40 days).

(13) All animals which have passed through an attack of anaplasmosis and redwater conveyed by inoculation and exposed to natural infection for over a year are still alive.

(14) The anaplasmosis transmitted by ticks was that of the type *centrale* and *marginale*.

(15) To judge by the blood smears obtained from the cattle exposed in the veld, a double infection is frequently met with.

(16) Animals immune to *Anaplasma* and *Babesia bigemina* infections could easily be infected with *Babesia mutans*.

(17) As an accidental occurrence in some instances the larval ticks of *Boophilus*, collected off immune cattle, transmitted an infection of *Spirochaeta theileri* in typical time. In one instance the inoculation with blood of a horse in which the spirochaetes were noted, the pure infection was transmitted to a susceptible animal.

(18) For the requirements of the conditions of South Africa, it is necessary to combine the immunization against anaplasmosis with an inoculation against redwater.

(19) The redwater inoculation can be done before or after the anaplasmosis inoculation, but it is practical to do both at the same time.

(20) The redwater, having a shorter incubation time, will develop first, and a recovery will usually be effected before the anaplasmosis reaction sets in.

(21) In applying this method to the practice, it is necessary to keep the animal during the redwater reaction (about fifteen days) under close observation and to check any unusual reaction by means of a trypan blue injection, and whilst the animal is undergoing the anaplasmosis reaction it will have to be well fed.