"B."—EXPERIMENTS WITH ENGLISH AND SOUTH AFRICAN REDWATER.

In order to overcome the mortality caused by ordinary redwater when exposing imported cattle on the South African veld, I decided to conduct some experiments in this connection, and was fortunate in obtaining the assistance of Mr. Stockman, Principal Veterinary Surgeon of England.

The experiments were performed with a three-fold object, namely, to test (1) whether the English and South African redwater are identical, (2) if English cattle immunised against English redwater would thereby acquire any immunity against the ordinary redwater of South Africa, and (3) if English heifers inoculated in England with South African redwater would be immune against our disease when exposed to natural infection in South Africa.

Accordingly six heifers were purchased on our behalf by Mr. Stockman, and were treated by him in England. One lot were injected with English redwater, the second batch with South African redwater, and the remaining two heifers with both English and South African redwater.

I am greatly indebted to Mr. Stockman for making careful examinations of these animals, and it is from his notes that the following particulars of temperatures and blood examinations have been obtained:—

Experiment No. 1.

English heifers injected with English redwater.

"*A*," *Heifer* 428.—Injected in England subcutaneously on the 25th July, 1906, with 5 c.c. defibrinated blood of a heifer (which had previously been inoculated with blood obtained from a natural case of redwater in Hampshire, but which did not react in any marked degree, and it is highly probable that she did not contract the infection).

Heifer 428 did not shew any reaction, and all smears examined from the date of injection to the 1st September gave negative results.

On the 1st September, 1906, 428 received an injection of 10 c.c. defibrinated blood from an English cow which had recovered from a natural attack of English redwater. Temperature rose on the 8th day to 105 F. in the morning and 106 in the evening, returning next day to normal. On the 24th day it reached 104.6 in the evening, but fell again to normal two days later.

Examinations of blood preparations from the 8th to 26th September gave negative results, and the blood count on the 4th October, 1906, shewed the number of red cells to be between six and seven millions per c.mm.

"*B*," *Heifer* 430.—Injected in England with 5 c.c. defibrinated blood of an English heifer (which had previously been inoculated with English redwater, but the injection probably failed to infect the beast).

430 did not shew any reaction, and no piroplasms were found in the blood preparations.

On the 1st September, 430 was inoculated subcutaneously with 10 c.c. defibrinated blood of an English cow which had recovered from a natural attack of English redwater.

Temperature of 430 rose to 102 on the 6th day and to 104 in the evening of the 8th day. Smears examined on the morning of this latter date shewed distinct, but only small numbers of piroplasms, and the evening examination shewed a few ring forms; the temperature now fluctuated between 102.6 and 105.2 for the next two days, and regained normal on the 11th September. Another, but slight, reaction ensued from the 23rd to the 28th September.

EXPERIMENT No. 2.

English heifers injected with South African redwater.

"*A*," *Heifer* 429.—Injected in England on the 1st September, 1906, with 5 c.c. defibrinated blood of an English heifer (which had been infested with the infected blue ticks I sent to Professor Sir J. M'Fadyean in 1905, and as a result developed South African redwater and recovered).

On the 9th day the temperature of 429 rose to 103, but examination of smears were negative. On the 10th day temperature recorded 105.2 in the evening, and piroplasms were found in the smears. The temperature fluctuated for the next four days, but returned to normal on the 15th September. Piroplasms were again noted on the 11th day.

"*B*," *Heifer* 432.—Injected on the 1st September, 1906, with 5 c.c. defibrinated blood from the English beast which had been infected with blue ticks and contracted South African redwater.

Temperature of 432 rose to 103 on the 6th day, but examination of smears were negative; regained normal on the 7th to 9th days, but recorded 105 on the 10th day, when blood examinations were again negative. Piroplasms were noted for the first time, and only on the following day. Temperature remained high for the next three days, and regained normal on the 16th September.

EXPERIMENT No. 3.

English heifers inoculated with English and South African redwater.

"*A*," *Heifer No.* 431.—Injected on the 25th July in England with 5 c.c. blood from an English heifer (which had previously been inoculated with English redwater, but this injection apparently did not cause the beast to contract the disease).

The temperature of 431 remained normal, and all blood examinations gave negative results.

On the 1st September 431 was injected subcutaneously with 10 c.c. blood of an English cow which had recovered from a natural attack of English redwater.

Temperature rose to 104.2 in the morning of the 8th day, and to 106 in the evening. Blood examinations on this date were negative. Temperature remained high for the next two days, but returned to normal on the 10th September.

On the 24th September 431 was injected subcutaneously with 10 c.c. defibrinated blood of an English heifer (which had been infested with infected South African blue ticks, causing her to contract South African redwater).

431 shewed a rise of temperature, consequent on this inoculation, to 105 in the evening of the 1st October, 1906—the 7th day. Piroplasms were noted in the blood on this date, and a high temperature was recorded for the next three days.

"*B*," *Heifer* 433.—Injected in England on the 5th July subcutaneously with 5 c.c. defibrinated blood of an English beast (which had been inoculated with English redwater, but apparently did not contract the infection).

The temperature of 433 remained normal, and all blood examinations were negative.

On the 1st September, 1906, 433 was injected with 5 c.c. defibrinated blood of the English beast, which had contracted South African redwater from the infestation of infected blue ticks. Temperature of 433 rose to 103 on the 6th day, but examinations of blood did not reveal any piroplasms. The temperature now fluctuated for the next four days, and no piroplasms were seen until the 11th and 12th days, when the temperature regained normal.

A short secondary reaction noted from the 22nd day, lasting for a few days, but no piroplasms were found.

On the 24th September, 433 was injected with 10 c.c. defibrinated blood of a beast which had recovered from a natural attack of English redwater.

Ten days later 433 shewed a slight reaction, and on this date and the following days piroplasms were present. Temperature regained normal on the 6th October.

Notes on heifers 428 *and* 430.

From Mr. Stockman's notes on the behaviour of heifer 428 in England, I am not inclined to consider it as immune against English redwater in view of the atypical reaction and the absence of piroplasms.

Heifer 430 undoubtedly underwent an English redwater reaction, and should therefore be immune against this disease.

Notes on heifers 429 *and* 432.

The injection of English heifers with a strain of virus originating from infected blue ticks sent from the Transvaal and passing through an English beast caused, in both instances, the appearance of piroplasms accompanied with a typical South African redwater reaction. Therefore these two heifers should be immune against South African redwater.

Notes on heifers 431 *and* 433.

The injection of English redwater into heifer 431 did not cause the appearance of piroplasms, and the reaction was atypical. A subsequent injection of South African redwater caused the appearance of piroplasms in the English heifer, together with a temperature reaction.

Heifer 433 was injected with South African redwater in the first instance, and as a result shewed piroplasms, accompanied with a temperature reaction.

Twenty-four days later it was injected with English redwater, and a slight reaction ensued, accompanied with piroplasms.

Heifer 433 should, therefore, have acquired immunity against both English and South African redwater.

Conclusions.

It would be seen from these notes that of four animals inoculated with English redwater, two failed to react or to shew piroplasms, and in the other two instances a reaction ensued, accompanied with the appearance of piroplasms. Therefore it is safe to say that English redwater is not always inoculable, and differs in this respect from South African redwater.

EXPERIMENT No. 4.

To note whether (1) *South African animals, susceptible to ordinary redwater, contract English redwater when injected with blood from animals previously inoculated with this disease, and* (2) *whether these South African animals prove immune against ordinary redwater when injected with piroplasma bigeminum.*

(The South African animals used in this experiment were born and bred in Cape Colony, in a district free of ordinary redwater, directly imported to the Transvaal; hence they were susceptible to the disease.)

Heifers Nos. 400, 418, 421 and 422 were all injected on the 13th December, 1906, with blood of English heifer 428. Heifer 428 (compare Experiment 1 A) had shewn a temperature reaction consequent on the inoculation with English redwater, but no piroplasms were noted in the blood.

These South African heifers were subsequently tested on their immunity against ordinary redwater by the injection of a strain of virus emanating from a natural case of ordinary redwater, complicated with piroplasma mutans. (See also "Further Notes on Piroplasma Mutans," Experiment No. 6 C, Heifer 425.)

"*A*," *Heifer* 400.—A two-year-old from Aliwal North, and susceptible to ordinary redwater. Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428. As there were no results from this injection, heifer 400 was again injected subcutaneously on the 3rd January, 1907, with 50 c.c. blood of heifer 428. Temperature remained quite normal, and, with the exception of the presence of poikilocytosis on rare occasions, all blood examinations were negative.

Tested on immunity against ordinary redwater by the subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum, on the 30th January, 1907. Reaction from the 5th day, reaching 104.6 four days later, and remaining high for the next three weeks. Poikilocytosis and the lesions of piroplasma mutans appeared, but piroplasma bigeminum was not seen.

On the 26th April, 1907, 400 was injected with 10 c.c. blood of heifer 435, which was an imported English heifer, and had been

rendered immune against ordinary redwater. This injection did not cause any temperature reaction in 400, nor did piroplasma bigeminum appear.

"*B*," *Heifer* 418.—Two-year-old heifer from Aliwal North, and susceptible to South African redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428. No reaction; all blood examinations proved negative. Accordingly on the 3rd January, 1907, heifer 418 was again injected subcutaneously with 50 c.c. blood of heifer 428. The temperature remained quite normal, and no piroplasms were found in the blood smears. Spirillum appeared on the 3rd day after the second injection, and five days later the lesions of poikilocytosis were noted, remaining for another two days.

Tested on the 26th March, 1907, by a subcutaneous injection of 10 c.c. blood of heifer 425, an animal which contained piroplasma bigeminum and piroplasma mutans in its blood. A slight temperature reaction followed, and on the 26th day piroplasma bigeminum appeared.

"*C*," *Heifer* 421.—Two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 31st December, 1906, intrajugularly with 5 c.c. blood of heifer 428. As no reaction ensued, and all blood examinations were negative, heifer 421 was again injected subcutaneously on the 3rd January, 1907, with 50 c.c. of heifer 428. With the exception of a sharp rise to 104 on the 3rd day after this latter injection the temperature remained normal, and no piroplasms were noted in the blood.

Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 426, containing piroplasma bigeminum.

Reaction from the 5th day, piroplasma bigeminum being noted four days later.

"*D*," *Heifer* 422.—Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 428.

Temperature remained normal until the 17th day, when a sharp rise occurred, reaching 105.2, but all microscopical examinations were negative. Heifer 422 was again accordingly injected on the 31st January, 1907, subcutaneously with 50 c.c. blood of heifer 428. Again no reaction ensued, the temperature consistently remaining about 102 to 103. All examinations negative, with the exception of the presence of rare Trypanosoma theileri on the 6th January, 1907. Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum and piroplasma mutans.

Reaction from the 15th day, reaching 104.2 the following day, and remaining high for about two weeks. Piroplasma bigeminum noted on the 25th day.

The following South African cattle Nos. 401, 419, 420, 423 and 424 were all injected with blood of English heifer No. 430. This heifer (compare Experiment 1, "*B*") had been inoculated in England with English redwater, and as a result gave a temperature reaction, accompanied with the presence of piroplasms.

"*E*," *Heifer* 401.—A two-year-old heifer, from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of English heifer 430. As this inoculation failed to cause a reaction, and no piroplasms were noted in the blood, heifer 401 was reinjected on the 3rd January, 1907, subcutaneously with 50 c.c. blood of 430. Again no temperature reaction ensued, and all blood examinations were negative.

Tested on the 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, containing piroplasma bigeminum.

Reaction from the 6th day, returning to normal seven days later. Secondary reaction from the 19th day, reaching 107.4 on the 33rd day, and during which piroplasma bigeminum appeared.

"*F*," *Heifer* 419.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of heifer 430.

No temperature reaction, and all examinations of blood negative. Heifer 419 was then reinjected on the 3rd January, 1907, subcutaneously with 50 c.c. blood of heifer 430, with the result that the temperature remained normal, and no piroplasms or blood changes were noted.

Tested on 26th March, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, an animal immune against ordinary redwater. Reaction six days later, reaching 104.2 on the 3rd April, 1907. Piroplasma bigeminum noted on the 9th day.

"*G*," *Heifer* 420.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. blood of English heifer 430.

No result, and 420 was accordingly reinjected on the 31st January, 1907, subcutaneously with 50 c.c. of heifer 430. Temperature remained quite normal, and daily examinations of the blood failed to reveal any piroplasms.

Tested on immunity on 31st January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 425, an animal immune to ordinary redwater.

Typical ordinary redwater reaction from 5th day, but piroplasma bigeminum not present. Secondary reaction from 17th day, lasting for about two weeks, and during which time piroplasma bigeminum and the lesions of anæmia appeared.

"*H*," *Heifer* 423.—A two-year-old heifer from Aliwal North, and susceptible to ordinary redwater.

Injected on the 13th December, 1906, intrajugularly with 5 c.c. blood of heifer 430.

As this injection failed to produce any results 423 received a subcutaneous injection of 50 c.c. from heifer 430 on the 3rd January, 1907.

With the exception of a sharp rise to 104.2 eight days later the temperature remained normal, and no piroplasms were seen in the blood preparations.

Tested on immunity on 30th January, 1907, by a subcutaneous injection of 10 c.c. blood of heifer 426, immune to ordinary

redwater. Reaction from the 6th day, piroplasma bigeminum appearing three days later.

"*I*," *Heifer* 424.—A two-year-old from Aliwal North, and therefore susceptible to ordinary redwater.

Injected on the 13th December, 1906, subcutaneously with 10 c.c. defibrinated blood of heifer 430. Temperature remained normal, and all blood examinations were negative. Accordingly 424 received a subcutaneous injection of 50 c.c. blood of heifer 430 on the 3rd January, 1907.

No result; the temperature remained normal, and no piroplasms were seen in the blood preparations.

Tested on immunity on 30th January, 1907, by subcutaneous injection of 10 c.c. blood of heifer 426, an animal containing piroplasma bigeminum in its blood.

Typical ordinary redwater from the 6th day, but piroplasma bigeminum not seen, although poikilocytosis and polychromatic cells were present.

Heifer 424 was again injected on the 8th March, 1907, with blood containing piroplasma bigeminum and piroplasma mutans from heifer 409.

No reaction, piroplasma mutans only appearing.

Notes on heifers 400, 418, 421, 422, 401, 419, 420, 423 *and* 424.

With regard to these nine heifers, two injections of English redwater blood failed in every instance to cause a reaction, and piroplasms did not appear.

A subsequent inoculation of South African redwater caused these injected heifers to contract this disease, and, with the exception of Nos. 400 and 424, piroplasma bigeminum appeared in every animal.

Heifers 400 and 424 were reinjected with blood containing piroplasma bigeminum, the result being that no reaction ensued, nor were piroplasms present.

Conclusions.

I do not feel justified in drawing any conclusions from the results of the injection of heifers 400, 418, 421 and 422 with blood of English heifer No. 428, for, as will be seen from Experiment 1, "*A*," this heifer did not appear to contract the infection in England, as no piroplasms were seen, due to an inoculation of English redwater blood, and the reaction was atypical. I therefore consider that the failure of heifer 428 to contract English redwater from the injection of virulent blood is another point in favour of my contention that English redwater is not always inoculable. The results obtained from the other five heifers, however, seem to be conclusive, as heifer 430 was certainly infected with English redwater.

Therefore English redwater was not inoculable in our five South African heifers, and accordingly when tested they all contracted ordinary redwater, proving that they had not acquired any immunity against South African redwater.

EXPOSURE EXPERIMENTS WITH THE IMPORTED HEIFERS.

Continuing on the lines of the arrangement made between Mr. Stockman and myself, the imported Ayrshire heifers were exposed on

the farm "Linwood," near Pretoria. The temperatures were taken daily, and the blood examined from time to time.

Heifer No. 428.—Exposed on the 5th January, 1907. Three days after the temperature commenced to rise, reaching 106, and constantly remaining high during the next 47 days. Nothing particular was noticed in the blood at the beginning of this reaction, but on the 35th day piroplasma bigeminum was noticed, remaining for some days, but disappeared from the 39th day. The lesions of poikilocytosis were occasionally noted, and the temperature returned to about normal on the 26th February. A second rise ensued on the 4th March, piroplasma bigeminum not being noticed, but poikilocytosis and marginal points appeared, and the animal remained very weak. Death occurred on the 17th March, with all the lesions of the sequel of ordinary redwater. The anæmia was so pronounced that the blood consisted almost entirely of basophile, polychromatic and nucleated cells.

Heifer No. 430.—Exposed at Linwood on the 5th January, 1907. Temperature commenced to rise on the 12th January, and then oscillated very irregularly for the next month; microscopical examination of the blood at repeated intervals failed to reveal piroplasma bigeminum, but the lesions of poikilocytosis were noted.

Recovered.

Heifer No. 429.—Exposed at Linwood on the 5th January, 1907. Reaction commenced six days after exposure, when the temperature rose to over 106, and remained high for the following 14 days. Spirillum, basophile cells, the lesions of poikilocytosis and marginal points were noted, but piroplasma bigeminum did not appear.

Recovered.

Heifer No. 432.—Exposed at Linwood on the 5th January, 1907. Irregular temperature noted soon after, and rose about three weeks later to a high elevation, touching 105.8; piroplasma bigeminum, the lesions of poikilocytosis, basophile granulations and polychromatic cells were present.

Recovered.

Heifer No. 431.—Exposed at Linwood on the 5th January, 1907. This animal also showed an irregular high temperature, reaching over 105, and as the maximum recorded 106. Poikilocytosis, basophile granulations and spirillum were noted. Piroplasma bigeminum was not present.

Recovered.

Heifer No. 433.—Exposed at Linwood on the 5th January, 1907. Irregular temperature noted on the 12th January, commencing with 106, maintaining high for the next 16 days, and touching 106.8 on the 31st January, 1907. Basophile granulations, polychromatic cells and poikilocytosis, accompanied with marginal points, were noted as the alteration in the blood. Piroplasma bigeminum was not noted, but the lesions of anæmia increased, and the animal died as the sequel of ordinary redwater.

RÉSUMÉ.

Heifer No.	Particulars.	Results when exposed to natural infection of Ordinary Redwater.
428	Not immune to English redwater ...	Reaction with piroplasma bigeminum ; died of ordinary redwater.
430	Immune to English redwater ...	Reaction ; recovered.
429	Immune to ordinary redwater ...	Reaction ; recovered.
432	Immune to ordinary redwater ...	Reaction with piroplasma bigeminum ; recovered.
431	Immune to ordinary redwater, but susceptible to English redwater	Reaction ; recovered.
433	Immune to both English and South African redwater	Reaction ; died of sequel of ordinary redwater.

As already stated, I am of the opinion that English redwater does not protect against South African redwater, and the above statement furnishes additional support to this conception. In every case a new reinfection occurred, in two instances accompanied with piroplasma bigeminum.

"C."—FURTHER TRANSMISSION EXPERIMENTS WITH EAST COAST FEVER.

In my Annual Report for 1903-4, I enumerated various experiments, the conclusions of which were as follows:—

Rhipicephalus decoloratus (the common blue tick) is not a host of piroplasma parvum.

Rhipicephalus evertsi (the red tick) is not a host of piroplasma parvum.

Rhipicephalus simus is a host of piroplasma parvum.

Amblyomma hebræum may be a host of piroplasma parvum.

Rhipicephalus appendiculatus (the brown tick) is the principal host of piroplasma parvum, and it was further stated that brown ticks transmit the disease principally in their imago stage, after having fed as nymphæ on sick beasts; less so as nymphæ, after having fed as larvæ, and not at all as larvæ originating from a mother tick removed from a beast infected with East Coast fever. In other words, piroplasma parvum does not pass from the female imago into the egg and from this into the larvæ, as is the case in Texas fever. These experiments were carried out almost simultaneously with those of Mr. Lounsbury, of Capetown, and as a result the facts were established that the blue tick under no conditions acts as a host of piroplasma parvum, and that the brown tick (the tick with three hosts) is the principal carrier of the disease.

In 1906 Mr. Lounsbury published a further series of experiments which proved that, besides the ticks mentioned, others transmit the disease, viz., rhipicephalus nitens, evertsi and capensis. In *Mense's Handbuch der Tropenkrank-heiten*, 1906, a contribution appeared by Luhe regarding the protozoa parasite in the blood, and in regard to my statement that "piroplasma parvum does not pass through the egg," he makes the objection that in my experiments the larvæ which were employed were too young, and he quotes a communication of Professor Koch, who exposed larval ticks hatched in the laboratory,