THE NEW BACTERIOLOGICAL LABORATORY AT ONDERSTEPOORT.

In continuation of my last annual report regarding the prevalency of enteric fever at the Laboratory, I am glad to say that the late Government decided to remove our quarters to a fresh site, and the present Parliament carried on the scheme, voting an additional sum for the purpose. We were able to purchase a suitable site in the neighbourhood, on the farm "Onderstepoort." This farm offers suitable conditions for our purpose, as it is within easy reach of Pretoria; a railway line passes quite close and a siding will be erected; the Aapies River forms one of the boundaries, and sufficient land can be irrigated for grazing and cultivation of our own crops.

The plans were designed by Mr. Eagle, Chief Architect of the Public Works Department; those of the main buildings, including the Laboratories and Administration Offices, have been approved, and these buildings were commenced at the close of the fiscal year.

In conclusion, I wish to place on record the good work performed by the staff, especially during the summer months.

I have the honour to be,
Sir,
Your Obedient Servant,

A. THEILER,
Government Veterinary Bacteriologist.

"A."—FURTHER NOTES ON PIROPLASMA MUTANS — A NEW SPECIES OF PIROPLASMA IN SOUTH AFRICAN CATTLE.

In my last Annual Report I established the fact that the rings and rods which sometimes appear in susceptible cattle after the injection of redwater blood have no connection with redwater (P. bigeminum), but must be considered as a separate piroplasm, which I have designated Piroplasma mutans.

The chief argument in favour of this is that an animal can be infected with redwater exclusively, and at any later period with piroplasma mutans. The incubation time of this latter parasite varies from 20 to 45 days, whereas the incubation time of redwater is from five days onwards. Naturally piroplasma mutans appears after the disappearance of piroplasma bigeminum.

I have in addition shown that not all the animals in the Transvaal are infected with piroplasma mutans, but those which are susceptible can easily be infected when injected with blood containing this new piroplasm.

The experiments I now bring forward will (1) add further proof of the duality of the two piroplasms, (2) show that the blue tick, which is the carrier of piroplasma bigeminum, does not transmit piroplasma mutans, (3) show that piroplasma mutans is distributed all over South Africa, and (4) show that the injection of blood containing piroplasma bigeminum and piroplasma mutans into cattle susceptible to ordinary redwater does not always cause the former parasite to appear.

EXPERIMENT No. 1.

Infection of animals known to be immune against redwater with piroplasma mutans.

The animals belonging to this experiment were originally considered as susceptible to redwater, but on examination of their blood it was found to contain piroplasma bigeminum, and therefore they were immune against this disease.

"*A*," *Calf* 382.—About one year old; was injected subcutaneously on the 11th June, 1906, with 10 c.c. blood of calf 359.

No reaction appeared consequent on the inoculation, and since in the meantime it was found that 382 was immune against redwater (piroplasma bigeminum) it was injected subcutaneously on the 6th July—25 days after the first inoculation—with 10 c.c. blood of heifer 316, an animal which contained both piroplasma bigeminum and piroplasma mutans in its blood. Nothing particular happened after this injection until the 36th day—11th August, 1906—when rings and rods were noticed, and a slight disturbance of the temperature ensued. These rings and rods increased slightly during the following days, but they were never present in great numbers. The lesions of poikilocytosis were noted on one occasion during their presence—viz., on the 20th August, 1906. Rings and rods were seen on the 11th and 12th September, and again on the 20th October, 1906—36 days after the second injection. The examinations were discontinued on the 20th October, 1906.

"*B*," *Calf* 384.—Injected on the 11th June, 1906, with 10 c.c. blood of calf 359 (*vide* EXPERIMENT No. 1, "*A*").

Nothing happened with this heifer for the same reason as in the former case. It was therefore decided on the 26th day—6th July—to inject 384 with 10 c.c. blood of heifer 316, containing both piroplasma bigeminum and piroplasma mutans. This inoculation produced a distinct reaction, during which piroplasma bigeminum was noticed on the 10th day, and again on the 14th day after the second injection. The reaction subsequently subsided, but from the 35th day another rise of temperature ensued, and three days later—13th August—rings and rods were noticed for the first time. These rings and rods increased, and during the time they were most frequent a febrile reaction was present; the lesions of poikilocytosis were also very pronounced. Rings and rods gradually decreased, and finally disappeared on the 71st day—15th September, 1906—when the examinations were discontinued.

"*C*," *Heifer* 386.—Belonging to the same lot as animals 382 and 384, and was considered susceptible to redwater, but examination proved the presence of piroplasma bigeminum in its blood.

Injected subcutaneously on the 29th June, 1906, with 10 c.c. blood of Cape animal 380 known to be infected with piroplasma bigeminum. On the 12th day after inoculation a reaction ensued, but examinations of blood proved negative. Irregular temperatures were noticed for some time after this. Fifty-six days later—19th October—it was decided to inject 386 with 5 c.c. blood of heifer 316—immune to piroplasma bigeminum and piroplasma mutans. Thirty-two days later—20th November—rings and rods were present; they

increased in number, and coinciding with their increase a rise of temperature ensued; the lesions of poikilocytosis became very pronounced. Rings and rods were daily present for over five weeks, and were still noticed in rare numbers on the 72nd day after the third inoculation—31st January, 1906—when the examinations were discontinued.

"*D*," *Heifer* 404.—About two years old, directly imported from Aliwal North, and therefore susceptible to redwater.

Was injected on the 16th October, 1906, subcutaneously with 5 c.c. blood of calf 396. This calf had been previously injected with piroplasma bigeminum blood (compare Experiment No. 3, "*E*"), and at the date of injection into heifer 404 was immune against redwater. After an incubation time of 16 days the temperature of heifer 404 rose to 105 F., and four days later piroplasma bigeminum and poikilocytosis were noticed. The temperature now oscillated, and basic cells were present on one occasion. The lesions of poikilocytosis were frequently noted from the 20th to the 37th day, and afterwards at rare intervals. On the 106th day after injection of blood of 396—30th January, 1907—heifer 404 was injected subcutaneously with 10 c.c. blood of heifer 425 immune to piroplasma mutans (see Experiment 6, "*C*"). Irregular reaction noted, probably due to spirillum; all blood examinations negative. Second reaction commenced on the 24th day, recording 106 on the 43rd, 44th and 45th days. Piroplasma mutans noted during the reaction on the 29th, 32nd, 34th and 36th days. Poikilocytosis and marginal points also frequently noted until the 47th day. All further examinations negative, and were accordingly discontinued from the 72nd day.

"*E*," *Heifer* 412.—This was an Aliwal North two-year-old animal, and susceptible to redwater. Accordingly on the 16th October, 1906, it was injected subcutaneously with 5 c.c. defibrinated blood of calf 387 (compare Experiment 3, "*B*"; calf 387 was immune against redwater, and although infested with blue ticks which had previously been feeding on animals containing both piroplasma bigeminum and piroplasma mutans in their blood, calf 387 did not contract piroplasma mutans, since, as I shall prove later, the blue tick is not the carrier of this new piroplasm).

Reaction commenced on the 8th day after injection, and four days later recorded 105.4. Examinations on the 9th and 12th days gave negative results. The temperature now fell and remained between 99 and 103 from the 19th to the 82nd days. Piroplasma bigeminum noted for the first time on the 13th day after injection; spirillum present the following day. Piroplasms again noted on the 15th, 16th and 18th days. The lesions of poikilocytosis, together with basophile cells, were present on the 21st day, and two days later basic cells appeared. Poikilocytosis was occasionally noted from the 27th to 60th days, and piroplasma bigeminum was again present on the 32nd and 36th days.

On the 83rd day—January 30th, 1907—heifer 412 was injected with 10 c.c. blood of heifer 425 immune to piroplasma mutans (compare Experiment 6, "*C*"). The lesions of poikilocytosis noted on the 8th and 9th days after this injection, and again on the 13th and 15th days; a slight reaction ensued from the 15th day, recording 105.8 two days later, and regaining normal on the

23rd day. All examinations during this reaction gave negative results. Poikilocytosis was frequently noted from the 24th to the 32nd days, and five days later (37th day) a rise of temperature from 101.4 in the morning to 103.8 twelve hours later was accompanied with the presence of piroplasma mutans for the first time. Piroplasma mutans and poikilocytosis were present the following day, and the temperature dropped. Another short reaction noted from the 43rd to the 50th days, when poikilocytosis and piroplasma mutans were again present for two days.

All further examinations were negative, although the temperature showed an irregular record. The experiment was discontinued on the 13th April, 1907, 71 days after injection of 425.

Conclusions.

The injection of blood of an animal which we knew did not contain piroplasma mutans failed to produce this parasite in the injected animal, but the same animals shewed piroplasma infection directly they were injected with blood containing piroplasma mutans. These parasites appeared within the typical incubation time, viz., in the first instance, after 36 days; secondly, after 35 days; thirdly, 32 days after injection; fourthly, after 29 days; and lastly, after 37 days. The lapse of time between the inoculation of blood known not to contain piroplasma mutans and the injection of blood containing this parasite is long enough to prove that piroplasma mutans infection is not due to the first injection. This period was in the first instance 61 days; secondly, 60 days; thirdly, 88 days; fourthly, 106 days; and lastly, 83 days.

EXPERIMENT No. 2.

Heifers infected with blue larval ticks in the first instance, causing a pure infection of piroplasma bigeminum, and subsequently injected with blood containing piroplasma mutans.

"*A*," *Heifer* 398.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infected on the 16th October, 1906, with numerous blue tick larvæ of heifers Nos. 314 and 316—animals immune to redwater and piroplasma mutans, therefore containing both piroplasma bigeminum and piroplasma mutans in their blood.

Nothing unusual occurred until the 22nd day, when a slight rise of temperature was noticed, and piroplasma bigeminum appeared and remained for the four following days; a slight poikilocytosis also resulted from this infestation.

Sixty-four days after the tick infestation, 398 was injected with blood of 316, an animal, as stated above, containing piroplasma mutans and piroplasma bigeminum in its blood. Twenty-five days after this injection the first flagellated form was seen and poikilocytosis was noticed as a result. The parasites increased in numbers, and remained present for some time.

"*B*," *Heifer* 402.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infested on the 16th October, 1906, with numerous blue tick larvæ of heifers 314 and 316, animals containing both piroplasma in their blood. On the 21st day a rise of temperature was noticed, accompanied with the appearance of spirillum for five days. On the 32nd day piroplasma

bigeminum was noticed and remained for six days, followed by a slight poikilocytosis; piroplasma bigeminum was again noticed on the 43rd and 45th days. On the 64th day this animal was injected with blood of heifer 316—containing both piroplasma bigeminum and piroplasma mutans. On the 23rd day after this injection the first rod-shaped parasite was seen. It increased in numbers, causing a more distinct poikilocytosis and a slight rise of temperature. The parasites remained present for some time.

"*C*," *Heifer* 405.—Aliwal North animal, and therefore susceptible to piroplasma bigeminum. Infested on the 16th October, 1906, with numerous blue tick larvæ of heifers 314 and 316—animals immune to piroplasma bigeminum and piroplasma mutans, therefore containing both these parasites in their blood. On the 20th day after this infestation a rise of temperature was noticed, which was succeeded by the appearance of spirillum. On the 34th day after infestation another rise of temperature was observed. This curve was succeeded by the appearance of piroplasma bigeminum, which was present for the four following days. Piroplasma bigeminum was again present on the 43rd and 45th days, and the poikilocytosis continued for some time. On the 64th day after infestation, the animal was injected with blood of heifer 316, containing both piroplasma bigeminum and piroplasma mutans. Twenty days after this inoculation the first rod-shaped parasite was seen; they were noticed in rare numbers, but four days later—24th day—increased, causing a slight rise of temperature, together with a slight poikilocytosis, and the appearance of marginal points. The rings and rods remained for some days.

"*D*," *Heifer* 407.—Aliwal North animal, about two years old, and susceptible to redwater.

Infested on the 16th October, 1906, with blue tick larvæ which had previously been feeding on Madagascar ox 347, immune to piroplasma bigeminum and piroplasma mutans (see Annual Report, 1905-6, page 59, No. "A"). The temperature reached 104.2 on the 6th day, but dropped and recorded from about 99.6 in the morning to 103.4 in the evening from the 10th to the 16th days, during which time all microscopical examinations gave negative results. A sharp rise was now noted from 101.2 in the morning of the 17th day to 105.6, 36 hours later, and returning to a normal record on the 21st day, from which date another rise was recorded, the temperature touching 104 on the 22nd and 23rd days. The lesions of poikilocytosis were noted on the 21st day, followed by the daily appearance of spirillum until the 25th day.

The blue tick adults commenced to fall on the 20th day, and for the two following days poikilocytosis was present. On the 32nd day the temperature rose from 101 to 103.8 in the evening, and coinciding with this, piroplasma bigeminum was noted for the first time. The temperature now fell and touched 99 F. on the 35th day, but piroplasma bigeminum was noted daily from the 33rd day to the 37th day. An irregular temperature record was shown from the 38th to the 105th day, occasionally touching 104 F. in the evening. Piroplasma bigeminum was again present

on the 39th day after infestation, and, with the exception of the occasional appearance of poikilocytosis, no further points were noted. On the 106th day (January 30th, 1907) heifer 407 was injected with 10 c.c. blood of heifer 425 immune against piroplasma mutans (compare Experiment 6, "*C*"). Reaction from the 12th to the 20th day, recording 104.2 on the 16th day; all examinations from the 8th to the 23rd day negative. Another febrile reaction noticed from the 24th day, and on this date slight poikilocytosis and a few ring forms were noted, these lesions being again present on the 26th and 27th days. During this time the temperature was slowly rising, and on the 29th day poikilocytosis and a few flagellated forms were noted, followed two days later by the presence of piroplasma mutans, the temperature on that date being 104.2. From the 34th and 35th days, when piroplasma mutans was still present, the temperature slowly fell, recording 98.8 in the morning of the 46th day, but rose sharply to 104.4 in the evening, the same record being noted the following morning, when piroplasma mutans and poikilocytosis were noted. The temperature remained high for the next eight days, on which latter date—54th after injection—piroplasma mutans was again present. All further examinations were negative, and the experiment was discontinued from the 71st day after injection of blood from 425.

"*E*," *Heifer* 408.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Infested on the 16th October, 1906, with blue tick larvæ, which had previously been feeding on ox 347. This was a Madagascar ox (compare Annual Report, 1905-6, page 59, No. "A") immune to redwater and piroplasma mutans. An irregular temperature was noted for the first 16 days, during which time all examinations were negative. A sharp rise from 100.4 in the evening of the 18th day to 106 48 hours later was followed by the appearance of spirillum on the 21st, 23rd and 25th days, by which time the temperature had regained normal. Another sharp rise was now noted, reaching 106 on the 27th day, and on the following day the blue ticks commenced to drop. The temperature now shewed a difference of about 4 F. between the morning and evening records for the next eight days, and on the 31st day after infestation piroplasma bigeminum was noted for the first time.

These piroplasms were present daily from the 32nd to the 37th day, and from this date the temperature became very irregular, recording between 100 and 104 for the next 69 days, during which time frequent microscopical examinations were made, but with negative results. On the 106th day after infestation (30th January, 1907) heifer 408 was injected subcutaneously with 10 c.c. blood of heifer 425, immune to piroplasma mutans (compare Experiment 6, "*C*"). Reaction noted from the 23rd day; all examinations from the 8th to the 26th days gave negative results, but on the following day the lesions of poikilocytosis appeared, and were present on the 29th day, accompanied with flagellated forms in rare numbers. Piroplasma mutans noted on the 31st day, accompanied with poikilocytosis, and were both present on the 34th and 36th days. From this date an irregular temperature record was noted, lasting until the 71st day, when the experiment was discontinued. With the

exception of the presence of poikilocytosis on the 51st and 55th days, all further examinations were negative.

"*F*," *Heifer* 409.—About two and a half years old; imported from Aliwal North, and therefore susceptible to redwater.

Infested on the 16th October, 1906, with blue tick larvæ previously feeding on heifers 314 and 316, animals immune to piroplasma bigeminum and piroplasma mutans (compare Annual Report, 1905-6, pages 44 and 45). Rise of temperature from 23rd day, reaching 105.6 three days later, and accompanied with spirillum during the 24th, 25th and 26th days. Adults dropped on the 28th day, and the tempearture slowly fell, recording 100 on the 33rd day, rising to 105 on the following morning, and accompanied with piroplasma bigeminum, which was present for the next three days. An irregular temperature record was now noted until the 105th day, poikilocytosis occasionally being noted. On the 106th day—30th January, 1907—heifer 409 was injected with 10 c.c. blood of heifer 425, immune to piroplasma bigeminum and piroplasma mutans (compare Experiment 6, "*C*"). Irregular reaction followed, poikilocytosis frequently being present, and on the 22nd day rings and flagellated forms were noted in rare numbers. These rings and flagellated forms increased during the next few days, but were present in rare numbers on the 29th and 31st days. The temperature record continued to be of an irregular character, until the 72nd day, when the experiment was discontinued.

Conclusions.

All the six animals—398, 402, 405, 407, 408 and 409—were susceptible to piroplasma bigeminum; they were all successfully infected with blue ticks, which caused the appearance of piroplasma bigeminum exclusively.

Sixty-four days after the infestation of animals 398, 402 and 405, and 106 days after that of 407, 408 and 409, they were injected with blood of an animal containing both piroplasma bigeminum and piroplasma mutans. This injection caused the appearance of piroplasma mutans after the typical incubation time—398 after 25 days, 402 after 23 days, 405 after 20 days, 407 after 29 days, 408 after 29 days and 409 after 22 days.

This experiment affords a preliminary proof that the blue tick is not a carrier of piroplasma mutans, although it transmits redwater (piroplasma bigeminum).

The injection of blood containing piroplasma mutans proved that all six animals were susceptible to piroplasma mutans, since the injection of blood caused the appearance of the parasite.

EXPERIMENT No. 3.

Susceptible heifers injected (1) *with piroplasma bigeminum exclusively,* (2) *infested with larval blue ticks feeding on animals containing piroplasma bigeminum and piroplasma mutans in their blood, and* (3) *injected with blood containing piroplasma bigeminum and piroplasma mutans.*

These experiments were intended in the first instance to note whether the blue tick would act as a carrier of piroplasma mutans; if so, it would then infect animals susceptible to piroplasma mutans.

If these animals failed to show a reaction consequent on the infestation, yet subsequent injections introduced the two piroplasms, it would prove that the blue tick is not a carrier of the disease.

"*A*," *Ox* 358.—About one year old; directly imported from Cape Colony and susceptible to redwater; was injected on the 6th July, 1906, with 10 c.c. blood of calf 359, containing piroplasma bigeminum exclusively in its blood. A temperature reaction ensued, and on the 20th day poikilocytosis and piroplasma bigeminum were noticed. The reaction subsided about the 25th day, and a normal curve resulted. On the 49th day after this inoculation—24th August—the ox was infested with numerous blue tick larvæ of ox 347, whose blood in previous experiments had proved to contain both piroplasma bigeminum and piroplasma mutans. Nothing resulted from the tick infestation; accordingly on the 19th October—56 days after the tick infestation—ox 358 was injected with 5 c.c. blood of heifer 316—immune to piroplasma bigeminum and piroplasma mutans; therefore containing both these piroplasms in its blood.

Nothing resulted from this inoculation until the 28th day, when one ring was noticed. These rings were very rarely met with up to the 38th day, on which date a slight febrile reaction occurred and the piroplasms slightly increased in numbers. The experiment was discontinued on the 18th December, 1906—60 days after the injection of blood of heifer 316.

"*B*," *Calf* 387.—This calf belonged to the same lot which subsequently proved to be immune against redwater.

Injected on the 6th July with 10 c.c. blood of calf 359 immune to redwater. Nothing resulted; the temperature remained normal, and examinations of blood constantly proved negative. Fifty-four days after this inoculation—29th August—387 was infested with numerous blue tick larvæ of heifers 314 and 316—whose blood contained both piroplasma mutans and piroplasma bigeminum. Nothing occurred from this infestation; the temperature again remained normal, and microscopical examinations constantly proved negative.

On the 4th November—67 days after the tick infestation—the animal was injected with 5 c.c. blood of heifer 316, immune to piroplasma bigeminum and piroplasma mutans. Negative results from this inoculation until the 7th December—33rd day—when rings and rods were noticed, but were very rare during the following days. The experiment was discontinued on the 18th December—44 days after the injection of blood of 316.

"*C*," *Heifer* 394.—Imported from Aliwal North, and therefore susceptible to redwater. Injected on the 24th August, 1906, with 10 c.c. blood of calf 359, whose blood contained piroplasma bigeminum exclusively. The temperature reaction commenced on the 10th day, and on the following day piroplasma bigeminum was noticed. This piroplasm was present for five days, when a slight poikilocytosis was observed, which continued up to the 20th day. A second reaction was noticeable between the 33rd and 45th days, although nothing particular was noticed in the blood. On the 54th day after the first inoculation—*i.e.*, 17th October, 1906—394 was infested with numerous blue tick larvæ of ox 347—an animal which contained piroplasma bigeminum and piroplasma

mutans in its blood. On the 13th day after infestation a temperature reaction was noticed which passed over after three days, but was succeeded by another curve, at the beginning of which piroplasma bigeminum and spirillum were noticed (ox 347 also being infected with spirillum). From the 30th to 37th day piroplasma bigeminum was noticed daily, and a very slight febrile reaction ensued. The temperature curve now remained normal.

Sixty-three days after the tick infestation—19th December—394 was injected with 10 c.c. blood of heifer 316, which contained both piroplasma bigeminum and piroplasma mutans in its blood. Twenty-four days after the injection the first rod-shaped parasite was seen. The parasites were now daily noticed in slightly increasing numbers, and accompanied with the appearance of a slight poikilocytosis and a considerable rise of temperature.

"*D*," *Heifer* 395.—Imported from Aliwal North, and therefore susceptible to redwater. Injected on the 24th August, 1906, with 10 c.c. blood of calf 359 immune to ordinary redwater. Temperature reaction began on the 8th day; piroplasma bigeminum was noticed on the 11th day only. The tepmerature curve continued until the 15th day, on which date marginal points and the lesions of poikilocytosis made an appearance. Poikilocytosis was noticed up to the 26th day, and between the 31st and the 38th days another temperature disturbance was noticeable, but examinations of blood proved negative. The temperature now remained normal until the 17th October, 1906, this being the 54th day after inoculation.

The animal was now infested with numerous blue tick larvæ of ox 347. On the 20th day after this infestation a temperature reaction commenced, on which day and the day following spirillum was noted—347 being also infected with spirillum. On the 31st day, and coinciding with a slight rise of temperature, piroplasma bigeminum was noticed; it was also present on the 31st, 35th and 44th days. Subsequent to this the lesions of a slight poikilocytosis were occasionally seen. Sixty-three days after the tick infestation the animal was injected with 10 c.c. blood of heifer 316—an animal which contained both piroplasma bigeminum and piroplasma mutans in its blood. Twenty-six days later a slight poikilocytosis was noticed, and on the 33rd day the first rod-shaped parasite was seen. These rod-shaped parasites were now daily noticed, accompanied by a slight poikilocytosis up to the 31st day.

"*E*," *Heifer* 396.—Imported from Aliwal North, and therefore susceptible to redwater.

Injected on the 24th August, 1906, with 10 c.c. blood of heifer 358, an animal infected with piroplasma bigeminum exclusively. A rise of temperature ensued on the 7th day; on the 9th day piroplasma bigeminum appeared, and was noticed daily for the three following days. On the 4th day poikilocytosis was well pronounced; polychromatic cells and basophile granulations were also noted. These blood changes were noticed for a considerable time, and were present at the time of the second reaction, which occurred between the 30th and the 36th day. No piroplasms were present during this period. The temperature again became normal. On the 54th day after inoculation—17th

October—the animal was infested with numerous blue tick larvæ of ox 347—an animal infected with both piroplasma bigeminum and piroplasma mutans. On the 19th day a slight rise of temperature became noticeable, and three days later spirillum was noticed, succeeded by the lesions of poikilocytosis. This slight poikilocytosis was constantly noticed up to the 63rd day slightly decreasing, whilst the temperature remained normal. On this day—19th December—the animal was injected with 10 c.c. blood of heifer 316, containing piroplasma bigeminum and piroplasma mutans. The temperature remained normal, and on the 21st day the first rod-shaped parasite was seen. Again, on the 24th day, another one was noticed, and from the 26th day onwards they slightly increased, together with a slight rise of temperature.

On the 23rd January, 1907, the animal died accidentally, it having become hoven.

Conclusions.

All these five animals—Nos. 358, 387, 394, 395 and 396—were treated in the same way, and the experiment had a similar result.

They were injected in the first instance with blood of an animal which was infected exclusively with redwater (piroplasma bigeminum).

From 49 to 54 days after this injection they were infested with numerous blue tick larvæ previously feeding on animals suffering from both piroplasma bigeminum and piroplasma mutans. In two instances—"*C*" and "*D*"—piroplasma bigeminum made its appearance, probably due to this infestation, and in the three cases—"*C*," "*D*," and "*E*"—was accompanied by the infection of spirillum (the animal from which the ticks were taken also being infected with spirillum).

If the blue tick is the carrier of piroplasma mutans it would have communicated the infection to these five animals. It did not do so within 56 to 67 days, when the animals were injected with blood containing both piroplasma bigeminum and piroplasma mutans, and, as a result of this injection, piroplasma mutans appeared with the typical incubation time (see appendix).

APPENDIX "A."

Animals.	Numbers of Days which elapsed between			
	Infection of piroplasma bigeminum exclusively and infestation of blue tick larvae.	Infestation of blue tick larvae and infection of both piroplasma bigeminum and piroplasma mutans.	Infection of both piroplasms and the appearance of rods and rings—piroplasma mutans.	Injection of blood containing piroplasma bigeminum and injection of blood containing both piroplasma bigeminum and piroplasma mutans.
358	49	56	28	105
387	54	67	33	121
394	54	63	24	117
395	54	63	33	117
396	54	63	21	117

EXPERIMENT No. 4.

To prove that the injection of blood of an animal immune to redwater which contracted the disease from ticks, is not followed by the appearance of piroplasma mutans.

About two years ago I forwarded blue ticks—taken from an animal which was suffering from ordinary redwater—to Professor Sir J. M'Fadyean, of London. These ticks were then placed on an animal "X," with the result that it passed through a typical reaction and shewed piroplasma bigeminum in its blood. The animal recovered, and its blood was utilised for the injection into heifer 429 in England, with the result that this animal also passed through a typical ordinary redwater reaction, and piroplasma bigeminum appeared. Heifer 429 was then sent to South Africa (see also article "B," Experiments with English and South African Redwater), and soon after its arrival at this laboratory the injection into 426 was made.

"*A*," *Heifer* 426.—Two years old; imported from Aliwal North and therefore susceptible to ordinary redwater. Injected on the 22nd December, 1906, subcutaneously with 10 c.c. blood of English heifer No. 429.

Slight rise on the 10th day, reaching 103.4 twenty-four hours later, when the presence of piroplasma bigeminum was noted, and remained for the following three days. The temperature shewed considerable fluctuations and marked differences between the morning and evening records were noted until the 77th day after this injection. On this date—8th March, 1907—heifer 426 was injected with 10 c.c. blood of heifer 409, immune to piroplasma mutans. No distinct reaction noticed, but the evening record remained fairly high, and poikilocytosis was occasionally present. Piroplasma mutans present for the first time on the 26th day after injection, and again noted four and seven days later.

All further examination negative, and the experiment was discontinued from the 59th day—136 days after injection of blood of English heifer No. 429.

Injections with blood of heifer 426, *an animal immune to piroplasma bigeminum.*

"*B*," *Heifer* 421.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (compare Experiment 4, "*A*").

Sharp rise noted from the sixth day, reaching 105.6 two days later, followed by the appearance of piroplasma bigeminum for the first time on the tenth day. The lesions of a strong poikilocytosis noted five days later and from this day until the 36th an irregular temperature record ensued. On this latter date polychromatic cells, accompanied with the lesions of a strong poikilocytosis, were noted. Next morning, 8th March, 1907, heifer 421 was injected subcutaneously with 10 c.c. blood of heifer 409—an animal containing both piroplasms in its blood (see Experiment 2, "*F*"). Reaction from the second day, accompanied with poikilocytosis, and reaching 107 on the 8th

and 9th days, when piroplasma bigeminum was noted on both occasions. The temperature now slightly fell, still accompanied with poikilocytosis, and on the 25th day piroplasma mutans was present for the first time, together with poikilocytosis and marginal points. These lesions were again noted three days later, and on the 30th, 31st and 34th days, Basic cells also being present on the 31st day.

"*C*," *Heifer* 423.—About two years old; directly imported from Aliwal North, and therefore susceptible to ordinary redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (compare Experiment 4, "*A*"). Reaction from the 6th day, reaching 103.8 three days later when piroplasma bigeminum was noted. The temperature remained fairly high until the 36th day. On the 37th day—8th March, 1907—heifer 423 was injected with 10 c.c. blood of heifer 409 (compare Experiment 2, "*F*"), immune to piroplasma mutans and piroplasma bigeminum. After an incubation time of 25 days, piroplasma mutans, accompanied with slight poikilocytosis, appeared, and were again noted on 27th, 31st and 34th days, during which time the reaction was very marked. Piroplasma mutans alone was present in fair numbers on the 32nd day. The experiment was discontinued on the 7th May, 1907, 60 days after the injection of 409.

"*D*," *Heifer* 424.—About two years old; imported from Aliwal North, and therefore susceptible to redwater. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 426 (see Experiment 4, "*A*"). Slight reaction from the 6th day, reaching 104 on the 10th and 11th days, and followed by the lesions of poikilocytosis on the 12th and 15th days. On the 30th day the temperature recorded 105.4, and this was again followed by poikilocytosis on the next day and accompanied with polychromatic cells on the 35th day. Two days later heifer 424 was injected subcutaneously with 10 c.c. blood of heifer 409 (compare Experiment 2, "*F*") and immune to piroplasma bigeminum and piroplasma mutans. Slight poikilocytosis and piroplasma mutans noted for the first time on the 25th day, and two days later these lesions were again present, accompanied with marginal points. Slight poikilocytosis and piroplasma mutans again noted on the 31st and 34th days. The temperature consistently remained between 99 and 104 until the 101st day, when the experiment was discontinued.

Conclusions.

When blue ticks obtained from a South African beast suffering from ordinary redwater were forwarded to England and placed on an animal, they transmitted piroplasma bigeminum; all the animals which were subsequently inoculated in South Africa with blood from a following generation of this strain developed piroplasma bigeminum exclusively, thereby proving that piroplasma mutans was not carried to England by the blue tick. The three South African animals injected with piroplasma mutans developed this infection within the typical period, thus proving that piroplasma mutans and piroplasma bigeminum are not connected with each other in any way.

Experiment No. 5.

SPONTANEOUS CASES OF PIROPLASMA MUTANS.

A.—Cases originating in Cape Colony.

"*A*," *Calf* 380.—This is an animal belonging to the Capetown lot, which was considered susceptible to piroplasma bigeminum, but experiments proved the presence of this parasite in its blood, therefore indicating that the animal was immune against red-water.

Injected subcutaneously on the 6th June, 1906, with 5 c.c. blood of calf 378, being one of the same batch of animals. Negative results, the temperature remained normal, and microscopical examinations for 83 days—until August 28th—proved negative. At the beginning of September, 1906, rings (piroplasma mutans) were noticed in scanty numbers, and disappeared after about ten days. The examination of smears was continued, but again without any positive results.

This calf was used for calf vaccine lymph on the 19th October, 1906—135 days after the first inoculation. The reaction from the vaccination concluded on the 4th November, and on that date 380 was inoculated with blood of heifer 316—containing piroplasma bigeminum and piroplasma mutans. Eight days after this inoculation rings and rods were again noticed. They became rarer and rarer during the following days, but were still present when the experiment was discontinued on the 18th December, 1906.

In this particular case I do not consider that the re-appearance of piroplasma mutans is due to the inoculation of blood of 316, but to the heavy reaction consequent on the vaccination.

"*B*," *Heifer* 411.—Injected on the 16th October, 1906, with 5 c.c. blood of the former animal No. 380, which, as shewn, contained piroplasma mutans. The object of the experiment was to note whether we had perhaps to deal with a pure infection of piroplasma mutans.

Following on the injection, on the 11th day a reaction ensued, and for the next three days piroplasma bigeminum was encountered. The usual changes of a piroplasma infection were noticed in the form of poikilocytosis.

On the 45th day after inoculation, rings and rods were noticed. They were, however, very rare, and hardly made an impression on the course of the temperature, which only shewed a slight rise in the morning.

The experiment was discontinued on the 60th day—16th of January, 1907.

Experiment No. 6.

B.—Cases originating in the Transvaal.

On the 1st October, 1906, Government Veterinary Surgeon Lindsay, of Middelburg, forwarded some smears of a dead cow, which although grazing in an East Coast fever infected area was not supposed to have died of this disease, but as the result of an accident.

Microscopical examinations proved the presence of endoglobular parasites corresponding to the description of piroplasma mutans, but since the animal was in an infected area a reservation was made as to the cause of death in order to make further investigations of the remaining in contact animals. Smears from two healthy animals running together with the one which had died were made, and examinations also shewed endoglobular parasites. It was now decided to prove whether this was a case of a pure infection of piroplasma mutans, and accordingly the animals were tapped by the Government Veterinary Surgeon, the blood forwarded to the laboratory on the 3rd November, and the following injections into calves 416 and 417 were made on the next day:—

"*A*," *Calf* 416.—Born on the station, and injected as above with 5 c.c. blood from a red ox. No rise of temperature or the presence of any parasites noticed for the first 28 days. On the next day marginal points appeared, and on the 30th day rings and rods (piroplasma mutans) were noticed for the first time. The symptoms of poikilocytosis increased; marginal points, rings and rods also increased, whereas the temperature showed fluctuations, but during the increase of piroplasma mutans a distinct rise ensued. The parasites were present up to the 61st day after inoculation in rare numbers, and on that day—January 4th, 1907—the examination was discontinued.

"*B*," *Calf* 417.—This calf was injected as above. Nothing happened until the 18th December, when one piroplasma bigeminum was noticed. On the 27th day rings and rods were seen. They were, however, very rare, and hardly increased up to the 25th December, when the examinations were discontinued.

In December, 1906, Mr. Dunning, then Government Veterinary Surgeon at Zeerust, forwarded blood preparations from a sick calf, and microscopical examinations revealed the presence of piroplasma mutans exclusively. It was then thought that this might prove to be a case of pure piroplasma mutans infection, and accordingly the calf was tapped, blood forwarded to this laboratory, and the following injection was made on the 21st December, 1906.

"*C*," *Heifer* 425.—About two years old; imported from Aliwal North. Injected as above with 10 c.c. blood from Zeerust calf. Irregular reaction from date of injection. Poikilocytosis noted on the 26th and 28th days, and on the 31st day, accompanied with flagellated forms for the first time. The rod and ring forms were also noted the following day. Slight poikilocytosis, ring and flagellated forms were occasionally seen until the 50th day, the temperature on the previous day recording 105. Experiment discontinued on the 181st day after inoculation.

Experiment No. 7.

To shew that blood containing piroplasma bigeminum and piroplasma mutans when injected into cattle susceptible to ordinary redwater does not always cause the former parasite to appear.

As heifer 425 did not shew piroplasma bigeminum consequent on the injection of blood from the Zeerust calf, the supposition that this might prove a pure case of piroplasma mutans infection received support. Accordingly the following experiments were made:—

Injections with blood of heifer 425, *supposed to be a case of pure piroplasma mutans.*

"*A*," *Heifer* 434.—About two years old, and imported from England. Injected on the 31st January, 1907, subcutaneously with 10 c.c. blood of heifer 425. A sharp rise noted on the 7th day, reaching 105.4 twenty-four hours later, and accompanied with poikilocytosis. Spirillum noted on the 14th day, and from the 23rd day a typical reaction was noted, rings being noted for the first time on this date. Piroplasma mutans, marginal points and poikilocytosis were noted on the 25th, 27th, 28th and 29th days. Basic and nucleated cells together with marginal points again accompanied with polychromatic cells. The heifer died on the 34th day, 5th March, 1907.

Post-mortem Report.

Condition:—Good. Rigor mortis not completely set in, blood still running from axillary vein. Beef somewhat pale. Subcutaneous and intra-muscular tissue yellow.

Lungs:—Interstitial emphysema. Lungs pale. Slight oedema.

Heart:—Pericard contained a little liquid. A few petechiae in heart bag. Epicard almost brick red. Endocard normal.

Spleen:—Enlarged to about 1½ times normal size. Pulpa soft.

Kidneys:—Normal but somewhat pale. Urine pale.

Bladder:—Gall bladder slightly distended; contained thick green bile.

Stomach:—Third stomach soft; mucosa pale, with bile stained streaks.

Intestines:—A few superficial haemorrhagic small spots on caecum. Mucosa of small intestines covered with a layer of a slight yellow tinge.

"*B*," *Heifer* 422.—About two years old; imported from Aliwal North. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Slight reaction from the 15th day. All examinations negative until the 24th day, when poikilocytosis was noted, and accompanied the following day with piroplasma bigeminum. Piroplasma mutans noted for the first time on the 32nd day, and again present two days later, together with poikilocytosis. Discontinued on the 71st day.

"*C*," *Heifer* 401.—About two years old; imported from Aliwal North. Injected on the 30th January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Slight reaction from the 6th day and a secondary one from the 19th day. All examinations negative until the 24th day, when the ring and flagellated forms accompanied with poikilocytosis appeared for the first time. These lesions were frequently present during the first four days, when a sharp rise to 107.4 on the 33rd day was accompanied with piroplasma bigeminum, which was also noted on the two following days. The temperature regained normal on the 42nd day, polychromatic cells being noted four days previously. The experiment was discontinued on the 69th day.

"*D*," *Heifer* 420.—About two years old; imported from Aliwal North. Injected on the 31st January, 1907, subcutaneously with 10 c.c. blood of heifer 425. Reaction from the 17th day, poikilocytosis being present, and five days later flagellated and ring forms were noted for the first time, and again on the following day, accompanied with piroplasma bigeminum.

Piroplasma bigeminum, rings, basic and polychromatic cells were noticed on the 24th day, and the latter lesions were again present on the 27th, 29th and 34th days. Another reaction noted from the 53rd day, accompanied with poikilocytosis and piroplasma mutans six days later. The experiment was discontinued on the 77th day.

"*E*," *Heifer* 445.—About two years old; imported from Aliwal North. Injected subcutaneously on the 26th March, 1907, with 10 c.c. blood of heifer 425. Poikilocytosis occasionally noted from the 22nd to the 38th day, and on the following day accompanied with piroplasma mutans; these lesions again present on the 43rd and 45th days, and from this date a typical reaction was noted, lasting for 15 days, and during which time piroplasma mutans was present on the 54th, 56th, 57th and 58 days. The experiment was discontinued on the 85th day.

"*F*," *Heifer* 418.—Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Irregular reaction. Poikilocytosis noted on the 26th day, and piroplasma mutans on the 33rd day. Poikilocytosis and piroplasma mutans again present on the 35th, 37th, 40th, 43rd and 47th days.

"*G*," *Heifer* 419.—About two years old, and imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Rise of temperature noted six days later, and on the 9th day piroplasma bigeminum was noted. Piroplasma mutans present on the 24th day, together with poikilocytosis, and were both frequently noted until the 47th day.

"*H*," *Heifer* 449.—About two years old; imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 425. Irregular temperature, poikilocytosis being noted on the 22nd, 23rd and 27th days, accompanied with piroplasma mutans for the first time on the 30th day. Piroplasma mutans, together with poikilocytosis, were noticed on the 33rd, 36th, 37th, 39th, 42nd and 44th days. Experiment discontinued on the 85th day.

"*I*," *Heifer* 400.—About two years old, and imported from Aliwal North. Injected on the 30th January, 1907, with 10 c.c. of heifer 425.

Reaction from the 13th day, and 10 days later flagellated forms noted. The lesions of a piroplasma mutans infection, frequently present from the 24th day, this piroplasm being noticed on the 30th and 35th days.

As no piroplasma bigeminum appeared in this animal, and in order to prove conclusively that the Zeerust calf was not a pure case of piroplasma mutans infection, heifers 448 and 453 were injected.

Injections with blood of heifer 400.

"*J*," *Heifer* 448.—About two years old, and injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of 400.

Sharp rise from the 6th day, reached 106 four days later, and on the 15th day basic and polychromatic cells were present, when piroplasma bigeminum was noted. This piroplasm was again noted two days later. Piroplasma bigeminum again noted on the 24th day. Poikilocytosis was occasionally noticed until the 40th day, and two days later piroplasma mutans appeared, together with the lesions of poikilocytosis. Piroplasma mutans and poikilocytosis were again present on the 45th day, and the experiment was discontinued from the 81st day.

"*K*," *Heifer* 453.—About two years old, and imported from Aliwal North. Injected on the 26th March, 1907, subcutaneously with 10 c.c. blood of heifer 400. A sharp rise to 106 on the 7th day was followed by the appearance of piroplasma bigeminum for the first time. The temperature record now shewed sharp oscillations for the next ten days, accompanied with basic, nucleated and polychromatic cells. Piroplasma bigeminum, basic and nucleated cells were again noted on the 22nd day. Basic and polychromatic cells, together with poikilocytosis, were occasionally noted for the next 16 days, and on the 39th day piroplasma mutans appeared for the first time. From the 48th day a febrile reaction was noted, the temperature reaching 106 on the 55th day, and accompanied with piroplasma mutans. The same record was observed on the 59th day, and again piroplasma mutans was present. Experiment was discontinued on the 80th day.

Conclusions from animals 425, 434, 422, 401, 420, 445, 418, 419, 449, 400, 448 *and* 453.

The inoculation with blood in which microscopically only piroplasma mutans was noted produced a reaction in animals Nos. 425 and 434 without the appearance of piroplasma bigeminum, but a subsequent inoculation into animals 422, 401, 420, 419, 448 and 453 proved the presence of piroplasma bigeminum, which, as these animals were susceptible to ordinary redwater, can only be traced to this injection of blood. Therefore, although the injection of blood containing piroplasma mutans is not always followed by the appearance of piroplasma bigeminum, no conclusion can be drawn as to the presence of piroplasma bigeminum in the injected animal, unless the experiment is carried out on a sufficient number of animals.

In the foregoing experiments it has been shewn that an animal can be exclusively infected with piroplasma bigeminum either by a subcutaneous injection of blood obtained from an animal immune against redwater or by blue ticks originating from animals infected with both piroplasma bigeminum and piroplasma mutans, but in no instance was piroplasma mutans transmitted by the blue tick. In every case where piroplasma bigeminum appeared, a subsequent injection with blood containing piroplasma bigeminum and piroplasma mutans caused piroplasma mutans to appear within the typical incubation time. This fact has to be regarded as a conclusive proof of the duality of the two piroplasms.

Proof has also been given that notwithstanding the non-appearance of piroplasma bigeminum after animals were injected with blood containing piroplasma mutans, yet piroplasma bigeminum proved to be present when this blood was subsequently tested.