

REPORT

OF THE

GOVERNMENT VETERINARY BACTERIOLOGIST.

Division of Veterinary Science,
Pretoria, 13th January, 1908.

TO THE DIRECTOR OF AGRICULTURE.

SIR,

I have the honour to submit the annual report of the Government Veterinary Bacteriological Division for the financial year ending the 30th June, 1907, and again take the opportunity of briefly commenting on the various articles comprising my investigations during the year, pointing out the achievements and the further points to be elucidated.

(A) FURTHER NOTES ON PIROPLASMA MUTANS (N. spec.)

In my last report I described *piroplasma mutans* (N. spec.), a piroplasm which in former years was identified with *piroplasma bigeminum* of the immune ox, and a series of experiments were adduced in support of my new conception which I considered sufficient to demonstrate the duality of *piroplasma mutans* and *piroplasma bigeminum*. The results of my investigations in 1905-1906 shewed that a successful infection with *piroplasma mutans* was possible at any period after a pure infection of *piroplasma bigeminum*, and it was chiefly this fact which convinced me that *piroplasma bigeminum* and *piroplasma mutans* were two distinct species. This year a similar experiment was undertaken, but the length of time between the two infections was sufficient to exclude any possibility whatever of a retarded appearance of *piroplasma mutans*. For this purpose calves immune against redwater, but not against *piroplasma mutans*, were utilised; this fact was ascertained experimentally, in as much as I observed that an inoculation of blood into susceptible cattle was followed by the exclusive appearance of *piroplasma bigeminum*; susceptible cattle were, therefore, rendered immune against *piroplasma bigeminum* by injecting them with blood taken from a case of pure redwater infection. After an interval, ranging from 25 to 106 days, a second inoculation was made with blood containing *piroplasma mutans*. In every instance after the typical incubation time this latter parasite made its appearance. Continuing with my endeavours to prove beyond doubt that *piroplasma bigeminum* and *piroplasma mutans* are two distinct species, experiments were conducted with ticks. *Piroplasma bigeminum* is carried by blue larval ticks—the offspring of blue adult females which have been sucking blood on an immune or sick animal—and it follows that if the two piroplasms are in any way connected, the blue tick would likewise transmit *piroplasma mutans*; but another possibility arises that the two parasites may be of different species, and yet be carried by the same tick. Therefore, if an infestation with infected blue larval ticks be followed by the exclusive appearance of *piroplasma bigeminum*, and, at a later period it is possible to transmit *piroplasma mutans* by means of a blood inoculation to the same cattle, the result would be of considerable value in support of my conception.

Six heifers, all derived from Aliwal North, a district free of redwater, and therefore susceptible to this disease, were infested with numerous blue larval ticks, previously feeding on animals which contained both piroplasms in their blood. In all six cases the infestation of ticks was succeeded by the

exclusive appearance of *piroplasma bigeminum*, and in four animals spirillum appeared, which, as shewn in previous reports, is also carried by blue ticks. In no instance did *piroplasma mutans* appear; after a period varying from 64 to 106 days, the heifers were injected with blood of an animal immune to *piroplasma mutans* and *piroplasma bigeminum*, and as a result *piroplasma mutans* appeared after the typical incubation period, thus proving that this piroplasm does not belong to the life cycle of *piroplasma bigeminum*.

A further series of experiments were now made, somewhat on the same lines, but under the assumption that if *piroplasma mutans* is a species of its own, yet is transmitted by blue larval ticks, then infected ticks would transmit *piroplasma mutans* to animals already immune against *piroplasma bigeminum*. Therefore, if *piroplasma mutans* failed to appear after this infestation, and a subsequent injection of blood containing *piroplasma mutans* causes this latter parasite to appear, it would prove that the blue larval tick is not a host of *piroplasma mutans*. Five animals were utilised for this experiment and were rendered immune against *piroplasma bigeminum* by the injection of blood containing this parasite exclusively. A slight attack of redwater followed, from which the beasts recovered, but *piroplasma mutans* did not appear. From 49 to 54 days after this injection the five heifers were infested with blue tick larvæ, collected from animals suffering from *piroplasma mutans* and ordinary redwater; in no instance did *piroplasma mutans* appear. It now remained to note whether these five heifers were susceptible to this piroplasm. This was done by injecting blood containing both *piroplasma bigeminum* and *piroplasma mutans*, after a period varying from 56 to 67 days after the tick infestation. In all cases *piroplasma mutans* appeared after the typical incubation time, this result, therefore, further emphasizing the fact that *piroplasma mutans* cannot be identified with *piroplasma bigeminum*, and that blue tick larvæ are not carriers of both piroplasms.

An interesting experiment in connection with this subject commenced in 1904, when I collected blue ticks from an animal suffering from ordinary redwater and forwarded them to Professor Sir John M'Fadyean of London, where the larvæ were placed on a steer, and South African redwater was promptly produced. Professor M'Fadyean was good enough to forward blood preparations of this animal, and I identified *piroplasma bigeminum*; the steer recovered and was therefore immune to South African redwater. Later the steer was bled and Stockman injected some heifers in England, which he ultimately exported to this Laboratory. (I shall again refer to this strain of virus in my notes under the title Experiments with "English and South African Redwater.")

In London one of these heifers shewed a typical South African redwater reaction, and on her arrival here blood was taken and injected into a susceptible South African heifer. My previous argument equally applies in this case, namely, if *piroplasma mutans* belongs to the life cycle of *piroplasma bigeminum*, the former must appear in the injected South African heifer. However, *piroplasma bigeminum* appeared, and although the examinations were continued for 77 days, *piroplasma mutans* was never present. Continuing with this strain, the South African heifer was bled, and 10 c.c. was injected into three other South African heifers, and again *piroplasma mutans* failed to appear. The precaution was taken of ascertaining that these four injected animals were not immune against *piroplasma mutans*, and for this purpose, after the lapse of a period varying from 36 to 77 days, they were injected with blood containing both piroplasms, when, after the typical incubation time, *piroplasma mutans* appeared.

In my last annual report I enumerated some experiments shewing that *piroplasma mutans* was also found in cattle imported from Madagascar. During the past year I have frequently met *piroplasma mutans* in practice, and this fact may give you an indication of the importance of this piroplasm from a practical point of view.

On the 1st October, 1906, Mr. Lindsay, Government Veterinary Surgeon, Middelburg, forwarded some smears from a cow which died at "Tonteldoos," in an East Coast fever area. Microscopical examination proved the presence of endoglobular parasites corresponding to the description of *piroplasma mutans*, but since the animal was in an infected area reservation was made as to the cause of death, in order to make investigations amongst the herd. Smears from two healthy oxen running on the same farm were forwarded, and microscopical examination of these also revealed the presence of similar endoglobular parasites. It was now thought that this might prove to be a case of a pure infection of *piroplasma mutans*; accordingly, at our request, Mr. Lindsay tapped the animals and forwarded the blood, which was injected into two susceptible calves at the Laboratory. The result was that both of these calves developed *piroplasma bigeminum* and *piroplasma mutans* in succession.

A further case was brought to our notice by Mr. Evans, Government Veterinary Surgeon at Zeerust, who forwarded smears from a sick calf, at the same time reporting that East Coast fever was not suspected. The examination of the preparations revealed the presence of *piroplasma mutans*, and again a case of a pure infection suggested itself. Blood was obtained and injected into an animal at the Laboratory; the result was that both *piroplasma bigeminum* and *piroplasma mutans* appeared. It is a general experience that an animal infected with *piroplasma mutans* is also infected with *piroplasma bigeminum*, and although animals can easily be infected with *piroplasma bigeminum* exclusively, yet I have not been able to obtain a case of pure *piroplasma mutans* infection, and thus afford the proof of the duality of the two piroplasms in a reverse order. The experiments previously enumerated prove that the blue tick is not a carrier of *piroplasma mutans*, and therefore I conclude that it will only be possible to produce a case of pure *piroplasma mutans* infection when the tick which acts as the host has been found.

You will have noticed in my previous notes of last year that (1) no African animals have died from the injection of blood which contained *piroplasma mutans* and *piroplasma bigeminum*; (2) that all these injected animals shewed double reactions, and therefore I have a right to conclude that such animals are immune to both diseases. And this is the case, inasmuch as all animals inoculated in this manner have withstood natural exposure and no deaths were noted due to a subsequent natural infection of either of these parasites. Thus, for susceptible African cattle, the inoculation of blood of immune animals is safe, but this is not the case with imported English cattle. In the notes on this subject you will notice that one imported heifer died on the thirty-third day after inoculation of blood containing *piroplasma bigeminum* and *piroplasma mutans*—that is to say, at the time typical for both *piroplasma mutans* and for the second reaction of *piroplasma bigeminum*, with such lesions as are found in both diseases, and therefore the conclusion suggests itself that the double infection was responsible. This case can be considered on a parallel with our first experience of inoculating freshly imported English cattle in Potchefstroom some years ago, when we lost a number due to what was thought at that time to be the second reaction of redwater, but which in the light of our present experience, and from the fact

that we have noted in the surviving Potchefstroom cattle ring-shaped parasites, we must conclude that death was due to a double infection. Therefore, if we succeed in separating both infections, we may also succeed in immunising animals against both diseases, provided that the second inoculation is not performed before the animal has thoroughly recovered from the effects of the primary injection, and I can hold out some hopes of success in the near future.

In my last annual report I appended a classification of the various piroplasmoses known to me up to that time. Since then more have been described of the type of *piroplasma parvum*, namely, by Stephens and Christophers in Madras, by Miyajima and Shibayama in Japan, by Schein in Annam, and Bettencourt found it in a deer in Portugal. Bettencourt is of opinion that the two types of *piroplasma bigeminum* and *piroplasma parvum* represent two different genus, and therefore he proposes to give them separate names. Accordingly he creates a new name for the type of *piroplasma parvum*, which he designates "Theileria."

(B) EXPERIMENTS WITH ENGLISH AND SOUTH AFRICAN REDWATER.

It would be of inestimable value if English cattle could be inoculated against South African redwater previous to exportation, since a great number—in fact, almost the majority—contract the disease after they have landed here and have been exposed to natural infection. It is only natural that in the past such attempts have been followed by disastrous results, for the reason that redwater is so thoroughly established that practically all Transvaal cattle are immune and retain *piroplasma bigeminum* in their blood. Consequently, immune and sick animals infect blue ticks, which in their turn transmit the disease to susceptible beasts. Accordingly the exposure of non-immune animals into a pasture where immune Transvaal cattle are running must be followed by the development of the disease in the susceptible animals. The only way out of the difficulty is to confer a certain amount of immunity on imported cattle before exposure. It is a general fact that susceptible cattle born in South Africa can be inoculated against redwater almost with impunity, but the same does not hold good with imported cattle, probably owing to the combined infection of *piroplasma mutans* and *piroplasma bigeminum*, as explained in my previous notes on *piroplasma mutans*.

Whilst staying in London some two years ago, Stockman and myself discussed this subject, and we thought it advisable to undertake the experiment. After my return to the Transvaal, I submitted the proposition to the then Acting Director of Agriculture, Mr. A. C. MacDonald, who supported the idea and furnished authority for the necessary expenditure. In England a disease exists, also known as redwater, which is caused by a piroplasm probably identical with *piroplasma bovis* of Europe. Cattle do not suffer so severely from redwater in England as is the case with our own disease in South African animals. Although we do not anticipate that the *piroplasma bovis* of Europe and the *piroplasma bigeminum* of South Africa are identical, yet they belong to the same type, and accordingly this fact gave grounds for expecting that the immunity obtained against one would protect against the other.

Stockman and myself decided to inoculate some cattle with English redwater and to expose them to the South African veld; another lot of cattle to be inoculated with South African redwater, and the remaining batch to be inoculated with both English and South African redwater. Six Ayrshire heifers were thereupon purchased on our behalf by Stockman, and

numbered Nos. 1 to 6. Of these heifers, Nos. 1 and 3 were inoculated exclusively with English redwater, Nos. 2 and 5 with South African redwater, and Nos. 4 and 6 with both piroplasmoses. The results of these inoculations were that No. 1 did not shew piroplasms and the reaction was atypical; No. 3 shewed a reaction in England, accompanied with piroplasms; Nos. 2 and 5 shewed typical reactions, together with piroplasms; the inoculation of English redwater into Nos. 4 and 6 gave negative results, and the subsequent inoculation of South African redwater produced a reaction, accompanied with *piroplasma bigeminum*.

Thus on first sight it would seem that South African and English redwater are not identical, but this can be interpreted in a different way. It will be noticed that three out of the four heifers did not shew reactions or piroplasms consequent on the inoculation of English redwater; therefore, in the first instance, it must be concluded that English redwater is not always inoculable. For the support of this view I quote an experiment which was performed with a view of obtaining the English strain in South African cattle for further immunisation purposes. Five African animals from Aliwal North, and therefore susceptible to ordinary redwater, were injected with blood of English heifer No. 1; four animals received the injection subcutaneously and one intrajugularly. As no result was obtained within three weeks of this inoculation, 50 c.c. blood of the same animal was injected subcutaneously into all five animals, again producing negative results. Another lot of five cattle from the same origin were inoculated with 10 c.c. blood of English heifer No. 3—the heifer which shewed *piroplasma bovis* in England—four animals received 10 c.c. subcutaneously and one 5 c.c. intrajugularly. In this instance nothing particular happened during the first three weeks, whereupon the animals received a second inoculation, subcutaneously, of 50 c.c. blood of the same animal, again with negative results. I refrain from drawing any conclusions with the results obtained from heifer No. 1, as in the first instance it was doubtful whether this animal was even immune against English redwater, since no piroplasms were noted in the blood and the reaction was not of a distinct character. But the interpretation of the experiment with the blood of English heifer No. 3 only allows of one conclusion, namely, that *piroplasma bovis* was not inoculable in our five beasts. All the animals utilised in these experiments were submitted to an inoculation with African redwater, with the result that four shewed *piroplasma bigeminum*, and in the other one the typical reaction, accompanied with the lesions of poikilocytosis, could only have been produced by the infection of *piroplasma bigeminum*. The conclusion from this experiment is that the previous inoculation with blood of an animal which in England contained *piroplasma bovis* did not protect against *piroplasma bigeminum*, and from the very peculiar behaviour of *piroplasma bovis* we may safely draw a further conclusion that English redwater is not always inoculable; South African redwater is easily inoculable, and therefore both diseases are not identical.

Exposure Experiments with the Imported Heifers.

Continuing on the lines of the arrangement made between 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. 1.—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. 3.—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. 2.—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. 5.—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. 4.—Exposed at Linwood on the 5th January, 1907.—This animal also shewed 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. 6.—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.

I have already expressed the opinion that English redwater does not offer protection against our ordinary redwater. Of the four animals which had piroplasma bigeminum in their blood, due to the primary inoculation, after exposure they all more or less shewed lesions which could be identified with a new reinfection of piroplasma bigeminum. It will be seen from the experiments quoted that the inoculation of English cattle with South African redwater did not give a complete guarantee against a natural infection of piroplasma bigeminum, and we may to some extent explain the reason for this apparent failure. The animals arrived in December, almost directly afterwards they were exposed, and naturally at a time when the tick infection was at its maximum, so that they had to become acclimatised under very adverse conditions. From previous experience we know that an animal immune against redwater may break down in immunity under the influence of a subsequent heavy tick infection, and taking this fact into consideration, together

with the loss of only one English heifer, I think you will agree with me that the experiment has afforded very encouraging results. I therefore propose that a further experiment be made, but with some slight variations, namely (1) to inoculate the cattle in England with South African redwater, (2) after their arrival in the Transvaal to again inoculate them against our disease, and keep them for the first month in a paddock to ensure complete acclimatisation, and (3) to perform this experiment so that the exposure would take place at the end of the winter, when the tick infection in the veld would be at its minimum, and, with the increase of the hosts of *piroplasma bigeminum*, the cattle would gradually obtain a better protection against the disease, so that by December they could arrive at a complete immunity.

(C) FURTHER TRANSMISSION EXPERIMENTS OF EAST COAST FEVER BY MEANS OF TICKS.

Under this heading I refer to a series of experiments, not exclusively undertaken for the purpose as indicated, but which lent themselves to various interpretations, as sundry points in the transmission of East Coast fever by means of ticks are settled to which objections have been made by some investigators, whilst others propounded new observations which I decided to control. Generally speaking the experiments were undertaken for the purpose of producing pure cases of East Coast fever on the station, in order to utilise the blood and material obtained from sick animals, for inoculation. You are aware that hitherto I have not succeeded in transmitting the disease except through the agency of ticks, and that accordingly the *sine qua non* for inoculation purposes is non-existent. I shall not enumerate all these inoculation experiments, sufficient to say that they were performed with many variations, and in analogy with other diseases but they completely failed, so that for the purpose of combating East Coast fever, no recourse can be had to artificial immunisation, and the sole hope of success lays in a strict adherence to the regulations which are based on the results of our previous experience.

A few experiments were carried out in connection with the transmission of *piroplasma mutans* by the same agency, but I do not propose to furnish details, as in my opinion the conclusions are incomplete. I can, however, safely state that *piroplasma mutans* was not carried by any of the stages of the brown and blue ticks.

I wish to refer to my previous notes in the Annual Report for 1903-1904, in which I supplied the following conclusions:—

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 hebraeum 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. These latter experiments were confirmed by Mr. Lounsbury of Cape Colony, and in 1906 he published a further series of experiments proving that other ticks, besides those mentioned above, act as hosts of *piroplasma parvum*, namely, *rhipicephalus capensis*, *rhipicephalus nitens*—both of which are closely allied to the brown tick, *rh. nitens* probably being

frequently confused with the latter—and above all *Rhipicephalus evertsi* (the common red tick)—which I did not consider a carrier of the disease—may also act as a host.

Luhe, in Mense's "Handbuch der Tropenkrankheiten," refers to my experiments with infected blue and brown ticks, expressing the opinion that the failure to transmit the disease may be due to the fact that the larvæ were utilised too soon after they had hatched. In support of his view he refers to the experiment of Professor Koch who created a new centre of infection by spreading larval ticks on a pasture.

Professor Schilling, in the "Handbuch der Pathogenen Mikroorganismen," also does not consider my experiments of the non-transmissibility of *Piroplasma parvum* as conclusive, and he refers to the same notes of Professor Koch.

Koch's experiment, in his own words, as published in the "Cape Agricultural Journal" for January, 1904, was as follows:—

"... In other directions we sought for a means of communicating the disease in its virulent form. For instance, intra-ocular injections with infected blood were tried without effect and we also endeavoured by means of tick infection experiments to imitate natural methods. For this purpose cultures of the various varieties of suspected ticks were prepared. At first much difficulty was experienced in hatching out such cultures on account of the coolness and dryness of the atmosphere, conditions which experience has shewn are unfavourable for work of this description. Ultimately, however, by the use of an incubator in whose interior the humidity of the air was artificially increased, the eggs laid by ticks collected from our animals were hatched out as expeditiously as they are under the most favourable natural conditions, but when the young ticks so hatched were placed on healthy animals we found that with the exception of certain doubtful cases we failed to produce a characteristic attack. Trials were made with broods of various varieties of ticks—with broods hatched out at different temperatures, with broods kept for various periods before being placed on the animals—and this work is still being continued. To approach natural methods still more closely broods of young ticks were liberated in various localities on the grass and susceptible animals were subsequently grazed in such places. That this method should be successful appeared somewhat doubtful as we expected that the drought, high winds, dust and sun, would speedily destroy the liberated ticks, but in spite of the unfavourable weather these larval broods remained where they were placed, being most abundant on the sheltered side of the grass stems away from the sun, and particularly plentiful at the extremity of the stalks, where they clustered together in small clumps apparently waiting for the passage of a suitable host to whom they might attach themselves. These larval ticks displayed no tendency to migrate or travel from place to place, but remained where they were placed for several months. High winds seemed to scatter them a little in the direction in which the wind was blowing, but no other atmospheric change appeared to affect them. Soon after sowing these broods of seed ticks in the veld we found that it became highly infective. *Previously only occasional cases of African Coast fever had occurred amongst animals grazing in these places, the natural veld infection appearing to be so slight that animals must graze there for many weeks without sickening, while latterly ticks had become exceedingly scarce and cases of sickness had been correspondingly few in number, apparently on account of the cold and drought.*" (The italics are mine.)

This experiment would tend to shew that the infection passes through the egg. Needless to say an experiment carried out in this manner cannot give forcible conclusions; *the larval ticks were distributed on an already infected area*, and with the introduction of fresh susceptible cattle the East Coast fever carrying ticks already present on the pasture, became infected and produced the broadcast infection.

It will be noticed that Professor Koch does not state in his article quoted that the blue tick was utilised, but simply mentions the fact that various varieties of ticks were used in his trials. However, at the "Conference on Diseases amongst Cattle," held at Bloemfontein in December, 1903, referring to this experiment he stated that he had only got these results with the blue tick, and therefore was of the opinion that the blue tick was of most importance in spreading the disease. He would not say that other ticks did not carry the disease, but he had never been able to produce infection with any but the blue tick. He had strong proof of his opinion, but still he would not be positive.

Our experiments were, therefore, undertaken again, in order to prove in an unmistakable way that the blue tick is not a carrier of the disease. For this purpose four animals were infested; I only utilised four animals, but the infestation was so heavy that no other interpretation is possible than that the blue tick did not act as the host of *piroplasma parvum*, and this experimental observation coincides with our experience in practice.

A further fact which forms the basis of our legislation is not accepted by Professor Schilling in his mentioned publication, namely, he refers to my statement that "an animal which is immune against East Coast fever does not act as a propagator of this disease," and as proof of his contention states that our view is contradicted by the history of the disease in Rhodesia. He asks the question "from which cattle the animals imported from New South Wales obtained the infection, if not from the cattle which were grazing in the neighbourhood of Beira and amongst which, as Koch has proved, were carriers of *piroplasma parvum*?" This statement does not of course constitute proof. The fact can, and must be, interpreted in a different way; East Coast fever was never in Beira; the Australian herd died of redwater, not of East Coast fever; they only died of East Coast fever after their arrival at Umtali. The best proof of this is that a mob of Madagascar oxen were grazing together with the Australian herd at Beira. Madagascar oxen are immune to ordinary redwater, and although a number of the Australian herd died, the Madagascar mob remained healthy. However, after the Madagascar mob were transported to Rhodesia, numbers died from East Coast fever, thus proving that these cattle were not immune to this disease. Having established this fact, it is quite clear that the disease responsible for the deaths amongst the Australian animals at Beira could not have been East Coast fever, otherwise the infection would have spread to the Madagascar herd. Therefore the infection of the Australian herd, after removal to Umtali, must have been contracted at that latter place.

I will now quote a statement made by Mr. J. M. Orpen, late Head of the Agricultural Department, Rhodesia, in the periodical "South Africa," dated March 18th, 1905, in support of my statements, and which supply the correct answer to Professor Schilling's question:—

"Before the arrival in Beira in November, 1900, of the shipment of a thousand head of cattle, which Mr. Rhodes introduced from New South Wales, as the first of an intended series of such importations by bought or chartered steamers, for the purpose of supplying the European settlers with stock, a gentleman in Umtali introduced by railway into that town a number of

slaughter cattle, which had come from German East Africa through Beira. Part of them he kept for sometime on Umtali commonage, and others he passed on by rail to Salisbury where they were slaughtered at the poles in the Makabusi Valley, on the commonage of that town. Not long after this, and just in those two places, some cases occurred of typical African Coast fever and then there began to spread from those two places along the various roads in Rhodesia a mixed infection of redwater and this new fever till then unknown to science, and indistinguishable from ordinary redwater, of which it was, of course, supposed to be only a severe form. No such outbreak occurred in the Portuguese territory through which the cattle had been brought quickly by rail."

"Soon after the introduction of these diseased animals, there arrived at Beira the splendid and healthy cattle from Australia which Mr. Rhodes was introducing. A breakdown occurred on the railway, and the imported cattle had, therefore, to be pastured on the Beira flats, as many others had been before. None previously pastured there had contracted African Coast fever which does not appear to exist there at all. But these Australian animals, coming from a country where no redwater exists and thus being susceptible, contracted that disease and began to die. . . . The cattle were taken to Umtali by rail and thence to a neighbouring Government farm, and isolated from all other animals. Most of them died there of ordinary redwater, but it is evident from the report (British South Africa's Company Shareholder Report for 1903), that many of them eventually died of African Coast fever, which as I have shewn had been brought to Umtali from German East Africa."

The objection raised by Luhe, certainly seemed to have some foundation. It may be that the brown tick which in the nymphal and adult stage is a carrier of *piroplasma parvum*, may carry it as larvæ of an infected female, when that larvæ is sufficiently old to allow for the development of the parasite. This point was also cleared up. Brown females were collected on the Coast near Durban in Natal from sick cattle, and from the same cattle brown nymphæ were also taken, which, after hatching in the Transvaal, promptly produced the disease. Bearing Luhe's observation in mind, tests were made to note if the larvæ of females taken off the same animals would transmit the disease. Altogether 19 animals were infested with brown tick larvæ, the progeny of these infected females, and the period which had elapsed after placing these ticks on the susceptible cattle and their hatching varied between 22 and 71 days. In no instance was the infestation followed by the disease and there is no reason to suppose that the animals were immune, because they all came from Aliwal North, and belonged to a group of animals of which some were utilised for infection with brown adults and promptly contracted the disease. Thus on investigation the objections of Luhe prove to be untenable, but another contingency offered itself by analogy with the transmission of piroplasmoses by other ticks, namely, that the future stage in the life cycle of the tick may transmit the disease. The engorged larvæ of these infected females were therefore all collected and after moulting were placed on 20 different animals, and again after a lapse of time sufficiently long to allow for the development of the parasite. In no instance was East Coast fever observed. The next stage (*i.e.*, the imago) had also to be tested; therefore after the nymphæ had hatched they were collected and the imagines placed on five different cattle, and in no instance did East Coast fever follow.

It so happened that an animal of this batch was utilised later, in which he succumbed to the infestation of infected brown ticks, thus proving that no immunity existed due to these previous infestations.

We may safely conclude, therefore, that the brown tick which as an adult fed on sick cattle, is not a possible factor in the propagation of the disease in any of its later stages, that is to say, the infection does not pass from the female through the egg, and infect either larvæ or nymphæ.

As already mentioned, Lounsbury stated that in his experiments the red tick proved to be a carrier of the disease. My previous experiment failed, some of his failed, so that the conclusion may be drawn that red ticks do not so readily lend themselves to the propagation of East Coast fever as the brown tick. I have repeated Lounsbury's tests, obtaining my ticks from different sources, and have been able to transmit the disease to three animals, whereas a large number failed.

With regard to the bont tick, the opportunity occurred to see whether these ticks also act as hosts of *piroplasma parvum*. Nymphæ were collected from the Natal cattle, and after moulting in the Laboratory, were placed on healthy cattle, but no results were obtained. I also experimented with larvæ, the offspring of females sucking blood on sick cattle, these experiments also gave negative results. It now remains to be seen whether the larvæ which as nymphæ fed on sick cattle, would transmit the disease in one of its later stages.

Mr. Lounsbury was good enough to forward me some infected rhipicephalus capensis ticks, which I placed on a beast. The result was that they transmitted the disease, as Mr. Lounsbury had anticipated, but only after an incubation time of 30 days, which is very long compared with the results given by the brown tick.

The résumé of the facts mentioned in my investigations is therefore as follows :—

- (1) *Rhipicephalus decoloratus* (the blue tick) is not a host of *piroplasma parvum* ;
- (2) *Rhipicephalus appendiculatus* (the brown tick) is a host of *piroplasma parvum* ;
- (3) *Rhipicephalus evertsi* (the red leg tick) is a host of *piroplasma parvum* ;
- (4) *Rhipicephalus capensis* (the cape tick) is a host of *piroplasma parvum* ;
- (5) *Rhipicephalus simus* is a host of *piroplasma parvum* ; and,
- (6) according to Lounsbury, *Rhipicephalus nitens* is a host of *piroplasma parvum*.

In the article by Mr. Lounsbury, *Cape Agricultural Journal*, May, 1906, page 638, he states : " Other observers have based their determination of African Coast fever chiefly on the occurrence of a certain rod-shaped intracorpuseular organism, named by Dr. A Theiler, *piroplasma parvum*, in the blood of the affected animal. The almost invariable presence of this organism in blood smears from very sick animals has led to the assumption that the disease is caused by it. Mr. Robertson's observations on the experimental cases, however, throw doubt on the correctness of this assumption. He permits me to say that he has been unable to find the organism in any of the smears taken from nine of the cases, and that it has been of doubtful occurrence or rare in almost as many others. In some cases, on the other hand, it has been as numerous as in cases of the disease contracted on the veld in Rhodesia or the Transvaal, indicating that the condition necessary for its appearance in the blood is sometimes present in Cape cattle."

From this extract it must be concluded that Mr. Robertson doubts that the *piroplasma parvum* is the actual cause of East Coast fever ; that in other words, *piroplasma parvum* may be an accidental occurrence due to the

presence of East Coast fever, which latter disease may be caused by an ultra-visible micro-organism. This objection had some foundation, and as long as we experimented with cattle in South Africa it would hold good, since it may be that cattle born in a South African redwater area may contain that organism which subsequently develops under the influence of East Coast fever. As I could not undertake the experiment in South Africa, I corresponded with Mr. Stockman in London, who at once kindly consented to carry out some experiments in his Laboratory for the purpose of elucidating this point. The object was to utilise cattle which had never in any way previously been in contact with any kind of piroplasmis infection, and this was naturally possible in England. I forwarded two batches of ticks, one of red imagines and the other brown imagines. Stockman placed the ticks on two animals, and in both cases was the infestation of the cattle followed by East Coast fever, and the piroplasms present in the blood preparations were as numerous as in any cases observed in the Transvaal. I therefore conclude that the tick which carries East Coast fever also carries *piroplasma parvum*, and that this piroplasm is the actual cause of East Coast fever. Mr. Robertson's observations suggest a different interpretation, and we only need refer to redwater of cattle, to the piroplasmis in the horse, and to the trypanosomiasis in cattle, the disease may develop in the animal itself with a minimum of parasites present in the blood. Therefore cases of East Coast fever may escape microscopical diagnosis for this reason.

As mentioned before in connection with *piroplasma mutans*, confusion with an infection of this latter parasite may render an accurate diagnosis extremely difficult and even impossible. The fact remains, however, that so far in our experience we have not diagnosed East Coast fever and found reason to correct our verdict at a later date.

(D) RESULTS OF HORSE-SICKNESS INOCULATION IN PRACTICE, 1906-1907.

The inoculation of mules against horse-sickness was recommenced in September, 1906. In the experiments at this Laboratory I noticed that the immunity obtained by the injection of virus of the ordinary strain, did not offer a complete protection against a subsequent spontaneous infection of horse-sickness; the mortality from relapses being 0.6 per cent.; further experimental cases proved that we were able to break this immunity by the injection of a virus obtained from Tzaneen and it was expected that the immunity from this latter strain would afford a better protection against the ordinary strain than *vice-versa*. I accordingly decided to introduce this new strain into practice. This was done in September, 1906, when the necessary instructions were given to the Government Veterinary Surgeons, and up to the new year the Tzaneen, virus was utilised in practice. This innovation seemed to prove satisfactory until about December, when reports came to hand, stating that the new strain did not produce the anticipated reaction. An investigation was therefore made, and the remainder of the virus in the hands of the Government Veterinary Surgeons was returned to the Laboratory, and tested on both horses and mules, the results proving that it was inert. This was contrary to all our previous experience, inasmuch as we had already ascertained that the virus could be preserved for several years without losing its virulency. This fact is therefore worth noting, but the reason cannot yet be given, although experiments to this end were immediately undertaken, and are still being continued.

It was apparent, however, that animals inoculated with the strain of virus which had proved inert, did not acquire any immunity. Fortunately

in some cases we could repair this mishap by the re-inoculation of the animals with the ordinary strain. For instance, in Rustenburg where 102 mules were inoculated with Tzaneen virus, 15 contracted horse-sickness and died, the owners being compensated, and the remaining 87 were re-inoculated free of charge; in Natal where the Tzaneen strain was also used, the re-inoculation was carried out before the animals were exposed. In Rhodesia, however, 80 mules were inoculated with the Tzaneen strain, and when our rows had reached the Chief Veterinary Surgeon it was impossible for him to collect the animals or to convince the proprietors of the necessity of a second inoculation; in this case the losses after exposure were rather heavy, and amounted to about 25 per cent. With this experience at my disposal I decided after the New Year to completely discontinue the use of the Tzaneen strain and re-introduce the ordinary strain which had been used in 1905-1906.

A deviation was made from our usual practice last year, by authorising the Government Veterinary Surgeons to inoculate mules on the owners' farms whenever requested, providing they were satisfied that the proprietors would give the necessary attention during the reaction, and this arrangement proved very satisfactory, especially in cases where the farm was situated at some considerable distance from the District Office.

During 1906-1907, in the various districts of the Transvaal, 3,155 animals were inoculated with a loss of 125, or 4 per cent.; Rhodesia inoculated 972 mules with a mortality of 21, or 2.0 per cent.—these figures do not include animals inoculated with inert virus. In the Orange River Colony, 24 were treated, with one death, or 4 per cent.; Natal inoculated 1,170, and 59 died—5 per cent.; Bechuanaland was rather unfortunate, and lost 3 out of 35 inoculated; 76 mules were inoculated in Swaziland, of which 4 died. The total for South Africa was 5,432 inoculated, with a mortality of 213, or 3.9 per cent.

The total number of mules immunised in South Africa, together with the mortality, since November, 1905, is 8,766 mules inoculated—329 deaths—3.7 per cent.

In addition to these figures, the mortality after exposure enters into consideration. Of the 8,325 immunised mules, 112 died after discharge, or 1.3 per cent., but it is improbable that horse-sickness was responsible for the death in every case, although in arriving at these figures all deaths after discharge were included, and considered as horse-sickness, unless certified by the owner or the Government Veterinary Surgeon to the contrary.

We may, therefore, conclude that the total percentage of deaths amongst mules treated by our immunisation method is 5 per cent., in other words, of every 100 animals inoculated, 95 survived and successfully passed through the worst horse-sickness season experienced for many years.

As far as the statistics are to hand, the total number of deaths from horse-sickness amongst horses and non-inoculated mules during last season amounted to 6,783, and although this return is incomplete, it affords an idea of the severe nature of the disease in 1906-1907.

(E, F, AND G) THE INVESTIGATIONS INTO HORSE-SICKNESS.

In the three articles on this subject detailed in my research experiments, *i.e.* (a) "Further notes on immunity in horse-sickness," (b) "Immunisation of mules with inadequate and adequate virus and serum and the immunity obtained therefrom," and (c) "Inoculation of mules with polyvalent virus," I am able to add to my previous notes regarding the immunity of mules and horses against horse-sickness. I pointed out in my former report that a

certain mortality occurred amongst immunised mules, amounting to 0·6 per cent., these relapses being known to the farmer under the name of "Aan-maning." I then expressed the opinion that it is not the animal which loses its immunity, but that several strains of horse-sickness exist in the various parts of the country—and even in one and the same locality—which do not protect reciprocally. I especially referred to a virus obtained from Tzaneen, and to another from Bulawayo, the former from an immunised horse, and the latter from an immunised mule, both of which were suffering from relapses. This year the deaths after exposure amongst 8,325 immunised mules amounted to 1·3 per cent., and the object of my experiments was to detect the extent of these relapses amongst immunised mules, when subsequently tested.

In order to avoid repetition, I will detail the practice followed in all these experiments: Mules were immunised against a particular strain of virus (such as ordinary, Tzaneen, etc.), the proof of their immunity being furnished by the temperature reaction; subsequently they were tested either by an injection of a few cubic centimetres of virus or by hyperimmunisation by means of a direct transfusion of about 9 litres of virulent blood; some mules were tested with several different strains. In detailing the results I have excluded all reactions accompanied with the presence of *piroplasma equi*, or if the temperature reaction was absolutely abnormal for horse-sickness, and suggested that the disease was of a different nature.

The term "Ordinary Virus" refers to the first virus used in practice and collected in Pretoria. The ordinary serum was obtained from animals injected with ordinary virus.

(1) ORDINARY VIRUS.

A.—Mules.

I will first of all deal with the mules immunised with the ordinary virus, that is the virus used in practice during 1905–1906.

(a) Mules immunised with ordinary virus, and tested with ordinary virus.

273 Mules were inoculated and tested, with the result that none contracted horse-sickness, nor did any shew a horse-sickness reaction. The conclusion, therefore, is that the immunity in mules obtained by the ordinary virus protects against a subsequent inoculation or infusion of the same virus.

(b) Mules immunised with ordinary virus, and tested with Tzaneen virus.

139 Mules were used, with the result that 12 shewed reactions, 4 reactions with dik-kop, and 1 died; the percentage being reactions—9 per cent., reactions with dik-kop—3 per cent., and deaths—0·7 per cent.

(c) Mules immunised with ordinary virus, and tested with Bulawayo virus.

Of 36 animals treated and tested in this way, 7 shewed distinct reactions, 6 reactions with dik-kop, 1 doubtful reaction, and 6 deaths; the percentage being reactions—19 per cent., reactions with dik-kop—16 per cent., and deaths—16 per cent.

(d) Mules immunised with ordinary virus, and tested with a mixture of Bulawayo and Tzaneen virus.

Of 6 animals, immunised with ordinary virus and tested with a mixture of Bulawayo and Tzaneen virus, 2 shewed reactions, or 33 per cent.

These statistics shew in a demonstrative manner the divergency of horse-sickness and the immunity obtained therefrom.

B.—Horses.

- (a) Horses immunised with ordinary virus, and tested with ordinary virus.

Referring to the experiments with horses, 104 were immunised with the ordinary virus, of which 88 were tested with the same virus; of these 1 shewed a reaction and dik-kop, or 1 per cent., and 3 a doubtful reaction—3·4 per cent. The one reaction with dik-kop is the only instance in which a horse immunised against the ordinary strain, has shewn a relapse with dik-kop, due to a subsequent injection of the same virus, and it is quite contrary to the result obtained from the corresponding experiment on mules. Shortly before testing, this horse was exposed at Onderstepoort, where some immunised horses and mules had contracted the disease spontaneously, so that it is quite possible that the horse was not suffering from a relapse due to the test, but to a natural infection of a different strain of virus.

- (b) Horses immunised with ordinary virus, and tested with Tzaneen virus.

40 Horses immunised with ordinary virus were tested with Tzaneen virus, producing 12 reactions—30 per cent.; 6 reactions with dik-kop—15 per cent.; 1 doubtful reaction, probably due to *piroplasma equi*, and 7 deaths—17 per cent.

- (c) Horses immunised with ordinary virus, and tested with Bulawayo virus.

26 Horses were immunised with ordinary virus, and tested with Bulawayo virus, the result being that 3 shewed reactions—12 per cent.; 4 reactions with dik-kop—16 per cent.; 1 doubtful reaction, and 5 deaths—20 per cent.

- (d) Horses immunised with ordinary virus, and tested with a mixture of Ordinary, Tzaneen and Bulawayo virus.

Of 10 horses immunised with ordinary virus, and tested with a mixture of O-T-B virus, none contracted horse-sickness or died.

- (e) Horses immunised with ordinary virus, and tested with virus obtained from spontaneous cases.

43 Horses were immunised with ordinary virus, and tested with virus obtained from spontaneous cases in the Zoutpansberg district, of which 2 shewed reactions—5 per cent.; 3 doubtful reactions, and 1 death—2 per cent.

In comparing the results obtained from immunised mules with immunised horses when tested with various vira it will be seen that only 1·5 per cent. of immunised mules died, whereas the mortality in immunised horses amounted to 6·2 per cent.

(2) TZANEEN VIRUS.

A.—Mules.

In order to overcome the possibility of subsequent relapses in immunised mules, and observing that the immunity obtained by the ordinary virus did not completely protect against an injection of the Tzaneen strain, the latter virus was introduced into practice last year. Control experiments were then carried out to note the protection it afforded against the other virus.

After it was ascertained that the mortality due to the simultaneous inoculation of mules with Tzaneen virus and serum obtained from animals hyperimmunised with the ordinary strain, was not higher than when ordinary virus was used in conjunction with ordinary serum, this alteration in the method was considered safe, and virus of various generations of the Tzaneen strain was utilised in conjunction with ordinary serum.

(a) Mules immunised with Tzaneen virus, and tested with
Tzaneen virus.

23 Mules were immunised with the Tzaneen strain (*i.e.*, Tzaneen virus and ordinary serum) and tested with the ordinary virus, with the result that 18 gave reactions—70 per cent.; 2 reactions with dikkop—9 per cent, and 3 deaths—11 per cent. It therefore appeared that the immunity obtained from the Tzaneen virus in conjunction with the serum of the ordinary strain, did not protect against a subsequent injection of the ordinary virus, in the same way as the immunity obtained from ordinary virus and serum resisted the Tzaneen virus.

The interpretation of this fact is probably that in inoculating mules with the ordinary virus and adequate serum, a complete immunity is obtained against the virus, but in using ordinary serum against Tzaneen virus—the virus and serum being inadequate—it is probable that not all the components of the Tzaneen strain are capable of making an impression on the system of the mule; in other words, the ordinary serum nullifies the effect of certain components of the Tzaneen strain, and consequently does not give complete immunity. This conception has a support in the following:—

(b) Mules immunised with Tzaneen virus and ordinary
serum and tested with Tzaneen virus.

26 Mules were immunised with the Tzaneen strain, and the test with the Tzaneen virus produced 19 reactions, or 73 per cent. The test was made with a higher generation of virus than that used for the immunisation, and possibly this may account for the relapses; but the fact can also be explained if we accept that the Tzaneen virus is already a complex virus, and the serum being of the ordinary strain neutralises some components of the Tzaneen virus and prevents the formation of antibodies in the injected animals which are active against the whole of the Tzaneen virus. When at a later period the effect of the serum has passed over and the same virus is again utilised, those components which could not produce anti-bodies are now able to cause a reaction.

It has, therefore, to be expected—as is the case with the ordinary virus—that the immunity obtained from a simultaneous injection of Tzaneen virus and the adequate serum would afford a better protection than that given by the injection of Tzaneen virus and ordinary serum.

(c) Mules immunised with Tzaneen virus, and tested with
Bulawayo virus.

Of 11 mules immunised with the Tzaneen virus and ordinary serum, and tested with Bulawayo virus 5 gave reactions—44 per cent.; 2 doubtful reactions were noticed, and 1 death—9 per cent.

(3) POLYVALENT VIRUS.

With all these particulars at my disposal I decided to experiment with a view of obtaining an immunity by a single injection which would protect against all three vira, namely, Ordinary, Tzaneen, and Bulawayo.

(In the following notes certain symbols, such as OTB and O-T-B appear, which must be explained. A virus or serum obtained by mixing the three strains, Ordinary, Tzaneen, and Bulawayo, was, for the sake of brevity, designated O-T-B. But if the three vira were consecutively injected and at the height of the fever reaction the animal was tapped and the blood utilised as virus, this trivalent virus was called OTB.

Thus the symbol OTBLPW means that eight different vira, Ordinary, Tzaneen, Bulawayo, Lydenburg, Piet Retief, Pietersburg (2), and Warmbaths were consecutively injected and an octovalent virus obtained from the animal during the fever reaction.)

(a) Virus O-T-B and Serum O-T-B.

Injections with this virus and serum produced reactions in all cases, but when the animals were subsequently tested with any of the constituents of the virus mixture separately, it appeared that the immunity was not complete.

A further experiment was made by injecting mules with a virus composed of the mixture of two virus, either ordinary and Tzaneen, or ordinary and Bulawayo, or Tzaneen and Bulawayo, the serum in each case being the mixture O-T-B. Again reactions were produced, but a subsequent test with either components of the virus mixture produced relapses.

These two failures must similarly be interpreted by admitting that the mixture of the various sera did not represent the adequate serum to the virus mixture, and one or the other virus was precluded from having any effect on the mule.

(b) Experiments with Virus OTB and Serum O-T-B.

The three strains of virus were now consecutively injected into a horse, and when the horse-sickness reaction ensued the horse was tapped and the blood utilised as virus. This virus was expected to be trivalent. The serum was obtained by mixing the sera corresponding to the three strains.

The simultaneous injection of this virus and serum into mules, produced reactions in all cases and immunity was apparently established, but when the animals were subsequently tested with one of the component strains, an unmistakable breakdown was noticed, probably for the same reason as explained previously.

Polyvalent immunity cannot therefore be obtained in this way.

(c) Virus OTBLPW and Serum O-T-B.

This virus was obtained on the same lines as in the previous case, but with eight different strains of virus obtained from Ordinary, Tzaneen, Bulawayo, Lydenburg, Pietersburg (two kinds), and Piet Retief.

The serum was O-T-B.

Again all injected mules reacted, but a test with any of the components of the virus mixture produced breakdowns.

(d) Virus OTB and Serum OTB.

The final experiments were now made by immunising animals with polyvalent virus and serum.

The virus used was OTB (*vide* Experiment 3b), and to which the serum corresponded. The result was that immunity given by virus OTB and serum OTB, with one exception, protected against a subsequent injection of any of the components of the virus mixture.

(e) Virus OTBLPW. Serum OTBLPW.

The simultaneous injection of virus OTBLPW (*vide* Experiment 3c) and a corresponding serum, did not completely protect against a subsequent injection of any of the components of the virus, but in the majority of cases no breakdowns were noted.

In comparing these results it will be seen that immunity obtained by the polyvalent virus and serum afforded a better protection against the constituents of the polyvalent virus than the immunity obtained by an injection of a mixture of the various virus and their adequate sera (O-T-B).

(H) CONTINUATION OF EXPERIMENTS FOR INOCULATION AGAINST EQUINE PIROPLASMOSIS.

In my last annual report a number of experiments were enumerated shewing that (1) the inoculation of mules with immune mule blood can be performed with a large prospect of success; (2) that a certain amount of risk is attached to the inoculation of donkeys with immune mule blood, and (3) that the inoculation of horses with immune donkey blood may be followed by disastrous results. During the past year these experiments have been continued on a somewhat different line, and based on the observation made in connection with redwater, namely, that the inoculation of cattle with blood of a calf immune against this disease is not so frequently followed by strong reactions and mortality as when the blood is derived from a full-grown beast.

Accordingly I decided to utilise the blood (*a*) from young immune weaned horse foals, and (*b*) from immune donkey foals which were still suckling. The experiments were classified according to the origin of the blood, namely, (1) from an immune horse, (2) from an immune mule, and (3) from an immune donkey. The various strains of virus were subsequently passed through horse foals and donkey foals, and blood of each generation used for inoculation purposes.

Four Transvaal horse foals were injected with blood from an immune horse and none died. In the second generation with this strain, eight Argentine horses were inoculated and five died; two horses were suffering from gangrenous pneumonia, probably the result of a disease contracted on board ship, called "ship's pneumonia," and died; one died from horse-sickness contracted spontaneously, and the other two were pregnant mares, and this fact, in conjunction with the piroplasmosis reaction, must be held responsible.

The second series of experiments refers to blood originating from an immune mule. The strain was passed through horse foals and continued for eight generations. The results were that five Argentine horses were inoculated and four died; 67 Argentine mules inoculated with one death, and 12 Argentine donkeys with one death; nine Transvaal horse foals and five Transvaal mules with no deaths.

With regard to the four Argentine horses which died, two were in very poor condition previous to inoculation, and death was caused by poverty complicated with piroplasmosis. A third animal died from syncope, and in the fourth instance broncho-pneumonia was responsible. The Argentine donkey slipped her foal and died, as a result of the complication with poverty and debility. The death of the Argentine mule was due to pneumonia, probably "ship's pneumonia".

Referring to the third class, where the strain emanates from a donkey, this was passed through a horse, horse foals, and donkey foals, and continued for six generations; in each generation blood from the horse foal or donkey foal was utilised with the result that

of 12 Transvaal horse foals inoculated	1	died
5 .. donkey foals	0	..
1 .. horse	0	..
7 .. mules	1	..
23 Argentine horses	3	..
62 .. mules	2	..
25 .. donkeys	0	..

The Transvaal mule died as a result of undoubted piroplasmosis; two Argentine horses died from rupture of the spleen; the third one was killed on account of pleuro-pneumonia. Of the two Argentine mules, one died of pure piroplasmosis and the other from the sequel of Piroplasmosis.

The conclusions, therefore, are that: (1) mares heavy in foal should not be inoculated; (2) animals in poor condition must not be inoculated; (3) animals imported from oversea should not be inoculated until all danger of an infection with ship's pneumonia has been removed; and (4) the contingency must always be expected that Argentine horses and mules may die of rupture of the spleen, as they are very wild and stabling often causes them to contract serious injuries.

Therefore, equines can now be inoculated against piroplasmosis with a small risk of mortality, providing the precautions previously mentioned are observed; and later I shall ask for your authority to introduce this method of inoculation into practice.

(I) INOCULATION AGAINST BLUE TONGUE, AND RESULTS IN PRACTICE.

In the article "Blue Tongue in Sheep," included in my annual report for 1904-1905, I enumerated several experiments carried out on the lines proposed by Mr. Spreull, M.R.C.V.S., of the Cape Colony, namely, a simultaneous inoculation of serum and of virus in certain proportions. In my experiments the dose of serum was probably too high, and the reaction necessary to produce an active immunity escaped notice. I therefore concluded that the serum had a very strong preventive action, and for future experiments proposed to reduce the quantity of serum injected. In continuing the experiments accordingly, and infecting sheep with virus for the purpose of hyperimmunisation, I noticed that after the virus had passed through several generations the mortality from inoculation completely ceased. All the animals had reactions typical for blue tongue, which, to judge from the temperature charts, were very severe, and yet they did not shew any clinical symptoms, so that the illness was hardly noticeable. The results of these observations are that of the first 10 generations, 10 sheep died out of 93, and from the 11th to 18th generations, none died out of 319; thus, out of a total of 412 sheep, 10 died, or 2·5 per cent. After I had ascertained that the immunity from this reaction was equal to that obtained from a natural attack, I decided to introduce the inoculation into practice and to utilise the virus obtained from the 11th to 18th generations. Accordingly in February, 1907, instructions were issued to all Government Veterinary Surgeons to inform the farmers that a vaccine for the prevention of blue tongue was ready, and would be issued free of charge, together with a syringe, as far as these instruments were available. Provision was also made for the full instructions to be thoroughly explained to the farmers, so that if they wished they could perform the operation themselves.

At the commencement of the inoculation a slight mishap was experienced owing to the rain, when mud entered into the puncture caused by the inoculation, causing blood poisoning, but steps were immediately taken to warn all concerned, and further instructions issued not to inoculate during the heavy rains, when the sheep would have to be exposed to wet pastures and muddy kraals. This is an important fact, and I have decided to recommend that the inoculation for the ensuing season be started before the rains have thoroughly set in.

The total number of doses issued in the Transvaal amounted to 31,087.

Towards the conclusion of the blue tongue season a circular was issued asking the various Government Veterinary Surgeons to obtain information regarding the success of the vaccination. These returns were unsatisfactory, as only 20 per cent. of the results came to hand, shewing that 5,875 sheep were inoculated in the Transvaal, and that 74 died within 14 days after the inoculation. It must be explained here that the whole course of the disease averages 14 days; therefore this period must be allowed for the reaction, consequent upon the vaccination, before immunity is finally established; it is, therefore, quite possible that an animal suffering from blue tongue, or naturally infected on the day of inoculation, would die within this period. Consequently deaths within 14 days after vaccination must be excluded, since the animals had not acquired immunity; neither can these deaths be considered as a result of the inoculation, since we have shewn in our experiments that out of 319 animals vaccinated, none died. The percentage of deaths occurring after 14 days from inoculation—breakdowns in immunity—is about 0.6 per cent. At the same time farmers were asked to furnish returns of mortality amongst their non-inoculated sheep. These returns shew that out of 16,218 sheep, 1,817 died, an average of 11 per cent. These figures speak for themselves and testify to the success of our vaccination method.

Generally speaking, last season was a very bad one for blue tongue in sheep; a complete return of the losses are not at my disposal, but in the Bethal District 5,076 sheep died; Lichtenburg shews deaths amounting to 7,096, out of a total number of 85,047.

The farmers are unanimous in testifying to the efficacy of the vaccine, and the following extract from the report of the Resident Magistrate, Ermelo, may be taken as a fair example:—"Mr. Turner, of Clifton, inoculated the whole of his flock (1,351) with the blue tongue vaccine, with apparently excellent results, as very few of his sheep have been lost, and since the inoculation the disease has apparently died out, whereas his neighbours are still losing sheep in considerable numbers."

From previous experiments, a certain amount of blue tongue serum was at my disposal, and which, as I considered it would be waste to throw away, I decided to utilise for curative purposes in practice. This curative treatment was also mentioned in the circular to Government Veterinary Surgeons, and over 1,000 doses were issued. I did not anticipate any good results from the curative treatment of blue tongue by means of serum, as I was fully aware that the injection of serum would, in the majority of cases, be too late to have any beneficial effect. To my great surprise, however, numerous reports came to hand stating that the serum proved successful in such cases where the disease was not too far advanced, and even in some cases where the animals were very ill. On the other hand, negative reports also came to hand.

However, I have decided not to prepare any more serum, as the preparation is very expensive, and the dose required extremely high, so that the charge would be prohibitive for the common sheep, and further, seeing that the vaccination is a complete success, no curative treatment should be required if the farmer takes the precaution of inoculating his flock before blue tongue makes its appearance.

I have not yet been able to ascertain how long the immunity obtained from either a natural attack or from our inoculation will last; I know that the immunity conferred by the vaccine may be broken down by a large dose of virus, but the immunity in practice seems to be sufficiently strong to protect against and prevent any great losses, and certainly seems to protect for at least a year.