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REPORT OF THE GOVERNMENT VETERINARY BACTERIOLOGIST.

Division of Veterinary Science,
Pretoria, 12th November, 1906.

To the Director of Agriculture, Pretoria.

Sir,

I have the honour to submit the annual report of the Government Veterinary Bacteriological Division for the year 1905-6.

In accordance with our usual practice this report is divided into two sections: (*a*) routine work, and (*b*) research experiments.

I take the liberty of briefly commenting on the various articles which formed the subjects of our investigations during the past fiscal year, pointing out our achievements and also the further research work necessary to elucidate doubtful points on the various subjects.

Piroplasma Mutans n.spec., and the Disease caused by it.

With this name I now designate the small piroplasma which, as a rule, is met with in cattle injected with the blood of a South African redwater immune animal. In laying stress on the origin of the immune animal, I wish to point out that a similar occurrence has not been met with in other parts of the world where Texas fever exists, and where the inoculation against this disease is used in practice. In my article on the "Piroplasma Bigeminum of the Immune Ox" I have largely dealt with the appearance of the small endoglobular parasites, and have also shewn that they cannot be identified with the piroplasma parvum of East Coast Fever. Immunity against the former does not protect against the latter. In my experiments I particularly noticed the regular appearance of these small endoglobular parasites, and I was inclined to believe that they were only a form in the life cycle of piroplasma bigeminum. The experiments of last year have, however, clearly disproved this theory and, further, indicate a new species of piroplasma for which I propose the above name. The two organisms—piroplasma bigeminum and piroplasma mutans—were separable, and this affords sufficient proof of their duality. It was accordingly shewn that an animal can be infected with piroplasma bigeminum by itself, and later with piroplasma mutans. The reverse experiment has not yet succeeded, as I have not yet noted an animal which was infected exclusively with piroplasma mutans.

Piroplasma mutans has all the characteristics of the piroplasma of the type of ordinary redwater, since it remains in the immune animal, and can be transmitted with the blood. Indeed when we inoculate against redwater, we usually inoculate against this latter parasite.

In my notes on Unclassified Diseases in Cattle several cases occurred which I now give *in extenso*, as they seem to be typical for the disease caused exclusively by piroplasma mutans. There is no name for this disease, and in the language of the farmer it would probably be called "galziekte." I have repeatedly pointed out the vagueness of this term, which is applicable to almost any disease in which there is a deranged condition of the liver and bile pro-

duction. In my experience the name has been applied to redwater, rinderpest, east coast fever, heartwater, gastro-enteritis due to vegetable poisoning, and other kinds of illness. The three former are diseases of recent occurrence and the term galziekte has been applied to them on account of one predominant symptom, namely, the deranged bile, which must have been noticed in a disease of the country previous to the introduction of the above-mentioned plagues. Formerly I have endeavoured to identify this gall sickness observed in earlier days with a trypanosomiasis due to the trypanosoma theileri (Bruce and Laveran), because I met similar lesions in animals inoculated with that parasite. I am now inclined to believe that this was due to the same disease described here, and that the trypanosoma was only a complication. There is, however, one difficulty in accepting this theory, as, so far, I have only noted piroplasma mutans in cattle, which at the same time are immune against piroplasma bigeminum, and not in cattle which are not immune against this latter parasite. In other words the geographical distribution of both parasites appears to be the same; thus where redwater is found, piroplasma mutans is also present and *vice versa*. This would infer that the introduction of piroplasma bigeminum and piroplasma mutans has taken place at the same time, and probably by the same tick. The experiments with Madagascar cattle seem to support this conclusion, as these cattle, which were directly imported from Madagascar, were infected with both parasites. Historically the introduction of redwater into South Africa can be traced back to the importation of Madagascar oxen, hence it is quite feasible that piroplasma mutans was also imported at that time, in view of the fact that the immune oxen experimented with contained both parasites. If it were possible to discover piroplasma mutans in districts of South Africa where piroplasma bigeminum is absent, then the identification of gall sickness with the former parasite would be practicable.

Piroplasma mutans plays a very important role, from a practical point of view, as its shape closely resembles piroplasma parvum, and a frequent occurrence of the former may lead to confusion with the latter. As far as I am aware this has not yet happened in our own experience, because we took the precaution to postpone the diagnosis of east coast fever in cases where only a few parasites were present, in order to obtain smears at a later date when piroplasma parvum would be more frequent. Another distinction is that as a rule animals infected with piroplasma mutans recover, whereas those infected with piroplasma parvum usually die. It is the imported animal, which survives or escapes an attack of redwater, that is liable to suffer from piroplasma mutans, and not the country-bred animal which becomes immune as it grows up. Another important point is that imported cattle have to be rendered immune against redwater and also this second piroplasmosis. Experience has proved that this may be accomplished by one and the same inoculation, as redwater, having a short incubation time, appears first and when it is over the second disease appears. This latter must not, however, be mistaken with the secondary reaction of redwater, due to piroplasma bigeminum, and from the effect of which an animal may die. An inoculation with redwater immune blood may have three reactions in its course when the blood of the immune animal contains the two parasites. Under these circumstances the inoculation of cattle in South Africa deserves further careful study.

Attention has also been drawn to a certain phenomenon which is frequently observed in connection with redwater inoculations, viz., the occurrence of marginal or peripheral points, which, although their nature is not yet clear, undoubtedly have a protozooic character.

They are sometimes encountered in blood preparations forwarded to this laboratory for diagnostic purposes, and until recently we have considered them to be a sequel of ordinary redwater infection, and recorded the diagnosis to that effect, but in view of the recent investigations it is advisable to devote more attention to these parasites in the future.

Inoculation against Horse Sickness.

At the end of last year the method of inoculating mules against horse-sickness consisted of a subcutaneous injection of serum together with a simultaneous injection of virus into the jugular vein. The various Government Veterinary Surgeons were instructed in this method, and carried it out in practice. The intrajugular injection was recommended, because we noticed that in the case of various horses the subcutaneous injection of virus, simultaneously with serum, failed to react, and when the animals were tested later they did not prove to be immune.

The experiments which are enumerated in my article, "Further Experiments with Mules" were then undertaken on a larger scale with these animals, which proved that the mules inoculated subcutaneously, reacted in the same way as those injected into the jugular vein, and mules which shewed very little or no reaction were nevertheless immune. It was found that the blood of a mule shewing a very slight reaction can be virulent; in other words the mule passes through a very mild disease. Under these circumstances, and since a subcutaneous injection is much simpler, it was found advisable to introduce this method into practice. The Government Veterinary Surgeons were informed to this effect, by a circular emanating from this division, and during the latter portion of the inoculation period all injections were made according to these directions. A further improvement to this method was the partial suppression of the second injection of serum, which, according to our original directions, had to be made on the fifth day. Experiments proved that this secondary injection could be omitted without increasing the first dose of serum, and accordingly the Veterinary Surgeons were further advised to this effect, with instructions to introduce it into practice. The second injection was recommended to be retained for the injection of large animals.

Up to the present time we recommended all inoculated animals to be kept well sheltered whilst they were undergoing reaction. Experience has shewn us that the shelter accommodation was not always of the best, and that, from a practical point of view, it would be more advantageous if inoculated animals could be turned out. An experiment was undertaken to this effect. The inoculated mules were kept in a paddock, day and night during the rainy season, and at a time during indifferent weather. The outside conditions did not influence the reacting animals in any way, so that in cases of urgency I am prepared to recommend the turning out of inoculated mules, especially when stabling means that animals are kept under hot-bed temperature, as is often the case in the badly built and unventilated tin sheds sometimes met with.

The inoculation of mules for the public was started at the end of November, after the various Government Veterinary Surgeons had undergone a course of instruction in the Laboratory. This course was intended not only to shew the Government Veterinary Surgeons the technique of the inoculation, but more especially to give them the necessary confidence for their practice, by an ocular demonstration of the development taken by the disease in inoculated animals, whilst undergoing the immunisation process. (During

the time these gentlemen were in Pretoria, advantage was taken of giving them—under the supervision of Mr. Dodd—a short instruction in microscopical and diagnostic work.) Mr. Sinclair, the Principal Veterinary Surgeon of Rhodesia, also attended one of the horse sickness courses, and subsequently introduced the inoculation in Rhodesia.

The inoculation had to be carried out by a Government Veterinary Surgeon, and the animals were retained for a period of three weeks on such a place as would allow the officer to inspect them in a proper manner. For this purpose the mules were brought to a central place. The hiring of stabling and feeding had to be borne by the proprietor of the mules. The inoculation fee was £1 10s. per animal, which included the cost of serum and an insurance guarantee value of £15 per animal in the event of any dying from the effects of the inoculation within the three weeks it was in our charge. Any death from piroplasmosis would be considered to be an accident of the inoculation. We were quite aware that the insurance not only covered animals against death due to the inoculation, but also against death contracted from spontaneous infection. The incubation time of horse sickness averages between 7 and 9 days, and it is therefore evident that animals infected previous to inoculation would. on the day of entry, pass as absolutely healthy and yet die on a date typical both for horse sickness contracted in the natural way, or by inoculation. There is no possibility of distinguishing the two, and the Government Veterinary Surgeon could only protect himself against inoculating an already sick animal, by taking the temperature previous to the inoculation, as animals during the beginning of the disease, although not visibly sick, would shew a fever, in which case the operation would not be performed

The collection of mules from various parts of a district into a central place involved a certain risk of spreading glanders, if occult cases happened to be amongst them. The Principal Veterinary Surgeon therefore insisted on all animals being malleined before they were inoculated, and in my opinion this was the only correct course to take.

During the last year the wishes and wants of the farmers in regard to the inoculation, were sounded by the various Government Veterinary Surgeons and laid before the Principal Veterinary Surgeon and myself, at a meeting in the Laboratory in June, 1906. It appeared therefrom that the great majority of farmers and proprietors preferred to bear the risk of loss from inoculation, provided a reduction could be made in the fees, and in the condition of keeping the animals. The proprietors desired that the immunisation be performed in the stables on the farms, where it would be easier to attend to animals and further that their feeding would be cheaper. With the experience of the previous year in front of us, it would be more advisable to meet the wishes of the farmers as far as possible. It must, however, be borne in mind that the production of serum is still a somewhat costly process owing to the fact that we have to feed all the animals during the greater part of the year, and, in the event of the insurance scheme being abandoned, the fee must not be reduced beyond the amount necessary to cover our actual expenses.

During the year 1905-6, in the various districts of the Transvaal 2,325 mules were inoculated with a loss of 3·8 per cent. from inoculation ; in Rhodesia 388 mules with a loss of 2·3 per cent. Thus in practice, mortality due to the operation resulted in the same average as in the Laboratory. In all 3,235 mules were exposed to horse sickness in the various parts of the Transvaal and Rhodesia ; included in this figure are 522 mules which were immunised at the Laboratory. The mortality from horse sickness after inoculation amounts to 0·6 per cent., but it should be noted that this includes the death of any mule

even when the cause of death could not be confirmed with certainty. The benefit of the doubt was given to the statements of the farmers. There was a return of 1.3 per cent of animals which shewed symptoms of the disease, the so-called "aanmaning." This latter statement must, however, be taken *cum grano salis*, as it is only natural that during the horse sickness season any illness of an immunised mule would be regarded as "aanmaning." These statistics were supplied to the Government Veterinary Surgeons by the proprietors of the mules. A return of almost every animal was obtained, so that the figures may be considered as reliable.

In the circular which was issued for the purpose of obtaining the above figures in connection with the mortality, etc., information was also requested regarding the severity of horse sickness in the various districts, together with a return of mortality amongst non-inoculated mules and horses. It appeared that horse sickness was scarcely observed in the districts of Pretoria, south of the Magaliesberg, Krugersdorp and Potchefstroom, but was very virulent in the northern and eastern districts. The mortality amongst horses and mules not inoculated was returned by the Government Veterinary Surgeons at 782 animals, but they all state that this number does not accurately represent the total mortality in their districts; the returns recorded only represented the cases which came to their personal knowledge. Mr. Sinclair, of Rhodesia, reported that the horse sickness in that country was a very bad one, indeed as bad as any on record, and that none of the inoculated mules which were exposed to very severe tests contracted the disease. Mr. Webb, of Lydenburg, also reported that in his district the disease was exceptionally virulent, quoting one particular case where an animal had salted in previous years; again contracted the disease. In other districts it was found that only inoculated animals died off, whereas the immunised ones remained.

The point now at issue is whether horse sickness is responsible for animals reported to have died from this disease. In order to settle the question, the various Government Veterinary Surgeons and other men who had immune animals in their charge forwarded to this Laboratory blood obtained from sick animals. In some cases, but not in all, this blood proved to be virulent for susceptible animals. We accordingly obtained from Tzaneen, Lydenburg, Piet Retief, Waterberg, Pretoria and Rhodesia, virus of immune animals which had either suffered or died from horse sickness, and it was found that these various kinds of virus were indeed more virulent than the one we had in use at the station, or than any other collected from spontaneous cases and obtained from Swaziland, Barberton, Zoutpansberg, Potchefstroom, Middelburg or Pretoria. This difference of virulency was found out in connection with the inoculation of serum, when virus of immune animals would always shew a more severe reaction than the one obtained from not immune animals. One kind of virus was more fully experimented with, and it was found that the immunity of an inoculated mule would not protect against this virus, a fact which hitherto had never taken place. By hyperimmunisation we were able to infect the same animal with horse sickness twice and even three times, and some of the infused animals died of this disease. In the light of this new experience we understand the phenomenon of a relapse, or "aanmaning." The truth is not that the animals lose immunity, but that various degrees of virulent horse sickness exist which do not immunise one against the other. The old farmers of the Transvaal knew this from practical experience when stating, "that an animal may be salted for one district and not for another." Our experience is the experimental proof of this observation, but if this is really the case, the question naturally arises why only 0·6 per cent. of our immune animals

contracted horse sickness. The reason is that under natural conditions smaller doses of virus are used than those utilised for infusion; that the reaction will only be a light one, and in all probability pass unnoticed, unless the animal worked hard. We have also every reason to believe that the extreme virulent virus is of rare occurrence. This extraordinary experience will naturally influence our future proceedings. Accordingly our first object is to immunise against this stronger virus in order to reduce future relapses. It will, therefore, probably become necessary to again alter our method of inoculation, inasmuch as I anticipate that a larger amount of serum will have to be injected to pass a mule through a reaction.

This new experience has, however, thrown us back in our efforts to adjust the inoculation to horses, whose immunity is obtained by ordinary virus—as shewn in the article on the subject—does not protect against the stronger virus.

Experiments in connection with the inoculation of horses against horse sickness will accordingly have to be repeated.

The knowledge obtained this year will be of considerable value; it was only gained through the present system of immunising mules.

On the Correlation of Various Diseases in Stock in South Africa.

In my article on the above subject a question has been dealt with on the identity of horse sickness with other stock diseases, principally heartwater. According to Dr. Edington, horse sickness would be inoculable into goats, sheep and cattle, producing heartwater, and from statements made by this scientist, one would be inclined to conclude that whilst inoculating goats with horse sickness the virus would become attenuated and act as a vaccine. It was, therefore, important to settle these points experimentally. Stockman and myself had previously taken exception to several of Dr. Edington's statements, and we maintained the duality of the two diseases on the grounds of both practical and experimental experience, the former for the reason that heartwater is a tick-borne disease and only occurs in such parts of the Transvaal where Amblyomma hebraeum (bont tick) is present; horse sickness appearing, however, also in such parts where both heartwater and the ticks are absent; the latter from the fact that we were never able to produce the disease by inoculation in goats or cattle. Dr. Edington's statement is, however, to the effect that the inoculation of horse sickness into goats is commonly met with numerous failures. I therefore repeated the experiments on a somewhat larger scale. For this purpose animals were selected in which, in all likelihood, immunity to heartwater or horse sickness could be excluded, and were obtained from such parts of South Africa where both diseases were absent. The net result is that I again failed to transmit the disease into oxen and sheep, but succeeded in producing febrile reactions in Angora goats. The blood of several goats taken during the febrile reaction proved to be virulent in only two cases, although almost every goat showed a reaction. From this it appears that fresh blood from a horse suffering from horse sickness produces a reaction when injected into goats whose blood in the majority of cases is not virulent for equines. I am unable to determine the cause of the reaction and more experiments will be necessary. It is, however, quite clear that the reaction did not cause any immunity in the goats against heartwater, all of which died when tested with heartwater virus, or exposed in a heartwater field. On the other hand, inoculated horses and mules which contracted the disease from goats' blood developed a deadly disease, and the animals which did not contract it proved to be susceptible to the ordinary horse sickness

virus of this station. The conclusion may accordingly be drawn that horse sickness and heartwater are not identical; that horse sickness virus, while passing through a goat does not lose its virulency; it is, however, quite exceptional that a goat can be infected with horse sickness.

A further interesting fact was elucidated, viz., that horse sickness is transmissible into dogs. Several dogs died when injected with virulent blood, and the disease runs a much quicker course in these animals. The pathological lesions are almost identical in both. It does not follow from this, however, that dogs suffer from horse sickness, but the fact that this disease is transmissible to both goats and dogs, points to the possibility that herbivorous and carnivorous animals may be susceptible to the disease.

These experiments throw some light on the existence of horse sickness virus under natural conditions. Both from a practical and experimental experience the theory was formed that the propagation of horse sickness takes place by means of insects, especially mosquitoes. Some difficulty, however, was experienced in explaining the fact that horse sickness may be contracted in parts of the low veld where an equine had not been for some years, but, in view of the fact that other animals besides equines can suffer from horse sickness, this difficulty is now overcome.

In co-operation with the Government Entomologist—Mr. C. B. Simpson—a series of experiments were undertaken with mosquitoes, some species of culex, anopheles and stegomya in order to see whether they would act as hosts for the horse sickness virus. Unfortunately the experiment failed, because it was not possible to keep mosquitoes alive in the stable, and only a few actually engorged. The conditions of a stable atmosphere seem to be adverse to the well-being of a mosquito. Guided by this experience a careful search was made in our stables for several evenings in order to discover these insects. Although the private dwellings were swarming with mosquitoes, none were encountered in the stables. This observation is worth recording, since it is in accordance with the theory of the transmission of horse sickness by mosquitoes; stabled horses do not as a rule contract the disease. It still remains to be proved whether a mosquito is the host of horse sickness and, if so, to discover the particular specie or species.

In concluding these commentary notes on the experiments with horse sickness, I wish to place on record the good work accomplished by the various Government veterinary surgeons who carried out the inoculations, and to whose attendance and care part of the success is undoubtedly due.

Equine Piroplasmosis.

This disease, commonly known as biliary fever, has formed the object of further research. The importance of the disease is illustrated in the various articles on this subject. We have noticed that this disease complicated the horse sickness inoculation in mules to the extent of 0·8 per cent. It was also responsible for 14·3 per cent. due to the hyperimmunisation.

The cause of the occurrence of this malady remains in the fact that an immune horse retains the piroplasma in its blood in the same way as all the hitherto known piroplasmoses, with the one exception of East Coast Fever. Hence the disease is produced in susceptible animals by the injection of fresh blood, which is utilised as virus and often contained in serum, both utilised in connection with horse sickness. This year's experience has shown that preserved serum containing red corpuscles may retain its virulency for nearly a month. In practice, however, we have completely excluded the possibility of transmitting equine piroplasmosis, by using well seasoned serum and virus,

which as a rule are over three months old. The appearance of biliary fever in practice cannot, therefore, be due to the infective power of the material used, but to the fact that under the influence of the horse sickness reaction, immunity against the piroplasma breaks down and piroplasma equi again meets with suitable conditions for its recrudescence. Several of the cases I have quoted in my article must be explained in this way. The fact that the disease is inoculable with immune blood was demonstrated last year. At that time the suggestion was made that this might be used with advantage to immunise mules and donkeys against this disease. This has been tested during 1905–6 to a certain extent. The inoculation of mules with immune blood has proved a complete success. The mortality in donkeys was five per cent., but some of the animals suffered somewhat severely, so that only under the most favourable conditions can the inoculation of imported donkeys be advocated. The inoculation of susceptible horses with immune donkey blood has proved a complete failure, whereas there is a better prospect in utilising the blood of immune mules. For the purposes of the laboratory, the inoculation of horses with mule blood has been introduced, but it was discovered that, due to the infusion of large quantities of blood, piroplasmosis may occur in animals which were immune, and did not react to the previous inoculation of immune blood. Nevertheless the danger of causing the disease has in this way been reduced, which for practical purposes is quite sufficient. Since the immunity under influence of adverse conditions can break down, we must always expect, after excluding all possibility of a direct transmission, a certain percentage of the animals to die from this disease.

We anticipated that equine piroplasmosis was a tick-borne disease, but it is only during the last year that we experimentally proved the fact. For practical reasons two species of ticks were considered to be the hosts of piroplasma equi, viz., (1) Rhipicephalus decoloratus, or the common blue tick; and (2) Rhipicephalus evertsi, or the so-called red leg tick. Experiments clearly demonstrated that the former has nothing to do with the dissemination of piroplasma equi, but that it is the latter one which is principally responsible. It was found that Rhipicephalus evertis takes the infection whilst it is in its larval and nymphal stage, and transmits it during the adult stage. Only one experiment was made—resulting in a failure—to see whether the infection will go through the egg. This one negative result must not be taken as conclusive. The proof that equine piroplasmosis is a tick-borne disease will undoubtedly give a further argument in favour of the eradication of ticks, which is advocated in certain quarters, as the panacea of clearing all South Africa of so many diseases. But here again a simple reflection will show that this supposed remedy will fail in connection with this disease. All, or most South African born equines, including the zebra, carry the piroplasma equi in their blood and therefore will infect the tick. The red tick on the other hand is not only found in all kinds of stock, but also on game. Its eradication will, therefore be impossible, and the immune animal will keep up its virulency. In my opinion a horse breeder would be unwise to attempt to eradicate this red tick at the present time. It is the very means by which his horse becomes immune and retains its immunity against piroplasmosis, because foals suffer very little from the disease. During all my experience I have never yet heard of a foal that died from this disease, and this is also another hint for horse breeders to import principally young horses, as they are better able to resist the disease than older ones.

We still hope, however, to overcome this drawback to horse breeding by improving the inoculation, so that it can advantageously be introduced into practice.

Treatment of East Coast Fever.

These experiments were undertaken in the first instance at the suggestion of Mr. Gray, the Principal Veterinary Surgeon, who at the time of Professor Koch's presence in Rhodesia assisted in experiments of a similar nature. This latter scientist observed that a microbicide serum could be produced by fortifying immune animals with defibrinated blood of sick animals, but such a serum had pronounced haemolytic action, and the injected animals died of haemolysis. Having overcome the haemolytic effect of horse sickness serum, principally by fortifying the animals by infusion, it was deemed advisable to apply the same process to east coast fever. If a haemolytic serum could be produced which would exercise a preventive action lasting only a fortnight, this would help considerably towards eradicating the disease. It was this object Mr. Gray and myself had in view.

The experiments, however, resulted in a complete reverse of Professor Koch's experience. His serum was haemolytic and microbicide, ours was neither one nor the other. From a scientific point of view this is very interesting, although from our side of the question very disappointing. The experiment, however, is not without practical value. The enormous extent an infusion from one animal into another can be carried out has been demonstrated, and is a fact well worth knowing in connection with the production of rinderpest serum, and which may be utilised in the latter with advantage.

Swine Fever and Swine Plague.

Until recently these diseases were considered to be caused by specific organisms belonging to the bacteria, the former being described as bacillus suipestifer (bacillus cholerae suis), and the latter as bacillus suisepticus. The swine fever bacillus was found in the caseated lymphatic glands of the diseased intestines and the swine plague parasite in the diseased lungs and its lymphatic glands and also in the blood. When swine fever was noticed in this country and attracted our attention we were looking for the confirmation of the diagnosis by tracing the specific bacillus. We failed in every instance, whereas in cases diagnosed as swine plague, we found bacillus suisepticus without exception. At about that period a publication appeared by three American scientists, Dorset, Bolton and McBryde, stating that American hog cholera was caused by an ultravisible micro-organism and could be inoculated with blood, which always contains this unvisible organism. As we identified our swine fever with hog cholera, the new facts noted by the Americans must also be traceable in our disease. It was accordingly our object to prove this.

The Americans' statements were confirmed, and we also arrived at another conception regarding the cause of swine plague in this country. For some time on the European continent, especially Germany, the idea was expressed that an epidemic disease of swine caused by the bacillus suisepticus does not exist, but that this bacterium rather complicates diseases due to other causes. Our observations seem to support this view, since we observed that, with the exception of one single outbreak, all our cases of swine plague were complicated with swine fever; in other words, the swine fever was the disease and the swine plague the complication.

These new developments in the etiology of swine fever will necessitate further investigations, in order to adapt legislation to the new scientific data.

The Congress in Buda-Pesth.

As the representative of the Transvaal Government, I attended the 8th International Veterinary Congress, which was held in Buda-Pesth from the 2nd to 9th September, 1905. This congress was particularly attractive for Colonial veterinary surgeons, inasmuch as one of the four sections was devoted to tropical diseases. The following papers were submitted for discussion :—

Ligniére, of Buenos Ayres	"Tropical Diseases."
Piet Bey, of Cairo	"Diseases of Egypt."
Theiler, of Pretoria	"Diseases of South Africa."
Laveran and Vallèe, of Paris; Motas, of Bucharest	"Protozooic Diseases of Domesticated Animals."

The meeting was attended by representatives of the various colonies co-operating on the congress, and a number of well-known scientists who interested themselves in the development of tropical veterinary science. Your representative was in the fortunate position of being able to place his knowledge of the three principal languages at the service of the meeting, by translating the discussions on the various subjects. The subject which seemed to be of most importance and interest was the different piroplasmoses, principally those of cattle. Dschunkowsky demonstrated both pathological specimens and blood preparations, and stated that the disease observed in the Trans-caucasus, called "Tropical Piroplasmosis," identified at one time with east coast fever, should no longer be considered as the same disease, as he had succeeded in transmitting tropical piroplasmosis into susceptible cattle derived from parts of the country where there were no piroplasma observed. Similar to our experience in South Africa, it was noted that the tropical piroplasmosis was very frequently accompanied by Texas fever, which is also met with in that country. A further communication was made that the trypanosoma, described in the first instance by myself as specific for cattle, was also encountered in the cattle of the Trans-caucasus, but this parasite did not appear to cause any specific disease.

Further interesting communications were made by Bitter, of Cairo, regarding a piroplasmosis in Egypt, which is exclusively observed in imported cattle, and distinct from Texas fever, this latter being also met with in Egypt.

From the descriptions and photographs produced by Bitter, this North African piroplasmosis seemed to be identical with either the tropical piroplasmosis of the Trans-caucasus, or the African Coast fever. The evidence seems to point to this latter one, as Bitter states that the disease is not inoculable. Ducloux, of Tunis, states that the tropical piroplasmosis of Dschunkowsky is also observed in his country, since he saw similar parasites and pathological changes as those demonstrated. (Reference is made to these various communications in my article on "Piroplasma Mutans.")

From these communications it is evident that the piroplasmoses deserve a good deal of attention, since they seem to form the principal cattle diseases of warm countries. Our knowledge regarding them is as yet very meagre, but sufficient to realise a great danger which would result from indiscriminate importation of cattle from tropical countries. Acquirement of knowledge in connection with the nature of the various tropical diseases becomes, therefore, a necessity for the Colonial veterinary surgeons.

The tropical section of the congress has seen the importance of the development of this particular branch of veterinary science in accepting the following resolution :—

"*Tropical Diseases.*"

" Tropical diseases should form a subject for special instruction in the veterinary schools of tropical countries, and of countries possessing tropical colonies.

" The individual Governments should investigate the sanitary condition of the domestic animals in their colony, and especially enquire into the existence of diseases produced by protozoa.

" It is desirable that the geographical distribution of different infectious diseases, and the general laws relating to their spread, should be defined.

" In every colony a central institution for parasitiology should be founded, provided with ample means and a technical staff of specially qualified investigators in bacteriology, mycology, parasitology and entomology.

" In colonies which are not yet provided with a veterinary police staff, such a body should be constituted. This staff would control the operations of experts appointed to deal with disease ; it should possess the most extensive powers for combating infectious diseases.

" The institute for parasitology, the sanitary police staff, and the staff for dealing with epizootics should possess the most complete autonomy, and should only be answerable to the highest civil power.

" Finally the scientific and administrative officers of these different institutions should pass their entire careers in the same colonies, or at least within colonies comprised within the same geographical region, inasmuch as success can only follow methodical work by those accustomed to the sanitary conditions of a given country."

It is very satisfactory to note that the principal South African colonies have acted in the spirit of the above resolution during the last few years.

In addition to the deliberations of the tropical section, which was of primary importance to your representative, the congress has offered several other discussions which deserve our interest. In the pathological section the principles of the application of the serotherapie were discussed, and considerable attention was given to the treatment of rinderpest by serum. There were two opinions expressed : (1) that the application of serum against rinderpest is followed by excellent results and therefore its application is to be recommended ; and (2) no noticeable result was observed from a preventive point of view, when serum was utilised.

These two opinions are, of course, in direct opposition to one another ; the former view was upheld by Dschunkowsky from Trans-caucasus, Bitter and Littlewood of Egypt, by Stockman, our late Principal Veterinary Officer, by Rassau of German South-West Africa, and by your representative. Piet Bey of Cairo, and Professor Arloing took the opposite view. It is noteworthy that this conflicting evidence emanates from Egypt, where serum of various origin was utilised. It is possible that this fact accounts for the difference in results.

Of the various resolutions which were passed at the congress and which embrace the majority of the subjects of the veterinary science, I select those which are of most importance to our Government veterinary surgeons, namely,

one dealing with the principles regarding the mallein reaction, and the other in connection with the estimating of the tuberculin reaction :—

"*Uniform Principles Regarding the Mallein Reaction.*

"(1) Unless the results following the injection of mallein exhibit the characteristics of a typical reaction, they must not be regarded as indicating the existence of glanders.

"(2) A typical reaction comprises a rise in temperature of at least 2°C. The rise would extend above 40°C; during the course of the first day, the temperature curve usually exhibits a plateau of two peaks, and on the second, and sometimes even on the third, a more or less marked rise. This rise in temperature is accompanied by a local and general reaction.

"(3) Any rise of temperature which falls short of 40°C., and higher atypical reactions, necessitate a second test.

"(4) A gradual rise of temperature sustained for some time is indicative of glanders, even though it differs from the ordinary type of diagnostic reaction.

"(5) The local typical infiltration at the point of injection is a certain indication of glanders, even when the rise in temperature and the general organic reaction fail.

"(6) Animals which have undergone the mallein test, whether or not without reaction, should always be tested a second time, after an interval of ten to twenty days.

"(7) The preparation of mallein should be entrusted to scientific Government institutions recognised and controlled by the State.

"(8) With the object of determining the full value of mallein, and of clearing up many still unexplained points in regard to the mallein reaction, the congress requests the various European Governments to appoint committees to study the question."

"*Uniform Principles for Estimating the Tuberculin Reaction.*

"(1) The preparation and supply of tuberculin should be controlled by the State.

"(2) No animal whose temperature exceeds 30·5°C. is a fit subject for the tuberculin test.

"(3) A rise of temperature to above 40°C. in any animal whose temperature at the moment of injection was below 39·5°C. is to be regarded as a positive reaction.

"(4) Any rise in temperature between 39·5°C. and 40°C. must be regarded as of doubtful significance; animals exhibiting such require special study."

Since rabies is threatening to invade this colony from Rhodesia, and probably will have to be dealt with in the future, I consider it opportune to repeat here the views expressed by the congress on this subject.

"*Measures against Rabies, including the Stamping Out Method.*

"(1) The struggle against and stamping out of rabies in a Continental State can only prove successful when appropriate veterinary police measures are strictly carried out in all the neighbouring countries. It is, therefore, urgently necessary that, as regards rabies, the work of the veterinary police should be undertaken in all countries simultaneously and on the same principles.

" (2) The duty of reporting, which up to the present has only been compulsory as regards rabid dogs and dogs suspected of rabies, must be extended to include all animals which have been bitten by rabid or suspected rabid dogs. Not only must the owner of the animal and the persons mentioned in Para. 9 of the German Contagious Diseases Act be required to give notice, but all those who have knowledge of animals which have been bitten by such dogs.

" (3) It is an open question whether it might not be desirable to extend the muzzling order over a larger area than hitherto, and for a longer time than three months.

" (4) It is desirable that a uniform law relating to the ownership of dogs and containing the following conditions should be introduced into all countries and severely enforced.

" (*a*) All dogs, both in towns and in the country, shall be reported, registered and taxed.

" (*b*) Every such animal shall be provided with a metallic ticket attached to the collar and bearing the name of the owner, and the number of the dog in the tax list.

" (*c*) Dogs without such metallic tickets and without muzzles shall be taken charge of by the police, and if not claimed within a stated time shall be killed."

Before concluding my remarks on the proceedings of the Veterinary Congress, I must express my high appreciation for the consideration which was shown to your delegate, both by the organising staff of the congress and the representatives of the Hungarian Government.

The Congress of Buda-Pesth gave me an opportunity of making the acquaintance of many distinguished scientists who were engaged in investigations of tropical diseases, an opportunity which only a congress of this kind is in a position to offer.

Routine Work.

A tabulated record is attached shewing the results of the examinations of pathological specimens and blood smears addressed to this Laboratory for diagnosis, shewing a total number of examinations amounting to 1,111. The list is headed by east coast fever, this being the principal disease we have to deal with. The return of negative results amounts to 708, and applies either (1) to a blood smear, or (2) to scrapings from goats or sheep. The former were taken in the majority of cases from suspects of east coast fever and the latter from animals suspected of suffering from acariasis (scab). The negative results are due to several causes; in some instances the blood smear is made too thickly, in other cases it is derived from an animal some time after death, when the blood corpuscles have dissolved. In both cases the return of the examination is given with the cause of the negative result, thus warning the man who sent it in to be on the look out for another opportunity, because, nevertheless, east coast fever may be present. In the majority of cases the smears are taken as precautionary measures from an animal reported to be sick. In these instances a return of negative result usually signifies absence of the disease. We have not yet heard of a single instance where, notwithstanding the absence of piroplasma parvum, east coast fever has appeared. Undoubtedly many of the smears were taken from sick animals, but naturally we are only able to diagnose such diseases as are caused by visible parasites, or in which certain

blood changes occur, and accordingly furnish the clue to the previous infection. These blood changes are also registered with the diagnosis of the disease the animal was probably suffering from. A return of basic, nucleated and polychromatic cells and poikilocytosis in all likelihood means that cattle were suffering from redwater, whereas the similar return in equines and dogs refers to a previous infection of piroplasma equi and piroplasma canis respectively.

It remains with the Government Veterinary Surgeons to elucidate symptoms and pathological lesions in such diseases of animals where the examination of the blood gave negative results. I have at repeated intervals drawn the attention of these gentlemen to this field of research.

A considerable number of smears are sent in by the farmers themselves, especially from districts in which east coast fever is present, or from farms from which the disease has disappeared. A large number of preparations are received from the South African Constabulary made by the troopers of that corps. The resourcefulness of farmers and South African Constabulary troopers is best demonstrated by the fact that in many cases of urgency and in the absence of proper slides, the smears are made on pieces of window glass or on portions of broken bottles. The farmer is thus proving not only his care for his stock, but also confidence in our knowledge. The South African Constabulary trooper shews his sense of duty and interest in the work he has to perform. Accordingly both classes of men have earned our highest appreciation.

Notwithstanding the difficulty in examining such preparations, in many cases we have been able to arrive at definite conclusions, confirming either a suspected diagnosis or allaying anxiety in doubtful cases.

During the past year we had occasion to make a diagnostic examination in a case of suspected rabies in two dogs, forwarded by the Government Veterinary Surgeon of the Zoutpansberg District, but fortunately the results proved negative in both cases. It is quite natural to expect the disease in this particular district, since the adjoining territory of Rhodesia has been infected for some years. The presence of rabies just beyond the border of the Transvaal is, however, a constant warning to be on the look out for its introduction, which is bound to occur sooner or later. It is for this reason that Mr. Gray and myself have to make arrangements for the necessary preparation of anti-rabies vaccine, in order to be ready for all emergencies.

The preparation of black quarter or sponziekte vaccine was undertaken this year at the request of various farmers who have had former experience; 450 double doses were issued. The inoculation is usually done by the farmer himself; the Government Veterinary Surgeon assists in the inoculation and gives the necessary instructions when requested. The preparation of this vaccine is under the charge of Mr. Dodd.

The issue of calf vaccine amounts to 566,717 tubes This constitutes a record issue since the manufacture was started. It is due to the fact that the Cape Colony, who in their former days used to prepare their own lymph, have now become our client. We are now supplying all South African States with calf vaccine.

In the statements given on the following pages you will find, in addition to the usual calf vaccine returns, a statistical table shewing the results obtained by the various vaccinators, according to the schedules received from them. These do not by any means represent the total number of vaccinations which have been performed, but it furnishes a medium for judging the effectiveness of the lymph. Attention is also drawn to a striking feature in the fact that

in a few instances the statistics of one and the same calf lymph shew a result of 100 per cent. in the hands of one vaccinator, and anything from nil to 50 per cent. in another. This is no doubt the result of the lymph deteriorating after it has left the laboratoy and whilst in the possession of the vaccinator.

The preparation of blue-tongue serum was also undertaken during this year, but so far little use has been made of it. I consider it necessary to undertake a test in practice, on a somewhat larger scale, in order to make the inoculation of this serum familiar to the farmer.

The amount of horse sickness serum for preventive purposes prepared during the year amounted to 6,871 litres; of this supply 2,127 litres were utilised in the Transvaal and in Rhodesia; 21 litres were despatched to German West Africa.

Increases and Alterations in Staff.

In November, 1905, Mr. Sydney Dodd, M.R.C.V.S., Demonstrator of Pathology and Bacteriology at the Royal Veterinary College, London, and assistant of Professor Sir J. M'Fadyean, M.B., B.Sc., M.R.C.V.S., was engaged to fill the new position of Assistant Government Veterinary Bacteriologist. Mr. Dodd is well acquainted with bacteriological research and also with the manufacture of quarter evil vaccine, the preparation of mallein and tuberculine. As the necessary incubators for the preparation of mallein and tuberculine have not been fixed up during the year, the manufacture of these has not, therefore, been started. Mr. Dodd has also undertaken the superintendence of some of the research work; he has worked out the etiology of a skin disease in pigs, the cause of which is the presence of a spirillum. The details of his investigations have been published in the "Journal of Comparative Pathology and Therapeutics."

The work in connection with swine fever was principally carried out by Mr. Dodd. This appointment has relieved me of a considerable amount of ordinary routine work, such as the veterinary supervision and inspection of the live stock necessary for the experiments.

The lay staff of this division was increased by the addition of three men, viz., Mr. R. White, Mr. W. D. van der Berg and Mr. Herman Oettli, whilst one member of the old staff, Mr. A. von Bergen, resigned. We were again unfortunate this year in losing one assistant from enteric fever, Mr. Herman Oettli.

On account of the prevalency of enteric fever in our establishment, which proved to be so virulent in the last three instances, the Medical Officer of Health for Pretoria, Dr. Boyd, has recommended the evacuation of the present premises.

Administration.

The increase of work in connection with the administration necessitated the creation of the post of Superintendent, whose duties consist of superintending the commercial side of the Laboratory work. This institution has also relieved me of a great amount of routine work which previously required my attendance. The position is filled by Mr. E. B. H. Parkes, who was appointed at the beginning of January, 1906.

During the year a considerable amount of correspondence was dealt with, and numerous interviews were granted to farmers and others who were interested in the work of the Laboratory and desired information regarding animals and their diseases.

The following is a return of the number of letters received and despatched, as compared with the previous year 1904-5 :—

	1904-5.	1905-6.	Increase.
Letters received	.. 2,257	2,517	260
Letters despatched	.. 1,857	1,952	95
Telegrams despatched ..	.. 581	885	304

Leave of Absence.

I was absent on leave in Europe from the latter end of the last fiscal year, and returned early in November, 1905. The superintending of the Laboratories during that period was undertaken by Mr. R. S. Garraway, the Government Veterinary Surgeon for the District of Pretoria ; the preparation of horse sickness serum and the hyperimmunisation of horses and mules, was carried out by Mr. Favre ; Mr. Heron attended to the diagnostic routine work ; Mr. Gray, the Principal Veterinary Surgeon, and Mr. Christy, the Assistant Principal Veterinary Surgeon, placed their assistance at the disposal of the Laboratory whenever it was required. It gives me great pleasure to acknowledge the good work performed by my staff during the whole year, more especially during the period of my absence.

My presence in Europe was made use of to visit the various laboratories in different countries, and in this way I was able to view the following laboratories :—In London, the Lister Institute and its dependence at Elstree the Bacteriological Laboratory of the Royal Army Medical Corps; the Pasteur Institute at Paris and its dependence in Garches; the Bacteriological Laboratory of the Veterinary College in Alfort, in Zurich; the Bacteriological and Therapeutic Institute of Berne; the Veterinary Colleges of Buda-Pesth and Vienna. These visits were made in order to collect information regarding the latest improvement in bacteriological institutions. I may state that my object was, in the main, achieved. New acquisitions were then made, based on the observations made at the other laboratories, which have already proved very successful.

I have the honour to be,

Sir,

Your obedient Servant,

A. THEILER,

Government Veterinary Bacteriologist.

Summary of Results of Microscopical and Pathological Anatomical Examinations for the Year ended 30th June, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	152
Doubtful, East Coast Fever, Piroplasma Mutans ?	„	1
Ordinary Redwater, Piroplasma Bigeminum	„	25
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	5
Marginal Points, Sequel of Ordinary Redwater	„	7
Basic Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	29
Poikilocytosis, Sequel of Ordinary Redwater	„	20
Piroplasma Equi :	Equines	11
Poikilocytosis, Sequel of Piroplasmosis	„	3
Piroplasma Canis	Dogs	2
Basic, Nucleated and Polychromatic Cells, sequel of Piroplasmosis	„	5
Swine Fever	Pigs	3
Swine Plague	„	1
Pneumonia (Swine Plague ?) Cysticercus Cellulosœ	„	1
Necrosis in Lung, Sequel Swine Plague ?	„	1
Traumatic Pneumonia	Ox	1
Bronchial Pneumonia	Sheep	3
Bronchial Interstitial Pneumonia	Mule	1
Pleuritis and Chronic Pneumonia	Goose	1
Pulmonary Oedema	Cattle	1
Basic, Nucleated and Polychromatic Cells, Anæmia, Sequel of Blue Tongue ?	Sheep	2
Abscess due to Bacterial Infection	„	10
Abscess due to Coccus Infection	„	1
Abscess of Spleen, due to Perforation by Spiroptera from Stomach	Horse	1
Acariasis, Sarcoptes of Africander Goats	Goats	21
Acariasis, Psoroptes of Sheep	Sheep	10
Actinomycocis	Cow	1
Aenurism of Aorta	Heifer	1
Amphistomum Conicum Cysticercus Tenuicollis } ?	Antelope	1
Anthrax	Cattle	9
Black Quarter	„	5
Callus	Rib Bone of Mare	1
Chronic Interstitial Nephritis	Pig	1
Cirrhosis (Liver)	Ox	1
Congestion of Lungs	Fowl	1
Dermoid Cyst	Horse	1
Epizootic Lymphangitis, Saccharomyces farciminosus	Equines	30
Echinococcus Cyst	Horse	1
Fatty Infiltration of Liver	Hen	1

Fibrous Tumour	Horse	1
Fibrous Tumour	Cow	1
Icterus	Calf	1
Mummified Foetus	„	1
Necrosis of Liver, due to Bacillus Necrophorus	Cattle	3
Parasitic Nodules in Lung	Equine	1
Parasitic Nodules in Muscle	Ox	1
Pthiriasis	Goats	3
Septicæmia Hemorrhagica	Antelope	1
Spirillosis	Heifer	1
Streptococcus Infection	Equines	3
Tuberculosis	Cattle	4
Necrosis of Liver	Equine	1
Traumatic Pericarditis	Cow	1
Basic, Nucleated and Polychromatic Cells, cause unknown, Worm Infection ?	Pigs	8
Negative Result	—	708
Total		1,111

Monthly Summary.

July, 1905.

East Coast Fever, Piroplasma Parvum.	Cattle	9
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	3
Aneurism of Aorta	Heifer	1
Anthrax	Cattle	1
Chronic Interstitial Nephritis	Pig	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	2
Pthiriasis	Goats	3
Septicæmia Hemorrhagica	Antelope	1
Negative Result	—	64
Total		85

August, 1905.

East Coast Fever, Piroplasma Parvum	Cattle	8
Abscess due to Bacterial Infection	Dog	1
Swine Fever	Pigs	2
Swine Plague	„	1
Amphistomum Conicum / Cysticercus Tenuicollis ?	Antelope	1
Congestion of Lungs	Fowl	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	3
Fatty Infiltration of Liver	Hen	1
Necrosis of Liver, due to Bacillus Necrophorus	Cattle	1
Pleuritis and Chronic Pneumonia	Goose	1
Tuberculosis	Cattle	1
Negative Result	—	72
Total		93

September, 1905.

East Coast Fever, Piroplasma Parvum	Cattle	21
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	1
Ordinary Redwater, Piroplasma Bigeminum	„	2
Piroplasma Equi	Equine	1
Piroplasma Canis	Dog	1
Basic, Nucleated and Polychr. Cells, Sequel of Piroplasmosis	Dog	2
Abscess due to Coccus Infection	„	1
Abscess due to Bacterial Infection	„	1
Acariasis, Sarcoptes of Africander Goats	Goats	5
Acariasis, Psoroptes of Sheep	Sheep	2
Anthrax	Cattle	1
Fibrous Tumour	Horse	1
Fibrous Tumour	Cow	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	7
Spirillosis	Heifer	1
Negative Result	—	67
Total		115

October, 1905.

East Coast Fever, Piroplasma Parvum	Cattle	15
Ordinary Redwater, Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	2
Poikilocytosis, Sequel of Ordinary Redwater	„	2
Abscess due to Bacterial Infection	„	1
Acariasis, Sarcoptes of Africander Goats	Goat	1
Anthrax	Cattle	1
Callus	Rib Bone of Mare	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equine	1
Streptococcus Infection	Equine	1
Basic, Nucleated and Polychromatic Cells, cause unknown, Worm Infection ?	Pigs	8
Negative Result	—	83
Total		117

November, 1905.

East Coast Fever, Piroplasma Parvum	Cattle	6
East Coast Fever, and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequal of Ordinary Redwater	„	1
Poikilocytosis, Sequel of Ordinary Redwater	„	1
Piroplasma Canis	Dog	1
Basic, Nucleated and Polychr. Cells, Sequel of Piroplasmosis	„	1
Swine Fever	Pig	1
Pneumonia in Pig, Cysticercus Cellulosœ, Swine Plague ?	—	1
Acariasis, Sarcoptes of Africander Goats	Goats	3
Acariasis, Psoroptes of Sheep	Sheep	1
Anthrax	Cattle	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equine	1
Negative Result	—	41
Total		60

December, 1905.

East Coast Fever, Piroplasma Parvum	Cattle	7
Ordinary Redwater, Piroplasma Bigeminum	,,	1
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	,,	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	1
Piroplasma Equi	Equines	2
Pulmonary Oedema	Cattle	1
Acariasis, Sarcoptes of Africander Goats	Goats	2
Necrosis in Liver, due to Bacillus Necrophorus	Cattle	1
Tuberculosis	,,	1
Negative Result	—	48
Total		65

January, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	9
Ordinary Redwater, Piroplasma Bigeminum	,,	6
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	2
Marginal Points, Sequel of Ordinary Redwater	,,	2
Doubtful, East Coast Fever, Piroplasma Mutans ?	,,	1
Piroplasma Equi.	Equine	1
Basic, Nucleated and Polychromatic Cells, Sequel of Piroplasmosis	Dog	1
Acariasis, Sarcoptes of Africander Goats	Goats	2
Anthrax	Cattle	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equine	1
Tuberculosis	Cattle	1
Traumatic Pericarditis	Cow	1
Negative Result	—	58
Total		86

February, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	17
Ordinary Redwater, Piroplasma Bigeminum	,,	4
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	3
Piroplasma Equi.	Equine	1
Necrosis in Lung, Sequel Pneumonia, Swine Plague ?	Pig	1
Abscess, due to Bacterial Infection	—	2
Dermoid Cyst	Horse	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	2
Necrosis in Lung of Bull, due to Bacillus Necrophorus	Bull	1
Parasitic Nodules in Muscle	Ox	1
Negative Result	—	56
Total		89

March, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	12
Ordinary Redwater, Piroplasma Bigeminum	„	3
Basic and Polychromatic Cells, Sequel of Ordinary Redwater	„	6
Marginal Points, Sequel of Ordinary Redwater	„	2
Poikilocytosis, Sequel of Ordinary Redwater	„	4
Poikilocytosis, Sequel of Piroplasmosis	Equine	1
Traumatic Pneumonia	Cattle	1
Bronchial Interstitial Pneumonia	Mule	1
Abscess of Spleen, due to Perforation by Spiroptera from Stomach	—	1
Basic, Nucleated and Polychromatic Cells, Anæmia Blue Tongue ?	Sheep	1
Anthrax	Cattle	2
Abscess due to Bacterial Infection	—	5
Black Quarter	Cattle	1
Cirrosis	Ox	1
Mummified Foetus	—	1
Negative Result	—	78
Total		120

April, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	7
Ordinary Redwater, Piroplasma Bigeminum	„	7
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	6
Marginal Points, Sequel of Ordinary Redwater	„	1
Poikilocytosis, Sequel of Ordinary Redwater	„	5
Piroplasma Equi	Equines	2
Poikilocytosis, Sequel of Piroplasmosis	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Piroplasmosis	Dog	1
Bronchial Pneumonia	Sheep	1
Acariasis, Sarcoptes of Africander Goats	Goats	2
Epizootic Lymphangitis, Saccharomyces Farciminosus	—	3
Negative Result	—	48
Total		85

May, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	21
Ordinary Redwater, Piroplasma Bigeminum	„	1
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	5
Marginal Points, Sequel of Ordinary Redwater	„	2
Poikilocytosis, Sequel of Ordinary Redwater	„	4
Piroplasma Equi	Equines	3
Poikilocytosis, Sequel of Piroplasmosis	„	1
Basic, Nucleated and Polychromatic Cells, Anæmia, Sequel of Blue Tongue ?	Sheep	1
Abscess, due to Bacterial Infection	—	1
Acariasis, Sarcoptes of Africander Goats	Goats	4
Acariasis, Psoroptes of Sheep	Sheep	2
Black Quarter	Cattle	1
Streptococcus Infection	Equines	2
Parasitic Nodules in Lung	Horse	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	7
Negative Result	—	41
Total		98

June, 1906.

East Coast Fever, Piroplasma Parvum	Cattle	20
Poikilocytosis, Sequel of Ordinary Redwater	„	4
Piroplasma Equi.	Equines	1
Bronchial Pneumonia,	Sheep	2
Acariasis, Sarcoptes of Africander Goats	Goats	2
Acariasis, Psoroptes of Sheep	Sheep	5
Actinomycosis	Cow	1
Anthrax	Cattle	2
Black Quarter	„	3
Ecinococcus Cyst	Equines	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	„	3
Icterus	Calf	1
Tuberculosis	Cattle	1
Negative Result	—	52
Total		98

District Summary.

Barberton.

East Coast Fever, Piroplasma Parvum	Cattle	4
Basic, Nucleated and Polychr. Cells, Sequel of Piroplasmosis	Dog	1
Negative Result	—	13
Total		18

Ermelo.

East Coast Fever, Piroplasma Parvum	Cattle	8
Ordinary Redwater, Piroplasma Bigeminum	„	2
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	„	2
Piroplasma Equi	Equines	2
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	Cattle	1
Poikilocytosis, Sequel of Ordinary Redwater	„	1
Traumatic Pneumonia	Ox	1
Abscess due to Bacterial Infection	—	1
Actinomycosis	Cow	1
Echinococcus Cyst	Horse	1
Icterus	Calf	1
Tuberculosis	Cattle	1
Negative Result	—	42
Total		64

Heidelberg.

Ordinary Redwater, Piroplasma Bigeminum	Cattle	3
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	5
Poikilocytosis, Sequel of Ordinary Redwater	„	3
Bronchial Pneumonia	Sheep	1
Abscess due to Bacterial Infection	—	1
Acariasis, Psoroptes of Sheep	Sheep	1
Callus	Rib Bone of Mare	1
Necrosis of Liver, due to Bacillus Necrophorous	Cattle	1
Streptococcus Infection	Equine	1
Tuberculosis	Cattle	1
Negative Result	—	20
Total		38

Johannesburg.

Doubtful, East Coast Fever, Piroplasma Mutans ?	Cattle	1
Ordinary Redwater, Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	2
Piroplasma Equi	Equine	1
Abscess due to Bacterial Infection	—	2
Anthrax	Cattle	4
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	10
Streptococcus Infection	—	2
Negative Result	—	72
Total		95

Krugersdorp.

Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	Cattle	1
Poikilocytosis, Sequel of Ordinary Redwater	„	1
Poikilocytosis, Sequel of Piroplasmosis	Equine	1
Abscess due to Coccus Infection	—	1
Cirrhosis	Ox	1
Negative Result	—	34
Total		39

Lichtenburg.

Fibrous Tumour	Cow	1
Traumatic Pericarditis	„	1
Negative Result	—	3
Total		5

Lydenburg.

East Coast Fever, Piroplasma Parvum	Cattle	2
Ordinary Redwater, Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	3
Poikilocytosis, Sequel of Ordinary Redwater	„	2
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	5
Negative Result	—	15
Total		28

Middelburg.

East Coast Fever, Piroplasma Parvum	Cattle	14
Ordinary Redwater, Piroplasma Bigeminum	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	4
Poikilocytosis, Sequel of Ordinary Redwater	„	1
Basic, Nucleated and Polychromatic Cells, Sequel of Piroplasmosis	Dog	1
Swine Fever	Pig	1
Pneumonia, Cysticercus Cellulosæ, Swine Plague ?	—	1
Dermoid Cyst	Equine	1
Negative Result	—	51
Total		75

Waterberg.

East Coast Fever, Piroplasma Parvum	Cattle	5
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	,,	1
Parasitic Nodules in Muscle	Ox	1
Negative Result	—	35
Total		42

Zoutpansberg.

East Coast Fever, Piroplasma Parvum	Cattle	57
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	,,	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	1
Poikilocytosis, Sequel of Ordinary Redwater	,,	2
Piroplasma Equi	—	4
Basic, Nucleated and Polychromatic Cells, Anæmia, Blue Tongue ?	Sheep	2
Abscess due to Bacterial Infection	—	2
Acariasis, Sarcoptes of Africander Goats	Goats	17
Acariasis, Psoroptes of Sheep	Sheep	4
Chronic Interstitial Nephritis	Pig	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	9
Fibrous Tumour	Horse	1
Pthiriasis	Goats	3
Negative Result	—	101
Total		205

Piet Retief.

East Coast Fever, Piroplasma Parvum	Cattle	15
Ordinary Redwater, Piroplasma Bigeminum	Cattle	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	2
Negative Result	—	15
Total		33

Potchefstroom.

Marginal Points, Sequel of Ordinary Redwater	Cattle	2
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	1
Poikilocytosis, Sequel of Ordinary Redwater	,,	2
Anthrax	,,	1
Mummified Foetus	—	1
Necrosis of Liver, due to Bacillus Necrophorus	Cattle	2
Necrosis of Lung, Sequel Pneumonia	Pig	1
Necrosis of Liver	Equine	1
Fatty Infiltration of Liver	Hen	1
Negative Result	—	29
Total		41

Rustenburg.

East Coast Fever, Piroplasma Parvum	Cattle	21
East Coast Fever and Ordinary Redwater, Piroplasma Parvum and Piroplasma Bigeminum	,,	1
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	1
Anthrax	,,	1
Black Quarter	,,	5
Negative Result	—	59
Total		88

Pretoria.

East Coast Fever, Piroplasma Parvum	Cattle	14
Ordinary Redwater, Piroplasma Bigeminum	,,	11
Marginal Points, Sequel of Ordinary Redwater	,,	5
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	,,	6
Poikilocytosis, Sequel of Ordinary Redwater	,,	8
Piroplasma Equi	Equines	4
Poikilocytosis, Sequel of Piroplasmosis	,,	1
Piroplasma Canis	Dogs	3
Swine Fever	Pigs	2
Swine Plague	,,	1
Bronchial Pneumonia	Sheep	2
Bronchial Interstitial Pneumonia	Mule	1
Pleuritis and Chronic Pneumonia	Goose	1
Pulmonary Oedema	Cattle	1
Abscess due to Bacterial Infection	—	3
Acariasis Sarcoptes of Africander Goats	Goats	2
Acariasis, Psoroptes of Sheep	Sheep	5
Aneurism of Aorta	Heifer	1
Amphistomum Conicum / Cysticercus Tenuicollis	Antelope	1
Anthrax	Cattle	2
Congestion of Lungs	Fowl	1
Epizootic Lymphangitis, Saccharomyces Farciminosus	Equines	6
Spirillosis	Cattle	1
Tuberculosis	,,	2
Basic, Nucleated and Polychromatic Cells, cause unknown Worm Infection ?	Pigs	8
Negative Result	—	144
Total		236

Standerton.

Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	Cattle	1
Abscess due to Bacterial Infection	—	1
Negative Result		9
Total		11

Swaziland.

East Coast Fever, Piroplasma Parvum	Cattle	10
Poikilocytosis, Sequel of Piroplasmosis	Equine	1
Basic, Nucleated and Polychromatic Cells, Sequel of Piroplasmosis	Dogs	2
Acariasis, Sarcoptes of Africander Goats	Goats	2
Parasitic Nodules in Lung	Equine	1
Septicæmia Hemorrhagica	Antelope	1
Negative Result	—	40
Total		57

Volksrust.

Ordinary Redwater, Piroplasma Bigeminum	Cattle	3
Anthrax	„	1
Negative Result	—	12
Total		16

Zeerust.

East Coast Fever, Piroplasma Parvum	Cattle	2
Ordinary Redwater, Piroplasma Bigeminum	„	2
Basic, Nucleated and Polychromatic Cells, Sequel of Ordinary Redwater	„	1
Abscess of Spleen, due to Spiroptera	—	1
Negative Result	—	11
Total		17

Cape Colony.

Negative Result	—	2

Madagascar.

Negative Result	—	1

VACCINE.

1905-6.	Transvaal.	Cape Colony.	Orange River Colony.	Portuguese Territory.	Mozambique.	Natal.	TOTAL.
	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.	Tubes.
July ...	41,550	6,000	2,000	6,000	—	—	55,550
August ...	38,215	18,000	1,000	6,000	—	—	63,215
September ...	46,645	24,000	10,500	6,000	—	—	87,145
October ...	16,015	3,000	5,000	6,016	—	—	30,031
November ...	8,115	18,000	2,000	6,000	50	—	34,165
December ...	10,045	6,000	2,000	—	—	—	18,045
January ...	14,321	12,000	—	6,000	—	—	32,321
February ...	10,315	24,000	1,000	6,000	—	300	41,615
March ...	15,615	60,000	1,000	6,000	—	350	82,965
April ...	15,215	42,000	1,500	6,000	—	250	64,965
May ...	9,325	6,010	1,000	6,000	—	300	22,635
June ...	9,065	18,000	1,000	6,000	—	—	34,065
TOTAL	234,441	237,010	28,000	66,016	50	1,200	566,717

566,717 tubes at 2d. each—£4,722 12s. 10d.

Calf Vaccine Lymph.

Analysis from Reports Received on Results of Vaccination with Calves Nos. 118 to 158, issued during the Year 1905-6.

Calf Number.	Primary Vaccinations.				Secondary Vaccinations.				Remarks
	Number Performed.	Number subsequently inspected.	Number found to be successful.	Percentage of successful vaccinations to No. inspected.	Number Performed.	Number subsequently inspected.	Number found to be successful.	Percentage of successful vaccinations to No. inspected.	
				%				%	
118									No returns received.
119	37	21	20	95	200	100	70	70	
120									" " "
121	116	96	95	99	168	90	70	78	
122	229	108	101	94	387	194	142	74	
124									" " "
125									" " "
126	33	4	4	100	11	11	4	36	
127									" " "
128									" " "
129									" " "
130									" " "
131	177	47	27	57	—	—	—	—	See below.
132									No returns received.
133	8	5	5	100	—	—	—	—	
134	13	3	3	100	—	—	—	—	
135									" " "
136	115	41	39	97	138	131	93	71	
137									" " "
138	75	4	4	100	—	—	—	—	
139	218	16	14	88	—	—	—	—	
140									" " "
141	30	27	25	91	65	65	35	54	
142	28	20	20	100	30	15	4	27	
143	194	21	19	90	77	77	62	80	
144	1	1	1	100	—	—	—	—	
145	18	1	1	100	—	—	—	—	
146	58	26	26	100	260	98	80	82	
147	222	88	86	97	1,044	446	333	75	
148	5	4	4	100	—	—	—	—	
149	72	72	71	98	40	39	14	36	
150	74	23	23	100	203	77	77	100	
151	20	20	13	65	23	23	10	43	See below.
152	25	14	13	93	—	—	—	—	
153									No returns received.
154	129	68	67	98	721	305	172	56	
155	2	2	2	100	4	4	2	50	
156	36	34	33	97	46	46	34	74	
157	199	138	132	96	286	144	72	50	
158	168	40	29	77	211	25	24	96	See below.
Total	2,302	944	877	93	3,914	1,890	1,298	69	

The following results from Calves Nos. 131, 151 and 158 are shown more fully, and it will be noticed that whereas one vaccinator obtains a very high percentage of successful results, another only obtains a very low figure, thus reducing the percentage on the total results :—

				%				%	
131	10	10	10	100					
	40	1	1	100					
	69	3	3	100					
	58	33	13	40					
Total	177	47	27	57					
151	11	11	4	36					
	9	9	9	100					
Total	20	20	13	65					
158	153	25	24	96	210	24	24	100	
	7	7	1	14	1	1	0	0	
	8	8	4	50	—	—	—	—	
Total	168	40	29	77	211	25	24	96	

Record of the Production of Blue Tongue Serum, Horse Sickness Serum and Quarter Evil Vaccine.

Blue Tongue Serum.—Amount manufactured, 77,487 c.c.

Horse Sickness Serum.—Manufactured from the 1/3/05 to the 30/6/06		*litres.* 6.871
Issued during the same period :—	*litres.*	
Transvaal	1,384	
Rhodesia	297½	
German West Africa	21	1,702½
		5,168½
Used on the Station		445½

Quarter Evil Vaccine.—The manufacture of this vaccine was started in March, 1906, and from that date to the 30/6/06, the total number of double doses issued was 450.

DR. THEILER'S RESEARCH.

PIROPLASMA MUTANS, n.spec., A NEW SPECIES OF PIROPLASMA AND THE DISEASE CAUSED BY IT.

In my annual report for the year 1904-5 I published an article on the piroplasma bigeminum of the immune ox in which I demonstrated that the inoculation of blood of an ox immune against ordinary redwater into susceptible animals caused the appearance of one or two reactions followed by the presence of endoglobular parasites resembling, in shape and size, the piroplasma parvum of East Coast fever. It has been repeatedly pointed out that the correct diagnosis of this latter disease is of the utmost importance from a legislative point of view, and, further, that the possibility of mistaking piroplasma parvum with those small parasites is considerable.

It was accordingly necessary to collect further evidence for differential diagnostic purposes, and a series of experiments with fresh susceptible animals were made. Captain Olver, of the A.V.D., who interested himself in microscopical work of this station, carried out part of this investigation, and his results are included in these notes.

The question of small endoglobular parasites in the shape of rods, rings and flagellated bacillary forms has lately assumed quite a new aspect.

At the last International Congress of Veterinary Surgeons (Section of Tropical Disease) held in Buda Pesth, Dschunkowsky from Transcaucasus, who (as I mentioned in the article referred to above) discovered " tropical piroplasmosis " in the Transcaucasus, stated that this disease should not, as we were formerly inclined to believe, be considered identical with the one commonly known in South Africa as East Coast fever. In their first publication, Dschunkowsky and Luhs stated that the disease studied by them and caused by small endoglobular parasites was not inoculable into cattle, and this observation was a further argument in favour of connecting the two diseases " tropical piroplasmosis " and " East Coast fever."

Subsequently, however, Dschunkowsky inoculated fresh cattle derived from other not infected regions, and found that he could inoculate susceptible cattle with the disease. This new discovery, accordingly separates the two diseases. Consequently, we have two distinct diseases due to small endoglobular parasites, one inoculable and the other not. Mr. Bitter, of Cairo, made a communication to the effect that in Egypt he noticed a piroplasmosis also due to small piroplasms and he exhibited photographs. These small endoglobular

parasites are found in imported cattle, especially in calves; country-bred cattle being immune. He further stated that this piroplasmosis could not be produced in susceptible cattle by inoculation of blood from sick cattle. He also mentioned a characteristic lesion found in calves, which succumbed to this disease, in the presence of numerous small hemorrhagic ulcers in the abomasum almost identical to those Dschunkowsky demonstrated *in natura*.

Ducloux, of Tunis, reported a similar disease in that country. Since then he made a communication to the Société de Biologie of Paris wherein he says the disease principally attacks young animals. The *post-mortem* lesions described resemble those of East Coast fever, especially those of the stomach and intestines. In my opinion, however, it has not yet been demonstrated with which disease that of Egypt and Tunis—which two are probably one and the same—are identical. To judge from the assertion of Bitter, who, when questioned on the subject, insisted on the non-inoculability of the disease, one is inclined to believe that this latter one, if not identical with East Coast fever, nevertheless belongs to that group which is caused by small piroplasms.

The piroplasmata of cattle would thus be grouped as follows:—

Type. *Piroplasma Bigeminum.*

Piroplasma bovis (Babes), found in the European hemoglobinuria of cattle.

Piroplasma bigeminum (Smith and Kilborne) of Texas fever.

Type. *Piroplasma Parvum.*

A. Inoculable piroplasmosis.

Tropical piroplasmosis of Transcaucasia. Piroplasma annulatum (Dschunkowsky).*

B. Non-inoculable piroplasmosis.

Piroplasma parvum (Theiler) of East Coast fever.

Piroplasma of the North African disease (Bitter and Ducloux.)

In the face of these new discoveries of various piroplasmoses due to small parasites, the question immediately arises whether ring and rod-shaped parasites found by us in calves after inoculation of blood derived from redwater immune animals are really forms of the piroplasma bigeminum—which, hitherto, I have considered them—or whether we have to contend with still another form of piroplasmosis caused by small piroplasmata and inoculable with the blood of immune animals, such as in the case of Texas fever. Accordingly, the animals from which we derived our redwater blood for the inoculation would have had the germs of both diseases in their blood, and, further, it would follow that cattle born and bred in redwater areas of South Africa would probably be immune to two different kinds of piroplasmoses.

* It was under this name that Dschunkowsky described the parasite as a new species at the Congress in Buda Pesth.

The point at issue at the present time is to determine whether such is the case.

It has been noted by Australian and American* investigators that the injection of blood from a Texas fever immune animal produces in a susceptible calf two more or less distinct reactions during which the number of red corpuscles decreases. Some of the animals may die during the first reaction and some during the second. The first rise of temperature, viz., at the beginning of the primary reaction, was usually noted to occur on or about the 8th or 9th day, sometimes a little earlier and sometimes a little later; it usually continued for seven or eight days. In some cases it may not exceed four days, and, in others, it may be prolonged to fifteen days. The daily average of the temperature during the primary fever period, counting all animals that had a distinct reaction, was about 104.5° F. The second reaction begins about the 25th to 30th day after inoculation and continues for seven to eight days. (This secondary reaction was not, as a rule, so severe as the primary.) In some animals which received but one inoculation no appreciable reaction appeared at the usual period of primary fever, but came up strongly at the secondary period. The suggestions arise that a tertiary and succeeding recurrences of fever take place, each milder than the preceding one, until immunity is finally attained. In my publication quoted earlier in these remarks, and also in an article on "redwater" which appeared in the "Agricultural Journal," I alluded to these observations, especially to the two reactions which also often occur in susceptible calves injected with South African redwater, and the observations were thus confirmed.

In the American investigations quoted above we miss a description of the changes the blood undergoes during these two reactions, and we must, therefore, conclude that no particular stress was laid on microscopical examinations. Hence we cannot expect to obtain information regarding the rings, rod-shaped and flagellated forms which I found, as a rule, in South African redwater.

In connection with our examination of the blood from cattle injected with blood of immune oxen, we occasionally noticed the appearance of coccus-like bodies almost without exception situated on the margin of the red corpuscle and deeply staining when any of the modern stain (Laveran, Giemsa) was applied, thus shewing that they consist of pure chromatine. On this account we have considered them to be of protozooic nature, and by this stain easily distinguishable from basophile granulations which may be present at the same time. These granula take the blue stain and are of various sizes. The "marginal points," as we call them, are almost of the same size, and, as mentioned, always situated on the periphery. These marginal points are frequently met with after inoculation of immune redwater blood and are sometimes present for a considerable time. I very often saw them by the side of the small piroplasmata, with which they cannot be mistaken, being easily distinguishable by their described characteristics.

* T. W. Connaway and M. Francis: "Texas Fever," Columbia, Missouri, 1899.

In perusing the literature on Texas fever, reference is made to the presence of these peripheral points by various authors, and this observation may be considered as a proof that the examination of blood after the appearance and disappearance of piroplasma bigeminum was continued for some time. The allusion to the presence, or otherwise, of these parasites in works on Texas fever put us in the position to say that those observers who noted the marginal points in the blood would also have seen the ring, rod and flagellated forms of the piroplasma which I have described to be present in South African inoculated cattle if they had been present in their cattle. Thus, the mention of the appearance of peripheral bodies on one hand, and not mentioning small endoglobular parasites on the other, can only be interpreted by the fact that these latter were not seen by the authors, hence they were not present. Thus we must indirectly accept that the rings, rods and flagellated forms have nothing to do with piroplasma bigeminum, and probably represent another species of piroplasma.

Smith and Kilborne were the first to notice the marginal points, and they refer to them as "peripheral bodies or peripheral coccus-like bodies." They found these forms in connection with a form of Texas fever called the mild type. Here they found from 5% to 50% of the red corpuscles in the circulating blood infected for a period of one to five weeks. In stained preparations, these coccus-like bodies, varying in size from 0.2 to 0.5 micro-millimetre in diameter, are seen situated within the corpuscle on its border. They sometimes appear situated on the border, but outside of the corpuscle, and, as a rule, only one is found in a single corpuscle. In many cases a division of the coccus-like bodies into two could be clearly made out, and these were found in

(1) Animals exposed to Texas fever late in the season (October and November);

(2) Animals which passed through an acute attack earlier in the summer (second attack or relapse in October and November);

(3) Animals which contract a mild disease during or previous to the season of the acute disease.

Smith and Kilborne also point out that these peripheral bodies must not be mistaken for the granules (punctate cells) which are found in connection with Texas fever, and with which these granules might possibly be mistaken. That they are different from these follows from the fact that they appear with, or immediately before, the destruction of the red corpuscles, whereas the granules appear after the number has fallen below half the original number, and when the destruction ceases the punctate cells still increase and the coccus-like bodies disappear. The bodies are all the same size in the same preparation of blood, but the granules differ considerably in this respect.

These two investigators leave the question open as to whether these bodies are stages of the Texas fever parasite or of another

parasite transmitted with it, but are inclined to believe in the unity of both. Ligniere, who studied Texas fever in the Argentine Republic, remarks, after quoting the statement of Smith and Kilborne regarding these punctiform bodies, that these scientists were probably mistaken in their observations, due to their method of staining, by which sometimes intraglobular bodies appear closely resembling parasites. Ligniere asserts that the benign form of Trystezza is due to the presence of the ordinary pear-shaped parasite.

Dr. Knuth, who studied Trystezza in the La Plata States in 1904, states that he also saw these peripheral bodies and devoted some attention to them. They appeared frequently, but principally after Texas fever. These points, when submitted to the Romanowsky method, stain red, and, consequently, consist of chromatin. Accordingly, they cannot be considered as either the products of destruction or as cocci. He did not see them in animals of countries free of ticks, but in animals after natural as well as artificial infection. He quotes the history of one observation of an animal in which he first saw the piroplasma bigeminum, when, on the 41st day after infection, these parasites appeared again accompanied with high fever. They were frequent in this case, having from two to four points in one and the same corpuscle. This animal died after an almost complete dissolution of the blood corpuscles accompanied with the appearance of many punctate cells (basophile granulations).

A second animal which had been inoculated with the same sample of blood shewed these parasites 46 days after infection, after which time they gradually disappeared. When the peripheral bodies were present in the blood of the former animal, it was tapped and injected into six fresh animals, four of which could be considered to be immune, whereas two were not. These two latter animals shewed peripheral bodies, but not piroplasma bigeminum, on the 10th and 18th day after infection. The punctiform parasites were very frequent, and, on the 28th day, the basophile granulations became very noticeable.

Dr. Knuth considers these peripheral bodies as belonging to the cycle of development of the piroplasma and not as a separate species.

Dschunkowsky and Luhs also describe these punctiform piroplasmata, and ascribe the cachectical form of tropical piroplasmosis to their presence. They appear as round or oval points, consisting of chromatin, frequently in the shape of a diplococcus. From 10% to 40% of all erythrocytes may be infected in the cachectical form of tropical piroplasmosis.

From the foregoing notes we see that the peripheral bodies which were noticed in North America by Smith and Kilborne, and in South America by Dr. Knuth, were considered to belong to the development of the piroplasma bigeminum.

Ligniere considers them as basophile granulations, and the Russian investigators identified them with a form of tropical piroplasmosis which they name the "cachectic form."

I have laid particular stress on the communications of these various observers because, as I have already stated, the fact that these

peripheral bodies were noticed by them means that the blood examinations have been carried out over a long period, during which, according to our South African experience, rings, rods and flagellated forms are present and would have been seen by the above-mentioned scientists if they had been present in their cases.

These peripheral points deserve, however, more attention than hitherto, since their exact nature has not yet been elucidated.

I consider it advisable to give the experiments undertaken for the purpose of the elucidation of this subject *in extenso.*

(1) *Heifer 278.*—Inoculated with blood of ox 347 which contained rods and flagellated forms in its blood.

The temperature rose the 7th day and remained high for about a week, after which it dropped to normal, but rose for the second time on the 21st day after inoculation and maintained a high level for five days. It again dropped to normal and a third reaction could be distinguished on the 29th day.

Piroplasma bigeminum appeared on the 7th day and was present in rare numbers during the fever period. It appeared during the second reaction on the 25th day, but disappeared with the drop of temperature.

Poikilocytosis was observed one day after the appearance of piroplasma bigeminum—8th day—and four days later basophile granulations made an appearance, the poikilocytosis increased, and, simultaneously, a marked drop in the count of red corpuscles took place. The number of these latter, on the second day of the appearance of poikilocytosis, was 5,600,000 per c.m.m., and dropped to 2,776,000 per c.m.m., which was the lowest record. For a considerable time the number of red corpuscles stood at a rather low mark, and, during this time, piroplasma was constantly present, only disappearing when the number of red corpuscles increased to about 5,000,000 per c.m.m. during the second reaction.

In this case the small endoglobular parasites in the shape of flagellated and bacillary forms began to appear on the 29th day, viz., at the beginning of the third rise of temperature. This third reaction surpassed in length the primary and secondary ones. The number of red corpuscles again increased during this reaction.

(2) *Heifer 270.*—Inoculated with blood of ox 347 as above.

Irregular temperature shortly after the inoculation.

Distinct reaction began on the 11th day; it reached the highest temperature—105° F.—on the 20th day, and gradually descended, marking the lowest morning temperature on the 26th day.

A second reaction followed almost immediately with the highest rise of temperature to 105° F. on the 29th day, from which date the curve again descended.

Neither piroplasma bigeminum or poikilocytosis—with its sequelæ of basophile granulations—were observed during these reactions.

The lowest reading of the red corpuscles at 5,300,000 per c.m.m. was noted on the 19th day.

The first ring and bacillary-shaped small piroplasmata were seen on the 35th day, almost at the time the second reaction was passing over.

(3) *Heifer 273.*—Inoculated with blood of calf 241.

First reaction began on the 7th day, the temperature reaching 106° F. on the 8th day. It dropped to normal on the 14th day.

A second reaction, although not very typical, began on the 21st day and lasted until the 34th day, whereupon a third rise of temperature became noticeable.

Piroplasma bigeminum and poikilocytosis appeared on the 9th day. These parasites were noticed in rare numbers during the apyretical stage, and disappeared with the third reaction. Rare basic cells put in an appearance on the 14th day after inoculation, viz., on the day the first reaction finished.

On this date the number of red corpuscles amounted to 4,554,000 per c.m.m., and the basophile granulations were seen daily in small numbers up to the 22nd day.

Poikilocytosis was observed up to the 25th day.

In this heifer the first ring-shaped piroplasma was seen on the 25th day—when the second reaction had already started. They considerably increased during the second reaction, notwithstanding which the number of red corpuscles gradually rose to 5,924,000 per c.m.m. on the 36th day, when the small piroplasma infested from about 6% to 7% of the red blood cells.

An analysis of the three preceding instances shews that, in each case, we are able to distinguish a primary and secondary reaction, and, in the first and last cases, even a third one.

They all differ, however, in the length of the second reaction, which, in the case of the last two, is much longer than in the first one.

With regard to piroplasma bigeminum, we distinguish three defined phenomena. It appeared in the first case twice, viz., with the typical reaction, in the second instance it did not appear at all, and in the third instance it was observed with the first reaction.

Poikilocytosis and its sequelæ were most pronounced and lasted the longest in the first case, which corresponds in respect of the length of the reaction to the typical case described by the Americans.

The small piroplasma appeared respectively on the 29th, 35th and 25th day, viz., in the first instance at the beginning of the third reaction, in the second instance at the beginning of the second reaction, and, thirdly, at the end of the second reaction.

There was no decrease of red corpuscles noticed corresponding to the appearance of the small piroplasmata.

(4) *Calf 284.*—Inoculated with blood of ox 347.

Irregular temperature started the first week after inoculation.

The first reaction began about the 10th day and lasted for a week, the second reaction started on the 23rd day and lasted about 12 days. Closely following this, a third and more pronounced reaction took place, lasting about five days.

There was nothing noticed during the first reaction, the number of corpuscles averaging from 4½ to 5½ millions per c.m.m. On the 25th day after inoculation—two days after the second rise of temperature—poikilocytosis was noticed and a drop of the corpuscles to about 4 millions per c.m.m. took place two days later. The poikilocytosis continued during the second reaction.

In this case ring-shaped small piroplasmata appeared on the 34th day after inoculation, viz., at the end of the second reaction. These small piroplasmata were still noticed on the 60th day.

Basic cells were also noticed after the third reaction and at the time when the number of red corpuscles had again decreased.

(5) *Calf 285.*—Inoculated with blood of ox 347.

Irregular temperature to begin with; the first reaction began on the 10th day and lasted 7 days.

A second reaction began about the 24th day and lasted until the 34th day.

A third reaction began about the 50th day.

Poikilocytosis was noticed for two days during the first reaction when the temperature was at its highest.

It appeared for the second time on the 26th day, viz., during the second reaction, and lasted for four days.

It was again noticed on the 36th day, between the second and third reactions, lasting for a few days.

In this case rings appeared on the 29th day and considerably increased during the third reaction, when polychromatic red corpuscles and basophile granulations appeared.

The drop in the number of red corpuscles was, during the last reaction, corresponding to the increase of small piroplasmata.

(6) *Calf 286.*—Inoculated with blood of ox 347.

Very irregular temperature started about three days after inoculation, but no definite primary reaction was noticeable. This irregular temperature kept on for the following three weeks, and, accordingly, a second reaction could not be distinguished.

Poikilocytosis was only noticed on one day—14th. The number of red corpuscles at that time was about 5,200,000 per c.m.m.

The second appearance of poikilocytosis was noticed on the 26th day, from which date it continued for the next ten days.

Piroplasma bigeminum was seen on the 32nd day, and again on the 47th day after inoculation.

In this case ring and bacillary-shaped parasites were noticed on the 41st day after inoculation. They increased considerably during the next 14 days, the number of red corpuscles decreasing with the appearance of the small piroplasmata, but, later again, increasing, and at a time when the small piroplasmata became more frequent.

Analysis of the Cases of Calves Nos. 284, 285 and 286.

Of the three animals inoculated with blood of ox 347 two shewed no piroplasma bigeminum, and the third one not until the 32nd day for the first time, and the 47th day for the second appearance.

In the first case there was a distinct reaction without any blood change, which only put in an appearance during the secondary reaction.

In the second case there was a primary reaction with blood change, and a secondary one in which the blood change increased.

In the third case there was no distinct reaction, but at the time when primary and secondary reactions appeared in other animals, blood changes were noticeable.

The small piroplasmata appeared on the 34th, 29th and 41st day respectively, either at the conclusion or following the conclusion of the second reaction. There was a decrease in the number of the red corpuscles either at the time of the appearance or during the increase of the small piroplasmata.

(7) *Heifer 281.*—Inoculated with the blood of heifer 200.

Irregular temperature the first week after inoculation.

A primary reaction was noticeable from about the 14th day, the temperature on that date reaching 105.4° F. It lasted about six days; a second reaction started about the 24th day, but only lasted three days; a third reaction began about the 37th day and continued for about 8 days.

There was a slight drop in the number of erythocytes during the time of the highest fever reaction, and, at the same period, poikilocytosis was noticed.

At the end of the second reaction—20th day—poikilocytosis was again visible and continued for some length of time, the number of red corpuscles being reduced to between $4\frac{1}{4}$ and $4\frac{3}{4}$ millions per c.m.m.

The small rings and bacillary-shaped forms appeared on the 34th day after inoculation, three days before the third rise of temperature became distinct.

Piroplasma bigeminum was noticed on the 47th day and occasionally after this. The small piroplasmata increased during this period.

Three days later—50th day—the number of red corpuscles dropped lower than on the previous occasion, maintaining at a lower average, whilst the rings were frequent, and rising again when the small piroplasma decreased.

(8) *Heifer 282.*—Inoculated with blood of heifer 200.

Irregular temperature became noticeable about the 10th day. It was not well marked, the morning temperature remaining normal.

A slight drop in the number of red corpuscles below 4 millions per c.m.m. occurred, lasting for the same period as the reaction.

Basophile red corpuscles and poikilocytosis were noticed on the 17th day.

The secondary reaction started about the 25th day and was of the same character as the first one; it continued with an intermission of two days into the third reaction, which started about the 35th day.

The number of red corpuscles during this time averaged about 4½ millions per c.m.m.

Poikilocytosis was noticed during the second reaction, and was again present after the third reaction had passed.

Piroplasma bigeminum was noticed on the 33rd day after inoculation, together with the small ring-shaped bacillary forms, viz., during the intermittent period of two days between the second and third reaction. These small parasites were present during the following weeks, and the red corpuscles dropped as the small piroplasmata increased in numbers.

(9) *Heifer 274.*—Inoculated with blood of heifer 200.

Irregular temperature started the first week after injection. There was no distinct primary reaction, but a slight drop of the red corpuscles from the 10th to the 14th day was noticeable, the number on the latter date being 4 millions per c.m.m.

There was no change in the blood corpuscles during this period, neither was there any distinct secondary reaction. From the 30th day, however, the morning temperature kept higher than usual, and, on the 35th day, the evening temperature rose to a distinct reaction, dropping to normal on the 41st day, when rings and bacillary forms appeared. These were not so frequent as in other cases, and the red corpuscles maintained at normal figures.

Analysis of the Three Cases of Heifers Nos. 281, 282 and 274, Inoculated with the Blood of Heifer 200.

Three reactions were noticed in the first case, and three could be distinguished in the second case, whereas in the third case, only one reaction was distinguishable.

Piroplasma bigeminum was not seen in heifer 281; in heifer 282 it appeared on the 33rd day, and in 274 on the 32nd day.

Poikilocytosis was noticeable in the first two cases at the time of the first and second reaction, and at the time of the second reaction in the third case.

There was a drop in the number of red corpuscles at the time when the first reaction is usually observed.

The small piroplasmata appeared on the 34th, 33rd and 41st day respectively, after inoculation. The presence of the small parasites was accompanied with a drop in the number of the red corpuscles in the two first cases.

(10) *Heifer 312.*—Inoculated with 10 c.c. blood of calf 241.

There was a primary reaction noticeable beginning about the 10th day marked by high evening elevations, the morning temperatures keeping about normal.

Poikilocytosis was observed for about six days during this reaction.

On one occasion a trypanosoma theileri was noticed.

There was no drop in the number of the red corpuscles until the reaction was over.

A second reaction started soon after the conclusion of the first one; it was not well pronounced in its course, but the evening temperatures kept, with only one intermission, at above 104° F.

At the beginning of the second reaction—on the 26th day—poikilocytosis was observed again, and, on the same day, the first ring and bacillary-shaped piroplasmata were seen. These small piroplasmata increased in number during the next fourteen days, while the rise of temperature still increased—so that the second reaction continued into the third one without any intermission.

The number of red corpuscles decreased to about $2\frac{1}{2}$ millions per c.m.m. on the 41st day after inoculation.

Basophile red cells were also noticed during this latter period.

(11) *Heifer 315.*—Inoculated with 10 c.c. blood of calf 241.

Irregular temperature curve noticed the first few days after the injection of the blood.

There was no rise of temperature to indicate a primary reaction, notwithstanding which piroplasma bigeminum made its appearance on the 15th day and a slight poikilocytosis was also noticed on the 13th day.

The number of red corpuscles decreased subsequent to this phenomenon.

No second reaction could be distinguishable, but poikilocytosis again appeared on the 24th day, and, two days later, the ring and bacillary-shaped forms were present and remained for the rest of the time the animal was under observation.

While these rings and rods were increasing, the morning temperature kept higher than normal; the evening temperature, however, maintained a normal average.

During this latter period the red corpuscles dropped to under 3 millions per c.m.m.

(12) *Heifer 313.*—Inoculated with blood of calf 241 to the extent of 10 c.c.

Irregular temperature reaction during the first few days. No distinct primary reaction was noticeable.

On the 13th day piroplasma bigeminum and marginal points were observed.

A slight reaction was observed which started about the 25th day, and, on the 26th day, poikilocytosis made its appearance.

Two days later the small rings and bacillary-shaped piroplasma were seen. No high temperature noticeable after this, but a tendency to a third reaction was marked.

During this last period basophile cells were seen, and a drop in the number of red corpuscles to below 4 millions per c.m.m. was noticed. The small piroplasms increased in number during the time poikilocytosis was present.

Analysis of the Three Cases of Heifers Nos. 312, 315 and 313, Inoculated with Blood of Calf 241.

Piroplasma bigeminum was only seen in one instance—heifer 313.

In all three cases changes in the blood were noticeable at the time of the first and second reaction which was not marked by a temperature elevation corresponding to those described in other cases.

The small piroplasmata appeared on the 26th, 24th and 28th day after inoculation, and in all three instances the number of red corpuscles dropped considerably whilst the piroplasms increased.

(13) *Heifer 314.*—Injected with blood of calf 242.

Irregular temperature curve followed, but not distinct to mark a primary reaction.

Poikilocytosis noticed on the 13th day after inoculation and lasted for a week.

Trypanosma theileri was noted on the 15th day.

Secondary rise of temperature—more distinct than the primary —about the 26th day; rings appeared on the same day for the first time.

Distinct tertiary reaction from about the 34th day and lasting 6 days.

The number of red corpuscles decreased during this last reaction and, on the last day, numbered 3 millions per c.m.m.

(14) *Heifer 316.*—Injected with blood of calf 242.

Irregular temperature followed, but no distinct rise to mark a primary reaction.

Poikilocytosis noticed on the 12th, 13th and 15th days.
Second and tertiary reactions, but slightly marked, were observed.
Poikilocytosis present for the second time on the 25th day and continued for about a fortnight.
Rings appeared on the 31st day after inoculation, and, on the 40th day, were fairly frequent.
The red corpuscles were but little affected during this period.

(15) *Heifer 317.*—Injected with blood of calf 242.

Irregular reaction for the first 7 days after this inoculation.
On the 15th day the morning temperature remained higher than normal.
Piroplasma bigeminum was noted on the 17th day.
Poikilocytosis was not observed until the 26th day.
Rings appeared the following day, and, from this date to the 41st day, a distinct reaction was noticeable.
At the time when the number of rings began to increase, chromatic and basophile cells made their appearance, and the number of red corpuscles dropped as low as 2,440,000 per c.m.m. on the 45th day.

Analysis of the Three Cases of Heifers Nos. 314, 316 and 317, Inoculated with Blood of Calf 242.

Only in one case piroplasma bigeminum appeared at the time of the primary reaction, which, however, was but little pronounced. In the other two cases, no distinct primary reaction was noticed, the usual blood change, however, being present.

The secondary reaction was but slightly pronounced in heifer 316 when poikilocytosis was again noticed. A second and tertiary reactions were found in the case of heifer 314, and, in both instances, poikilocytosis was noticed. The secondary reaction continued into the tertiary one in the third case—317. Rings and rods appeared on the 26th, 31st and 26th day respectively. In two instances the increase of rings was accompanied by the decrease of the number of red corpuscles.*

Remark.—The fifteen calves were inoculated with blood of cattle which we were certain to be immune against redwater. Their blood had either been utilised before for similar experiments or on microscopical examination proved to contain ring forms.

The blood of ox 347 was utilised for ox 6 (compare article on the "Piroplasma Bigeminum of the Immune Ox," Case No. 4); it was an animal which had recovered from redwater, and its blood produced piroplasma bigeminum and rings in the ox 6; it was also utilised for calves 240 and 241 (compare same article, Case No. 5),

*The preceding experiments with twelve animals, Nos. 284, 285, 286, 281, 282, 274, 312, 315, 313, 314, 316, 317, were carried out in this Laboratory by Captain Olver of the Army Veterinary Department.

both calves shewing piroplasma bigeminum and rings from the injection.

Heifer 200 (compare same article, Case No. 10) shewed the small piroplasms as the result of a heartwater reaction.

Calf 241 is mentioned above.

Calf 243 had been injected with blood of calf 240 whilst it was undergoing the secondary reaction. It shewed piroplasma bigeminum, and, later, rings, as the result of the injection. The calves which were used for these experiments were purposely selected from a district—Aliwal North—in which we were certain that redwater did not exist.

Conclusions to be Drawn from the Inoculation of the Fifteen Calves.

A distinct primary reaction was noticed in nine cases, three of which were accompanied with the appearance of piroplasma bigeminum and eight with the appearance of poikilocytosis. Six animals shewed no primary reaction, notwithstanding which poikilocytosis was noticed in five cases.

A secondary reaction could be distinguished in thirteen cases; two had no distinct reaction and shewed either piroplasms or poikilocytosis at the time of the second reaction.

With the exception of one case, where there was a distinct secondary reaction—calf 270—poikilocytosis was always present.

The first fever reaction started in the shortest instance at the seventh day—two cases; the average was 10 days, and the longest 15 days—one case. The poikilocytosis of the first reaction started in the shortest instance on the 8th day, and, in the longest instance, on the 17th day; the usual period was from 12 to 14 days.

The second reaction began, in the shortest instance, on the 22nd day, and, in the longest, on the 30th day. Its usual appearance was after the 24th, 25th and 26th days.

The poikilocytosis, which disappeared after the first reaction, began again on the 25th and 26th day, and the piroplasma bigeminum, when it appeared, did so at the same time. The only exceptions to this were in two cases, firstly, when it appeared on the 32nd day, and, secondly, when the secondary reaction started on the 30th day.

The tertiary reaction—when it could be distinguished—was, in the shortest instance, noted to start on the 29th day, and, in the longest, on the 37th day; it was accompanied in three cases with piroplasma bigeminum, and, in all cases, the period corresponding to the third reaction, whether the fever reaction was noticeable or not, was accompanied with the appearance of poikilocytosis, basophile granulations, polychromatic cells, normo and megalo-blasts, and marginal points.

During this period—but not always coinciding with the rise of temperature—the small piroplasms made an appearance, sometimes

before, and sometimes after; only in two cases, however, no rise of temperature was noticed. The piroplasmata appeared as under:—

On the	25th	day in	1 case.
„	26th	„	4 cases.
„	28th	„	1 case.
„	29th	„	2 cases.
„	31st	„	1 case.
„	33rd	„	1 case.
„	34th	„	2 cases.
„	35th	„	1 case.
„	41st	„	2 cases.

The most remarkable phenomenon with the appearance of the small piroplasms was the decrease of the red corpuscles in the majority of cases, a decrease much more marked than in the primary and secondary reaction due to piroplasma bigeminum. Although there was not a pronounced fever curve during this period, there was, notwithstanding, a febrile elevation in the course of the temperature during that period in the majority of animals. The fever was the highest in the cases where the decrease of the red corpuscles was most pronounced and the small piroplasms were very numerous. A tertiary reaction as a sequel to the inoculation of redwater immune blood has been described to occur occasionally by the Americans. The third reaction observed by these investigators cannot be due to the appearance of small piroplasms, and we must accordingly admit that, in some cases, the tertiary reaction may be observed after injection of Texas fever immune blood where small piroplasmata are absent.

In South Africa this tertiary reaction may be accompanied with the appearance of a small endoglobular parasite. We are quite justified in accepting that the tertiary reaction due to the infection with piroplasma bigeminum is sometimes overlapped by the tertiary reaction due to the small piroplasmata which frequently appears before that time, so that a distinction between the secondary and tertiary reactions is impossible. With this we express the view that the small piroplasmata, hitherto described as rings and rods, have nothing to do with the piroplasma bigeminum, but represent a species of its own. Thus we change our former theory that rings and rods are the immune form of piroplasma bigeminum. We are led to this conclusion by (1) the occasional typical fever, following the second reaction, due to piroplasma bigeminum; (2) the increase and subsequent decrease of small piroplasmata after their first appearance; (3) the marked decrease of the red corpuscles during the third reaction, and at the time when the small piroplasmata are increasing; and (4) the very pronounced alteration of blood during this period.

A still more convincing phenomenon is the appearance of small rosettes similar to those described in piroplasma equi. They must be considered as multiplying forms of the piroplasmata, and are not very frequent, but were found, in each case, sometimes more often than in others.

Dschunkowsky, at the congress of Buda-Pesth, has already drawn attention to these crosses, and, lately, Professor Koch, who observed them in what he considered East Coast fever in German East Africa, is of the opinion that they are typical, and proposes to distinguish a group of piroplasmoses due to the small endoglobular parasites and found along the Mediterranean, Trans-Caucasus, New Guinea, and the East Coast in which these are present.

In my previous article on the same subject, I have shewn that animals which have these rings and rods in their blood, very closely resembling piroplasma parvum, could easily be infected with pathogenic brown ticks, thus shewing that piroplasma parvum was not identical with the disease in question, and, in view of the fact that the new piroplasma is neither identical with the Dschunkowsky piroplasma of tropical piroplasmosis, and also on account of the various shapes it takes, viz., rings, rods and flagellated forms, I propose to call it "piroplasma mutans" n. spec.

In the experiments quoted, blood of immune animals was used for inoculation, and which blood we were certain contained the piroplasma bigeminum and piroplasma mutans. The former was not always observed in the inoculated animals, but there was a febrile reaction, or blood lesion, as occur after inoculation of redwater blood. The latter was present in every case.

The question now arose whether the piroplasma mutans is really so frequently met with as piroplasma bigeminum, and whether an animal which is immune to redwater is also immune to this new piroplasmosis. Since both diseases are inoculable, this question can easily be settled by the inoculation of blood from animals born in redwater areas, and which had never before been injected with immune blood. If such an animal is immune to ordinary redwater, then piroplasma bigeminum will only appear in the inoculated animal. On the other hand, if it is immune to piroplasma mutans, then this parasite will only appear. If immune to both diseases, both piroplasmata will appear. In the first instance, the animal which supplied only piroplasma bigeminum must be susceptible to piroplasma mutans when injected, and, in the second instance, the reverse holds good.

Bull calf 269, Africander, about two years old, born in the Transvaal, was tapped and 10 c.c. defibrinated blood was injected into

(16) *Calf 341.*—Born on the premises, and 8 months old.

Primary reaction from the 9th day; piroplasma bigeminum seen on the 20th day. From the 22nd day, poikilocytosis was noticed, and a secondary reaction, marked in the elevation of the morning temperature, ensued.

Poikilocytosis was observed during this period and continuing until the 67th day.

Marginal points were seen on the 27th and 28th day, and again on the 54th and 55th days.

Piroplasma mutans was not noticed.

Bull Calf 269.—Injected with 10 c.c. blood of ox 347, which contained both piroplasma bigeminum and piroplasma mutans.

No reaction, blood parasites or changes of the blood were found in this ox after the injection.

Result.—Calf 341 shewed only piroplasma bigeminum in its blood. Piroplasma mutans was absent, therefore 341, when re-injected with blood containing piroplasma mutans, should develop this parasite after the usual incubation time.

(17) *Calf 341.*—Injected on the 1/2/06 with 10 c.c. defibrinated blood of heifer 317, which at that date, had still a few ring forms in the blood.

No characteristic change in the temperature curve ensued during the time it was under observation—1/2/06 to 18/4/06—except that the morning and evening records sometimes shewed remissions varying 5° F. Changes in the blood in the shape of poikilocytosis were noticed from the third week after inoculation, and, from the 30th day, the small endoglobular parasites made their appearance, beginning as flagellated forms.

With their appearance the morning temperature rose above the usual morning curve and continued above normal for over a week.

The flagellated forms were soon associated with rings; rosettes were also noticed. These small piroplasmata were present for over three weeks, when they became rarer, but had not completely disappeared when the examination of the blood was discontinued.

The blood of bull 269 did not cause the appearance of piroplasma mutans in calf 341, hence it should be concluded that his blood did not contain the parasites; therefore, bull 269, when injected with blood containing piroplasma mutans, should contract an infection from such an injection. It proved, however, to be refractory to the injection, and there is some difficulty to explain why this should be, unless it is by the fact, which is admitted in East Coast fever, namely, that some animals cannot be infected and prove completely immune.

The appearance of the small piroplasms in the blood of calf 341 should demonstrate that the injection from bull 269 did not produce any immunity against the small piroplasms, and this fact is in favour of the theory that the small piroplasma has nothing to do with piroplasma bigeminum, but is a species of its own.

Texas calf 322, two years old, and born in Potchefstroom, was tapped and 10 c.c. of its blood injected into

(18) *Calf 343.*—Africander, about one year old and born on the station. At the same time, calf 322 was injected with blood of calf 241, which blood had previously been utilised for inoculation and contained rings and rods.

The temperature of calf 343 rose three days after injection and shewed slight poikilocytosis on the 6th day. The fever reached 106° F. on the 8th day.

The first temperature curve ended about 15 days after inoculation. On the 21st day a second reaction began, and, on the 23rd day, piroplasma bigeminum was noted for the first time. This second reaction was marked by an increase of the poikilocytosis, which was very strong on the 26th day after inoculation. From the 34th day after inoculation, marginal points and basophile granulations were observed, which, together with poikilocytosis, increased during the next few days.

The marginal points were noticed for about 20 days.

A third reaction appeared 42 days after injection, and basophile granulations, marginal points and poikilocytosis were all present, remaining until the 60th day.

No small piroplasmata were observed during this period.

Calf 322.—which was injected at the same time as 343—shewed on the 27th and following days after injection a slight poikilocytosis, and on the 27th day a few rings were present, but very rarely.

Result.—The same phenomenon, as in calf 341, was noticed here.

Piroplasma bigeminum, poikilocytosis, and the other blood changes were present during the second reaction, but piroplasma mutans was not noticed.

The appearance of rings and rods in calf 322 on the 34th day, coincides with the period observed in the other calves; these rings and rods were, however, only present for one day.

The same argument, as in calf 341, holds good here, namely, if the blood changes in 343 are not due to the presence of the piroplasm mutans then a second injection of blood containing this piroplasma should produce them.

(19) Accordingly, calf 343 was injected on the 1/2/06 with 10 c.c. blood of heifer 317, which at that time still had a few ring forms in the blood. 343 did not shew any change in its temperature curve during the first few weeks after inoculation.

At the end of this time the morning temperature shewed a continual rise, lasting 10 days and representing a typical curve.

Flagellated forms and rings made their appearance from the 26th day.

They were preceded by the usual blood changes, such as poikilocytosis. Rosettes were noticed when the number of the parasites were most frequent. These parasites were still present 49 days after their first appearance, when the examination of the blood was discontinued.

No marginal points were noticed as a result of the second injection.

If the blood of calf 322—which after its injection causes the appearance of piroplasma bigeminum in calf 343—had contained piroplasma mutans they would, in all probability, have also appeared, but such was not the case.

The injection of blood containing the piroplasma mutans caused this parasite to appear in calf 343 which, by the first injection, had become immune against piroplasma bigeminum. The appearance of rings in calf 322, due to the injection of blood of calf 241, may be interpreted in accepting that it was neither previously infected by this parasite nor immune.

Texas calf 323, born in Potchefstroom District, was tapped and 10 c.c. defibrinated blood was injected into Africander

(20) *Calf 344*, 4 months old, and born on the premises.

Irregular temperature ensued 3 days after injection.

Poikilocytosis was present on the 7th day.

Low fever reaction started from the 22nd day, during which poikilocytosis marginal points and basic cells were noted. The poikilocytosis was very pronounced, and increased from about the 34th day after injection; a fever reaction was also noticed during this period.

The marginal points were noticed for one day on the 24th and again for the 37th day, continuing for 24 days.

Poikilocytosis was still present on the 60th day.

No rings and rods were observed.

(21) *Calf 323.*—Injected with blood containing the small piroplasms from calf 242.

Irregular reaction ensued lasting for about three weeks, but no blood changes were observed during this period.

In the course of the next three weeks a reaction was noticeable, but no changes took place in the blood.

From the 42nd day a very irregular reaction started with high evening excursions. During this reaction—on the 49th day—slight poikilocytosis was observed, which was present for the following 14 days, and a few rings were noted on the 56th day.

The appearance of blood changes in calf 344 is explained in the same manner as in the two previous cases, viz., due to piroplasma bigeminum, although not seen.

The presence of rings and rods and blood changes in calf 323 is probably due to the same cause as in calf 322, viz., it was not previously infected with piroplasma mutans, and, consequently, not immune.

(22) *Calf 344.*—This animal only shewed blood changes and no small parasites.

Inoculated with 10 c.c. blood of heifer 317 on the 1/2/06, and should, consequently, shew rings.

The temperature shewed nothing abnormal during the following four weeks.

On the 29th day after inoculation, flagellated forms and rings were seen and the typical changes of blood made an appearance.

Rosettes were also noticed.

The temperature rose a little higher in the morning—29th day—and, later, shewed greater fluctuations from morning to evening.

The parasites were noticed for 27 days, after which they disappeared, and the examination of the blood was discontinued.

Result.—Calf 344 shewed neither piroplasma bigeminum or piroplasma mutans in its blood due to the first injection.

It shewed, however, blood changes such as are met with in an infection with the former parasites, viz., marginal points and basic cells. The second injection of blood, containing piroplasma mutans, caused the appearance of these parasites, hence again the conclusion that the animal had not become immune against this parasite from the first blood injection.

Calf 323 could also be successfully infected with the small piroplasms as we had anticipated.

Texas heifer 324, born in the Potchefstroom District, was tapped on the 15/11/05, and 10 c.c. defibrinated blood was injected into Africander

(23) *Calf 345.*—About 8 months old and born on the premises.

High temperature reaction started on the 9th day, the fever reaching 106° F.

Piroplasma bigeminum was noticed on the following day and remained for two days.

Poikilocytosis appeared 3 days after the appearance of piroplasma bigeminum—13 days after the inoculation. It did not disappear but increased, basophile cells and marginal points appearing later; these appeared on the 25th day and remained 11 days, and again, for the second time, on the 39th and 40th day.

Distinct secondary reaction during which poikilocytosis became stronger, the marginal points especially increasing.

Third reaction was observed from about the 42nd day, from which date flagellated small piroplasms and ring forms appeared.

Piroplasma bigeminum was noticed for the second time on the 55th day after injection.

(24) *Texas Heifer 324*, whose blood was used for the above inoculation, was injected with 10 c.c. blood of calf 312 (compare the 10th experiment).

There was a pronounced reaction after this inoculation, and, for 40 days following; the 7th day after inoculation, poikilocytosis was noticed.

Distinct secondary reaction about 24 days after the inoculation, when poikilocytosis was again noticed, and rings and flagellated forms were present for 4 days. They disappeared after this, but, from the 45th day, a third reaction ensued during which poikilocytosis and rare rings were again noticed for 10 days.

The Africander calf 345 shewed, what we may call, a more or less typical reaction due to the inoculation of immune redwater blood.

The Texas calf, of which the blood for the former one was taken, also shewed similar symptoms, and we must, therefore, conclude that either (1) the immunity of calf 324 was not sufficient to protect against the piroplasms of calf 312; or (2) that we have to contend with different varieties or species of piroplasms; or (3) that both piroplasma bigeminum and piroplasma mutans again appeared due to some other influence not controllable, a fact known to us from previous experience.

Texas calf 325, about two years old, was tapped on the 15/11/05 and 10 c.c. blood was injected subcutaneously into

(25) *Africander Calf 346.*—Six months old and born on the premises.

Irregular temperature curve for the following three weeks; the examination of the blood during this time gave negative results.

Poikilocytosis was noticed from the 23rd day after inoculation.

Marginal points were also noticed during this period.

Elevation of the morning temperature for some days and basophile cells were present.

The marginal points—noticed for the first time on the 23rd day —were present daily from the 32nd to 48th day.

Flagellated forms and rings were noticed from the 44th day and were noted daily for the remainder of the observation period —over three weeks—and during this time a febrile reaction was present.

(26) *Texas Calf 325* was injected on the same day as 346 with 10 c.c. blood of calf 317 (compare experiment 15).

Irregular reaction ensued, but a slight continuous rise was noticeable for about 15 days after inoculation, the temperature at that period reaching 106° F.; poikilocytosis was noticed on one day.

Secondary reaction was noticeable 25 days after the inoculation, but only lasting a few days. No changes in the blood took place.

A distinct tertiary reaction started about 47 days after inoculation, when a slight poikilocytosis was noticed; rings appeared and were present for 8 days.

The same explanation as in the previous case holds good here.

Conclusion.—The injection of blood taken from calves born in the Transvaal into susceptible stable-born calves was, in all cases, followed by the appearance of piroplasma bigeminum or such blood changes as are typical to its presence.

As a result of the injection, piroplasma mutans appeared in two cases, viz., 324 and 325; it did not appear in the other three animals, 341, 343 and 344, but these latter could, subsequently, be successfully infected with blood of an ox which contained piroplasma mutans.

We may, therefore, conclude that a Transvaal calf can be immune to piroplasma bigeminum and not to piroplasma mutans. Immunity against the former does not protect against the latter, hence a further support to the duality of the two piroplasmata.

Clinical Observations.

In the experiments quoted before, none of the injected animals died from the effects of the inoculation, and, naturally, the question arises whether the piroplasms have really such a slight virulency that it does not cause death. It must be borne in mind that only young animals under two years old were used, animals which we were certain would not die from redwater due to the same injection of blood. Since the animals did not die from the second piroplasmosis, we may, therefore, infer that young animals have about the same resistance against piroplasma mutans as they have against piroplasma bigeminum.

I have, however, some clinical observations shewing that adult cattle may contract this second piroplasmosis and die. These observations were made some time before East Coast fever had reached Pretoria, but, nevertheless, were for some time mistaken for this latter disease. They were noticed amongst cattle which had arrived from the border of Basutoland in the Orange River Colony. Amongst this lot of oxen, redwater had broken out and carried off most of them. It was now noticed that, towards the end of the epidemic, the sick animals no longer shewed red urine, although, on *post-mortem*, the typical lesions of redwater were present. An examination of the blood of these animals shewed the absence of the typical forms of piroplasma bigeminum and the presence of small endoglobular rod-like parasites. It was about this time that the report of Gray and Robertson appeared, describing a virulent form of redwater in Rhodesia, together with similar parasites as those referred to above. This report and the communication of Professor Koch in regard to Texas fever in East Africa induced us to identify our cases with those described by the above-mentioned gentlemen.

It was not until later that we discovered our mistake. At the time of the observation, East Coast fever had not reached Pretoria, and it had never been on the pasture on which our cattle had grazed. We were not able to identify this disease, and were, later, under the

impression that the small parasites were identical with what we have described as forms of piroplasma bigeminum in the immune ox.

Ox "Stomphorn."—Owners, S.A.C., stationed near the Laboratory; was found to be very ill on the 4/6/02.

Redwater was suspected and the blood was examined.
Basophile granulations, megaloblasts and poikilocytosis were noticed.
Piroplasma bigeminum was not seen.
On the 5/6/02 a count of the red blood cells was made and the number found to be 1,300,000 per c.m.m.
Basophile granulations and poikilocytosis were still present.
The count on the following day was 1,400,000 per c.m.m., and the same changes of blood were noticed.
The ox died on the night of the 6-7/6/02, and *post-mortem* was made early on the following morning—7/6/02.

Post-mortem.

Carcase in an emaciated condition.
Jaundice well pronounced throughout the whole of the body.
Blood waterish and had a brownish hue.
Jelly-like infiltrations were present in the adipose tissue.
Lungs shewed fibrous lesions of a former attack of pleuro-pneumonia, otherwise normal.
Pericardium was thickened by a jelly-like infiltration and contained hæmorrhagic patches.
Epicard had a brick-red appearance; one of the auricles was impregnated with blood throughout its walls, forming one continuous mass of blood.
The hemorrhagic patches were present on the endocard of both ventricles.
Liver was very much enlarged, contained a great amount of blood, and was also stained deep yellow.
The gall bladder was somewhat distended with viscid thick yellow greenish bile.
The surroundings of the gall bladder were also infiltrated with yellow bile stain.
The spleen was enlarged from twice to three times its normal volume.
Pulpa soft.
Kidneys also shewed yellow discolouration, but were otherwise normal.
The urine was clear, and, when tested according to Esbach's method, shewed but a slight precipitation of albumen.
Reaction neutral.
Specific weight, 1,010.
All four stomachs and the intestines had a normal appearance.
The rectum contained dung of normal consistency and colour.
The marrow of the big bones was yellow, containing several

hemorrhagic patches, and, on microscopical examination, the blood shewed nucleated blood cells.

A small preparation made from the cut surface of the liver shewed threads of liver cells lined with engorged bile ducts.

A careful examination of the blood from different parts of the body shewed the presence of the rod-like endoglobular parasite.

Ox B. 331.—Owners, S.A.C.

On the 9/6/02 this ox was observed to be very ill, the symptoms pointing to some lung troubles, and the diagnosis of pleuro-pneumonia suggested itself.

The animal succumbed during the night, and *post-mortem* was made on the morning of the 10/6/02.

Post-mortem.

A general jaundiced condition of the skinned carcase was noticed.

The lungs were in the state of the inspirium—œdematous—and the trachea was filled with foam. There were small greyish areas of varying size up to the circumference of a sixpence and surrounded by a reddish zone.

Lung tissue was soft and friable.

Emphysematous visicules were present under the pleura of the lung.

Pericardium contained a fair amount of brown yellow liquid. The epicard was of a brick-red colour and contained subendocardial hemorrhagic patches.

Blood was coagulated and of a brownish hue.

The spleen was enlarged and soft.

The liver was also enlarged, of a brownish yellow colour.

Gall bladder was filled with yellow bile, and its surroundings were discoloured with bile stain.

Lymphatic glands were engorged.

Kidneys œdematous but of normal colour.

Urinary bladder was filled with clear urine of a brown colour; it contained traces of albumen.

Reaction, neutral.

Specific weight, 1,016.

Mesentry was infiltrated throughout its whole length with œdematous liquid, and the lymphatic glands were enlarged. The outer walls of the intestines were also infiltrated by the same liquid.

Contents of the third stomach were somewhat dry.

Mucosa of the abomasum pale, the folds were œdematous and thickened, slightly reddened and covered with viscous mucous.

The mucosa of the colon had a slate colour in patches; that of the cæcum was quite black.

Blood shewed basophile granulations and contained the small endoglobular parasite in small numbers.

Ox No. 3.—Owners, S.A.C.

On the 5/6/02 this animal was pointed out to have been ill for a few days, not to have fed well, and to lose condition. The blood was examined and basophile granulations were found.

This ox was found dead in the morning of the 17/6/02.

Post-mortem was immediately made; rigor mortis was present.

Post-mortem.

Symptoms of anæmia and slight icterus were present.

Lungs in a state of œdema, and liquid was observed in the pericardium.

Epicard contained hemorrhagic patches, and there were also a few on the endocard.

Peritoneal cavity contained some clear liquid.

Symptoms of a muskat liver; it was enlarged and contained but little blood.

The spleen was not enlarged, the pulpa soft.

Kidneys pale, the cortex yellowish, and the urine normal. Blood smears were made from the various organs, and the small endoglobular parasites were present in all of them.

Ox No. 114.—This was a full grown Cape ox, and was injected on the 19/11/02 with 50 c.c. blood into the jugular vein and subcutaneously with 10 c.c. containing the piroplasms of East Coast fever.

The blood had been taken from an ox which had died in Belfast and had been forwarded by a trooper of the S.A.C.

The blood was sent in for diagnostical purposes, as the ox was sick previous to its death.

Ox 114 died on the 7/1/03, viz., 47 days after inoculation, and is the same animal whose *post-mortem* has been mentioned in my article on "Spirillosis of Cattle."

I alluded to this same case when quoting my doubts as to whether this was really a case of East Coast fever.

In the light of our experience gained in connection with East Coast fever and the inoculability of piroplasma mutans, I am now prepared to consider this case as being due to the inoculation with the blood from Belfast, which contained both parasites piroplasma parvum and piroplasma mutans. The former, we know, is not inoculable; the latter may be borne by any ox of Transvaal origin.

The lapse of 47 days from inoculation corresponds to about the time when these small endoglobular parasites were most frequently met with in the blood of our experimental calves.

Post-mortem of Ox 114.

Cadaver was very poor and the flesh pale.

The subcutaneous tissue of the shoulder, breast and abdomen was infiltrated with gelatinous liquid.

Blood was pale.

The pleural cavity contained a large quantity of yellow liquid.

Lungs were œdematous.
Pericardium was thickened by infiltration with a gelatinous liquid and there was an increased quantity of serous liquid in the pericardium.
The base of the heart and the sulci trans versales, together with the longitudinales, were also thickened with a similar gelatinous looking fluid.
There was a coat of white fibrous-looking tissue on the pericardium, giving the surface a white patchy appearance.
The myocardium was very pale.
Endocardium normal.
A well-formed clot was found in the ventricles.
Spleen, slightly congested and soft, weighing about 2½ lbs.
The liver weighed 7 lbs. 12 ozs.; it was hard and the section had a glossy appearance.
Gall bladder was contracted and of a brown colour.
Kidneys studded with white spots about the size of a pin's head.
Urine of a normal colour, and, when tested according to Esbach's method, a slight precipitation was discovered.
Specific gravity, 1,012.
The omentum was covered with numerous hemorrhages averaging about the size of the diameter of a pea and containing a small clot of coagulated blood; in other parts it was infiltrated with liquid.
The mesentry, serosa of the intestines, connective tissue of the kidneys and of the pelvis were all infiltrated with the same liquid.
The first two stomachs were normal, the third contained soft food.
The mucosa of the fourth stomach was very pale.
Duodenum, jejunum and ilium were slightly congested; at some places superficial necrosis of the mucosa were present.
The mucosa of the colon and cæcum was somewhat thickened.
The contents of the bowels were soft.
The peritonial cavity contained an enormous quantity of serous fluid.
All lymphatic glands were enlarged.
Smears were made of the different organs.
There was a well-pronounced poikilocytosis, basophile granulations and the bacillary piroplasms were also present.

Experiments with Madagascar Oxen.

It is a well-known fact in South Africa that cattle imported from Madagascar, and exposed in any part of this sub-continent, shew very strong immunity against redwater and other indigenous diseases, generally termed gall sickness.

If we interpret this general experience scientifically, we must say that in Madagascar redwater is prevalent; the cattle born and bred there become vaccinated by the ticks whilst young, and, accordingly, become immune, and retain the immunity against South African red-

water. If this is the case, then Madagascar and South African redwater are identical.

The introduction of the disease into Natal—as far back as the beginning of the seventies of the last century—can be traced to the importation of Madagascar oxen, which, being immune, infected the hitherto clean ticks of Natal. At that time, the causus connexus between the importation of Madagascar cattle and the originating of the disease was not even surmised, and this is only natural, since no one knew of a precedent of health cattle acting as propagators of a disease.

The very fact that our redwater is traceable to Madagascar leads us to surmise that piroplasma mutans may also be traced back to that country, viz., in other words, when we inject blood from a Madagascar ox into susceptible South African cattle, not only redwater, but also piroplasma mutans, will result.

I do not hesitate to state that, if this be so, the very fact may be utilised as an argument in favour of the theory that the piroplasma mutans is nothing else than another stage of the piroplasma bigeminum, as I was inclined to believe originally. But all the facts brought against this idea and gathered from experience of South African redwater may also be laid against Madagascar redwater. Indeed, since we are able to trace the introduction of our redwater to Madagascar, we are also justified in believing that piroplasma mutans also hails from there. In support of this idea would be the observation that, where redwater has not yet appeared, piroplasma mutans is also unknown, which, so far, seems to be the case.

Accordingly, in inoculations of blood from Madagascar oxen into susceptible cattle, we must be prepared, in the first instance, for the appearance of piroplasma bigeminum, and, in the second, for the piroplasma mutans. On the other hand, the injection of blood of a South African animal which contains piroplasma bigeminum and also piroplasma mutans into Madagascar cattle produces neither of these parasites in them.

For the purpose of these experiments, four oxen were directly imported from the Madagascar Cattle Importation Company, and, by the courtesy of the Principal Veterinary Officer of Natal—Mr. Woollatt—the oxen were directly entrained and forwarded to Pretoria where they were stabled in the Laboratory.

"*A.*" *Madagascar Ox 347.*

On the 16/3/06 this ox was first tapped and the blood was injected into Cape ox 355.

At the same time, ox 347 was injected with 10 c.c. of ox 241, whose blood was known to contain both piroplasma bigeminum and piroplasma mutans.

Ox 347.

On the 10th day after inoculation, one single small ring form was detected in its blood, and also one flagellated form on

the 30th day. The former observation already indicates that piroplasma mutans would be present in the blood.

No variation in the temperature during the first three weeks; the evening records usually reached 102° F., and the morning ones varied between 98.4° F. and 100° F.

The number of red corpuscles during this period never dropped below 6,500,000 per c.m.m. During the following three weeks the temperature record was much the same, but a rise to 103° F. was noted on the 27th, 30th and 32nd day after injection.

The number of red corpuscles was constantly above 6 millions per c.m.m., and this figure—the lowest mark—was reached on the 40th day, when trypanosoma theileri was noticed in the blood for a few days, which infection must have been contracted naturally. Hippobosca rufipes was very frequent during that time, and a further drop in the number of red corpuscles to 5,200,000 per c.m.m. on the 42nd day was probably due to the presence of this latter parasite.

Slight poikilocytosis of then blood was noticed.

With the disappearance of the trypanosoma the increase of erythocytes again followed. There was no distinct temperature curve after the sixth week, although, on one occasion, the evening temperature reached 105° F. and remained at 103° F. during the next three days.

Taken as a whole, the temperature during the 75 days after the blood injection shewed no noticeable change.

With the exception of the microscopical result already mentioned, there was nothing else noted.

"*B.*" *Madagascar Ox 348.*

On the 16/3/06 this ox was first tapped and its blood injected into susceptible Africander ox 354.

At the same time ox 348 was injected with blood containing both piroplasma bigeminum and piroplasma mutans from Africander heifer 314.

Ox 348.

There was no definite temperature reaction during the first three weeks; the blood count was at its lowest mark on the 19th day after injection, recording 5,200,000 per c.m.m. It remained at 6 millions per c.m.m. during the following three weeks, whilst the temperature was oscillating between 98.4° F. and 103° F. The two extremes, however, were not the rule.

The same observation was again made during the following three weeks, the number of red corpuscles never dropping below 6 millions per c.m.m.

Considered as a whole, the temperature curve during the 75 days of observation kept normal.

No microscopical changes were noted in the blood.

Control Experiments.

In order to make sure that the blood of cattle 241 and 314 which were used for the injection into Madagascar oxen 347 and 348, the same amount of blood—10 c.c.—was injected into two susceptible Cape oxen, 18 months old, obtained from Aliwal North.

"C." Cape Ox 351.

Injected on the 16/3/06 with 10 c.c. blood of ox 341.

On the 26/3/06—10 days after injection—piroplasma bigeminum was noted.

A distinct, although slight, temperature reaction followed; both morning and evening readings slightly higher than normal, reaching in the evening of the 1/4/06, 104.2° F.

After the appearance of piroplasma bigeminum, the lesions of poikilocytosis were noticed in the red corpuscles, and there was a distinct decrease in the number of erythocytes, the lowest record being 5,300,000 per c.m.m. on the 2/4/06.

During the second three weeks after inoculation the temperature became very irregular, especially after the 35th day. This irregular curve was present for the rest of the observation —75 days.

Flagellated and ring forms were seen in very rare numbers for the first time on the 32nd day after injection—17/4/06— but they increased daily from the 26/4/06 until the 2/5/06.

The reading of the blood counts during this period reached the lowest mark of 4,300,000 per c.m.m. on the 29/4/06, and the numbers increased henceforward, not to drop below 6 millions per c.m.m.

Coinciding with the increase of the red corpuscles, the small endoglobular parasites decreased and became very rare, but were still present on the 75th day after injection—30/5/06 —when the observations were discontinued.

"D." Cape Ox 352.

Injected on the 16/3/06 with 10 c.c. blood of heifer 314.

Slight disturbance in the temperature after the 7th day, both morning and evening temperatures being slightly higher than usual.

There was no alteration in the blood count, nor did the microscope reveal any blood changes.

The number of corpuscles during the first three weeks did not drop below 7 millions. At the beginning of the 4th week some extraordinary oscillations between 98.4° F. and 104.8° F. were noticed for two days, and a slight poikilocytosis became noticeable.

The temperature continued oscillating for the next three weeks, averaging about 103° F. in the evening and 99° F. in the morning.

On the 34th day after injection—19/4/06—the flagellated forms were seen for the first time; they increased during the next 10 days, but were never numerous.

During this period the blood count reached its lowest mark of 4½ millions per c.m.m. on the 19th day, when endoglobular parasites appeared for the first time. The corpuscles remained at this figure—4½ millions—but rose to six millions per c.m.m. on the 50th day after injection—5/5/06—and when the endoglobular parasites had become very rare.

These latter were, however, still present on the 75th day of observation—30/5/06.

Conclusions.—The blood of animals 341 and 314 still contained the piroplasms in their blood, although there may be some doubt in regard to heifer 314. Nevertheless, sufficient proof is given that piroplasma mutans was present. It will again be noticed that, coinciding with the increase of this parasite, a marked decrease of red corpuscles ensued.

The injection of the same blood into two Madagascar oxen did not cause either the appearance of piroplasma bigeminum or piroplasma mutans, hence they must have been immune against both piroplasmoses. This fact could be anticipated since ox 347 shewed one single ring form in its blood the 10th day after injection, thereby indicating the presence of p. mutans.

Injection of Blood from Madagascar Oxen into Susceptible African Cattle.

The cattle used for this purpose consisted of 18 month old, cross bred shorthorn oxen, born and bred in the neighbourhood of Aliwal North, a stretch of country free of redwater.

"E." Ox 353.

Injected on the 16/3/06 with 10 c.c. blood of Madagascar ox 350 belonging to the same lot as oxen 347 and 348.

Disturbance in the course of the temperature starting on the 8th day, both morning and evening readings being above normal, the latter reaching 104.6° F. on the 10th day.

Towards the end of the reaction, and on the 12th day after injection, piroplasma bigeminum was noticed in rare numbers for two consecutive days.

Poikilocytosis was noticeable after this, notwithstanding which the number of red corpuscles kept at its normal average. This parasite was present for some time, during which the excursions of morning and evening temperatures were more irregular than before.

A few marginal points were visible on the 35th day after injection, and on the 42nd day rings and flagellated forms were noticed for the first time.

Coinciding with these latter, a decrease in the number of red corpuscles took place. Marginal points and small endoglobular parasites were noticed alongside for 20 days, when the former completely disappeared, and the piroplasma became very scarce; the latter were, however, still present, although in very rare numbers, on the 75th day after injection.

With the increase of the parasites, the temperature became very oscillating, on one occasion reaching 105° F. in the evening.

"F." Ox 354.

Injected on the 16/3/06 with 10 c.c. blood of Madagascar ox 348.

A slight disturbance in the morning temperature ensued for the 11 days following the injection. The evening records were normal.

Piroplasma bigeminum was found for two consecutive days.

There was no decrease in the number of red corpuscles.

A very slight poikilocytosis followed and was noticeable for some time.

The small piroplasms were met with for the first time in the shape of flagellated forms on the 34th day after inoculation. They were rare during the following days and increased but slightly.

There was also a slight decrease in the number of corpuscles during that period, although hardly noticeable.

The temperature again became very oscillating during this period.

The endoglobular parasites were still present in very rare numbers on the 75th day after observation.

"G." Ox 355.

Injected on the 16/3/06 with 10 c.c. blood of Madagascar ox 347.

Temperature disturbance began on the 10th day, the evening record on the following day being 105° F.

Piroplasma bigeminum was present on the 12th day and remained for three days.

Poikilocytosis was apparent after the piroplasma bigeminum had disappeared.

The number of red corpuscles slightly decreased for a few days to below 6 millions per c.m.m., but soon rose again above this number.

Marginal points were visible on the 30th day and were present for 22 days, after which they disappeared.

Poikilocytosis was also noticed during this latter period.

The small endoglobular parasites only became noticeable on the 47th day after inoculation, and were but rarely met with in the subsequent days.

Their appearance was indicated by a decrease of the red corpuscles which, although not very marked, were, nevertheless, distinct.

The temperature during this period was very oscillatory.
The endoglobular parasites were still noticed in large numbers, but gradually decreased and were present in rare numbers on the 75th day after inoculation.

"*H.*" *Ox 356.*

Injected on the 16/3/06 with 10 c.c. blood of Madagascar ox 349.
Distinct temperature reaction, chiefly noticeable by the evening exacerbations, which reached 104° F. on the 12th day.
Piroplasma bigeminum appeared on the following day and was present for two days, after which the poikilocytotic changes appeared and were constantly noticed during the next few weeks.
Coinciding with the appearance of piroplasma bigeminum was a slight decrease of red corpuscles, the number of which again increased after the lapse of a week; the temperature during this period became very irregular.
Endoglobular parasites were noticed for the first time on the 28th day.
Marginal points were observed on the 37th day after inoculation.
These latter were preceded by a decrease in the number of erythocytes.
Marginal points were present for 11 days.
During this time the small endoglobular parasites and marginal points were present; the temperature became strongly oscillating and passed 104° F.
The parasites were still noticed in small numbers 75 days after inoculation.

Conclusion.—The inoculation of blood from directly imported Madagascar oxen produced the same alteration in the blood of susceptible African cattle as that produced by blood from redwater immune African cattle.

It, therefore, follows that the Madagascar cattle are immune against both piroplasmoses, the one caused by piroplasma bigeminum, and the other by piroplasma mutans.

The microscopical examination thus supports the observation, made in practice, of the immunity of these cattle against our indigenous diseases.

"Marginal Points."

These bodies require some special consideration, inasmuch as, in the first instance, they might be mistaken with the piroplasma mutans, or with the basophile granulations; and, secondly, that it has not, as yet, been ascertained what they really are.

They are easily distinguishable from piroplasma mutans by their position, shape and staining character. They are situated on the periphery of the corpuscle, round or oval, without piroplasms, and exclusively take the chromatic stain.

Sometimes two are met together, which have probably arisen from the partition of one body. The authors, who have been working in America, consider these forms to belong to piroplasma bigeminum; the Russians identify them with the cachectical form of tropical piroplasmosis, but it must be remembered that Dschunkowsky and Luhs also met piroplasma bigeminum in their investigations.

The results of my own investigations may be classed into three groups:—

(1) *Piroplasma bigeminum and marginal points appearing in one and the same animal.*

(Calves 343, 345, 353, 355 and 356.)

(2) *Piroplasma bigeminum alone.*

(Calves 351, 352 and 354.)

(3) *No piroplasma bigeminum but marginal points.*

(Calves 341, 344 and 346.)

Considering the length of time which elapsed between the injection of redwater blood and of the appearance of marginal points, we notice the following periods:—

Calf	*346.*	(1st	appearance).	After	23	days	to remain for	2	days.
„	„	(2nd	„).	„	~~32~~	„	„	17	„
„	*345.*	(1st	„).	„	25	„	„	21	„
„	*344.*	(1st	„).	„	24	„	„	1	„
„	„	(2nd	„).	„	~~35~~	„	„	24	„
„	*343.*	(1st	„).	„	34	„	„	20	„
„	*341.*	(1st	„).	„	27	„	„	2	„
„	„	(2nd	„).	„	~~54~~	„	„	2	„
„	*353.*	(1st	„).	„	35	„	„	20	„
„	*355.*	(1st	„).	„	30	„	„	22	„
„	*356.*	(1st	„).	„	37	„	„	11	„

Seeing that some of the animals did not shew marginal points as a result of the injection, the question naturally arose whether these animals would shew marginal points when injected with blood which had previously produced them.

For this purpose we selected calves 351 and 354 which had only shewn piroplasma bigeminum and calf 352 in which no piroplasma bigeminum was observed.

Accordingly, on the 5/6/06, all three animals were injected with 10 c.c. blood of Madagascar ox 347 whose blood had produced marginal points in calf 355.

A careful count and examination was made for six weeks, but no marginal points were noticed nor was there any decrease in the number of the red corpuscles.

The inference to draw from these experiments is that a previous injection of redwater blood into an animal gives immunity against marginal points; the fact that the injected blood does or does not contain piroplasma bigeminum makes no difference whatever. These

marginal points, therefore, seem to be connected with the development of piroplasma bigeminum.

In my opinion, however, the experiments were not sufficient to arrive at any definite conclusion, and I am inclined to leave the question open.

From a practical point of view we identify marginal points with piroplasma bigeminum, and their appearance is returned as a sequel of ordinary redwater.

Resumé.—In the Transvaal, at the present time, there are three different piroplasmoses known to exist in cattle: (1) one due to piroplasma bigeminum, and commonly called "redwater"; (2) one due to piroplasma parvum, and known by the name of East Coast fever; and (3) one due to piroplasma mutans, for which a specific term for this disease does not exist, but it probably ranges under the name of "gall sickness."

The first and third of these diseases are inoculable—piroplasma bigeminum and piroplasma mutans. Immune cattle contain the parasites in their blood. In both diseases calves easily recover from the infection, whereas, under natural conditions, adult cattle suffer more severely. Cattle born in the Transvaal usually acquire immunity against both diseases, hence the imported ones suffer principally in this respect.

Piroplasma bigeminum causes a disease after a short incubation time, and, being deadly for imported cattle, destroys a large number before piroplasma mutans has time to develop, hence cases due to this latter disease are comparatively rare. It is also probable that this second disease is constantly mistaken for redwater, and will continue unless microscopical examinations of blood are made.

Piroplasma mutans has a practical importance in connection with East Coast fever.

Piroplasma parvum may easily, and has constantly, been mistaken at various times for piroplasma mutans. The presence of small piroplasmata in rare numbers is, therefore, not always indicative of East Coast fever.

For diagnostical purposes in such cases, examinations of blood must be repeated. In East Coast fever the piroplasms will usually rapidly increase in numbers, whereas piroplasma mutans increases slowly and is never present in large numbers.

See Plates Nos. 1-4.

ON THE CORRELATION OF VARIOUS DISEASES OF STOCK IN SOUTH AFRICA.

Under this title Dr. Edington, then Director of the Bacteriological Laboratory, Grahamstown, in 1903, made a communication at a meeting of the South African Association for the Advancement of Science, held in Johannesburg, containing certain statements to which Mr. Stockman and myself, present at the meeting, had to take exception for reasons of absolutely contrary experience to that quoted by the above investigator. Dr. Edington's article appeared later in the "Journal of Comparative Pathology and Therapeutics," Volume XVIII., Part 2, June, 1904. Mr. Stockman and myself found it advisable to also lay our views before the readers of that journal, repeating our previous arguments which, since the first publication, were again tested by experiments. Shortly after the publication of our joint article refuting Dr. Edington's statement, his annual report for the half-year ended 30th June, 1904, appeared, containing the same article and thus maintaining the same statements. From a second article of the same report entitled "Protective Inoculation against Horse Sickness in Mules" it would appear that the outcome of this statement is an inoculation method of mules against horse sickness by means of the injection of heartwater blood.

In short, the statements of Dr. Edington are to the effect that

(1) Horse sickness is inoculable into cattle, sheep and goats, and

(2) Heartwater of goats is inoculable into horses and cattle.

The conclusion is that horse sickness inoculated in cattle, sheep and goats correspond to the description of heartwater, that is to say, the two diseases "horse sickness" and "heartwater" are identical.

We admitted the susceptibility of cattle, sheep, and goats to heartwater, but refuted the inoculability of horse sickness into other than equine animals, and hence disapproved of the statement that horse sickness and heartwater are identical. One of our reasons was that, in a series of experiments, we were unable to inoculate horse sickness into cattle, sheep or goats, whilst these species of stock are usually very susceptible to heartwater and quickly die from the inoculation.

In his report for the year 1904, page 2, Dr. Edington, while referring to the inoculability of the various diseases which he identified as one and the same, stated that "the transference of horse sickness from horses to cattle or goats is commonly attended with numerous failures."

In the face of this statement, and accepting that Dr. Edington's views were correct, I deemed it advisable to again repeat the experi-

ment, taking particular care to utilise only fresh imported stock preferably of young age. For this purpose, cattle and goats were obtained in the Cape Colony from regions where neither horse sickness or heartwater are known to exist. The sheep were all young animals born on the stud farm at Standerton, where both diseases are absent.

Experiment No. 1 with Angora Goats.

"A." The following goats were all injected on the 14th November, 1905, subcutaneously with 10 c.c. fresh virus horse 382.

Goat 371.—A slight reaction was noticeable, starting on the 5th day —19/11/05—and lasting about 15 days.
Bled on the 10th day after injection—November 24th—and on the 24th day after injection—December 8th.

Goat 372.—A slight temperature reaction indicated by a higher elevation of the morning temperature, the evening temperature remaining normal.
Bled on the 14th day after injection—November 28th—and on the 24th day after injection—December 8th.

Goat 373.—Temperature reaction began on the 9th day after injection. This reaction, however, was slight and principally indicated by a higher elevation of the morning temperature. There was a drop to normal about the 18th day.
Bled on the 14th day after injection—28th November—and on the 24th day after injection—8th December.

"B." The following goats were both injected on the 14th November, 1905, subcutaneously with 20 c.c. fresh virus of horse 382.

Goat 374.—A reaction began about the ninth day and lasted for nine days—December 2nd, 1905. The temperature during this reaction was somewhat irregular.
Bled on the 10th—14th day after injection—24th and 28th November.

Goat 375.—The temperature rose on the 5th day after injection. Reaction lasted until the 14th day and was well pronounced.
Bled on the 8th day after injection—November 22nd—the 10th day after injection—November 24th—and on the 24th day after injection—December 8th.

"C." The following goats were injected on the 14th November, 1905, intrajugularly with 10 c.c. fresh virus horse 382.

Goat 376.—A rise of temperature started on the 8th day—November 22nd—and returned to normal on the 17th day—December 1st. The reaction was somewhat irregular.
Bled on the 14th day after injection—November 28th—and on the 24th day after injection—December 8th.

Goat 378.—A distinct reaction started about the 7th day and lasted until the 17th day after injection—December 1st. Both morning and evening temperatures remained high on the 14th, 15th and 16th days between 105° and 106° F.

Bled on the 8th day after injection—November 22nd—and on the 24th day after injection—December 12th.

Goat 379.—No indication of a temperature reaction.

Bled on the 14th day after injection—November 28th—and on the 24th day after injection—December 8th.

"D." The following goats were injected on the 14th November, 1905, intrajugularly with 18 c.c. fresh virus horse 382.

Goat 380.—Irregular temperature observed.

Bled on the 10th day after injection—24th November— and on the 24th day after injection—December 8th.

Goat 381.—Irregular reaction; the temperature rose on the 7th day after injection—November 21st.

Bled on the 8th day after injection—November 22nd—and on the 24th day after injection—December 8th.

Remarks.—It has to be stated that none of the goats shewed symptoms of any disease. They were all feeding well during the observation time, and nothing was noticed amiss in any of them.

Conclusions to be Drawn from the Results of Experiment No. 1, "A," "B," "C," "D."

The foregoing experiments demonstrate that the blood of a horse suffering from horse sickness injected into young Angora goats bred in a country free from horse sickness produces a fever reaction in some goats. This reaction appears after an incubation time and lasts for some days.

It now remains to be seen whether the blood taken from these goats during the fever reaction is virulent for horses and mules.

Experiment No. 2.

To shew whether the blood of Angora goats injected with horse sickness virus and tapped during the reaction is virulent.

For the above purpose the blood of goats Nos. 375, 378 and 381, tapped on the 22/11/05—8 days after injection—was utilised. These three goats shewed the most distinct fever reaction.

Horse 495.—Injected intrajugularly on the 22nd November, 1905, with 5 c.c. of a mixture of defibrinated blood of the above goats.

The temperature rose on the second day after injection and reached 106.6° F. on the 4th day—November 26th.

This animal died on the 5th day—November 27th, 1905—with symptoms of horse sickness.

Post-mortem.—The pulmonary and gastric lesions of this disease were well pronounced.

Mule 487.—Injected intrajugularly on the 22nd November, 1905, with 5 c.c. of a mixture of defibrinated blood of goats 375, 378 and 381.

The temperature rose the second day after injection and reached 106.2° F. on the fourth day—November 26th.

This animal died the next day—fifth day after injection.

Post-mortem revealed pulmonary and gastric lesions.

Conclusion.—The blood of three goats which have been injected either subcutaneously or intrajugularly with blood from a horse suffering from horse sickness, and which have shewn a febrile reaction, proved to be virulent for a horse and a mule.

It follows, therefore, that goats can be successfully infected with horse sickness; that this procedure does not cause either illness or death, and that no attenuation of the virus ensues whilst passing through a goat.

Experiment No. 3.

To prove that the blood of goats injected with horse sickness blood which, freshly drawn, produced horse sickness in a horse and a mule, is still virulent when kept for fourteen days.

Horse 526.—Injected on the 7th December, 1905, intrajugularly with 5 c.c. of a mixture of defibrinated blood of goats Nos. 375, 378 and 381, tapped on the 22nd November, and, consequently, 14 days old.

Reaction began two days after inoculation, the fever reached 106° F. on the third day—10th December.

This animal died on the fifth day—12th December—from horse sickness. Pulmonary, gastric and cardiac lesions were found on *post-mortem.*

Conclusion.—The blood of goats injected with horse sickness blood which, freshly drawn, produced horse sickness in a horse and mule, is still virulent when 14 days old.

Experiment No. 4.

To shew the effect of a subcutaneous injection of a small quantity of virus taken from goats inoculated subcutaneously with horse sickness blood.

Mule 531.—Injected on 4th December, 1905, subcutaneously with 1 c.c. preserved mixture of blood of goats Nos. 375, 378 and 381. (Corresponds to 0.5 c.c. pure blood.)

No reaction was present.

This mule was tested on its immunity with 5 c.c. virus of horse 529. A horse sickness reaction was noted from which the mule recovered.

Conclusion.—Virus taken from goats when injected subcutaneously in a small dose did not produce the disease in a susceptible mule.

Experiment No. 4a.

To note whether preserved blood of goats Nos. 375, 378 and 381, which produced horse sickness in horses and mules after intrajugular injection, will produce horse sickness after subcutaneous injection, but in smaller quantities.

The following animals were both injected on the 12th December, 1905, subcutaneously with 3 c.c. preserved virus of goats Nos. 375, 378 and 381, tapped on the 22nd November:—

Mule 544.—After an incubation time of five days, the reaction started and lasted four days—21st December, 1905.

This animal died on the evening of the ninth day—21st December—from horse sickness.

Horse 537.—Incubation time of five days, the reaction lasting 10 days. Died on the evening of the 10th day—22nd December—from horse sickness.

The following animal was injected with 5 c.c. of above mixture:—

Mule 545.—Incubation time of five days, the reaction starting immediately after and lasting 3 days.

Died on the evening of the 8th day—20th December—from horse sickness.

Conclusion.—The virus of horse sickness from a horse after passing through goats is equally virulent after subcutaneous injection for mules and horses as it is after intrajugular injection.

Experiment No. 5.

To note whether the blood of goat 375, which was included in the previous mixture (Experiment No. 4a), is virulent by itself.

This goat was injected subcutaneously and had a pronounced reaction. The first tapping of blood took place on the 22nd November and was used in the previous experiment; the second took place on the 24th November, viz., 10 days after injection and during the distinct febrile reaction.

Horse 631.—Injected on 19th December, 1905, intrajugularly with 5 c.c. preserved blood of goat 375.

No reaction took place within the first eight days. It was accordingly tested on its immunity with 20 c.c. virus of donkey 593. It now contracted horse sickness and died on the 31st December, 1905.

Conclusion.—Although goat 375 shewed a reaction to a subcutaneous injection of horse sickness virus, yet the blood taken during this reaction did not prove to be virulent when injected into a susceptible horse 24 days after it was tapped.

The virulency of the mixture 375, 378 and 381 must, therefore, be due to one of the latter two animals.

Experiment No. 6.

To note whether the mixture of virulent goat's blood, which proved to be virulent for a horse and a mule, will produce a reaction when injected into fresh susceptible goats.

Goats 384 and 390.—Injected on the 12th December, 1905, with 5 c.c. preserved mixture of goats 375, 378 and 381 into the jugular vein.

There was no reaction in goat 384, and a doubtful one in 390. The latter was accordingly bled on the 8th day—15th December, 1905—and 5 c.c. of this blood was injected into the jugular vein of mule 489. No reaction ensued in this mule which was tested on its susceptibility to horse sickness on the 12th January, 1906, with 20 c.c. virus of donkey 599 injected intrajugularly. The mule died 7 days after this latter injection.

Both goats were injected on the 27th December, 1905—20 days after the first injection—with virus of horse 529. No reaction followed from this injection.

Conclusion.—The injection of goat's blood, virulent for horse and a mule, did not produce a reaction when injected into goats.

The subsequent inoculation with horse sickness virus did not cause any distinct reaction in these animals.

Experiment No. 7.

To note whether the blood of goat 375, which had a very marked reaction due to the injection of virulent horse sickness blood, will produce a reaction in other susceptible goats.

Goats 382 and 383.—Injected on the 28th November, 1905, with 5 c.c. defibrinated blood of goat 375 into the jugular vein. (The blood from this goat (375) was tapped on the 24th November, 1905.)

Both goats shewed irregular temperature reactions.

Bled on the 12th day after injection—December 10th.

Horse 1504.—Injected on the 28th November intrajugularly with 5 c.c. mixture of blood of goats 382 and 383.

No reaction ensued.

This horse was tested on its immunity to horse sickness on the 23rd January, 1906, with virus of mule 489. It contracted horse sickness and died on the 29th January, 1906.

Goats 386 and 389.—Injected with 5 c.c. mixture of goats 382 and 383—goat 386 into the jugular vein, and goat 389 under the skin. No reaction noticed.

All four goats, 382, 383, 386 and 389 were injected into the jugular vein with 10 c.c. virus of horse 529 on the 27th December, 1905.

Irregular reactions were again noticed. The most typical one was in goat 389 which was tapped on the 3rd January, 1906.

Horse 916.—Injected with 5 c.c. blood of goat 389 on the 19th April, 1906.

No reaction.

Tested later on the 9th June, 1906, with 5 c.c. virus horse 727, and died on the 11th June from horse sickness.

Conclusion.—The blood of goat 375 was not virulent, although a typical reaction was present in this animal. (Compare Experiment No. 5.)

Experiment No. 7a.

To note whether the mixture of virulent goat's blood, which proved to be virulent for horses and a mule, will cause a reaction when injected into susceptible goats. (A repetition of the previous experiment on a larger scale.)

Goats 379, 398, 399, 400 and 409.—Injected into the jugular vein with 10 c.c. mixture of blood of goats 375, 381 and 378; bled on the 22nd December.

Date of injection, 24th December, 1905.

Goats 397 and 400 shewed reaction and were accordingly bled on the 11th day after injection—January 3rd. Horse 584 was injected with 20 c.c. of this mixture into the jugular vein.

No reaction ensued.

Tested on its susceptibility to horse sickness by the injection of 5 c.c. virus of horse 542 into the jugular vein. This horse contracted horse sickness and died on the 6th day after injection—29th January.

Conclusion.—The blood of goats 397 and 400, taken 11 days after inoculation and during the reaction, proved not to be virulent for a susceptible horse.

Experiment No. 7b.

(Repetition of previous experiments Nos. 7 and 7a.)

Angora Goats 403, 404, 406, 408 and 410.—Injected with mixture of blood of goats 375, 381 and 378; tapped on the 22nd November, 1905.

Date of injection, 24th January, 1906.

There was a more regular febrile reaction in goats 403 and 408 than in the other three. All five goats were bled on the 9th day after injection—2nd February, 1906.

Horse 785.—Injected with 25 c.c. defibrinated blood of goats 403, 404, 406, 408 and 410 on 2nd February, 1906.

No reaction ensued in this horse, and it was accordingly tested on its susceptibility to horse sickness by the injection of 20 c.c. virus of a horse sent in from Barberton. Horse 785 died from horse sickness on the 6th day after injection—21st February, 1906.

Mule 772.—Injected on 8th February, 1906, with a similar mixture as that used in horse 785.

No reaction ensued from this injection and the mule was accordingly tested on its susceptibility to horse sickness by the injection of 20 c.c. virus of a mule which had succumbed to horse sickness in Warmbaths.

A horse sickness reaction was noticeable from this injection, but the mule recovered.

Conclusion.—The blood of goats taken nine days after injection of virulent goat's blood did not prove to be virulent for a susceptible horse and mule.

Experiment No. 8.

To note whether the blood of goats 371, 374, 380 and 375, all of which had a more or less pronounced reaction due to the injection of horse sickness blood, will prove virulent for horses.

Horse 527.—Injected into the jugular vein with 5 c.c. blood of the above goats tapped on the 24th November—10 days after injection of horse virus, and at the time of a febrile reaction.

Date of injection, 12th December, 1905.

No reaction followed.

Horse 527 was accordingly tested on January 23rd, 1906, with 5 c.c. virus of mule 542 injected into the jugular vein. This animal contracted horse sickness and died on the 30th January, 1906.

Conclusion.—The blood of sheep tapped during a febrile reaction due to the injection of horse sickness blood from a horse did not prove to be virulent for one susceptible horse.

Experiment No. 9.

To note whether the blood of goats 371, 373, 374, 375, 376, 377 and 378 (compare Experiment No. 1) injected with virulent horse sickness blood, and which goats all had a more or less pronounced reaction, is virulent for horses when tapped 24 days after the inoculation. It should be noted that, at this period, most of the goats still had high temperatures.

Horse 398.—Injected on the 3rd December, 1905, with 20 c.c. mixture of blood of goats Nos. 371-378.

No reaction.

This animal was tested on March 8th by an intrajugular injection of 10 c.c. virus of a mule which died of horse sickness at Tzaneen Estate.

Horse 398 contracted horse sickness and died 13 days after the injection—16th December, 1905—from horse sickness.

Horse 448.—Injected with 15 c.c. mixture of blood of goats 371-378 on December 8th, 1905.

No reaction ensued.

This horse was utilised on January 6th, 1906, for a horse sickness experiment, it contracted horse sickness, but recovered.

Conclusion.—The blood of goats taken 24 days after injection of horse sickness virus and during a reaction did not prove to be virulent for horses.

Experiment No. 10.

To note whether the injection of virulent donkey blood into goats will produce a reaction.

Goats 385 and 387 were injected on the 5th December, 1905, into the jugular vein with 2 c.c. mixture of virulent blood of donkeys.

Neither goat shewed reaction.

Goat 385 and 387 were injected on the 27th December intrajugularly with 10 c.c. virulent blood of horse 529.

A reaction started in both animals which was identical with those observed previously.

Both these animals were bled on the 30th January, 1906.

Horse 938.—Injected on the 17th April, 1906, with 5 c.c. blood of above goats—385 and 387.

No reaction followed.

This horse was consequently tested on the 5th May with virus of horse 863.

It contracted horse sickness and died on the 11th May, 1906.

Conclusion.—Virulent donkey blood injected into the jugular vein of goats in quantities of 2 c.c. did not produce a reaction.

Experiment No. 11.

To note whether the injection of virulent blood of a mule suffering from horse sickness will produce a reaction in Angora goats.

The following goats were all injected on the 24th December, 1905, intrajugularly with 5 c.c. virus of mule 532.—

Goat 392.—Reaction started about 8 days after the injection and lasted for six days. It was bled on the 3rd January, 1906.

Goat 393.—No reaction.

Goat 394.—Doubtful reaction.

Goat 395.—Reaction. This goat was bled on the 3rd January, 1906, or ten days after the injection.

Goat 396.—Reaction. The goat died on the 31st December, 1905. *Post-mortem* revealed the death to be due to a hœmorrhagic gastritis.

Horse 937.—Injected subcutaneously with 5 c.c. mixture of blood of goats 392 and 395.

No reaction.

This animal was injected with horse sickness virus from horse 893 on the 5th May, 1906. It contracted horse sickness and died 7 days later—12th May, 1906.

Conclusion.—The injection of virulent blood of a mule suffering from horse sickness produces a reaction in Angora goats, but the blood taken during this reaction did not prove to be virulent when injected into a horse.

Experiment No. 12.

To note whether virulent horse blood injected into sheep will produce a reaction, and whether the blood taken during this reaction is virulent for horses.

The following sheep were injected on the 14th November, 1905, intrajugularly, with 10 c.c. virus of horse 382:—

Merino Sheep 354.—Irregular reaction.

Bled on the 24th November—10 days after injection.

Merino Sheep 355.—No reaction.

Bled on the 28th November—14 days after injection.

The following sheep were injected on the 14th November, 1905, subcutaneously, with 20 c.c. virus of horse 382:—

Merino Sheep 356.—No reaction.

Bled on the 28th November—14 days after injection.

Merino Sheep 363.—Slight reaction, but not regular.

Bled on the 24th November—10 days after injection.

Merino Sheep 368.—Doubtful irregular reaction.

Bled on the 24th November—10 days after injection.

Conclusion.—Irregular reaction was observed in some of the sheep after the injection of virus.

Experiment No. 13.

To note whether the blood of sheep injected on the 14th November with virulent horse blood and tapped on the 24th and 28th November, 1905, will prove to be virulent for horses.

Horse 504.—Injected into the jugular vein with 25 c.c. mixture of blood of sheep 354, 355, 356, 363 and 368 on the 28th December, 1906.

No reaction ensued.

It was accordingly tested on the 26th December, 1905, by an intrajugular injection of 10 c.c. virulent blood of donkey 592.

Horse 504 contracted horse sickness and died on the fifth day after injection—31st December, 1905.

Conclusion.—The blood of sheep injected with virulent horse blood did not prove to be virulent for a susceptible horse.

Experiment No. 14.

To note whether the blood of goats 371, 374, 375, 380, and of sheep 354, 363 and 368—animals which all had a more or less pronounced reaction after the injection of virulent horse blood—will prove to be virulent for a horse and mule.

Horse 383.—Injected with 5 c.c. of above mixture into the jugular vein on the 24th November, 1905.

No reaction followed.

Tested with 2 c.c. virus of donkey 427 on 17/1/06.

Died of horse sickness on the 20th February, 1906—34 days after the injection.

Mule 1489.—Injected as above on the 24th November, 1905.

No reaction.

Tested on the 12th January, 1906, with an injection intrajugularly of 20 c.c. virus of donkey 599.

Mule 1489 contracted horse sickness and died on the 19th January, 1906—7 days after the injection.

Conclusion.—The blood of sheep and goats, injected with virulent horse blood—animals which had a more or less pronounced reaction—did not prove to be virulent for a horse and a mule.

Experiment No. 15.

To note further whether the blood of goats 371, 374, 375 and 380, and of sheep 354, 363 and 368—animals which shewed a more or less febrile reaction after the injection of virulent horse blood—will prove to be virulent for a horse.

Horse 526.—Injected intrajugularly with 5 c.c. mixture of defibrinated blood of above animals on the 7th December, 1905.

No reaction.

Tested by an intrajugular injection of 5 c.c. virus of mule 489 on the 23rd January, 1906.

Horse 526 contracted horse sickness and died six days after the latter injection—29th January, 1906.

Conclusion.—The blood of sheep and goats—animals which shewed a more or less febrile reaction after the injection of virulent horse blood—did not prove to be virulent for a horse.

Note.—Blood of goat 375 has been in mixture 375, 378 and 381 which proved to be virulent for horses and mules. It was also used

in other mixtures, proving not to be virulent, hence in the mixture of 375, 378 and 381 blood of goat 375 must be excluded as not virulent.

Experiment No. 16.

To note whether the injection of virulent horse blood will produce a reaction in cattle.

The cattle for this purpose were imported from Aliwal North, Cape Colony, a country free from horse sickness, and, accordingly, they could be considered to be susceptible to horse sickness, if this disease *is* inoculable into cattle. The heifers were all about one year old.

Heifer 312.—Injected subcutaneously with 10 c.c. virus of horse 363. No reaction.

Heifer 313.—Injected intrajugularly with 10 c.c. virus of horse 363. No reaction.

Heifer 314.—Injected subcutaneously with 10 c.c. virus of horse 363. No reaction.

Heifer 315.—Injected subcutaneously with 20 c.c. virus of horse 363. No reaction.

Heifer 316.—Injected subcutaneously with 20 c.c. virus of horse 363. No reaction.

Conclusion.—None of the inoculated heifers shewed any reaction due to this inoculation, and, consequently, no animal was tapped for testing purposes.

Résumé.

(1) The inoculation of horse sickness virus into goats produced in the majority of these a febrile reaction without any other clinical symptoms. The blood taken during this reaction proved to be virulent for only two animals out of fifteen (13%).

(2) No attenuation of virus had taken place by passing it through the goats.

(3) It was impossible to cause a horse sickness reaction in goats with goat's blood which had proved to be virulent for horses and mules.

(4) Sheep and cattle could not be infected with horse sickness virus.

(5) Virulent goat's blood retained its virulency in the same way as virulent horse blood.

These statements do not allow us to draw the conclusion whether the febrile reaction observed in goats after the injection of virus was that of a modified type of heartwater, nor can this febrile reaction of the majority of animals be interpreted as a modified form of horse sickness.

It must be taken into consideration that in all cases fresh horse sickness blood was injected into goats. A reaction in these, therefore,

may be due to some other influence not yet exactly known. In support of this theory the fact may be quoted that a successful infection of goats with virulent goat's blood was never possible.

The nature of the febrile reaction still remains to be investigated. The point at issue, however, is to know whether the febrile reaction was not a virulent form of heartwater. If such was the case, it would follow that the injected animals which reacted would have contracted some immunity to protect them against a subsequent artificial or natural attack of heartwater.

The following notes furnish the results of experiments undertaken for this purpose:—

Goat 371 (compare Experiment No. 1a) was injected on the 5th July, 1906, with 50 c.c. virulent heartwater blood of goat 542.

Died on the 16th July, 1906.
Post-mortem revealed the lesions of heartwater.

Goat 374 (compare Experiment 1b) was injected on the 20th July, 1906, subcutaneously with 10 c.c. blood of sheep 515 suffering from heartwater.

Died on the 6th August, 1906, from heartwater.

Goat 375 (compare Experiment 1b) was injected on the 5th July, 1906, with 50 c.c. blood of goat 542, which was suffering at that date from heartwater.

Died on the 16th July from heartwater.

Goat 376 (compare Experiment 1 c).—This goat was sent to Komatie Poort on the 11th March, 1906; brought back on the 22nd June, 1906. On the 13th July the animal was found dead with all the symptoms of heartwater on *post-mortem*.

Goat 381 (compare Experiment 1d) was injected subcutaneously on the 5th July, 1906, with 5 c.c. blood of goat 542 which was suffering from heartwater.

Died on the 18th July, 1906.
Post-mortem shewed lesions of heartwater to be present.
Note that goat 381 was one of the animals whose blood proved to be virulent for horses.

Goat 382 (compare Experiment No. 7) was injected on the 3rd August, 1906, with 10 c.c. virus of goat 374 which was suffering from heartwater.

Died on the 16th August, 1906, with all the symptoms of heartwater.

Goat 389 (compare Experiment No. 7) was exposed on the 17th May, 1906, at Sjambok's Kraal.
Died on the 7th June, 1906.
Post-mortem revealed the lesions of heartwater.

Goat 398 (compare Experiment No. 7a) was exposed on the 17th May, 1906, at Sjambok's Kraal.

Died on the 13th June, 1906.
Post-mortem revealed the lesions of heartwater.

Goat 400 (compare Experiment No. 7a) was exposed on the 17th May, 1906, at Sjambok's Kraal.

Died on the 8th June, 1906.
Post-mortem shewed the lesions of heartwater.

Conclusions.

All goats which died from heartwater, contracted either by inoculation or exposure to natural infection, were animals which had been injected on one or two occasions with horse sickness blood, and some of which had shewn distinct reaction.

If horse sickness is identical with heartwater, it is only natural to expect that animals which reacted to the injection of horse sickness blood would be immune against heartwater. This, however, was not the case, and we have to conclude that the injection of horse sickness blood into goats followed by a reaction does not protect these animals against heartwater. Hence we deduce that heartwater and horse sickness are not identical.

Referring back to Dr. Edington's statement, it is undoubtedly correct that goats can be infected with horse sickness, but that the transference of this disease is commonly attended with numerous failures.

In our experiments no attenuation of virus after passing through a goat had taken place. The virus was quite as virulent as that obtained from equines suffering from horse sickness.

EXPERIMENTS WITH SERUM AGAINST EAST COAST FEVER.

I.

In the second report by Professor Koch on the above subject, he mentioned that he was conducting experiments with a view of obtaining a curative serum. For the purpose of obtaining an anti-toxic serum which would tend to neutralise the toxic products of the organism, the animal whose serum it is proposed to use is gradually inoculated with increased doses of virulent blood.

Since it was discovered that healthy animals are also able to resist large doses of virulent blood, animals were prepared by inoculation with successive large doses of virulent blood for the purpose of obtaining a cytolytic serum. Such a serum possesses the property of directly attacking the specific parasite instead of neutralising its products as an anti-toxic serum would.

For the production of anti-toxic serum and cytolytic serum, only immune animals can be used, as susceptible animals tend to break down under repeated doses of virulent blood.

In this way immune animals were injected with increasing doses of blood taken from sick animals, starting with doses of 5 c.c., and concluding with a maximum of 2,000 c.c., while others received a succession of doses of 2,000 c.c. each of sick blood injected subcutaneously, or of 1,000 c.c. injected intravenously. Care was taken to utilise sick blood which contained a large number of organisms for fortifying purposes. After three or four large injections given at intervals of from two to three weeks, the serum of these animals was found to possess very remarkable properties, which may be summarised as follows:—

Injection of such serum into healthy animals in doses up to 150 c.c. resulted in no systematic disturbance. Injection of the same serum into sick animals caused a striking change in the African coast fever parasites circulating in their blood. The parasites became smaller, their outline was lost; sometimes they were scarcely visible, and, in the course of a few days they disappeared. Unfortunately, however, this specific serum also possessed an undesirable property in a very high degree which exerted a solvent action upon the blood cells of sick animals, whilst healthy animals remained unaffected.

Injection of 50 c.c. of hæmolytic serum into sick animals has nearly always been fatal, death being primarily due to its solvent action upon the blood cells. In sick animals treated in this way, there

is a sudden rise of temperature, which is associated with collapse and death. On *post-mortem* the blood is found to be hæmolytic, and the fat, subcutaneous tissues and mucous membranes are intensely yellow from staining, with altered blood pigment.

This hæmolytic effect could also be noted in animals even before any parasites are present in the circulation, and while the only indication of approaching indisposition is an elevation of the temperature.

The employment of serum for therapeutic purposes having been found to be highly dangerous when large doses were given, the administration of repeated small doses was tried. These, however, were not followed by any better results, the animals dying notwithstanding the disappearance of the parasites.

Preventive treatment was also attempted by means of serum inoculation. Three animals treated with large single doses of serum all sickened and died, although a prolonged duration of this illness shewed that, in their case, the serum exercised a certain protective action.

Repeated injections of 5 c.c. had no beneficial result. Repeated injections of 10 and 20 c.c. had marked but varied results.

Another complicating factor in these serum experiments was the appearance of redwater in animals approaching convalescence after serum treatment, from which the animals died.

For these various reasons Professor Koch discontinued the serum treatment.

It thus appears from Professor Koch's report—which I have freely quoted above—that it would be possible to produce a curative and preventive serum if the hæmolytic action could be nullified.

If such a serum would simply prevent the infection of an animal in an infected area for about a fortnight it would prove to be of immense value from many points of view, and the eradication of the disease would be considerably simplified. This consideration led us to continue the experiments of Professor Koch, and the object was to produce a serum which has no hæmolytic action on the blood of sick animals, and acts as a preventive on healthy ones.

II.

In my experiments for the immunisation of horses and mules against horse sickness, I have encountered a somewhat similar difficulty, inasmuch as the serum of horses and mules injected with large doses of virulent blood acquired, in many instances, hæmolytic properties, dissolving the blood corpuscles of a healthy horse or mule into which it was injected. There is a difference between the serum produced

by Professor Koch against coast fever, and with my own against horse sickness; the former only develops its hæmolytic action on blood corpuscles of infected animals, the latter on the corpuscles of healthy animals. In the case of horse sickness, a hæmolytic serum is produced by the injection of healthy red corpuscles into healthy immune animals; and, in the case of coast fever serum, by the injection of sick red corpuscles. From a scientific point of view, this difference is very interesting, since, in our former experience in connection with the production of rinderpest serum, which is produced on the same principle, hæmolysis was never observed. It would accordingly follow from these remarks that hæmolysines only develop when the red corpuscle is in a stage of disease or disintegration.

Having overcome the difficulty of producing hæmolysines in equines by abandoning the subcutaneous or intrajugular injection of defibrinated virulent blood and introducing the direct transfusion of virulent blood from sick into immune animals, it was thought advisable to apply the same method to the hyperimmunisation of immune oxen against east coast fever.

The following tabulated form shews the records of the transfusion. There were eight immune oxen utilised. The infusion was made as high as possible in order to obtain, at the same time, some information regarding the extent an animal will stand this.

The time of transfusion was carefully noted, and it will be observed that, in one session, the transfusion was continued for twenty minutes. Needless to say that from time to time, the tubing was disconnected in order to ascertain whether the blood was still running. By collecting the blood into a measure glass, it was found that a fast running current would yield about 500 c.c. per minute, so that, in twenty minutes, about 10 litres of blood would be transfused. It will also be noticed that all the animals stood the first transfusion of twenty minutes very well; the second infusion lasted 10 minutes for one and 20 minutes for the remainder, still with good results; the third infusion, however, killed ox 98—which had been infused for 30 minutes—and also ox 99, 20 minutes' infusion. A fourth infusion was made into ox 101, which he stood well.

Our oxen were accordingly hyperimmunised to the extent of a minimum of 20 litres, and a maximum of 40 litres.

The sick blood of the infusion was first examined, and only used when piroplasma parvum were present in large numbers. The two oxen which died from the transfusion shewed extensive heart lesions in the form of hemorrhagic patches on the endocard and myocard, and exudation of blood into the myocard. They died of heart failure without any febrile reaction preceding, thereby indicating no specific disease, and the examination of the blood did not shew any blood changes.

The heavy infusion of blood did not cause any after effect in the remaining oxen, and no signs of any illness were noted.

HYPERIMMUNISATION OF SALTED OXEN.

Number of Oxen.	NUMBER OF TIMES HYPERIMMUNISED.																Total Extent.
	FIRST.				SECOND.				THIRD.				FOURTH.				
	Date.	Time.	Speed.	Virus No.	Date.	Time.	Speed.	Virus No.	Date.	Time.	Speed.	Virus No.	Date.	Time.	Speed.	Virus No.	
	1906.	minutes.			1906.	minutes.			1906.	minutes.			1906.	minutes.			litres.
101	23/2	18	slow	1	24/2	22	slow	1	1/3	20	fast	8	1/3	20	fast	10	40
98	23/2	20	slow	2	24/2	10	slow	2	1/3	30	fast	8	Died	1/3/06.			30
105	23/2	20	fast	3	25/2	20	fast	3	2/3	20	very fast	8					30
102	24/2	20	fast	4	25/2	20	very fast	1	2/3	20	very fast	5					30
99	25/2	20	very fast	4	25/2	20	very fast	4	2/3	20	fast	8	Died	2/3/06.			30
87	25/2	20	very fast	3	26/2	20	fast	7	2/3	20	very fast	5					27
106	26/2	20	fast	7	27/2	20	very fast	5	4/3	10	fast	9					25
103	26/2	20	fast	4	27/2	20	very fast	5	4/3	10	fast	9					25

EXPERIMENTS WITH SERUM OF THE HYPERIMMUNISED OXEN.

On the 20/3/06—not less than 16 days after infusion—the oxen were bled for serum. It was now noted that the yield of serum was less than was usually found in hyperimmunised rinderpest oxen, and it was thought that the enormous infusion was responsible for this.

As is the rule with our horse sickness serum, this ox serum was tested in vitro by mixing 2 c.c. immune serum with about 10 drops of a 5% emulsion of red corpuscles. The following table shews the result:—

Experiment on the 31/3/06.

		Immune Oxen Numbers.					
		87	101	102	103	105	106
Healthy Ox	269 ...	clear	clear	traces	clear	clear	clear
,,	325 ...	,,	,,	,,	,,	,,	,,
East Coast Fever Ox	361 ...	,,	,,	,,	,,	,,	,,
,, ,,	362 ...	,,	,,	,,	,,	,,	,,

NOTE.—For the emulsion of blood, that from two healthy oxen and also two oxen suffering from east coast fever was used.

The serum of ox 102 was hæmolytic, but this was the case before it was mixed with corpuscles.

Experiment on the 31/3/06.

		Immune Oxen Numbers.					
		87	101	102	103	10 5	106
East Coast Fever Ox	363 ...	clear	clear	clear	clear	clear	clear
,, ,,	364 ...	,,	,,	,,	,,	,,	,,

The result was that no hæmolysis took place in vitro. (Serum 102 was clear this time.)

It therefore follows that the hyperimmunisation by infusion of immune oxen with blood of oxen suffering from east coast fever does not cause a hæmolytic serum which would shew its blood solving action in vitro.

Having ascertained this fact, it now remained to prove whether —as in the case of horse sickness serum—which serum when injected into both sick and healthy oxen will not produce hæmolysis.

Experiment of the 22/3/06.—Injection into healthy cattle.

Ox 325.—Injected both subcutaneously and intrajugularly with 250 c.c. (total 500 c.c.) serum mixture of oxen 87, 101, 102, 103, 105 and 106.

Result.—There was not the slightest disturbance in ox 325.

Experiment of the 21/3/06.—Injection into sick cattle.

Cow 361.—Suffering from east coast fever, and far advanced in the disease, was injected both under the skin and into the jugular vein with 250 c.c. (total 500 c.c.) serum mixture of oxen 87, 101, 102, 103, 105 and 106.

Result.—This animal died during the night of the 22-23/3/06.

Post-mortem made on the following morning, 23/3/06.

General condition—fair.
Lungs—oedematous.
Trachea—full of foam. The base of the trachea was infiltrated with yellow gelatinous exudate.
Heart—numerous petechiæ on the epicardium.
Endocard of left ventricle echymosed.
Spleen—normal.
Liver—very icteric; numerous small areas of necrosis.
Kidneys—a few white infarcts in each.
Urine—normal.
Stomach—abomasum intensely congested; a few superficial ulcers on mucous membrane.
Intestines—deeply congested throughout, except rectum, which was almost normal; a few hemorrhages in duodenum.

NOTE.—The typical lesions found on *post-mortem*, due to hæmolysis—tumour of the spleen and red urine—were absent.

The examination of the blood, before and after the injection of the serum, revealed absolutely no difference in the shape of the piroplasma parvum, neither did their number decrease. The same statement holds good with the parasites in the blood corpuscles mixed with serum in vitro.

Experiment on the 31/3/06.—Injection of serum into a healthy ox which had previously been infused with sick blood.

Ox 260.—Infused on the 21/3/06 for 15 minutes with blood of an animal suffering from east coast fever. The infusion was repeated for 5 minutes on the 31/3/06 from a sick animal, and, immediately after, 500 c.c. serum mixture of immune oxen Nos. 87, 101, 102, 103, 105, and 106 was injected subcutaneously and into the jugular vein.

No immediate result from this reaction.

The ox died from heartwater on the 17/4/06. The diagnosis was proved by subsequent injection of blood into goats, which animals all contracted the disease.

It follows, therefore, that one of the animals used for the purpose of infusing ox 260 must have been infected with heartwater at the time.

No hæmolysis was noted in this case.

Experiment on the 2/4/06.—Injection of serum into sick animal.

Cow 363, suffering from east coast fever, and far advanced in the disease, was injected both subcutaneously and intrajugularly with 500 c.c. serum of immune oxen Nos. 87, 101, 102, 103, 105, and 106.

Result.—Died during the night.

Post-mortem made on the morning of the 3/4/06.

Lungs—old fibrous lesions on pleura; lymphatic glands swollen.
Heart—flabby.
Spleen—normal.
Liver—numerous necrotic foci; icteric.
Kidneys—a few red and white infarcts.
Stomach—Intense congestion of abomasum, moderate congestion of omasum.
Intestines—Deeply congested as far as caecum; onward patches of congestion, with echymoses.
Bladder—urine scanty but normal in colour.

NOTE.—The typical lesion due to hæmolysis was also absent in this case.

The examination of blood both before and after the injection of serum did not reveal any change, either in the shape or number of the parasites.

Conclusion.

Although our experiments were not numerous, and, perhaps, the animals were too far advanced in the disease for the injection of serum, we may conclude that no hæmolysis had taken place in vivo. It is possible that the sick animals did not live long enough, and that the hæmolysis had no time to develop.

No changes were noticed in the shape of the parasites, or was there any noticeable increase in their number. Here again it may be argued that the animal died before the serum had time to act on the parasites.

If any conclusion may be drawn with safety, it is—that the injection of the immune serum had no effect on the sick animals.

III.

EXPERIMENTS TO TEST PREVENTIVE VALUE OF SERUM.

Two courses were open to us for this purpose: firstly, to inject the serum into susceptible healthy cattle, and then infect them with ticks, or, secondly, to expose injected healthy cattle in an infected area together with an equal number of control animals, which would not be treated in any way. An excellent opportunity occurred in the Native Location at Sjambok's Kraal, where coast fever had for some time destroyed a large number of cattle and was still rampant.

The conditions for an early decision regarding the preventive qualities of the serum were present, viz., a thoroughly infected farm on which every bovine animal would, sooner or later, contract the disease. It was decided to purchase, for the experiment, cattle which had been running on the farm. In all, twelve head of cattle, mostly yearlings, were obtained and divided into lots of six; one set to be inoculated with serum and the other six to serve as controls.

I. Cattle Injected with Serum.

(*a*) Injection of serum to be repeated every fourth week.

Bull 371.—Injected on the 6/4/06 with 200 c.c. serum mixture oxen 87, 101, 102, 103, 105, 106.

Thirteen days after this injection the temperature began to rise and a regular coast fever reaction ensued which culminated in death on the 3/5/06.

Piroplasma parvum was noted for the first time on the 24/4/06, and, four days later, it was fairly frequent; on the 29/4/06, it was recorded as medium; and on the 30/4/06, it was strong, increasing daily until death three days later.

Bull Calf 373.—Injected on the 6/4/06 with 200 c.c. serum.

Temperature rose six days later.

Death ensued 15 days later—27/4/06.

Piroplasma parvum noted for the first time on the 15/4/06; a medium frequency was recorded three days later, and, on the 22/4/06, a strong injection was noted.

Piroplasma bigeminum appeared on the 23/4/06 and remained until death—four days later.

Heifer Calf 375.—Injected on the 6/4/06 with 200 c.c. serum.

Immediate rise of temperature.

Piroplasma parvum noted on the 10/4/06.

Basophile cells and poikilocytosis noted on the same day.

Piroplasma parvum increased and was recorded as numerous on the 15/4/06; the rise continued until death, which occurred on the 19/4/06.

Smears of this date shewed both piroplasma parvum and piroplasma bigeminum.

(*b*) Injection of serum to be repeated every fourteen days.

Cow 366.—Injected on the 6/4/06, 20/4/06, and on 6/5/06 with 200 c.c.

No reaction.

Killed on the 11/4/06.

Bull 372.—Injected as Cow 366.

Temperature rose on the 26/4/06, and death resulted on the 9/5/06.

Piroplasma parvum noted for the first time on the 30/4/06; a medium frequency was recorded on the 2/5/06 and until death.

Cow 369.—Injected as Cow 366.
No reaction ensued.
Slaughtered on the 26/5/06.

II. Controls.

Cow 365.—No reaction.
Slaughtered on the 18/5/06.

Cow 367.—Reaction started on the 19/4/06, and death took place on 1/5/06.
Piroplasma parvum noted on the 22/4/06; it was fairly frequent three days later, and increased until death.
Piroplasma bigeminum noted on the 30/4/06.

Cow 368.—Died on the 9/4/06. Death due to an accident.

Cow 370.—Reaction started on the 19/4/06.
Piroplasma parvum noted two days later; on the 24/4/06 it was frequent, and rapidly increased until death on the 29/4/06.

Heifer 374.—No reaction.
Killed on the 23/5/06.

Heifer 376.—Reaction began on the 17/4/06.
Piroplasma parvum noted on the 24/4/06; increased daily, and, on the 28/4/06, a strong infection was recorded.
Piroplasma bigeminum present on the 2/5/06, and remained until death on the 3/5/06.

Result.—Of the animals injected with serum, four contracted coast fever and died, two were complicated with piroplasma bigeminum.

Of the control animals, three contracted coast fever and died, two were complicated with piroplasma bigeminum.

The course of the disease in injected and non-injected animals did not differ; no change in the shape, form or number of parasites was noticed. The secondary development of piroplasma bigeminum was noted to the same extent in the two sets of animals.

Conclusion.

Comparing Professor Koch's experiments with those enumerated on the preceding pages, the following conclusions may be drawn:—

1. Fortifying immune animals with defibrinated blood by means of subcutaneous or intrajugular injection produces a serum which is (*a*) hæmolytic for coast fever infected animals, and (*b*) microbicide for piroplasma parvum (Koch).

2. Fortifying immune animals with blood of coast fever animals by infusion, a serum is produced which has neither (*a*) hæmolytic action on coast fever or healthy animals, (*b*) microbicide influence on piroplasma parvum, or (*c*) any preventive value.

3. The value of these experiments is the fact that by transfusion from sick to immune animal an enormous amount of virus can be incorporated which may be utilised with expediency in the case of fortifying animals for rinderpest serum.

INOCULATION AGAINST EQUINE PIROPLASMOSIS.

In the annual report of last year a series of experiments were quoted which elucidated the fact that equine piroplasmosis could be transmitted by the inoculation of blood from an animal which had recovered from an attack of piroplasmosis (immune animal). We also proved that, in this way, the disease could be transmitted (1) from immune horse into susceptible horse, mule and donkey; (2) from immune mule into susceptible horse, mule and donkey; and (3) from immune donkey into susceptible horse, mule and donkey. There was a variation in the degree of virulency of the disease shewn by the various classes of animals, so that the deduction was made that immune blood might advantageously be used for inoculation purposes. The following recommendations were suggested as likely to be successful:—

1. Inoculation of mules with blood of immune donkeys.
2. Inoculation of donkeys with blood of immune mules.
3. Inoculation of horses with blood of immune donkeys.

It was, therefore, our next object to discover the extent to which the above suggestions were applicable into practice, and with what result. Accordingly, horses, mules, and donkeys directly imported from the Argentine and stabled in the premises of the Laboratory after their arrival were utilised for this purpose.

Inoculation of Horses.

I.

Inoculation of Horses with Immune Donkey Blood.

(*a*) Blood of donkey 306 (compare Annual Report, 1904-5, page 101, G., horse 600 and 723).

"*A.*" *Horse 404*, about 3 years old.

Injected on the 17/11/05 with 10 c.c. defibrinated blood, subcutaneously.

A first and second reaction developed, the former after 11 days incubation, and continuing for 4 days; piroplasma equi was very numerous. The number of red corpuscles decreased to 3,000,000 per c.m.m. After an interval of 7 days the second reaction started, continued for 6 days, and caused the death of the horse.

(Compare temperature and red corpuscle curve, Plate No. 5.)

"*B.*" *Horse 406*, about 3 years old.

Injected as above.

Irregular temperature reaction, beginning on the 10th day and lasting for 4 days.

Piroplasma equi was not noticed during this reaction.
Red corpuscles decreased in numbers.
The intermittent irregular temperature reactions continued.
Piroplasma equi noted on the 20th day.
Red corpuscles reached the minimum number of 3,000,000 per c.m.m. on the 24th day. A low record of the blood cells continued for some time.
The animal finally recovered.
(Compare temperature and red corpuscle curve, Plate No. 5.)

Note.—After 40 days another reaction ensued lasting for 10 days, the nature of which could not be determined.

(*b*) Blood of donkey 739 (compare Annual Report, 1904-5, page 100).
This donkey had been injected with horse blood, and shewed a typical reaction with piroplasma equi.

"*C.*" *Horse 449*, about 3½ years old.
Injected on the 17/11/05 with 10 c.c. defibrinated blood.
Slight temperature reaction occurred one day after injection and could not be accounted for.
The typical primary reaction began about the 9th day and lasted for about 10 days.
In about the middle of this reaction the number of red corpuscles decreased to 2,800,000 per c.m.m.
Second reaction began on the 22nd day, coinciding with the minimum decrease of red corpuscles to 2,300,000 per c.m.m. This second reaction only lasted 3 days, culminating in the death of the horse.
Piroplasma equi was frequently met with.
(Compare temperature and red corpuscle curve, Plate No. 5.)

"*D.*" *Horse 435*, about 4 years old.
Injected on the 17/11/05, as above.
Temperature rose after an incubation period of 8 days.
The number of red corpuscles decreased and reached the minimum of 3,600,000 per c.m.m. on the 12th day.
The horse collapsed on this date and died.
Piroplasma equi was noted from the 8th day, increasing daily.
(Compare temperature and red corpuscle curve, Plate No. 5.)

(*c*) Blood of donkey 741 (compare Annual Report, 1904-5, page 101).
This donkey had been injected with immune mule blood; it shewed a reaction and piroplasma equi.

"*E.*" *Horse 450*, about 4 years old.
Injected on the 17/11/05 subcutaneously with 10 c.c. defibrinated blood.
Temperature rose on the 10th day after injection.
The number of red corpuscles began to decrease about the same date.

Piroplasma equi also appeared simultaneously; it increased rapidly and the horse died after 5 days' reaction, the number of red corpuscles having dropped as low as 2,400,000 per c.m.m.

(Compare temperature and red corpuscle curve, Plate No. 5.)

"*F.*" *Horse 451*, about 4 years old.

Injected as above.

A somewhat irregular temperature reaction began on the 9th day.

Piroplasma equi was noted on the 12th day.

The number of red corpuscles decreased from this latter date, and, on the 18th day, reached 3,200,000 per c.m.m.

No secondary reaction.

This horse rallied again when the number of corpuscles increased, and finally recovered.

(Compare temperature and red corpuscle curve, Plate No. 5.)

(*d*) Blood of donkey 744 (compare Annual Report, page 103, year 1904-5). This donkey had been injected with immune donkey blood; it shewed a reaction, and piroplasma equi was present.

"*G.*" *Horse 452*, about 4 years old.

Injected on the 17/11/05 with 10 c.c. defibrinated blood.

Reaction started after 9 days' incubation, and continued for 13 days.

Piroplasma equi was met with in rare numbers.

A sudden destruction of red corpuscles occurred on the 19th day followed by a rapid regeneration, notwithstanding which the animal died on the 24th day.

(Compare temperature and red corpuscle curve, Plate No. 5.)

"*H.*" *Horse 453*, about 4 years old.

Injected as above.

Reaction started on the 10th day after inoculation; it lasted for 16 days with a slight interval of two days after the 6th day.

The number of red corpuscles decreased during the incubation time; the minimum number reached was 3,600,000 per c.m.m. shortly after the temperature curve. A second drop took place at the end of the reaction.

The horse recovered.

(Compare temperature and red corpuscle curve, Plate No. 5.)

Results.—Of eight horses injected with immune donkey blood all contracted piroplasmosis, and five died: four from the effect of the first reaction and one from the second. There was no apparent difference in the virulency of the disease produced by immune blood of donkeys injected previously with either horse, mule or donkey blood.

Conclusions.—It is very dangerous to inject susceptible horses with blood of immune donkeys, and this procedure cannot be utilised as a means of immunising horses against piroplasmosis.

II.

Inoculation of Horses with Immune Mule Blood.

(*a*) Blood of immune mule 589 (compare Annual Report, 1904-5, page 98). This mule had been injected with immune horse blood and shewed a reaction together with piroplasma equi.

"*A.*" *Horse 454*, Argentine, about 4 years old.

Injected on the 20/3/06 with 5 c.c. defibrinated blood, subcutaneously.

Temperature rose after an incubation time of 8 days.

Piroplasma equi appeared at the same time.

Red corpuscles decreased in numbers from this date, and reached their lowest number of 3,800,000 per c.m.m. on the 17th day.

A slight secondary reaction was noticeable which affected the red corpuscles but slightly.

(Compare temperature and red corpuscle curve, Plate No. 5.)

Note.—This horse was subsequently used for horse sickness experiments.

"*B.*" *Horse 830*, Argentine, about 4 years old.

Injected as above.

Slight reaction of an irregular nature ensued.

The number of red corpuscles decreased to 3,800,000 per c.m.m. on the 15th day.

The horse recovered.

(Compare temperature and red corpuscle curve, Plate No. 5.)

Note.—This horse was subsequently used in horse sickness experiments and also hyperimmunised.

"*C.*" *Horse 832*, Argentine, about 4 years old.

Injected as above.

Reaction started after an incubation time of 10 days.

Piroplasma equi appeared.

The number of red corpuscles dropped to 2,800,000 per c.m.m.

Secondary reaction started after an interval of about a week.

The number of red corpuscles—which had increased during the interval between the primary and secondary reaction—again dropped very low.

Piroplasma appeared again but very rarely.

Chromatic points were seen in the red corpuscles in large numbers.

The phenomenon of poikilocytosis was very marked.

Although the temperature returned to normal, the animal failed to rally and died.

Post-mortem revealed the lesions of an advanced piroplasmosis.

(Compare temperature and red corpuscle curve, Plate No. 5.)

"*D.*" *Horse 833*, Argentine.
Injected as above.
Reaction commenced after an 8 days incubation.
Piroplasma was only found later, and but rarely.
The number of red corpuscles dropped below 4,000,000 per c.m.m. after the reaction had finished. There was also a second reaction, during which poikilocytosis was noticed.
The animal recovered.
(Compare temperature and red corpuscle curve, Plate No. 5.)

Result.—Of four horses which were injected with immune blood from mule 589, all contracted piroplasmosis, and one died from the effect after the second reaction.

Conclusion.—The inoculation of immune mule blood into susceptible horses causes the appearance of piroplasmosis, but this disease seems to be less virulent than that caused by immune donkey blood.

Inoculation of Mules.

I.

Inoculation of Mules with Immune Donkey Blood.

(*b*) Blood of donkey 306.

"*A.*" *Mule 414*, Argentine, 3-4 years old, immunised against horse sickness on the 10/11/05.
Injected on the 29/12/05 with 5 c.c. defibrinated blood.
There was hardly any reaction.
Slight decrease of red corpuscles noticed.
Piroplasma equi was present.
(Compare temperature and red corpuscle curve, Plate No. 5.)

"*B.*" *Mule 415*, Argentine, 4 years old.
Injected as above.
There was hardly any reaction.
Chromatic points were noted, but no piroplasma was seen.
Slight decrease in the number of red corpuscles, but these regenerated very quickly.
(Compare temperature and red corpuscle curve, Plate No. 5.)

(*b*) Blood of donkey 740 (compare Annual Report, 1904-5, page 100).
This donkey had been injected with immune horse blood; piroplasma was present during the reaction due to this injection.

"*C.*" *Mule 416*, Argentine, about 4 years old, immunised against horse sickness on the 10/11/05.
Injected on the 29/12/05 with 5 c.c. defibrinated blood.
There was hardly any reaction noticeable, and only a slight decrease in the number of red corpuscles.
Chromatic points were noted.
Piroplasma equi was not noted.
(Compare temperature and red corpuscle curve, Plate No. 5.)

"*D.*" *Mule 417*, Argentine, 4 years old.
Injected as above.
Very slight temperature reaction.
Red corpuscles shewed a decrease during that period.
Piroplasma equi was present.

(Compare temperature and red corpuscle curve, Plate No. 5.)

Result.—Of four mules injected with donkey blood, all shewed lesions due to the injection, which was indicated by a slight decrease in the number of red corpuscles. The temperature reaction was very slight; piroplasma equi and chromatic points were both observed on two occasions.

Conclusion.—The injection of immune blood of donkeys into mules only causes a mild attack of piroplasmosis in the latter. Immune donkey blood can, therefore, be used as a means of immunising mules against piroplasmosis.

II.

Inoculation of Mules with Immune Mule Blood.

Blood of mule 589.

"*A.*" *Mule 420*, Argentine, 4 years old, immunised on the 10/11/05 against horse sickness.
Injected on the 29/12/05 with 5 c.c. defibrinated blood.
Irregular temperature reaction, during which the number of red corpuscles dropped to 4,600,000 per c.m.m.
No piroplasms were noticed.
Chromatic points visible on one occasion.

(Compare temperature and red corpuscles, Plate No. 5.)

"*B.*" *Mule 421*, Argentine, 4 years old.
Injected as above.
Slight and irregular temperature reaction.
The number of red corpuscles decreased to 4,500,000 per c.m.m.
No piroplasms were seen.
Chromatic points were present on two occasions.

(Compare temperature and red corpuscle, Plate No. 5.)

"*C.*" *Mule 422*, Argentine, 4 years old.
Injected as above.
Irregular but slight temperature reaction.
The number of red corpuscles decreased on one occasion to 4,200,000 per c.m.m.
Piroplasma equi seen for one day.

(Compare temperature and red corpuscle curve, Plate No. 5.)

Result.—The injection of immune mule blood into susceptible mules causes in these latter a reaction which is more pronounced than the reaction caused by the injection of immune donkey blood.

Piroplasma equi appeared on one occasion only, and chromatic points were seen twice.

Conclusion.—The injection of immune mule blood into mules causes a mild attack.

Immune mule blood may, therefore, be utilised as a means of immunising mules against this disease.

Inoculation of Donkeys.

Inoculated with Blood of Immune Mule 589.

N.B.—These donkeys were all Argentines, and from 3-5 years old.

"*A.*" *Donkey 591.* Injected on the 27/1/06 with 5 c.c. blood.

Result.—

Slight temperature reaction.

Considerable decrease of number of red corpuscles to 3,700,000 per c.m.m.

No piroplasms noted.

Chromatic points visible on one occasion.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*B.*" *Donkey 592.* Injected as above.

Result.—

Slight temperature reaction.

Decrease of the number of red corpuscles to 3,800,000 per c.m.m., maintaining at this figure for some days.

Piroplasma noted on the 21st day after injection.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*C.*" *Donkey 593.* Injected as above.

Result.—

Distinct but not high temperature reaction distinguishable into primary and secondary.

Decrease of number of red corpuscles to 3,200,000 per c.m.m. in the interval between the two reactions.

Piroplasma equi noted on two occasions, viz., 21st and 23rd day after inoculation.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*D.*" *Donkey 594.* Injected as above.

Result.—

Distinct but somewhat irregular primary and secondary reactions.

Decrease of number of red corpuscles to 3,400,000 per c.m.m.

Piroplasma equi noted on five occasions, viz., 10th, 17th, 19th, 21st and 24th days after injection.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*E.*" *Donkey 595.* Injected as above.

Result.—

Distinct primary reaction, less severe than the secondary one, which was more pronounced.

Decrease of number of red corpuscles to below 4,000,000 per c.m.m., and continuing at that figure for some time.

Piroplasma equi noted on the 17th and 21st days after injection.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*F.*" *Donkey 596.* Injected as above.

Result.—

Distinct reaction with rapid and strong increase of piroplasma equi, and very rapid decrease of number of red corpuscles to 1,200,000 per c.m.m. The animal died.

Post-mortem.

General condition—fair.
Blood—very watery.
Muscles—pale.
Lungs—normal.
Heart—normal.
Spleen—slightly swollen.
Kidneys—pale.
Liver—slightly icteric.
Stomach—normal.
Intestines—duodenum and jejunum contained only blood-stained mucous. Other parts contained bile-stained ingesta.
Bladder—distended with blood-stained urine.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*G.*" *Donkey 597.* Injected as above.

Result.—

Distinct but slight primary and secondary reactions.

Number of red corpuscles decreased to 3,600,000 per c.m.m. at the end of the first reaction.

Piroplasma equi noted on the 14th and 17th days.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*H.*" *Donkey 598.* Injected as above.

Result.—

Distinct but not very high temperature reaction.

Number of red corpuscles decreased to 3,000,000 per c.m.m.

Piroplasma equi noted on the 17th day.

Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*I.*" *Donkey 599.* Injected as above.

Result.—

Distinct and high reaction with numerous piroplasma equi, and a very low reading of the number of red corpuscles at 1,700,000 per c.m.m.
Poikilocytosis marked.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*J.*" *Donkey 600.* Injected as above.

Result.—

Irregular temperature reaction.
Medium decrease of number of red corpuscles.
Piroplasma equi noted on the 17th day.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*K.*" *Donkey 601.* Injected as above.

Result.—

Distinct irregular primary and regular secondary reaction.
Slight decrease of number of red corpuscles to 4,500,000 per c.m.m.
Piroplasma rare on the 20th, 23rd and 24th days.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*L.*" *Donkey 602.* Injected as above.

Result.—

Distinct primary and secondary reactions.
Number of red corpuscles decreased moderately.
Piroplasma equi noted at the beginning of the first and secondary reaction.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*M.*" *Donkey 603.* Injected as above.

Result.—

Pronounced primary and secondary reaction.
Number of red corpuscles dropped to 2,600,000 per c.m.m. at end of first reaction.
Poikilocytosis marked just previous to the low reading of the corpuscles, and for several days afterwards.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*N.*" *Donkey 604.* Injected as above.

Result.—

Long reaction with high fever.
Number of red corpuscles dropped to below 4,000,000 per c.m.m. shortly after the appearance of piroplasma equi.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*O.*" *Donkey 605.* Injected as above.

Result.—

Prolonged irregular reaction.
Number of red corpuscles decreased below 4,000,000 per c.m.m.
Piroplasma equi noted on two occasions—in rare numbers—on the 13th and 18th day.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*P.*" *Donkey 606.* Injected as above.

Result.—

Slight and irregular temperature reaction.
Moderate decrease of the number of red corpuscles, maintaining for some time.
Piroplasma equi noted on the 23rd day after inoculation.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*Q.*" *Donkey 607.* Injected as above.

Result.—

Irregular temperature reaction distinguishable into primary and secondary.
Decrease of number of red corpuscles to 3,600,000 per c.m.m. during primary reaction.
Piroplasma equi not noted.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*R.*" *Donkey 608.* Injected as above.

Result.—

Slight and irregular reactions.
Moderate decrease of number of red corpuscles.
Piroplasma equi noted on the 16th day.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*S.*" *Donkey 609.* Injected as above.

Result.—

Slight and irregular reactions.
Moderate decrease of number of red corpuscles.
Piroplasma equi noted on the 16th and 18th days after injection.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

"*T.*" *Donkey 610.* Injected as above.

Result.—

Slight and irregular reactions.
Very slight decrease of number of red corpuscles.
Piroplasma equi noted on the 17th and 23rd days.
Recovered.

(Compare temperature and red corpuscle curve, Plate No. 6.)

Result.—The inoculation of young Argentine donkeys with immune mule blood caused a reaction in all of them, during which time there was a noticeable decrease of red corpuscles.

Piroplasma equi was not always noted.

One death occurred out of 20 inoculations = 5%.

The decrease of red corpuscles in some cases was so considerable that it is questionable whether, under the conditions of exposure and dry veld, they would have recovered. It is also likely that the young age had an influence on their recovery.

Conclusion.—From a practical point of view the inoculation of imported donkeys is only advisable when they can be sheltered and well fed during the reaction.

INOCULATION OF HORSES—ORIGIN UNKNOWN—AGAINST PIROPLASMOSIS.

(*a*) Subcutaneous injection of 5 c.c. defibrinated blood of immune Mule 589.

"*A.*" *Horse 781.* Gelding, aged. Immunised against horse sickness on the 31/1/06.

Injected on the 10/3/06 as above.

No reaction.

Hyperimmunised against horse sickness on the 23/6/06 with virus, origin Tzaneen.

Typical horse sickness reaction followed from which it recovered, but developed an attack of piroplasmosis, and died from this latter attack 14 days after the first infusion.

Note.—Mule 589 did not cause a reaction, yet the horse could be infected by infusion.

"*B.*" *Horse 823.* Gelding, aged. Immunised against horse sickness on the 8/2/06.

Injected on the 16/3/06 as above.

Reaction with high fever started on the 10th day after latter injection; piroplasma equi present.

Secondary reaction noted on the 19th day.
Piroplasma equi again present.
On the 26th day after piroplasmosis inoculation the horse was hyperimmunised—13/4/06.
Severe horse sickness reaction ensued.
Piroplasma equi and poikilocytosis were noted.
Horse 823 died from piroplasmosis on the 12th day after infusion.

Note.—Mule 589 did not cause a reaction; infusion could cause a second attack of piroplasmosis.

"*C.*" *Horse 672.* Gelding, aged.
Immunised against horse sickness on the 17/1/06.
Inoculated on the 16/3/06 as above.
No reaction.
Hyperimmunised on the 6/4/06, but did not shew piroplasmosis.

"*D.*" *Horse 837.* Gelding, aged.
Immunised on the 28/2/06 against horse sickness.
Injected on the 24/3/06 as above.
No reaction.
Hyperimmunised 18 days after injection.
Reaction followed, more typical for horse sickness than for piroplasmosis, during which time chromatic points were noted.

"*E.*" *Horse 667.* Gelding, 6 years old.
Immunised against horse sickness on the 17/1/06. No reaction.
Injected as above on the 24/3/06.
No reaction.
Hyperimmunised on the 10/4/06 without complication of piroplasmosis.

"*F.*" *Horse 666.* Gelding, aged.
Immunised against horse sickness on the 26/1/06.
Injected on the 24/3/06 as above.
No reaction.
Hyperimmunised on the 10/4/06 without the complication of piroplasmosis.

"*G.*" *Horse 586.* Gelding, aged.
Immunised on the 6/1/06 against horse sickness.
Injected, as above, on the 24/3/06.
No reaction.
Hyperimmunised on the 10/5/06 without complication of piroplasmosis.

"*H.*" *Horse 902.* Gelding, aged.
Not immune to horse sickness.
Injected, as above, on the 10/4/06.
No reaction.
Injected on the 24/4/06 with 5 c.c. defibrinated blood of donkey 306 (immune against piroplasmosis).
No reaction.

"*I.*" *Horse 903.* Gelding, aged.
Not immune to horse sickness.
Injected, as above, on the 10/4/06.
No reaction.
Injected on the 24/4/06 with 5 c.c. blood of donkey 306 (immune).
No reaction.

(*b*) Immune blood injected into jugular vein.

"*A.*" *Horse 656.* Gelding, aged.
Immunised against horse sickness on the 26/1/06.
Injected on the 20/4/06 with 5 c.c. defibrinated blood of mule 589.
Reaction started 6 days later.
Piroplasma equi noted at the beginning of the reaction.
Secondary reaction on the 23rd day, lasting 7 days with a very high fever (106° F.).
Piroplasms were numerous.

"*B.*" *Horse 670.* Gelding, aged.
Immunised against horse sickness on the 26/1/06.
On the 20/4/06 injected with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised on the 9/5/06 without complication of piroplasmosis.

"*C.*" *Horse 829.* Gelding, aged.
Immunised against horse sickness on the 23/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised, without complication of piroplasmosis, on the 9/5/06.

"*D.*" *Horse 866.* Gelding, aged.
Immunised against horse sickness on the 24/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised on the 2/5/06 without complication of piroplasmosis.

"*E.*" *Horse 867.* Gelding, aged.
Immunised against horse sickness on the 23/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
Slight abortive rise of temperature on the 8th day.
Distinct reaction from the 18th to 28th day, during which time piroplasma equi was found.
Hyperimmunised on the 7/6/06 without complication of piroplasmosis.

"*F.*" *Horse 868.* Gelding, aged.
Immunised against horse sickness on the 23/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised on the 2/5/06 without complication of piroplasmosis.

"*G.*" *Horse 872.* Gelding, aged.
Immunised against horse sickness on the 24/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised on the 10/5/06 without complication of piroplasmosis.

"*H.*" *Horse 881.* Gelding, aged.
Immunised against horse sickness on the 29/3/06.
Injected on the 20/4/06 with 5 c.c. blood of mule 589.
No reaction.
Hyperimmunised on the 10/5/06 without complication of piroplasmosis.

"*I.*" *Horse 536.*
Immunised against horse sickness on the 25/4/06.
Injected on the 14/6/06 with 5 c.c. blood of mule 589.
No reaction.

"*J.*" *Horse 1,900.* Gelding, aged.
Not immune against horse sickness.
Injected on the 22/5/06 with 5 c.c. blood of mule 589.
Reaction after 17 days; piroplasma equi not found.

"*K.*" *Horse 1,905.* Mare, aged.
Not immune against horse sickness.
Injected on the 22/5/06 with 5 c.c. blood of mule 589.
Reaction started after 17 days.
Piroplasma equi not found.

"*L.*" *Horse 1,907.* Mare.
Not immune against horse sickness.
Injected on the 22/5/06 with 5 c.c. blood of mule 589.
Reaction immediately after injection, but not typical for piroplasmosis.

"*M.*" *Horse 1,958.* Gelding.
Immunised against horse sickness on the 25/4/06.
Injected on the 22/5/06 with 5 c.c. blood of mule 589.
Temperature rose from the 6th day, continuing into a long reaction.
No piroplasms were seen during this reaction.

Results.—The subcutaneous injection of blood of mule 589 produced a reaction in one instance—823. No immunity was produced by this reaction, the subsequent infusion again causing the appearance of piroplasmosis. It is, however, most probable that the infusion was made too early after the piroplasmosis reaction.

When the blood of mule 589 failed to cause a reaction, a subsequent infusion nevertheless resulted in the rise of a typical reaction and appearance of piroplasma equi—cases 781 and 837.

The intrajugular injection of blood of mule 589 caused piroplasmosis with the appearance of parasites in two cases—656 and 1,857—and reactions without parasites in a similar number of cases —1,900 and 1,905.

Horses which were injected with blood of mule 589, and which shewed a reaction due to this injection, proved to be immune for a subsequent infusion.

Inoculation of Zebra Blood into a Susceptible Horse.

A young zebra mare, obtained through Mr. Turnbull, Government Veterinary Surgeon for Barberton, and which was caught in the low veld, was tapped on the 20/1/06, and 20 c.c. blood was injected into

Horse 405, Argentine, and directly imported.

A rise of temperature began after seven days; high fever resulted with the appearance of piroplasma equi which was very frequently noticed during this reaction.

Strong poikilocytosis was present and the animal had some difficulty to recover.

Conclusion.—The zebra must be considered as one of the carriers of piroplasma equi, in the same way as horses, donkeys and their bastards.

It is probably in this species—zebra—that the parasite first developed and found its host before horses and asses came to South Africa.

Resumé. (*a*) The inoculation of horses with immune donkey blood resulted in heavy mortality.

(*b*) Inoculation of horses with immune mule blood was followed by only one death out of nine cases in which a reaction was noticeable after injection.

(*c*) Inoculation of mules with immune mule blood caused only a slight attack of piroplasmosis, hence an inoculation of mules can be done with a great prospect of success.

(*d*) Inoculation of donkeys with immune mule blood caused the death of one animal. The inoculation has a certain amount of risk which is probably greater in old than in young animals. Under favourable conditions this inoculation may be utilised as a means of immunisation against piroplasmosis.

TRANSMISSION OF EQUINE PIROPLASMOSIS BY TICKS IN SOUTH AFRICA.

It was a matter of course to expect that equine piroplasmosis would be transmitted by ticks; it remained to find the species of ticks which acts as a host of piroplasma equi. Taking into consideration the prevalence of the disease in the various parts of the country—high and low veld—three separate species of ticks had to be regarded as possible hosts, namely, Rhipicephalus decoloratus (Koch), the "common blue tick"; Rhipicephalus evertsi (Neumann), the "red leg tick"; and Hyalomma ægyptium (Koch), the "bont pot tick." This latter changes its host in its intermediate stages; in its larval stage it seeks birds and small mammalia (rabbits), and leaves them as engorged nymphæ. It was for this reason that I excluded it from the experiments. The remaining two species are found in the high veld, where equine piroplasmosis is also found in freshly imported horses, and, under these circumstances, I decided to experiment firstly with these two species. It is quite possible that other species, for instance, Rhipicephalus appendiculatus, simus, capensis and nitens may act as hosts. The observations I made on the veld, however, exclude these as principal propagators of the disease. The experiments were, therefore, only made with those species, which, from observations in practice, proved to be most conspicuous.

Experiments with Rhipicephalus Decoloratus (Koch).

1. To note whether the larvæ of blue ticks—the progeny of females which have been sucking on a horse during the piroplasmosis reaction—transmit the disease to susceptible horses.

(*a*) Infection of ticks with piroplasma equi.

Horse 598. Argentine, 6 years old; was infested on the 14/10/04 with blue tick larvæ which had engorged on ox 262. The eggs were laid on the 30/9/04.

Re-infestations with larvæ of the same lot were made daily from the 16/10/04 to 23/10/04.

The first adult blue tick male was noticed on the 3/11/04—20 days after the first infestation.

This horse was injected on the 4/11/04 with a mixture of defibrinated blood of horses 442, 523 and 599, all of which had passed through an attack of biliary fever at some earlier date. (Compare article "Further Notes on the Piroplasmosis of the Horse, Mule and Donkey" in the "Transvaal Agricultural Journal," Volume XVIII., 1905, No. 12, page 703.)

Engorged adult ticks began to drop on the 6/11/04, and continued daily until the 22/11/04.

The febrile reaction due to the injection of immune blood started on the 8/11/04.

Piroplasma equi was noticed for the first time on the 13/11/04, on which date the typical rosettes were present.

The piroplasms were noticed during four days, viz., 13th, 14th, 15th and 16th November, but none were noticed from the 17/11/04 until the 23/11/04, during which time the fever reaction was not present, and engorged ticks were collected in great numbers.

The collected ticks were separated into four different lots, viz:

(1) Those which dropped from the 7th to the 10th November, at a time when no piroplasms were seen in horse 598, although a slight fever reaction was noticed;

(2) those which dropped from the 10th to the 12th November, during which time no piroplasms were found, but a distinct reaction was present;

(3) those which dropped from the 12th to the 14th November, during which time piroplasms were present; and

(4) those which dropped from the 16th to the 22nd November. The piroplasms were only present at the beginning of this stage—16/11/04.

(*b*) Infestation of horses with infected blue ticks.

The larvæ of lot No. 3 were placed on

Horse 604. Argentine; daily from the 16th to the 19th January, 1905, in large numbers.

Rise of temperature ensued from the fourth day after tick infestation of an irregular character and continued for about 8 days.

The adult blue ticks started to drop on the 22nd day after first tick infestation and continued for 8 days—7th to the 14/2/05.

Temperature again rose from the 20th day after tick infestation and reached 104.8° F. and 105.6° F. on the 29th and 33rd days respectively.

Examination of the blood during the fever reaction shewed neither the presence of piroplasms or any other blood parasite, and the reaction was put down to the heavy infestation with the blue ticks.

This horse was submitted to the simultaneous injection of serum and horse sickness virus on the 17/4/05. Preserved blood of horse 726, several months old, was used as virus; the serum was fresh and contained red corpuscles, thus piroplasma equi.

Horse sickness reaction followed, and, on the 14th day after inoculation, piroplasma equi was noticed.

The horse died on the 3/5/05.

Post-mortem revealed the typical lesion of biliary fever.

Horse 718. Argentine; infested with ticks of lot No. 3 on the 17th, 18th and 19th January, 1905.

Irregular temperature noticed from the 8th day after infestation.

A high reaction started on the 18th day and lasted for about three weeks.

Examinations of the blood proved to be negative.

The reaction was adduced to the infestation of the ticks, which were present in enormous numbers, and the horse shewed a well marked oedematous swelling under the stomach. (Compare Plate No. 9.)

The engorged ticks dropped from the 21st day after infestation, and continued dropping for 9 days.

The further history of this horse is very interesting, inasmuch as it developed an attack of biliary fever on the 15/3/06, the temperature rising on this date to 105.6° F., and piroplasma equi were present in great numbers. The animal did not recover from this attack; it became very poor and had to be killed on account of debility.

When the attack of biliary fever was noticed, a search was made for other than blue ticks, and an adult red leg tick was discovered under the tail. This tick must have been picked up in the paddock where the horse had been turned into after the experiment with blue tick larvæ had expired.

Donkey 743. Argentine; infested on the 17th and 18th January, 1906, with larvæ of lot No. 3.

Adults began to drop on the 7th February, and were collected until the 14/2/06.

Irregular temperature elevations ensued during the time the females were dropping, and continued even after the females had left the animal.

Horse 725. Argentine; infested on the 17th, 18th and 19th January, 1905, with blue tick larvæ of lot No. 4.

Aduts began to drop on the 17/2/05 and continued detaching up to the 25th.

No reaction was noticeable. (Compare experiments with Rhipicephalus evertsi.)

The horse died on the 27/7/05 from horse sickness.

Horse 601. Argentine; infested on the 17/1/05 with blue larval ticks of lot No. 4.

Engorged females dropped from the 7-15/2/06.

Irregular temperature elevations were noticeable at the time the adults were dropping.

Secondary reaction was observed three weeks after the ticks had finished to drop.

Temperature reached 105.4° F. during this reaction.

Examinations of the blood proved negative.

This horse was used for horse sickness experiments on the 17th April, 1905. It developed a horse sickness reaction which was complicated by piroplasmosis, and piroplasma equi was noticed on 1/5/06—14 days after injection of serum.
Died during the following day.

Horse 722. Argentine; infested on the 3/2/05 with blue tick larvæ of lot No. 4.
Engorged females began to drop on the 25/2/05—22 days after infestation.
Temperature rise was observed shortly before, and during, the dropping of adults.
Examinations of the blood proved negative.
(For further history of this horse compare experiments with the red leg tick.)

Mule 588. Argentine; infested with larval blue ticks of lot No. 4 on the 3/2/05.
The mule was able to rub the ticks off, and only a few engorged females were collected.
Was injected on the 12/3/05 with 10 c.c. defibrinated blood of horse 422 (immune against biliary fever).
No reaction.
Re-injected on the 21/3/05 with 10 c.c. defibrinated blood of horse 722.
Reaction was noticeable 12 days later.
Examinations of the blood proved negative.

Donkey 742. Argentine; infested on the 3/2/05 with blue tick larvæ of horse 598, lot No. 4.
Adults started dropping on the 21st day after infestation.
Irregular temperature ensued during this time, but, otherwise, the animal was normal.

Conclusions.

In the foregoing experiments, Rhipicephalus decoloratus (Koch) did not transmit piroplasma equi through its progeny from sick horse to healthy susceptible horse, mule or donkey. As Rhipicephalus decoloratus passes all its three stages on one and the same host, no other way of transmission would be possible.

We may, therefore, conclude that this tick does not act as a host of piroplasma equi.

EXPERIMENTS WITH RHIPICEPHALUS EVERTSI (NEUMANN).

(1) To note whether adult red leg ticks, which as larvæ and nymphæ had been feeding on a horse suffering from biliary fever, will transmit the disease to healthy animals.

(*a*) Infection of red leg ticks with piroplasma equi.

Horse 721. Argentine, 4 years old; was infested on the 9/2/05 with red leg larvæ, the offspring of females taken from oxen in Nelspruit on the 4/12/04.

On the 15/2/05 injected with 10 c.c. blood of mule 592, which passed through the biliary fever reaction in November and December, 1904.

Rise of temperature started six days after this injection and continued for 7 days, on which date—26/2/05—the animal died from piroplasmosis.

Piroplasma equi appeared for the first time on the 23/2/05, and was subsequently noticed daily.

Engorged nymphæ began to drop on the first day of the appearance of the piroplasms, or 14 days after tick infestation; they continued dropping until death, whereupon those which were still attached were collected.

These nymphæ were placed in glass dishes and began to moult on the 26/3/05, when the adults were used for the following experiments:—

(*b*) Infestation of horses with infected red leg ticks.

Horse 719. Argentine, 4 years old; infested on the 30/3/06 with red leg adults which, as larvæ and nymphæ, had been feeding on horse 721 then suffering from biliary fever. A second, third, and fourth infestation took place on the 1st, 5th and 8th April, 1905, respectively.

Piroplasma was found on the 17th day after the first infestation.

Fever reaction was present and the clinical diagnosis was certain.

The disease lasted 6 days, when the horse recovered.

Horse 725. Argentine, 4 years old; infested on the 31/3/05 with red leg ticks, adults, which, as larvæ and nymphæ, had been feeding on horse 721 during the piroplasmosis reaction.

Four re-infestations took place on the 3rd, 5th, 6th and 8th days.

The adults fed well.

Twenty days after the first tick infestation, or 12 days after the last one, a rise of temperature was noticed.

Examinations of the blood gave negative results.

Died later from horse sickness. (This animal is referred to in the experiments with Rhipicephalus decoloratus. Q.V.)

Horse 722. Argentine, 4 years old; infested with red adult ticks on the 3/4/05, which, as larvæ and nymphæ, had been feeding on horse 721.

A second and third infestation took place on the 5th and 8th days afterwards.

Sixteen days after the first infestation, or 9 days after the last, the temperature began to rise, and a reaction of 6 days in length ensued, at the end of which the animal died.

Piroplasms were noted from the 19th April until death.
Post-mortem revealed the typical lesions of biliary fever.

Donkey 742. Argentine, 3 years old; infested on the 30/3/05 with adult red leg ticks which, as larvæ and nymphæ, had been feeding on horse 721 during the fever reaction due to the presence of piroplasma equi.
Re-infestations took place on the 3rd and 5th April, 1905.
The adults were found attached and feeding on the 31/3/05.
No fever reaction ensued.
The donkey was killed on the 5/11/05 on account of glanders.

Conclusions.

Of three horses infested with adult Rhipicephalus evertsi (Neumann), which, in their larval and nymphal stage, were feeding on a horse suffering from piroplasmosis, two contracted the disease. One horse and one donkey which must be considered susceptible, although proof to that effect is wanting, were not infected by ticks belonging to the same lot.

(*a*) Infection of red leg ticks with piroplasma equi.

(2)

Horse 403. Argentine, 4 years old, was infested on the 9th, 10th, and 11th January, 1906, with numerous red larvæ ticks the progeny of adults taken from cattle at Linwood, and with adults taken from a horse suffering from piroplasmosis.
Injected subcutaneously on the 14/1/06 with 10 c.c. blood of horse 406, which had recently suffered from biliary fever.
Temperature rose on the 23/1/06, viz., 9 days after injection of blood.
Piroplasma equi was noticed on the same day; it remained for the four following days, but in rare numbers.
This horse recovered.
Nymphæ began to drop on the 24/1/06 and continued for 6 days. They were placed in glass dishes and kept at the temperature of the room.
The moulting in adults began on the 16/2/06—after a lapse of 22 days.

(*b*) Infestation of a horse with infected red leg ticks.

Horse 1,833. Argentine, 3 years old; infested with adults from horse 403 on the 1/3/06, viz., 12 days after moulting.
Engorged females began to drop on the 8/3/06—7 days later—and were collected in considerable numbers.
No reaction noticeable.
Injected subcutaneously on the 20/3/06 with 5 c.c. blood of mule 589, which had recently passed through an attack of piroplasmosis.

Temperature rose after the lapse of 9 days, following which a reaction lasted 7 days.

Piroplasma equi noted during this reaction for the two days the 3rd and 4th April, 1906.

The horse recovered after it had passed through a second reaction which started on the 16th April, 1906.

Conclusion.

The red adults infected on horse 403 did not transmit piroplasmosis to susceptible horse 833. No reason can be given for this failure. (Adult red legs were only 12 days old when placed on susceptible horse 1,833, viz., older than in previous experiments.

(*a*) Infection of red leg ticks with piroplasma equi.

(3).

Horse 1,401. Argentine, directly imported, and 4 years old.

Infested on the 16/2/06 with numerous red leg larvæ, the progeny of adults collected from cattle on Linwood farm.

Injected subcutaneously 24 hours later with 10 c.c. blood of mule 589, immune against piroplasmosis.

Temperature reached 103.2° F. in the evening of the 26/2/06.

Piroplasma equi was present in the blood on the same and following days, but was not seen again.

The horse recovered.

Nymphæ began to drop on the 25/2/06—10 days after infestation. They continued for five days during the fever reaction and were subsequently placed in glass dishes and kept at the temperature of the room.

These nymphæ moulted into adults on the 22/3/06, viz., 25 days after collecting.

The adults were used for the following experiments.

(*b*) Infestation of horses with infected red leg ticks.

"*A.*", *Horse 1,904.* Argentine, five years old; supposed to have been recently imported and on station since 14/3/06.

Infested on the 5/4/06 with adults collected on the 26/2/06, viz., on the first day of the presence of piroplasma equi.

Engorged females began to drop on the 11/4/06.

No reaction ensued from this infestation.

Re-infested on the 23/4/06 with adults off horse 401—32 days after moulting—and collected on various dates from the 25/2/06 to the 3/3/06.

Engorged females began to drop on the 17th day after this infestation.

Reaction noticeable on the 22nd day.

Piroplasma equi was noticed on the 23rd day; it was present for the next four days, and the horse recovered from the attack.

"*B.*" *Horse 1,906.* Argentine, aged; supposed to have been recently imported and on station since the 14/3/06.

Infested on the 5/4/06 with red adult ticks from horse 401, collected on the 28/2/06—on the third day after the appearance of piroplasma equi.
Engorged females dropped on the 7th day.
No reaction.
On the 23/4/06 this animal was re-infested with red adult ticks from horse 401, collected on the various days between the 26/2/06 and the 2/3/06, viz., 32 days after they had moulted.
Engorged females dropped on the 7th day.
No reaction.
This horse tested on its immunity to piroplasmosis by an injection of 5 c.c. blood of mule 589—immune against piroplasmosis—into the jugular vein on the 22/5/06.
Temperature rose after an incubation time of 16 days.
Reaction lasted 8 days.
Blood was examined during this reaction, but piroplasma equi was never found.

"*C.*" *Horse 1,910.* Argentine, 8 years old; supposed to be recently imported and on station since 15/3/06.

Infested on the 5/4/06 with red adults collected on the second day of the appearance of piroplasma equi from horse 401—27/2/06.
Engorged females dropped on the 7th day.
No reaction.
On the 23/4/06 red adults off horse 401 collected on various dates between the 25/2/06 and 3/3/06, and which had moulted, 32 days earlier, were placed on horse 1,910.
Engorged females began to drop on the 7th day.
Temperature began to rise on the 16th day after infestation.
It was very high for 6 days, culminating at 106° F.
Piroplasma equi was noticed for the first time on the 12/4/06, viz., 19 days after tick infestation, and was present for 8 days.
The animal recovered.

"*D.*" *Horse 1,905.* Argentine, aged; supposed to have been directly imported and on this station since 14/3/06.

Infested with adults off horse 401 on the 17th and 18th April, 1906; the adults were collected on the 26th, 27th and 28th February, 1906, and moulted 26 days previously.
Engorged females dropped on the 9th day.
On the 22nd day a reaction was noticeable lasting for 10 days.
Microscopical examinations gave a negative result.

Temperature reached 104.2° F. This was probably quite an accidental occurrence, and cannot be adduced to the tick infestation.

Injected on the 22/5/06 with 5 c.c. defibrinated blood of immune mule 589 into the jugular vein.

On the 18th day after this inoculation, a reaction occurred lasting 6 days, the temperature rising to 104° F.

The blood was examined microscopically during this reaction, but no piroplasma equi was found.

"*E.*" *Horse 1,913.* Argentine, 2 years old; supposed to have been directly imported from the Argentine, and on station since 14/3/06.

Infested on the 17th and 18th April with red adults off horse 401, collected on the 1st, 2nd and 3rd March, 1906, viz., after piroplasma equi had disappeared.

Engorged females began to drop on the 8th day.

On the 19th day after infestation the temperature rose and a typical reaction resulted.

Piroplasma equi appeared for five days subsequent to the reaction, viz., 8th to the 12th May, 1906.

The animal recovered.

"*F.*" *Horse 1,907.* Argentine, aged; supposed to have been directly imported from the Argentine, and on station since the 14/3/06.

Infested on the 27/4/06 with red adults off horse 401, collected on various dates between the 25th February and the 3rd March, 1906, and 35 days after moulting.

Engorged females dropped on the 8th day.

No reaction.

Tested on the 22/5/06 by an injection of 5 c.c. defibrinated blood of immune mule 589.

No reaction.

"*G.*" *Horse 1,908.* Argentine, aged; supposed to have been directly imported from the Argentine, and on station since the 14/3/06.

Infested on the 27/4/06 with red adults off horse 401, collected on various dates between the 25th February and 3rd March, 1906, and 35 days after moulting.

No reaction.

Tested on the 22/5/06 by an injection of 5 c.c. defibrinated blood of immune mule 589.

Reaction ensued 14 days after this injection, and continued for 11 days.

Examinations of the blood were frequently made, but no piroplasma equi could be found.

Conclusion.

Of six horses, supposed to be susceptible to piroplasmosis, infested with adult red leg ticks feeding in their larval and nymphal stage on a sick horse, three contracted the disease. The remaining three, which did not contract the disease, were tested with immune blood, but piroplasma equi was not found in any of them.

Remark.

It was discovered later that the aged Argentine horses were not directly imported, but had been resident in South Africa for some considerable time.

(4). (*a*) Infection of red leg ticks with piroplasma equi.

Horse 831. Argentine, 4 years old.

Infested on the 1/3/06 with numerous larvæ of the red tick hatched on 31/1/06; the mother ticks originated from cattle and were collected on the 17/12/05.

Injected subcutaneously on the 4/3/06 with 10 c.c. blood of horse 653, which, at that time, was suffering from biliary fever and had piroplasma equi in its blood.

Temperature rose after an incubation time of 6 days—1/3/06.

Piroplasma equi appeared on the 11/3/06 and was present until the 20/3/06.

Engorged nymphæ dropped on the 11/3/05—10 days after infestation, and continued dropping for 9 days.

This horse died on the 24/3/06 from piroplasmosis.

(*b*) Infestation of horses with infected red leg ticks.

Horse 2,062. Two year old gelding, directly imported from Argentine to Capetown and immediately transported by rail to the Laboratory stables.

Infested on the 17/6/06 with 25 red leg adult ticks from horse 831. Collected on the 13/3/06 and moulted on the 20/4/06.

Engorged females began to drop on the 30/6/06.

Further infestations on the 20/6/06 with same lot of ticks.

Temperature rose on the 6/7/06—21 days after first infestation.

Piroplasma equi noted on the 10/7/06. Temperature rose higher.

First reaction on the 15/7/06.

Second reaction on the 24/7/06.

This horse died from piroplasmosis, complicated with septic pneumonia, on the 27/7/06.

Horse 2,064.

Infested on the 17/6/06 with 25 red leg adult ticks from horse 831. Collected on the 13/3/06 and moulted on the 20/4/06.

Only a few attached themselves.

Re-infested on the 4/7/06—7 days after first infestation.

Temperature rose and piroplasma equi was noted on the 5/7/06; it was present until the 13/7/06.

This horse died from piroplasmosis on the 14/7/06.

Conclusion.

The two horses recently imported and susceptible to piroplasmosis, when infested with adults feeding in their larval and nymphal stages on horse 831 suffering from piroplasmosis, contracted the disease and died.

(5).

EXPERIMENTS WITH LARVÆ OF RHIPICEPHALUS EVERTSI, THE PROGENY OF FEMALES COLLECTED FROM HORSES SUFFERING FROM BILIARY FEVER.

Horse 832. Argentine, 4 years old; directly imported and on station since 12/2/06.

Infested on the 15/2/06 with numerous red larvæ the progeny of adults taken from a military horse suffering at that time from biliary fever, by Captain Olver, A.V.D.
Engorged nymphæ began to drop on the 27/2/06—12 days after infestation.
No reaction.
Injected subcutaneously with 5 c.c. blood of immune mule 589 on the 20/3/06.
Reaction began after 11 days.
Piroplasma equi was noticed during the reaction on three occasions.
Second reaction took place 25 days after infestation, again accompanied with piroplasma equi.
This horse died on the 4/5/06.
Autopsy revealed biliary fever.

Conclusions.

Red larvæ, the progeny of adults feeding as such, on a horse suffering from biliary fever, did not transmit the disease to a susceptible horse.

(6).

EXPERIMENTS WITH RED ADULTS FEEDING IN THEIR LARVAL AND NYMPHAL STATE ON A HORSE IMMUNE AGAINST EQUINE PIROPLASMOSIS.

(*a*) Infection of red leg ticks with piroplasma equi.

Horse 405. Three years old; freshly imported from the Argentine on the 20/1/05.

Injected intrajugularly with 20 c.c. defibrinated blood of a Burchell's zebra taken on the same day.
After an incubation period of 9 days, a typical piroplasmosis reaction ensued, during which piroplasma equi was very frequent.

On the 1/3/06, infested with numerous red larvæ, the progeny of adults taken from cattle.
Adults began to drop on the 14/3/06—13 days after infestation.
Moulted on the 20/4/06.

(*b*) Infestation of horses with infected red leg ticks.

Horse 968. Not freshly imported from the Argentine.
Infested with numerous adults of above lot from horse 405.
No reaction.

Horses 2,065 and 2,068. Freshly imported from the Argentine to Capetown and directly entrained to Pretoria, where they were kept in the stables of the Laboratory. Age, 2 years.
Infested with numerous adults off above horse 405.

Horse 2,068. No reaction.

Horse 2,065. Soon after its arrival it shewed a high fever reaction, and all the symptoms of pneumonia developed and were present 35 days after infestation with the ticks.
This animal died on the 22/7/06, having shewn on this day and the two previous days piroplasma in its blood. It is difficult to say when the fever reaction started, as the horse constantly had a high temperature and the diagnosis of pneumonia somewhat delayed the microscopical examination.

Post-mortem revealed the typical lesions of septic pneumonia and piroplasmosis.

Conclusion.

Of two horses which had to be considered as susceptible to biliary fever one contracted the disease from adult red leg ticks, which, in their larval and nymphal stage, had been feeding on a horse immune against piroplasmosis. This case was somewhat masked by the appearance of pneumonia, but the animal was always kept stabled, so that no other infection was possible.

Résumé.

In no instance did Rhipicephalus decoloratus transmit equine piroplasmosis, and it cannot, therefore, be considered as a host of piroplasma equi.

Rhipicephalus evertsi transmitted it in its adult stage after feeding as larvæ and nymphæ on a sick horse, in 7 out of 9 horses which had to be considered as susceptible to equine piroplasmosis.

Notwithstanding the fact that the only experiment failed, there is not yet sufficient proof to shew that piroplasmosis is transmitted through the egg of a tick.

Rhipicephalus evertsi is a host of piroplasma equi.

PIROPLASMOSIS IN HORSES DUE TO HYPERIMMUNISATION.

The hyperimmunisation of horses and mules for the purpose of fortifying against the disease, and thereby for the production of serum, is carried out by infusion.

The jugular vein of the sick animal is connected with the corresponding vein of the immune animal, and the blood is allowed to flow for about six minutes at one session. This infusion is usually repeated about three times, so that a horse receives about 8-9 litres of blood. Virulent blood is obtained from any broken down horse or mule which is healthy and in fair condition. It is quite natural to expect that amongst these virus animals a large proportion, and probably the greater part, to be immune against piroplasmosis; in other words that they contain piroplasma equi in the blood. It is, therefore, only logical to expect that some of the infused animals will contract piroplasmosis.

For the purpose of obtaining these serum animals, it has been our practice to select only such horses which, to judge by their appearance or age, could be considered as natives of South Africa, or by their long residence in this country to have attained immunity.

We have since discovered that it is more judicious to inoculate these animals previous to hyperimmunisation. This practice is more fully dealt with in another article. From a practical point of view, therefore, it is interesting to know to what extent this accident happens. The following list gives the details of the observations:—

Horse 1874.—First hyperimmunisation on 10/4/06.

Contracted piroplasmosis. Piroplasma equi present on 20-22/4/06. Second reaction with piroplasma on 11/5/05. Recovered.

Horse 1054.—First hyperimmunisation on 15/5/05.

Contracted piroplasmosis. Piroplasma equi present on 21-27/5/05.
Died of piroplasmosis on 29/5/05.

Horse 1062.—First hyperimmunisation on 15/5/05.

Immediate reaction. Piroplasma equi seen on 27/5-29/5/05.
Died on 4/6/06 from piroplasmosis.

Horse 1084.—First hyperimmunisation on 13/6/05.
Intermittent reactions (1) from 23/6-27/6/05.
„ „ (2) „ 3/7- 6/7/05.
„ „ (3) „ 11/7-19/7/05.
„ „ (4) „ 22/7-27/7/05.
„ „ (5) „ 29/7-31/7/05.
Piroplasma equi was noted on the 20/7/05—between third and fourth reactions.

Horse 719.—First hyperimmunisation on 9/7/05.
Second hyperimmunisation on 24/7/05.
Third „ 27/10/05.
Fourth „ 27/1/06.
Fifth „ 18/5/06.
Contracted piroplasmosis after the fifth infusion.
A long reaction was noticed during which piroplasma equi was present—3/6/06—5/6/06.
A second reaction was observed and piroplasma equi was present on 20/6/06.
The horse recovered.

Horse 1167.—First hyperimmunisation on 9/7/05.
Second hyperimmunisation on 18/7/05.
Contracted piroplasmosis.
Piroplasma equi was present on the 26-30/7/05.
Died on the 17/8/05 from piroplasmosis.

Horse 1216.—First hyperimmunisation on 15/8/06.
Contracted piroplasmosis.
Piroplasma equi present on 21/8/05-29/8/05.
The horse recovered.

Horse 1223.—First hyperimmunisation on 16/9/05.
Contracted piroplasmosis.
Shewed three reactions with piroplasma equi.
Killed on the 4/10/05 on account of bad condition.

Horse 1254.—First hyperimmunisation on 30/8/05.
Second hyperimmunisation on 5/9/05.
Contracted piroplasmosis on 24/10/05.
Piroplasma equi present on 30-31/10/05.
Killed on 6/11/05 on account of debility, a sequel of piroplasmosis.

Horse 1291.—First hyperimmunisation on 17/9/05.
Contracted piroplasmosis on 24/9/05.
Short reaction noticeable.
Piroplasma equi present on 4/10/05 and on 17/10/05.
Died on 17/10/05 from piroplasmosis.

Horse 1347.—First hyperimmunisation on 27/9/05.
Contracted piroplasmosis.
Piroplasma equi not seen during the reaction, but was observed on the 26/10/05.
Died on the 1/11/05 from piroplasmosis.

Horse 1309.—First hyperimmunisation on 3/10/05.
Second hyperimmunisation on 20/1/06.
Contracted piroplasmosis on the 3/2/06, on which date piroplasma equi was present.
Three distinct reactions observed.
Died on the 23/2/06 from piroplasmosis.

Horse 1322.—First hyperimmunisation on 5/10/05.
Second hyperimmunisation on 17/10/05.
Piroplasmosis and piroplasma equi were observed on the 7th day after the second hyperimmunisation—24/10/05.
The horse recovered.

Horse 1350.—First hyperimmunisation on 5/10/05.
Second hyperimmunisation on 22/2/06.
Contracted piroplasmosis.
Piroplasma equi present on 14th, 15th, and 17th day after second hyperimmunisation.
Killed on 1/5/06 on account of debility, a sequel of piroplasmosis.

Horse 1581.—First hyperimmunisation on 26/1/06.
Contracted piroplasmosis after an incubation time of six days.
Piroplasma equi present on 8th and 9th days—3-4/2/06.
Recovered from this attack, but died on the 8/3/06 with lesions of piroplasmosis.

Horse 1653.—First hyperimmunisation on 24/2/06.
Contracted piroplasmosis.
Piroplasma equi present on the 5th, 6th, and 7th day.
Recovered.

Horse 1661.—First hyperimmunisation on the 27/2/06.
Contracted piroplasmosis after an incubation period of 18 days.
Piroplasma equi present on the 21st day.
Recovered.

Horse 1837.—First hyperimmunisation on 2/4/06.
Contracted piroplasmosis.
Piroplasma equi was not seen, but chromatic points were noticeable.
Recovered.

Horse 1823.—First hyperimmunisation on 13/4/06.

Contracted piroplasmosis.
Piroplasma equi seen on the 4th day.
Died on the 25/4/06 from piroplasmosis.

Horse 1881.—First hyperimmunisation on the 11/4/06.

Second hyperimmunisation on the 12/7/06.
Contracted piroplasmosis from the second infusion, and died on 7/7/06.
Piroplasma equi was present on the 13th day—25/7/06.

Analysis.

The total number of horses hyperimmunised amounts to 147; of these 20 contracted piroplasmosis—13.6%.

This number may be divided as under:—

(1) Horses contracting piroplasmosis from the first hyperimmunisation—13 = 8.8%.

(2) Horses contracting piroplasmosis from the second hyperimmunisation—6 = 4.1%.

(3) Horses contracting piroplasmosis after three hyperimmunisations—1 = .7%.

Conclusion.

The greatest risk of causing piroplasmosis by infusion is in animals which are hyperimmunised for the first time.

There is still a certain amount of risk in subsequent infusions, probably due to the first virus horse not being immune against piroplasmosis.

Referring to the article on inoculation of piroplasmosis, it will be noticed that a horse which has undergone an inoculation and shewn piroplasma equi during the reaction may still contract the disease from hyperimmunisation. We shall, therefore, have to expect this contingency whenever we utilise piroplasmosis immune animals for hyperimmunisation purposes.

OBSERVATIONS AND EXPERIMENTS ON SWINE FEVER AND SWINE PLAGUE.

The question of the etiology of these two diseases has entered on a new phase, as a result of two publications, one by the Americans Dorset, Bolton and McBryde, on "Hog Cholera," and the other by Grips, Glage and Nieberle, on "Swine Plague," which have furnished the necessary impulse to pursue further investigations.

As a result of the introduction of the "Contagious Diseases of Animals Act" in the Transvaal, embodying compulsory notification of outbreaks of diseases, we have obtained possession of some knowledge regarding the prevalence of contagious diseases amongst swine, which, as far as circumstances have permitted, were investigated by us very closely.

The following experiments are not by any means complete, but they, nevertheless, allow us to make certain deductions about the nature of contagious diseases in the Transvaal. The name swine fever is given to a disease in pigs in England, and is identical with the disease hog-cholera in America. This malady—swine fever—was observed in the Transvaal, and diagnosed with certainty for the first time in November, 1903, in the neighbourhood of Johannesburg. Previous to this, reports were received from various parts of the country in connection with outbreaks of diseases amongst swine, killing large numbers, but the true nature of this disease was never ascertained.

Before we diagnosed swine fever in the Transvaal, the same disease was found in the Cape Colony—Western Province—where it caused considerable damage before it was recognised. We were inclined to connect the outbreaks in the Transvaal with those in the Cape, and, therefore, prohibited the importation of swine from that Colony. Since that time several outbreaks occurred, all of which have been diagnosed as swine fever. In the majority of cases the diagnosis was controlled in the Bacteriological Laboratory from pieces of intestines which were forwarded. Whenever lung lesions appeared in the sick pigs these were also forwarded. Since the pathological anatomical diagnosis of swine fever was beyond doubt, it was our primary object to trace the bacillus suipestifer in the diseased organs, and for this purpose the lymphatic glands of the caecum and the colon were opened; cultures were made on various occasions; also the spleen was utilised for these cultures. It was noticed that, from the very beginning, no case of swine fever came to our notice in which we could find the bacterium as described to occur in outbreaks of swine

fever in Europe. There was no doubt in our minds, however, that the disease was Swine Fever notwithstanding our failure to discover the bacillus, as the absence of this parasite was in accordance with the known information regarding this disease, namely, that bacillus suipestifer may disappear from the body of the sick animal shortly before death.

During the year 1905 our attention was drawn to the report of the Americans on the etiology of hog cholera wherein it was stated that this disease can be produced when the blood of a sick animal is injected into a healthy susceptible animal; that the disease produced in this way is propagated by simple contact with healthy pigs and those which have recovered from the disease prove to be immune. The blood of a sick animal produces the disease notwithstanding the absence of bacillus suipestifer, and this fact was proved by passing virulent blood serum through the Berkefeld and Chamberlain filters, the filtrate promptly producing the disease. The presence of bacillus suipestifer is not necessary to produce that malady designated by the Americans as hog-cholera, but notwithstanding this, the bacillus suipestifer seems to be found in connection with hog cholera. A subcutaneous inoculation of a culture made from bacillus suipestifer which proves fatal for rabbits and guinea pigs only produces a disturbance in a relatively small number of pigs, whereas the intravenous injection of even small quantities produces a deadly disease with great regularity, and the feeding of the culture occasionally results in death also; the lesions caused by bacillus suipestifer are similar to those found in pigs which contracted the disease by contagion; indeed, a distinction is hardly noticeable. The main difference, however, is that the disease produced by bacillus suipestifer is not contagious; it cannot be produced by contact or inoculation of blood, neither does recovery give immunity against hog cholera produced under the natural conditions by contagion. Bacillus suipestifer, therefore, does not play an important *rôle* in the etiology of the disease; it can complicate hog cholera, but does not produce it. These publications were so startling that, in the first instance, one could not help thinking that the American scientists were dealing with a new disease, but the data given by them are too exact to suspect that a mistake has arisen. Indeed, if we peruse the European literature on this subject, we notice that inoculation of blood from sick animals has not been carried out in Europe. The disease was naturally considered to be confined to the intestines, and, consequently, the bacterium was expected to be present in the lymphatic gland, where one was found with great regularity.

We identified swine fever in South Africa with hog cholera of the Americans, and we concluded that the observations made by the latter should also be made in South Africa. As a criterion of the disease, we considered (1) its inoculability with blood of sick animals in the absence of bacillus suipestifer; (2) the occurrence of the disease in healthy animals due to contact with sick animals infected

with blood free of bacteria; and (3) immunity of the recovered animal to injections of virulent blood.

The following experiments were made in connection herewith:—

Inoculation of the Disease with Blood.

At the beginning of November, 1905, the Government Veterinary Surgeon of Middelburg forwarded to the Laboratory organs from pigs suffering from swine fever. An assistant was immediately despatched to the scene of the outbreak with instructions to kill one or more pigs, to collect the blood, and to bring the intestines in order to verify the diagnosis. The blood from four sick animals was mixed in the Laboratory, and three pigs, "A," "B," and "C," about five months old, were subcutaneously injected.

Transmission by the First Generation.

(1) *Pig "A."*

Injected on the 19/11/05 subcutaneously with 20 c.c. mixture as indicated above.

Pig died on the 2/12/05, and the autopsy was made soon after death.

Post-mortem.

General condition—poor.

Large necrotic and caseating local lesion at the seat of inoculation behind the left shoulder.

Lungs—normal, with the exception of a few areas of atelectasis.

Heart—normal.

Spleen, kidneys and liver—normal.

Stomach—deeply congested with areas of commencing necrosis.

Caecum—slightly congested.

Ileo-caecal valve—several advanced areas of necrosis.

Small intestines—congested; contained several ascarides.

Diagnosis: Swine fever.

(2) *Pig "B."*

Found dead in the morning of the 13/12/05.

Post-mortem.

General condition—very emaciated.

Local lesion at seat of inoculation behind the right shoulder, about 3 inches square, necrotic but apparently in process of healing up. The centre contained a small quantity of pus. Right axillary gland slightly congested.

Lungs and heart—normal.

Spleen and kidneys—normal.

Stomach—normal.

Intestines—caecum contained a number of typical swine fever ulcers. Other parts of surface of caecum show well-marked diphtheric deposits.
Ileo-caecal valve showed marked ulceration and necrosis.
Small intestines—normal, with the exception of a few ascarides.
Diagnosis: Swine fever.

(3) *Pig "C."*
Died on the 2/12/05.

Post-mortem.

General condition—poor.
Large local lesion, necrotic and caseating, about 3 inches by 2 inches at the seat of inoculation behind the left shoulder.
Lungs—normal, with a few areas of atelectasis.
Pharyngeal glands—congested. Others normal.
Heart, spleen, liver and kidneys—normal.
Stomach—two patches of deep congestion, about 2 inches in diameter each. Commencing necrosis in parts.
Duodenum—slightly congested.
Caecum—deeply congested with diphtheric areas about the size of a pea, scattered all over the surface.
Ileo-caecal valve—one or two commencing ulcers in the neighbourhood of the valve.
Diagnosis: Swine fever.

TRANSMISSION OF THE SECOND GENERATION.

(4) *Pig 13.*
On the 3/12/05 an emulsion was made with the coagulated blood of Pig "A," and 5 c.c. was injected into a young pig No. 13. The blood of Pig "A" was examined microscopically. Polychromatic cells, but no bacteria, were noted. After an incubation time of 13 days the temperature of Pig 13 began to rise; the fever continued during the following 7 days, and the pig died in the afternoon of the 20th day.
On the first and last day of the reaction, the blood was tapped from the femoral artery.

Post-mortem, made on the 22/12/05.

General condition—poor.
Skin of loins—diffusely reddened. Haemorrhage under the skin at the place of the operation when the blood was tapped. Numerous petechiae under peritoneum.
Lungs—very pale ; four small haemorrhages under the pleura.
Lymphatic glands—congested and swollen.
Heart—normal.
Spleen—swollen.
Liver and kidneys—pale.

Stomach—deeply congested.

Intestines—the caecum contained a number of ulcers the size of a threepenny piece distributed over the surface. On and in the neighbourhood of the ileo-caecal valve the ulcers were confluent and stained with bile. The small intestines and the colon contained numerous haemorrhages ; Peyers patches all show punctiform haemorrhages along colon and duodenum.

Diagnosis : Swine Fever.

(5) *Pig 18.*

On the 3/12/05 Pig "B," which was undergoing reaction, was tapped from the femoral artery, and blood injected into Pig 18.

The fever started after an incubation time of 6 days ; it lasted 6 days and the pig died during the night of the 14-15/12/05.

Post-mortem, made on the 15/12/05.

General condition—very poor.

Lymphatic pharyngeal glands—congested.

Lungs—the whole of the right apex was consolidated, and contained several necrotic patches ; some of these latter were becoming caseous. The lower margin was collapsed, and there were also several collapsed patches in the lung tissue. The right bronchial gland was enlarged and necrotic.

Heart—normal.

Kidneys—congested; surfaces showed numerous punctiform haemorrhages.

Spleen—slightly swollen.

Liver—enlarged and congested.

Stomach—congested.

Intestines—caecum showed numerous small ulcers about the size of a pea. Ileo-caecal valve congested without ulceration. The mucosa of the colon was congested with numerous punctiform haemorrhages.

(Compare later with cultures made from the lungs.)

Diagnosis: Swine fever, complicated with swine plague.

Transmission of the Third Generation.

(6) *Pig 15.*

Inoculated on the 18/12/05 with 2 c.c. serum of blood of Pig No. 13, mixed with 18 c.c. boullion. This serum was derived from blood which had been taken on the 16/12/05—14 days after inoculation—and at the beginning of the febrile reaction. Cultures had been made on an agar-agar, but remained sterile. After an incubation time of 10 days the fever reaction started ; this fever had the character of

a febris continua, and reached 108° F. The animal died on the 20th day after inoculation—5/1/06.

Post-mortem, made on the morning of the 6/1/06.

General condition—fair. Recent haemorrhages on both ears. Abdomen shows numerous echymoses under the skin.

Lungs—a few punctate haemorrhages on surface ; otherwise normal.

Heart—pericardial sac contained about 3 ozs. of clear fluid. endocardium contained a few petechiæ in both auricles; three present in left ventricle.

Spleen—enlarged.

Liver—normal.

Kidneys—cortices showed numerous punctiform haemorrhages.

Stomach—patches of marked congestion.

Intestines—duodenum, jejunum and ileum showed numerous punctiform haemorrhages. Peyer's patches congested. Caecum and large colon markedly congested, and thickly scattered over the whole surface, specially in the caecum, small circular areas of necrosis were present. Caecum contained several well defined swine fever ulcers, also in neighbourhood of ileo-caecal valve. Small colon intensely congested. Rectum moderately congested.

Lymphatic glands—all very congested and swollen.

Compare later for cultures made from heart blood, spleen and mesenteric glands.

Diagnosis: Swine fever.

(7) *Pig 6.*

Inoculated on the 22/12/05 with 6 c.c. blood of Pig No. 13, taken during *post-mortem* examination.

After an incubation time of 6 days a high fever started, which continued for 14 days. The pig died during the night of the 12-13/1/06—22 days after inoculation.

Post-mortem, made in the morning of the 13/1/06.

General condition—poor. A diffuse reddening of skin in region of loins. Haemorrhage under skin at the seat of inoculation.

Lungs—very pale ; three or four haemorrhages under pleura.

Lymphatic glands—inguinal, pharyngeal, bronchial, &c., all congested and swollen.

Heart—normal.

Spleen—swollen.

Liver and kidneys—pale.

Stomach—deeply congested in parts.

Intestines—caecum contained a number of ulcers about the size of a threepenny piece scattered over the surface. On, and in neighbourhood of ileo-caecal valve the ulcers were

confluent and bile-stained. Peyer's patches all show punctiform hæmorrhages. Numerous hæmorrhages along colon and duodenum.

Diagnosis: Swine fever.

(8) *Pig 9.*

Inoculated on the 3/1/06 subcutaneously with 5 c.c. blood of pig No. 4 taken on the same day during the *post-mortem* examination.

High continuous fever developed after an incubation time of 10 days, and the pig was killed in order to obtain the blood.

Post-mortem made on the same day.

General condition—good. Very little reddening of skin in any part. Local lesion about the size of a walnut on the seat of injection. Skin was intact, but the swelling contained a soft cheesy material.

Lungs—left lung adherent to chest wall for about ½ inch in diameter. The base of both lungs were consolidated, each area being about the size of the palm of a hand. On incision these areas were seen to be of pure necrosis; no caseation was observed anywhere.

Heart—a few petechiae on right auricle.

Spleen and liver—normal.

Kidneys—a few punctate hæmorrhages on the cortex.

Stomach—normal.

Intestines—normal.

Lymphatic glands—slightly congested.

Diagnosis: Swine fever, complicated with swine plague. This latter diagnosis was not controlled bacteriologically.

Experiments to Transmit Swine Fever by Contact.

On the 6/1/06, pigs Nos. 10 and 11 were placed into the stall occupied at that time by pig No. 6. This latter pig, as already noted, had been inoculated on the 22/12/05 with blood of pig No. 13. On the day of the first contact pig No. 6 was in high fever and died six days later. The contact during six days was, therefore, a very close one.

(9) *Pig 10.*

Rise of temperature noted 6 days after contact which continued into a febris continua, and lasted until the 24/1/06, *i.e.*, incubation time of 6 days and a fever period of 12 days. On this latter date the pig was killed for the purpose of obtaining blood.

Post-mortem, made on the 24/1/06.

General condition—fair. No discolouration of skin.
Lungs—in right lung a patch of consolidation about the size of the palm of a hand, containing a number of necrotic areas.
Heart—a little fluid in pericardial sac.
Spleen, liver, kidneys, and stomach—normal.
Intestines—Ileum contained a few punctate hæmorrhages scattered throughout the whole length. Caecum showed a few necrotic black-stained areas, mainly around the ileo-caecal valve. These areas appeared to be healing up.
Lymphatic glands—slightly swollen, but not congested.
Diagnosis: Swine fever, complicated with swine plague. This latter diagnosis was not controlled bacteriologically.

(10) *Pig 11.*

Fever developed on the 8th day after contact with No. 6; it lasted until the 20/1/06—7 days. Pig died during the night of the 20-21/1/06.

Post-mortem made on the 21/1/06, in the morning.

General condition—fair. Skin reddened over scrotum, abdomen, sternum, and submaxillary space. Submaxillary glands slightly enlarged and congested.
Lungs—oedematous. Both interior lobes hepatised, and contained yellow necrotic areas about the size of a sixpence.
Heart—normal.
Stomach—mucous membrane shewed a deep red patch about the size of the palm of a hand. Mucosa throughout the jejunum, and ileum dotted with fine punctiform hæmorrhages, especially marked in lower end of ileum. Caecum and first third of colon uniformly covered with necrotic membrane, under which were hæmorrhagic patches. Mesenteric glands were enlarged and deeply congested.
Spleen—slightly enlarged.
Liver—normal.
Urinary bladder full.
Diagnosis: Swine fever, complicated with swine plague. This latter diagnosis was not controlled bacteriologically.

Immunity.

On the 2/12/05, pig No. 12 was inoculated with 1 c.c. blood of pig "C." After an incubation time of six days, the fever developed and lasted six days; normal temperature was observed after this.

This pig was injected at various times subsequently, as under:—

On the 17/1/06 with 10 c.c. blood of pig 15.
„ 1/2/06 „ „ „ „ 9.
„ 26/2/06 „ 15 „ „ from a spontaneous case of swine fever.

The result was that none of these injections produced a febrile reaction, therefore the pig had obtained a strong immunity from the first attack of swine fever.

Bacteriological Investigations.

In order to trace the bacillus suipestifer, the following cultures were made:—

(1) Pig No. 18: Blood and lymphatic glands of the intestines.
(2) Pig 13: Blood and lymphatic glands of the intestines.
(3) Pig No. 6: Lymphatic glands of the intestines.
(4) Pig No. 9: Blood and lymphatic glands of the intestines.
(5) Pig No. 10: Blood and lymphatic glands of the intestines.
(6) Pig No. 7: Blood and lymphatic glands of the intestines.

Several test tubes containing agar-agar were inoculated with the blood; the lymphatic glands were touched with a red hot iron; a piece was then torn off and rubbed over the slanting surface of the agar test tubes. It is evident that in every case we obtained colonies from the lymphatic gland. All cultures were examined microscopically, and no similarity to bacillus suipestifer was noted. Sub-cultures were made to prove or disprove the identity with this bacillus. Bacteriological investigations for bacillus suisepticus were only made in pig No. 18; in no case, either in the blood or in the lymphatic glands of the intestines, could bacillus suipestifer be traced.

Conclusions.

The pathological and anatomical description given in the ten *post-mortem* reports correspond with those described by the American investigators on hog cholera. The South African swine fever corresponds in all the criterions which we tested with those given by the latter scientists.

On comparison of our *post-mortem* reports with those published by the European investigators, we must admit that no difference could be established between our disease and the swine fever of Europe. The striking point in our investigations is the fact that we were never able to trace bacillus suipestifer. We may confidently state that it was never present in the pigs at the time of the examination, but we do not draw the conclusion therefrom that it is not present in South Africa. We were able, however, to produce the disease with blood which did not contain any visible bacteria, and, therefore, we conclude further that the presence of bacillus suipestifer is not necessary for the development of the disease. Accordingly, swine fever is not caused by bacillus suipestifer.

Swine Plague.

(1) The existence of a bacillus corresponding to the description of bacillus suisepticus in South Africa was proved for the first time in September, 1904. At that time Government Veterinary Surgeon Edgar, of Pietersburg, forwarded the lungs, heart, and intestines of a

pig. We found extended hepatisation in the lungs, with a few necrotic areas; there was also a very pronounced congestion of the mucosa of the caecum and colon. Suspicion of swine plague was immediately pronounced and the Government Veterinary Surgeon was informed to that effect. Six weeks after the death of the first pig, a second pig died; the cause of death was not, however, ascertained. An investigation was made, but no further deaths occurred. On the 7/9/04—that is on the day the sick organs were received—a rabbit was subcutaneously injected with the blood of the heart; the rabbit died on the 9/9/05, and the *post-mortem* revealed oedematous lungs and a pleuritis serofibrinosa. A few bipolar bacteria were found in the blood. Cultures were made on an agar; they proved to be pure. On the 23/9/04, a second rabbit was injected subcutaneously with 5 c.c. of the pure culture obtained from rabbit No. 1. It died on the 26/9/04, and *post-mortem* lesions were found of pleuritis serofibrinosa, pericarditis, and pneumonia. The blood contained a few bipolar organisms. On the 12/10/06, a pig was inoculated subcutaneously with 5 c.c. culture, but without success.

(2) On the 1/2/05, Government Veterinary Surgeon Dunphy, of Krugersdorp, forwarded a living pig, which died soon after its arrival.

Post-mortem revealed the lesions of a pericarditis, pleuritis, serofebrinosa and a croupous pneumonia.

Cortex of kidney—contained several petechiæ.

Liver and spleen—normal.

Stomach—contained lesions of acute gastritis, and in the caecum were encountered five ulcers of the size of a sixpenny piece.

Lymphatic glands—infiltrated with blood, and swollen.

Diagnosis: Swine fever, complicated with swine plague.

On the 18/2/05, an investigation was made *in loco*, and the *post-mortem* of three pigs all revealed swine fever ulcers and one also shewed the lesions of pneumonia; this latter proved to be swine fever complicated with swine plague.

(3) On the 4/8/05 a sick pig was brought into the Laboratory which came from "Swartkopjes." It died the next day.

Post-mortem made on the 5/8/05.

Livid discolouration of the skin. Peritoneal cavity contained a fair amount of dark coloured liquid. Pericard was filled with the same liquid, and both lungs contained hepatised areas.

Mucous membrane of the stomach—small hæmorrhages present.

Intestines—contained petechiæ throughout the whole length.

Mucous membrane of the lungs—congested, and contained numerous ulcers.

Lymphatic glands—swollen and hepatised.

On the surface of the kidney were numerous small spots.

Diagnosis: Swine fever, complicated with swine plague.

Bacteriological investigation: With the blood taken from the heart, and the fluid from the mediastinal lymphatic gland, cultures were made which all appeared as pure bacillus suisepticus. A series of feeding experiments were made with this culture, for which compare later.

(4) Under the ten inoculation experiments of swine fever, in five instances the lesions were found to be of a mortifying inflammation of the lungs which we diagnosed as swine plague, based on the pathological anatomical change. One pig, No. 18, was examined bacteriologically, and this case is especially important because swine plague was complicated with swine fever, which latter disease was produced in the blood of a pig which, on *post-mortem*, only shewed the lesions of swine fever. The swine plague, therefore, could not have been produced by the inoculation of the blood, which has also to be excluded in the remaining cases. Cultures were made from the necrotic area in the lungs of pig No. 18.

On the 21/12/05, 5 c.c. boullion was inoculated into a rabbit, which died two days later. *Post-mortem* was made on the same day —23/12/05.

A hæmorrhagic oedema at the seat of inoculation.

Numerous blood points in the lungs.

The heart blood contained numerous bacteria which, on agar-agar, appeared as pure cultures. They proved to be bacillus suisepticus.

The Characters of the South African Bacillus Suisepticus.

The characters of the bacillus suisepticus are as follows:—

Staining.—Does not stain according to Gram.

Form and Shape.—Bipolar bacterium in the blood smears and tissues. An ovid shape in the culture.

Motility.—None.

Growth on Ordinary Agar.—Begins as small colonies which extend later; colour—white to greyish-white.

On Grape Sugaragar.—Does not produce gas.

Gelatine.—Does not liquify.

Boullion—At the beginning homegeniously cloudy; clears up later and forms a precipitate.

Milk.—No coagulation.

Indol.—None produced.

The pathogeny has already been demonstrated for rabbits, and we again refer to it in the following experiments on Experimental Swine Plague.

Experimental Swine Plague.

Pig 1. Fed on the 18/9/05, with 300 c.c. boullion culture of bacterium suisepticus which was isolated from the heart blood of pig "Swartkopjes." Temperature began to rise on the 20/9/05, reaching 104.8° F. in the evening; the following morning it recorded 105.4°F.,

and in the evening 107.6° F. The temperature subsequently remained between 103.1° F. in the morning, and 105° F. in the evening. On the 28/9/05 it dropped below 102.2° F., and the pig died during the night.

Post-mortem made in the afternoon of that date.

Stomach and intestines—in a state of very strong congestion. Numerous ascarides were present.

Owing to the advanced state of decomposition of the cadaver no cultures were made.

Pig 2. On the 18/9/05, this pig was fed with 300 c.c. boullion culture from the pulmonary lymphatic gland of pig "Swartkopjes." The temperature began to rise two days later, and reached 104.8° F. in the evening of that day—20/9/05. The following morning it reached 106°F., but gradually dropped and became sub-normal on the 27/9/05. The pig died on the 28/9/05.

Post-mortem.

Heart, lungs, kidney and spleen—normal.

The mucosa of the stomach and intestines strongly congested.

Caecum—shewed patchy congestion.

Numerous ascarides were present.

Cultures were made from the blood of the heart, and colonies were obtained which resembled those described in every respect.

Rabbit.—On the 12/10/05, 2 c.c. of a boullion culture was injected into a rabbit. It died during the night of the 16/10/05.

Post-mortem.

Strong congestion throughout the intestines.

The blood contained numerous bacteria, and the phenomenon of phagocytosis was very pronounced.

The cultures which were made proved to be pure.

Pig 4. Fed on the 10/10/05, with pure boullion culture obtained from pig No. 2. Rise of temperature was noted in the morning of the 13/9/05, and reached 105.3° F.; it continued at this figure in the evening, but oscillated during the following day between that figure—105.3° F.—as the evening maximum and 103.2° F. as the morning minimum. The pig died during the night of the 19-20/10/05.

Post-mortem revealed extended hepatisation in both apices of lungs; necrotic areas were present.

Heart, spleen, liver, and kidney were all healthy.

The pylorus was strongly congested.

Mucous membrane of the small intestines—diffusely reddened, especially the necrotic patches.

Caecum and colon normal.

Ascarides were found in the intestines.

Pig No. 2a. Fed on the 10/10/05, with 300 c.c. of a boullion culture obtained from the mediastinal lymphatic gland of pig "Swartkopjes." The temperature began to rise after three days, and shewed a very irregular curve. The pig was found dead in the morning of the 28/10/05.

Post-mortem made the same day.

Heart—normal.
Kidney, liver and spleen—normal.
Mucous membrane of the stomach and of the small intestines —strongly congested. Symptoms of diarrhœa very pronounced.
Ascarides were found in large numbers.
Cultures were made from the heart, and the colonies were found to be pure.

NEGATIVE RESULTS.

Pig 5. Further feeding experiments were made with pig No. 5. On the 28/10/05, this animal was fed with 200 c.c. boullion culture obtained from the heart of rabbit No. 4. No rise of temperature ensued, and the animals were alive 14 days later.

Pig 6. Fed on the 28/10/05 with 200 c.c. boullion culture obtained from bacterium of heart of pig No. 2a. No rise of temperature; the animal was still alive 42 days later.

Pig 7. Fed on the 11/11/05, with 200 c.c. boullion culture obtained from blood of heart of pig 11. No rise of temperature ensued, and the animal was utilised 35 days later for another experiment.

Conclusions.

The characters typical for bacillus suisepticus of Europe are also found in South Africa. The pathological lesions with which this bacterium is associated, and which in Europe are considered to be typical for swine plague, are also noted in South Africa. Contrary to European experiments, we were able to kill four out of seven pigs, of which one shewed lesions typical for swine plague, by means of feeding large quantities of bacteria. It is possible that the presence of ascarides has something to do with it, since a similar occurrence is reported by Salmon in America. But what is of special interest, and is really the point at issue, is the fact that, in South Africa under natural conditions, the lesions of swine plague are found, as a rule, associated with those of swine fever. We have only had one observation of a sporadic case of swine plague in which bacillus suisepticus was found and where swine fever had to be excluded. The South African experiences will, therefore, have to be interpreted as follows: Epidemics caused by bacillus suisepticus amongst pigs do not exist whenever the presence of swine plague was noted; it was associated with swine fever. The conception, therefore, that bacillus suisepticus is generally a saprophyte, and only develops on an animal under favourable conditions, seems also to hold good in South Africa.

FURTHER EXPERIMENTS WITH IMMUNISATION OF MULES AGAINST HORSE SICKNESS.

PROPORTION OF MIXED HORSE AND MULE SERUM IN EQUAL QUANTITIES. VIRUS INJECTED INTO JUGULAR VEIN.

Experiment 232. 31/5/06.

First injection 350 c.c. serum mixture No. 5 (horse serum), and No. 6 (mule serum) dated 3/5/05, in equal quantities.
Second injection 100 c.c. on the 4/6/06. Virus 726.

Remark.—Serum mixture 5 consisted of serum of 12 horses.
Serum mixture 6 consisted of serum of 29 mules.

Nos.		Result.
104	Reaction, dikkop, 10th day	Recovered.
105	„	„
106	„	„
107	„	„
108	„	„
109	„	„
110	Signs of dikkop on the 9th day	Died of dikkop 10/4/06.
111	Reaction	Recovered.
112	„	„
113	„	„
114	„	„
115	„	„
119	„	„
120	„	„
121	„	„
122	„	„
123	„	„
124	„	„
125	„	„
126	„	„
127	„	„
128	„ dikkop on the 12th day	„
129	„	„
130	No reaction	„

Result.—Of 24 mules one died of horse sickness.

Experiment 233. Dated 31/5/06.

First injection 300 c.c. of mixture 5 and 6; date, 3/5/06.
Second injection 150 c.c.
Virus 726.

Nos.		Result.
131 ..	Reaction	Recovered.
132 ..	„	„
133 ..	„	„
134 ..	„ dikkop on the 12th day	„
135 ..	„	„
136 ..	„	„
137 ..	„	„
138 ..	„	„
139 ..	„	„
140 ..	„	„
141 ..	„	„
142 ..	„	„
143 ..	„	„
144 ..	„ dikkop on the 12th day	„
145 ..	„	„
146 ..	„	„
147 ..	Shewed signs of dikkop on 9th day	Died 10/6/05.
148 ..	Reaction	Recovered.
149 ..	„ dikkop 10th day ..	„
150 ..	„	„
151 ..	„	„
152 ..	„ dikkop 9th day ..	„
153 ..	„	„
154 ..	„	„

Result.—Of 24 mules, one died of horse sickness.

Experiment 234. Date, 14/7/05.

First injection 300 c.c. of serum mixtures 19 and 20, in equal parts, and dated 27/6/06.
Serum mixture 19 consisted of serum of 16 horses.
Serum mixture 20 consisted of serum of 12 mules.
Second injection, 150 c.c., on the 18/7/06.

Nos.		Result.
212 ..	Reaction	Recovered.
213 ..	„	„
244 ..	„	„
245 ..	„	„

Conclusion.—None of the mules died of horse sickness.

Experiment 235. Date, 28/8/05.

First injection on the 28/8/05 with 300 c.c. serum mixture 31 and 32, dated 8/8/05.

Serum mixture 31 consisted of serum of 14 horses.

Serum mixture 32 consisted of serum of 27 mules.

Nos.		Result.
202 ..	Reaction, dikkop on the 17th day	Died on 15/9/05.
305 ..	„	Recovered.
306 ..	„	„
295 ..	„	„
296 ..	„	„
297 ..	„	„
298 ..	„	„

Result.—Of 7 mules inoculated, 1 died of horse sickness eighteen days after inoculation.

Experiment 236. Date, 2/9/05.

First injection, 300 c.c. of serum mixtures 29 and 30, equal parts, dated 1/8/06.

Serum mixture 29 consisted of serum of 17 horses.

Serum mixture 30 consisted of serum of 31 mules.

Second injection, 150 c.c., on the 6/9/05.

Virus 726.

Nos.		Result.
323 ..	Reaction	Recovered.
324 ..	„	„
325 ..	„ dikkop, 13th day ..	„
326 ..	„	„
327 ..	„	„
328 ..	„	„
329 ..	„	„
330 ..	„	„
331 ..	„	„
332 ..	„	„
333 ..	„ dikkop, 13th day ..	„
334 ..	„	„
335 ..	„	„
336 ..	No reaction	„
337 ..	Reaction	„
338 ..	„	„
339 ..	„ dikkop, 17th day ..	„
340 ..	„	„
341 ..	„	„
342 ..	„	„
343 ..	„	„
344 ..	„	„
345 ..	„	„
346 ..	No reaction	„

Result.—None of the 24 mules inoculated died from horse sickness.

Experiment 237. Date, 20/10/05.

First injection, 300 c.c. of serum mixture 41 and 42, in equal parts, dated 11/9/05.
Serum mixture 41 consisted of serum of 21 horses.
Serum mixture 42 consisted of serum of 38 mules.
Second injection, 150 c.c., on the 24/10/05.
Virus 726.

Nos.		Result.
407	Reaction, dikkop, 13th day	Recovered.
408	„	„
409	„	„
410	„ dikkop, 17th day	„
411	„	„

Result.—No mule died from horse sickness.

Experiment 238. Date, 2/11/05.

First injection, 300 c.c. of serum mixture 43 and 44, in equal parts, dated 18/9/05.
Serum mixture 43 consisted of serum of 20 horses.
Serum mixture 44 consisted of serum of 42 mules.
Second injection, 100 c.c., on the 7/11/05.
Virus 726.

Nos.		Result.
412	Reaction	Recovered.
413	„	„
441	„	„
442	„	„

Result.—None of the four mules died from horse sickness.

Experiment 239. Date, 10/11/05.

First injection, 300 c.c., serum mixture 49 and 50, in equal parts, dated 9/10/05.
Serum mixture 49 consisted of serum of 25 horses.
Serum mixture 50 consisted of serum of 55 mules.
Second injection, 100 c.c., on the 14/11/05.
Virus 726.

Nos.		Result.
456	Reaction	Recovered.
457	„	„
458	„	„
459	„	„
460	„	„

Result.—None of the four mules died.

Experiment 240. Date, 10/11/05.

First injection, 300 c.c. serum mixture 51 and 52, in equal parts, dated 16/10/05.
Serum mixture 51 consisted of serum of 26 horses.
Serum mixture 52 consisted of serum of 60 mules.
Second injection, 100 c.c., on the 14/11/05.
Virus 726.

Nos.		Result.
461	Reaction	Recovered.
462	„	„
463	„	„
464	„ dikkop, 15th day	„
455	„	„

Result.—None died from horse sickness.

Experiment 241. Date, 17/11/05.

First injection, 300 c.c. serum mixture 37 and 38, in equal parts, dated 28/8/05.
Serum mixture 37 consisted of serum of 20 horses.
Serum mixture 38 consisted of serum of 35 mules.
Second injection, 100 c.c., 21/11/05.
Virus 726.

Nos.		Result.
465	Reaction	Recovered.
466	„ dikkop, 11th day	Killed, suspected glanders.
467	„	Recovered.
468	„ dikkop, 12th day	„
469	„	„

Result.—None died from horse sickness.

Experiment 242. Date, 17/11/05.

First injection, 300 c.c. serum mixture 45 and 46, in equal parts, dated 27/9/05.
Serum mixture 45 consisted of serum of 22 horses.
Serum mixture 46 consisted of serum of 50 mules.
Second injection on the 21/11/05.
Virus 726.

Nos.		Result.
472	Reaction	Recovered.
473	„	„
474	„	„
475	„	„
476	„	„

Result.—None died.

Experiment 243. Date, 17/11/05.

First injection, 300 c.c. serum mixture 53 and 54, in equal parts, dated 23/10/05.
Serum mixture 53 consisted of serum of 26 horses.
Serum mixture 54 consisted of serum of 52 mules.
Second injection, 100 c.c., on the 21/11/05.
Virus 726.

Nos.		Result.
477	Reaction	Recovered.
478	„	„
479	„	„
480	„	„
481	Dikkop on 11th day	Died on the 30/11/05.

Result.—Of five mules, one died of dikkop.

Experiment 244. Date, 17/11/05.

First injection, 300 c.c. serum mixture 55 and 56, in equal parts, dated 30/10/05.
Serum mixture 55 consisted of 25 horses.
Serum mixture 56 consisted of 66 mules.
Second injection, 100 c.c., on the 21/11/05.
Virus 726.

Nos.		Result.
482	Reaction, dikkop, 13th day	Recovered.
483	„	„
484	„	„
485	„	„
486	„ dikkop, 11th day	„

Result.—None died of horse sickness.

Experiment 245. Date, 18/11/05.

First injection, 300 c.c. serum mixture 47 and 48, in equal parts, dated 2/10/05.
Serum mixture 47 consisted of serum of 22 horses.
Serum mixture 48 consisted of serum of 58 mules.
Second injection, 100 c.c., on the 22/11/05.
Virus 726.

Nos.		Result.
496	Reaction	Recovered.
497	„	„
498	„ dikkop, 11th day	„

Result.—None died of horse sickness.

Experiment 246. Date, 20/11/05.

First injection, 300 c.c. serum mixture 33 and 34, in equal parts, dated 14/8/05.
Serum mixture 33 consisted of serum of 20 horses.
Serum mixture 34 consisted of serum of 34 mules.
Second injection, 100 c.c., on the 24/11/05.
Virus 726.

Nos.		Result.
499	Reaction	Recovered.
500	„	„

Result.—None died.

Experiment 247. Date, 24/11/05.

First injection, 300 c.c. serum mixture 43 and 44, in equal parts, dated 18/9/05.
Serum mixture 43 consisted of serum of 20 horses.
Serum mixture 44 consisted of serum of 42 mules.
Second injection, 100 c.c., on the 28/11/05.
Virus 726.

Nos.		Result.
501	Reaction	Recovered.
502	„	„
503	„	„

Result.—None died.

Proportion of Mule to Horse Serum as 2 : 1; One Injection.

Experiment 248.—To note whether mixture of serum of horses and mules in proportion 1-3rd : 2-3rd in one inoculation is sufficient to pass mules through a horse sickness reaction.

Date, 10/11/05.
First injection, 300 c.c. serum mixture 11 and 12, dated 30/5/05.
Mixture 11 consisted of serum of 12 horses.
Mixture 12 consisted of serum of 22 mules.
Virus 726.

Nos.		Result.
414	Reaction, dikkop, 15th day	Recovered.
415	„	„
416	„	„
417	„	„
419	„ dikkop, 13th day	Died 25/11/05.

Result.—Of five mules one died of horse sickness.

Experiment 249.—To note whether mixture of serum of horses and mules in proportion 1-3rd : 2-3rds in one injection is sufficient to pass mule through a horse sickness reaction.

Injection of 300 c.c., on the 17/11/05, of serum mixture 23 and 24 dated 11/7/05.

Serum mixture 23 consisted of serum of 14 horses.

Serum mixture 24 consisted of serum of 23 mules.

Virus 726.

Nos.		Result.
420	Reaction	Recovered.
421	„	„
422	„	„

Result.—None died of horse sickness.

Experiment 250.—To note the effect of a serum mixture of horses and mules hyperimmunised (1) once; (2) twice; and (3) three times, in the proportion of one horse serum to two mule serum.

One injection of 300 c.c. subcutaneously.

Virus subcutaneously for one half, and intrajugularly for the remainder.

Remark.

Serum mixture I. (horse and mule) consisted of the serum of horses and mules hyperimmunised to the extent of 8.5 litres. Date, 18/12/05.

Serum mixture II. (horse and mule) consisted of the serum of horses and mules hyperimmunised to the extent of 17 litres in two operations at intervals of from 4-6 months. Date, 18/12/05.

Serum mixture III. (horse and mule) consisted of the serum of horses and mules, hyperimmunised to the extent of 26.5 litres, in three operations at intervals of 3-6 months. Date, 18/12/05.

Serum Mixture I. (Animals hyperimmunised once.)

Consisted of 1-3rd serum mixture I. (horse) and 2-3rds serum mixture I. (mules).

(*a*) Virus injected subcutaneously.

Mule 554.—Injected on the 19/12/05 with 300 c.c. serum and 2 c.c. virus 726 (horse).

Result.—Incubation time, 7 days; reaction, 12 days; dikkop on the 16th day.

Mule 616.—Injected as above. Virus X. (virus passed through a goat).

Result.—Incubation time, 7 days; reaction, 8 days.

Mule 573.—Treated as above. Virus, donkey 427 (virus passed through a donkey).

Result.—Incubation time, 7 days; reaction, 12 days.

Mule 570.—Treated as above. Virus, mule 487 (virus passed through a mule).
Result.—Incubation time, 7 days; reaction, 8 days.

Conclusion.—Of four mules treated with above serum mixture and virus of various origin injected subcutaneously, all passed through the horse sickness reaction.

(*b*) Virus intrajugularly.

Mule 568.—Injected on the 19/12/05 with 300 c.c. serum and 2 c.c. virus 726.
Result.—Incubation, 7 days. Died on the 12th day from horse sickness.

Mule 615.—Treated as above; 3 c.c. virus X.
Result.—Reaction.

Mule 612.—Treated as above. Virus, donkey 427.
Result.—Incubation, 7 days; dikkop, 13th day; died, 15th day.

Mule 559.—Treated as above. Virus, mule 487.
Result.—Incubation, 7 days; dikkop, 14th day; reaction, 12 days.

Conclusions.—Of four mules injected with serum I., and injected with virus of various origin intrajugularly, two died from horse sickness.

Serum Mixture II. (Animals hyperimmunised twice.)

Consisted of 1-3rd serum mixture II. (horses) and 2-3rds serum mixture II. (mules).

(*a*) Virus injected subcutaneously.

Mule 566.—Injected on the 19/12/05 with 300 c.c. serum mixture and 2 c.c. virus 726.
Result.—Incubation, 7 days; dikkop, 15th day; reaction, 12 days.

Mule 556.—Treated as above. 3 c.c. virus X.
Result.—Incubation, 7 days; reaction, 12 days.

Mule 548.—Treated as above. Virus, donkey 427.
Result.—Incubation, 6 days; reaction, 12 days.

Mule 550.—Treated as above. Virus, mule 487.
Result.—Incubation, 7 days; reaction, 12 days.

Conclusion.—Of four mules treated with serum mixture II., and injected with virus of various origin subcutaneously, all passed through the horse sickness reaction.

(*b*) Virus intrajugularly.

Mule 560.—Injected on the 19/12/05 with 300 c.c. serum mixture II. and 2 c.c. virus horse 726.
Result.—Incubation, 7 days; dikkop, 18th day; reaction, 12 days.

Mule 555.—Treated as above. Virus donkey 427.
Result.—Incubation, 7 days; reaction, 12 days.

Mule 617.—Treated as above. Virus mule 487.
Result.—Incubation, 7 days; reaction, 11 days.

Mule 572.—Treated as above. 3 c.c. virus X.
Result.—Incubation, 7 days; dikkop, 18th day; reaction 12 days.

Conclusion.—Of four mules treated with serum mixture II., and virus of various origin intrajugularly, all passed through horse sickness reaction.

Serum Mixture III. (Animals hyperimmunised three times.)

(*a*) Virus subcutaneously.

Mule 561.—Injected on the 19/12/05 with 300 c.c. and virus 726.
Result.—Incubation, 7 days; reaction, 12 days.

Mule 577.—Treated as above. Virus X.
Result.—Incubation, 7 days; dikkop, 14th day; reaction, 8 days.

Mule 551.—Treated as above. Virus, donkey 427.
Result.—Incubation, 8 days; reaction, 9 days.

Mule 567.—Treated as above. Virus, mule 487.
Result.—Incubation, 8 days; reaction, 10 days.

Conclusion.—Of four mules injected with serum mixture III. and virus of various origin subcutaneously, all passed through horse sickness.

(*b*) Virus intrajugularly.

Mule 576.—Injected on the 19/12/05 with 300 c.c. serum and virus 726.
Result.—Incubation, 8 days; reaction, 10 days.

Mule 614.—Treated as above. Virus X.
Result.—Incubation, 7 days; dikkop, 14th day; reaction, 12 days.

Mule 564.—Treated as above. Virus, donkey 427.
Result.—Incubation, 7 days; dikkop, 15th day; reaction, 12 days.

Mule 563.—Treated as above. Virus, mule 487.
Result.—Incubation, 7 days; reaction, 10 days.

Conclusion.—Of four mules injected with serum mixture III. and virus of various origin intrajugularly, all passed through horse sickness.

One Injection of Virus and Serum subcutaneously.

Experiment 251.—To note the effect of a serum mixture consisting of 1-3rd horse serum mixture and 2-3rds mules serum mixture. Second injection of serum to be abolished.

Virus, horse 726 subcutaneously.

(1) Serum 0 and 00, being old serum collected previous to 18/12/05, and recently mixed.

Mule 613.—Injected on the 19/12/05 with 300 c.c. mixture and 2 c.c. virus.
Result.—Incubation, 6 days; reaction, 10 days.

Mule 553.—Treated as above.
Result.—Incubation, 4 days; died on the 9th day from horse sickness.

Mule 562.—Treated as above.
Result.—Reaction after 2 days incubation; died on the 9th day.

Mule 574.—Treated as above.
Result.—Incubation, 3 days; died on the 11th day.

Conclusion.—Of four mules treated with serum mixture 0 and 00 in proportion of 1 : 2, three died of horse sickness; old serum appears to lose its efficacy.

(2) Serum 000 and 0000, being serum mixture numbers 1, 3, 7 and 11 horses; and 2, 4, 6, 8 and 10 mules, recently mixed. Date, 18/12/05.

Mule 549.—Injected on the 19/12/05 with 300 c.c. serum mixture and 2 c.c. virus 726 intrajugularly.
Result.—Dikkop on the 16th day; recovered.

Mule 557.—Treated as above.
Result.—Incubation, 3 days; reaction, 12 days.

Mule 571.—Treated as above.
Result.—Incubation, 6 days; dikkop, 18th day; recovered.

Mule 575.—Treated as above.
Result.—Incubation, 4 days; reaction, 14 days.

Conclusion.—Of four mules treated with serum mixture 000 and 0000 in proportion of 1 : 2, all passed through horse sickness.

One Injection of Virus and Serum Intrajugularly.

(3) Serum 12 and 21. Date, 30/5/05 and 4/7/05 respectively.

Mule 558.—Injected on the 19/12/05 with 300 c.c. serum mixture subcutaneously and 2 c.c. virus 726 intrajugularly.
Result.—Reaction; recovered.

Mule 621.—Treated as above.
Result.—Reaction, 16 days.

Mule 628.—Treated as above.
Result.—Reaction; died on the 9th day from horse sickness.

Mule 629.—Treated as above.
Result.—Incubation, 7 days; reaction, 8 days.

Conclusion.—Of four mules injected with serum 12 and 21 in proportion of 1 : 2 and virus intrajugularly, one died of horse sickness.

(4) Serum 57 and 20. Date, 6/11/05 and 27/6/05 respectively.

Mule 626.—Injected on the 19/12/05 with 300 c.c. serum mixture subcutaneously and 2 c.c. virus intrajugularly, 726.
Result.—Reaction; died 10th day.

Mule 624.—Treated as above.
Result.—Incubation, 6 days; reaction, 11 days.

Mule 618.—Treated as above.
Result.—Incubation, 7 days; reaction, 11 days.

Mule 630.—Treated as above.
Result.—Incubation, 6 days; reaction, 10 days.

Conclusion.—Of four mules injected with serum 57 and 20 in proportion of 1 : 2, one died of horse sickness.

(5) Serum 59 and 30. Date, 12/11/05 and 1/8/05 respectively.

Mule 619.—Injected on the 19/12/05 with 300 c.c. serum mixture subcutaneously and 2 c.c. virus 726 intrajugularly.
Result.—Incubation, 7 days; dikkop, 16th day; reaction, 10 days.

Mule 622.—Treated as above.
Result.—Incubation, 3 days; died, 11th day.

Mule 625.—Treated as above.
Result.—Incubation, 5 days; reaction, 10 days.

Mule 627.—Treated as above.
Result.—Incubation, 6 days; dikkop, 14th day; reaction, 10 days.

Conclusion.—Of four mules injected with serum mixture 59 and 30 in proportion of 1 : 2, one died of horse sickness.

(6) Serum 61 and 62. Date, 20/11/05.

Mule 620.—Injected on the 19/12/05 with 300 c.c. serum mixture subcutaneously and 2 c.c. virus 726 intrajugularly.
Result.—Incubation, 7 days; dikkop, 13th day; reaction, 10 days.

Mule 623.—Treated as above.
Result.—Reaction.

Mule 569.—Treated as above.

Result.—Incubation, 7 days; dikkop, 17th day; reaction, 11 days.

Conclusion.—Of three mules treated with serum mixture 61 and 62 in proportion of 1 : 2, none died of horse sickness.

Inoculated Mules, Stabled and Not Stabled. One and Two Injections of Serum.

Experiment 252.—With various sera in one and two injections (*a*) in proportion of equal quantities of horse and mule serum; (*b*) in proportion of 1-3rd horse serum to 2-3rds mule serum. With various virus, and some of the inoculated animals to be exposed to out-of-door conditions.

A. Virus Horse 726. Injected Subcutaneously.

Serum 0 and 00.

Mule 686.—Injected on the 11/1/06 with 300 c.c. serum mixture 0 and 00, and 2 c.c. virus. Animal kept in stable.

Result.—Incubation, 6 days; reaction, 8 days. Tested with 20 c.c. virus intrajugularly on the 1/2/06.

Mule 681.—Treated as above. Kept exposed.

Result.—Incubation, 5 days; reaction, 10 days. Tested with 10 c.c. virus intrajugularly on the 8/2/06.

Mule 703.—Injected on the 11/1/06 with 300 c.c. serum mixture 0 and 00; 2 c.c. virus 726 subcutaneously.

Second injection of serum on the 15/1/06. Kept in stable.

Result.—Irregular reaction; tested with 10 c.c. virus intrajugularly on the 8/2/06.

Mule 696.—Treated as above. Kept exposed.

Result.—Incubation, 8 days; reaction, 10 days. Tested with 10 c.c. virus intrajugularly on the 8/2/06.

Serum 59 and 18. Date, 12/11/05 and 20/6/05 respectively.

Mule 683.—Injected on the 11/1/06 with 300 c.c. serum mixture 59 and 18 and 2 c.c. virus 726 subcutaneously. Kept in the stable.

Result.—Incubation, 10 days; reaction, 4 days. Tested with 10 c.c. virus on the 8/2/06.

Mule 688.—Treated as above. Exposed.

Result.—No reaction. Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 699.—Treated as above; second injection of serum on the 15/1/06. Kept in stable.

Result.—Very slight reaction.

Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 698.—Treated as above; two injections. Exposed.
Result.—Incubation, 7 days; reaction, 6 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Serum 13 and 14. Date, 6/6/05.

Mule 682.—Injected on the 11/1/06 with 300 c.c. serum 13 and 14 in proportion of one-third of horse (13) to two-thirds of mule (14). One injection. Virus, 726 subcutaneously. Kept in stable.
Result.—Slight reaction; incubation, 9 days; reaction, 5 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 694.—Treated as above. Exposed.
Result.—Slight reaction of 5 days; incubation, 11 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 687.—Treated as above; two injections. Kept in stable.
Result.—Died on the 21/1/06, *not* horse sickness.

Mule 707.—Treated as above; two injections. Exposed.
Result.—Incubation, 8 days; reaction, 8 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Serum 23 and 16. Date, 11/7/05 and 13/6/05 respectively.

Mule 706.—Injected on the 11/1/06 with 300 c.c. serum mixture 23 and 16 in proportion of one-third to two-thirds; one injection. Virus, subcutaneously, 726. Kept in stable.
Result.—Incubation, 4 days; reaction, 9 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 691.—Treated as above; one injection. Exposed.
Result.—Incubation, 7 days; reaction, 8 days.
Tested on the 8/1/06 with 10 c.c. virus intrajugularly.

Mule 692.—Treated as above; two injections. Kept in stable.
Result.—Irregular reaction.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 697.—Treated as above; two injections. Exposed.
Result.—Incubation, 8 days; reaction, 7 days.
Tested on the 8/1/06 with 10 c.c. virus intrajugularly.

B. Virus 529. Injected Subcutaneously.

Virus 529 (passed through donkey 427 into horse 529).

Serum 65 and 26. Date, 4/12/05 and 18/7/05 respectively.

Mule 680.—Injected on the 11/1/06 with 300 c.c. serum 65 and 26 in equal parts; one injection. Kept in stable.
Result.—Incubation, 7 days; slight reaction, 6 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 693.—Treated as above; two injections. Exposed.
Result.—Irregular reaction.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 684.—Treated as above; two injections. Stabled.
Result.—Incubation, 6 days; reaction, 7 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 701.—Treated as above; two injections. Exposed.
Result.—Incubation, 7 days; reaction, 8 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Serum 67 and 40. Date, 11/12/05 and 4/9/05 respectively.

Mule 702.—Injected on the 11/1/06 with 300 c.c. serum 67 and 40 in equal quantities, mixed; two injections. Stabled.
Result.—Incubation, 6 days; reaction, 10 days.
Tested 1/2/06 with 20 c.c. virus intrajugularly.

Mule 678.—Treated as above; one injection. Exposed.
Result.—Incubation, 6 days; reaction, 8 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 689.—Treated with above serum in proportion of 1 : 2; two injections. Exposed.
Result.—Incubation, 6 days; reaction, 8 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 704.—Treated as above; one injection. Stabled.
Result.—Incubation, 7 days; reaction, 10 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

C. Virus 427 Donkey. Subcutaneously.

Virus (passed from horse into donkey).

Serum 61 and 22. Dated 20/11/05 and 4/7/05 respectively.

Mule 705.—Injected on the 11/1/06 with 300 c.c. serum mixture 61 and 22 in equal quantities; one injection. Stabled.
Result.—Incubation, 7 days; reaction, 8 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 690.—Treated as above; two injections. Exposed.
Result.—Incubation, 9 days; reaction, 8 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 695.—Treated as above; two injections. Exposed.
Result.—Incubation, 9 days; reaction, 8 days.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 696.—Treated as above. Serum in proportion of 1 : 2; one injection. Stabled.
Result.—Incubation, 6 days; reaction, 12 days; dikkop, 17th day.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Mule 779.—Treated as above; two injections. Exposed.
Result.—Irregular reaction.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Serum 65 and 24. Dated 4/12/05 and 1/7/05 respectively.

Mule 685.—Injected on the 11/1/06 with 300 c.c. serum mixture 65 and 24 in equal proportions; one injection. Exposed.
Result.—Irregular reaction.
Tested on the 8/6/06 with 10 c.c. virus intrajugularly.

Mule 700.—Treated as above; two injections. Stabled.
Result.—Incubation, 8 days; reaction, 9 days.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 636.—Treated as above. Serum in proportion of 1 : 2; one injection. Exposed.
Result.—No reaction.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Mule 637.—Treated as above; two injections. Stabled.
Result.—Irregular reaction.
Tested on the 8/2/06 with 10 c.c. virus intrajugularly.

Conclusion.—Of 32 mules injected subcutaneously with serum in equal proportions and proportion of 1 : 2; double and single injections of serum; exposed and not exposed; none died of horse sickness.

When tested with virus intrajugularly, three and four weeks later, all proved to be immune.

Experiment 253.—Experiment with various serum mixtures mixed in equal proportions.

Virus of horse 726 to be injected subcutaneously in doses of 2 c.c. The second dose of 100 c.c. serum to be injected on the 4th day.

(1) Serum 67 and 68. Date, 11/12/05.

Mule 710.—Injected on the 11/1/06 with 300 c.c. serum mixture and 2 c.c. virus 726; second injection on the 15/1/06.
Result.—Incubation, 6 days; reaction, 8 days.

Mule 711.—Treated as above.
Result.—No reaction typical for horse sickness.

Mule 712.—Treated as above.
Result.—Incubation, 8 days; reaction, 7 days.

Mule 713.—Treated as above.
Result.—No distinct reaction.

Mule 714.—Treated as above.
Result.—Indistinct reaction.
Tested on the 1/2/06 with 20 c.c. virus intrajugularly.

Remark.—Mules 711 and 713 were tapped on the 14th day after injection. The blood was mixed and injected into two susceptible mules Nos. 708 and 648. These two mules did not contract horse sickness from this injection.

Mules 711 and 713 were again submitted on the 6/2/06 to the inoculation with serum 75 and 76 subcutaneously, and 300 c.c., one inoculation, also 2 c.c. virus 726 intrajugularly. No reaction.

It must, therefore, be concluded that the two mules were salted before they were subjected to the simultaneous injection.

Experiment 254. Date, 12/1/06.

Virus 726. Serum 41 and 42. Date, 11/9/05.

Nos. Mules.	Quantity Injected. c.c.	Incubation. Days.	Reaction. Days.
763	300	7	7
758	300	7	9
759	300	5	9
760	300	5	9
761	300	5	7
762	300	5	9
755	300	5	Dikkop 12th day, died 14th day.
756	300		Irregular reaction.
757	300		,,
	Serum 61 and 62.		
714	300	7	7
715	300		Irregular reaction.
716	300		,,
717	400	8	9
693	350	6	8
719	300	5	7
720	350	5	8
721	300	7	6
722	300	7	8
723	300	6	8
	Serum 63 and 64.		
724	300	6	6
725	350	5	9
726	300	6	10
727	300	6	9
728	300		Irregular reaction.
729	300		,,
730	350	6	8
731	300		Irregular reaction.
732	300	6	11
733	300	7	9

Serum 65 and 66.

Nos. Mules.	Quantity Injected. c.c.	Incubation. Days.	Reaction. Days.
734	300	6	9
735	300	5	9
736	350	6	7
737	350	6	8
738	300	5	10
739	300	7 Dikkop, 14th	9
740	300	5	7
741	300	5	8
742	300	6	8
743	300	5	8

Serum 67 and 68.

744	350	6	9
745	300	6	7
746	300	6	7
747	300	Reaction not distinct.	
748	300	5	7
749	300	Irregular reaction.	
750	300	6	8
751	300	Irregular reaction.	
752	350	6	9
753	300	5	9

Remark.—Mules 726, 728, 740 and 743, whose reactions were but mildly pronounced, were tapped on the 14th day, and two susceptible mules were injected. Mixture 726 and 728 into mule 647, and mixture 740 and 743 into mule 709. Both mules contracted horse sickness.

Conclusion.—The inoculation of 50 mules with virus, subcutaneously, resulted with one death due to horse sickness. The animals which had a slight reaction—so slight that doubts might have been entertained as to whether it was really a reaction—proved to have passed through an attack of horse sickness, their blood having proved virulent when injected into susceptible mules.

Experiment 255.—To note effect of various horse and mule sera mixed in the proportion of 1 : 2, and injected in equal quantities. Virus subcutaneously of 2 c.c. No second injection.

Serum 0 and 00. Mixed in equal proportion.

Mule 793.—Injected on the 6/2/06 with 300 c.c. serum and virus 726.
Result.—Irregular reaction.

Mule 812.—Treated as above.
Result.—Incubation, 6 days; died on the 10th day of horse sickness.

Serum 0 and 00. Mixed in proportion of 1 : 2.

Mule 807.—Injected on the 6/2/06 with 300 c.c. serum and virus 726.
Result.—Incubation, 6 days; died on the 11th day of horse sickness.

Mule 817.—Treated as above.
Result.—Incubation, 4 days; died on the 11th day of horse sickness.

Conclusion.—Of four mules treated with serum in equal proportions, one died; in proportion of 1 : 2, two died from horse sickness. Serum 0 and 00 was accordingly considered as ineffective and destroyed.

Serum 63 and 64. Equal proportions. Date, 27/12/05.

Mule 810.—Injected on the 8/2/06 with 300 c.c. serum and 2 c.c. virus 726.
Result.—Incubation, 5 days; slight reaction.

Mule 813.—Treated as above.
Result.—Incubation, 5 days; slight reaction.

Serum 63 and 64; proportion of 1 : 2. Date, 27/12/05.

Mule 809.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 7 days; slight reaction.

Mule 811.—Treated as above.
Result.—Incubation, 6 days; pronounced reaction, lasting 10 days.

Conclusion.—Of four mules treated with horse and mule serum 63 and 64 and in proportion of 1 : 2, none died of horse sickness.

Serum 65 and 66; equal proportion. Date, 4/12/05.

Mule 798.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus subcutaneously.
Result.—Incubation, 7 days; slight reaction; dikkop on the 15th day.

Mule 804.—Treated as above.
Result.—Incubation, 6 days; slight reaction.

Serum 65 and 66; proportion of 1 : 2. Date, 4/12/05.

Mule 794.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus subcutaneously.
Result.—Incubation, 7 days; reaction, 9 days.

Mule 815.—Treated as above.
Result.—Incubation, 6 days; reaction, 10 days.

Conclusion.—Of four mules treated with horse and mule serum 63 and 64 in equal proportions and in proportion of 1 : 2, none died of horse sickness.

Serum 67 and 68; equal proportion. Date, 11/12/05.

Mule 806.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 9 days; slight reaction.

Mule 814.—Treated as above.
Result.—Incubation, 7 days; reaction, 7 days.

Serum 67 and 68; proportion of 1 : 2. Date, 11/12/05.

Mule 816.—Injected on the 8/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 7 days; dikkop on the 15th day; reaction lasted 10 days.

Mule 808.—Treated as above.
Result.—Incubation, 8 days; reaction, 8 days.

Conclusion.—Of four mules treated with horse and mule serum 67 and 68 in equal proportion and of 1 : 2, none died of horse sickness.

Serum 69 and 70; equal proportion. Date, 18/12/05.

Mule 818.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 6 days; reaction, 10 days.

Mule 819.—Treated as above.
Result.—Incubation, 6 days; reaction, 10 days.

Serum 69 and 70; proportion of 1 : 2. Date, 18/12/05.

Mule 796.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Slight reaction.

Mule 799.—Treated as above.
Result.—No reaction.

Conclusion.—Of four mules treated with horse and mule serum 69 and 70 in equal proportions and in proportion of 1 : 2, none died of horse sickness.

Serum 71 and 72; equal proportion. Date, 27/12/05.

Mule 791.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 7 days; dikkop, 15th day; reaction, 10 days.

Mule 800.—Treated as above.
Result.—No reaction.

Serum 71 and 72; proportion of 1 : 2. Date, 27/12/05.

Mule 552.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 6 days; reaction, 8 days.

Mule 565.—Treated as above.
Result.—Slight reaction.

Conclusion.—Of four mules injected with horse and mule serum 71 and 72 in equal proportions and of 1 : 2, none died of horse sickness.

Serum 73 and 74; equal proportion. Date, 2/1/06.

Mule 790.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 7 days; reaction, 8 days.

Mule 795.—Treated as above.
Result.—Incubation, 6 days; dikkop on the 15th day; reaction lasted 9 days.

Serum 73 and 74; proportion of 1 : 2. Date, 2/1/06.

Mule 797.—Injected on the 6/2/06 with 300 c.c. serum and 2 c.c. virus 726 subcutaneously.
Result.—Incubation, 6 days; reaction, 10 days.

Mule 801.—Treated as above.
Result.—No reaction.

Conclusion.—Of four mules treated with horse and mule serum 73 and 74 in equal proportion and of 1 : 2, none died of horse sickness.

Serum 75 and 76; equal proportion. Date, 8/1/06.

Mule 711.—Injected on the 6/2/06 with 300 c.c. serum subcutaneously and 2 c.c. virus intrajugularly.
Result.—No reaction.

Mule 713.—Treated as above.
Result.—No reaction.

Remark.—Compare Experiment 253. Mules 711 and 713 had undergone subcutaneous injection without reaction. They were again tested to see whether intrajugular injection of virus produces a reaction.

Serum 75 and 76; proportion of 1 : 2. Date, 8/1/06.

Mule 792.—Injected with 300 c.c. serum subcutaneously and 2 c.c. virus subcutaneously.
Result.—Slight reaction; incubation, 8 days; reaction lasted 7 days.

Mule 802.—Treated as above.

Result.—Incubation, 6 days; dikkop, 11th day; died on the 13th day.

Mule 803.—Treated as above.

Result.—Incubation, 6 days; reaction lasted 8 days.

Conclusion.—Of three mules injected subcutaneously with serum 75 and 76 in proportion of 1 : 2, one died of horse sickness.

Virus obtained from animals which had contracted horse sickness spontaneously.

Experiment 256.—To note effect of virus horse, first generation, and virus mule, first generation, in connection with simultaneous injection of serum mixture, horse and mule in equal proportion and in 1 : 2.

Serum mixture 77 and 78. Date, 15/1/06.

Mule 919.—Injected on the 4/4/06 subcutaneously with 300 c.c. serum mixture in equal proportion and 2 c.c. virus horse 785. (First generation.) Origin, a sick horse at Barberton.

Result.—Incubation, 6 days; reaction, 9 days.

Tested on its immunity with 20 c.c. virus on the 26/4/06 intrajugularly.

Mule 920.—Treated as above. Virus mule 788 (first generation), obtained from a sick mule at Potchefstroom.

Result.—No reaction.

Tested on its immunity on the 26/4/06 with a simultaneous injection of virus 785 intrajugularly and serum subcutaneously. No reaction. Tested on the 12/5/06 with 10 c.c. virus 918. No reaction.

Mule 921.—Injected on the 4/4/06 with serum mixture in proportion of 1 : 2. Virus horse 785.

Result.—No reaction.

Tested on the 26/4/06 with a simultaneous injection of virus 785 into the jugular vein and serum subcutaneously. No reaction. Tested on the 12/5/06 with 10 c.c. virus horse 918. No reaction.

Mule 922.—Treated as above. Virus mule 788.

Result.—No reaction.

Tested on the 26/4/06 with a simultaneous injection of virus 785 into jugular vein and serum subcutaneously. No reaction. Tested on the 12/5/06 with 10 c.c. virus horse 918. No reaction.

Conclusion.—Of four mules injected with virus horse and mule (first generation), none died of horse sickness. Mules 920, 921 and 922 must be considered to have been immune previous to injection.

Serum mixture 79 and 80. Date, 22/1/06.

Mule 923.—Injected subcutaneously on the 4/4/06 with 300 c.c. serum mixture in equal proportions and 2 c.c. virus horse 785 subcutaneously.

Result.—Incubation, 8 days; reaction, 8 days.

Injected with virus 20 c.c. intrajugularly on the 26/4/06.

Mule 924.—Injected as above with virus mule 788.

Result.—No reaction.

Tested on the 26/4/06 by a simultaneous injection of virus 785 into jugular vein and serum subcutaneously. No reaction. Tested on the 12/5/06 with 10 c.c. virus horse 918. No reaction.

Mule 925.—Treated as above with serum in proportion of 1 : 2. Virus horse 785.

Result.—Incubation, 6 days; reaction, 8 days.

Tested on the 26/4/06 with 20 c.c. virus into jugular vein.

Mule 926.—Treated as above with virus mule 788: 100 c.c. serum.

Result.—Incubation, 7 days; reaction, 11 days.

Tested on the 26/4/06 with 20 c.c. virus 785 into jugular vein.

Conclusion.—Of four mules injected with virus horse and mule (first generation), none died of horse sickness.

Serum mixture 61 and 82. Date, 20/11/05 and 29/1/06.

Mule 927.—Injected on the 4/4/06 subcutaneously with 300 c.c. serum mixture in equal proportion, and 2 c.c. virus horse 785 subcutaneously.

Result.—Incubation, 7 days; reaction, 8 days.

Tested on the 26/4/06 with 20 c.c. virus 785 into jugular vein.

Mule 928.—Treated as above. Virus mule 788.

Result.—Incubation, 8 days; reaction, 8 days.

Injected on the 26/4/06 into jugular vein with 20 c.c. virus 785.

Mule 929.—Treated with serum mixture in proportion of 1 : 2. Virus horse 785.

Result.—Incubation, 8 days; reaction, 9 days.

Tested on the 26/4/06 by injection of 20 c.c. virus 785 into jugular vein.

Mule 930.—Treated as above. Virus mule 788.

Result.—Incubation, 7 days; died of horse sickness on the 13th day.

Conclusion.—Of four mules injected with virus horse and mule (first generation), one died of horse sickness.

Serum mixture 57 and 58. Date, 6/11/05.

Mule 931.—Injected subcutaneously on the 4/4/06 with equal proportion of serum mixture. Virus, horse 785.

Result.—Incubation, 6 days; reaction, 10 days.

Tested on the 26/4/06 by injection of 20 c.c. virus 785 into jugular vein.

Mule 805.—Treated as above. Virus mule 788.
Result.—No reaction.
Tested on the 26/4/06 by simultaneous injection of virus into jugular vein. Virus 785. Serum subcutaneously. Reaction. Incubation, 3 days; reaction lasted 9 days. Injected on the 12/5/06 with 10 c.c. virus horse 918.

Experiment 257.—To note effect of various sera mixtures on mules, virus injected subcutaneously and simultaneously.

Serum mixture 85 and 86. Date, 12/2/06.

Mule 978.—Injected on the 16/5/06 with 300 c.c. serum and 2 c.c. virus 785 subcutaneously.
Result.—Reaction.
Tested on the 5/6/06 with 10 c.c. virus 785.

Mule 979.—Treated as above.
Result.—Reaction. Tested on the 5/6/06.

Mule 980.—Treated as above. Virus 785.
Result.—Reaction. Tested on the 5/6/06.

Mule 981.—Treated as above. Virus 785.
Result.—Died of horse sickness; on the 14th day, dikkop.

Conclusion.—Of four mules injected with virus horse (first generation) one died of horse sickness.

Serum mixture 87 and 88. Date 19/2/06.

Mule 982.—Injected on the 16/5/06 with 300 c.c. serum mixture and 2 c.c. virus 785 subcutaneously.
Result.—Reaction. Tested on the 5/6/06 with 10 c.c. virus.

Mule 983.—Treated as above.
Result.—No reaction. Tested on the 5/6/06 with 10 c.c. virus 785.

Mule 984.—Treated as above. Virus 785.
Result.—Reaction. Tested on the 14/6/06.

Mule 985.—Treated as above. Virus 785.
Result.—Reaction. Tested on the 5/6/06.

Conclusion.—Of four mules injected with virus horse (first generation) none died of horse sickness.

Serum mixture horses 1 to 23 and mules 2 to 26. Date, 11/7/05.

Mule 986.—Injected on the 16/5/06 with 300 c.c. serum mixture and 2 c.c. virus 785.
Result.—Reaction. Tested on the 5/6/06 with 10 c.c. virus 785.

Mule 987.—Treated as above.
Result.—Reaction. Tested on the 5/6/06 with 10 c.c. virus 785.

Mule 933.—Treated as above.

Result.—Reaction. Tested on the 5/6/06 with 10 c.c. virus 785.

Mule 934.—Treated as above.

Result.—Reaction. Tested on the 5/6/06 with 10 c.c. virus 785.

Conclusion.—Of four mules injected with virus subcutaneously and simultaneously, none contracted horse sickness.

SUMMARY OF PREVIOUS EXPERIMENTS SHEWING THE NUMBER OF ANIMALS WHICH CONTRACTED DIKKOP, THE DATE, AND THE NUMBER WHICH DIED.

Experiments 232 to 249.

138 mules injected, of which 23 contracted dikkop, as under:—

Nos.	Dikkop appeared.	Mule died.
419	13th day	14th day.
414	15th „	—
498	11th „	—
482	13th „	—
486	11th „	—
481	11th „	13th „
466	11th „	—
468	12th „	—
464	15th „	—
407	13th „	—
410	17th „	—
325	13th „	—
333	13th „	—
339	17th „	—
202	17th „	17th „
134	12th „	—
144	12th „	—
147	9th „	10th „
149	10th „	—
152	9th „	—
104	10th „	—
110	9th „	10th „
128	12th „	—

Experiments 250 to 257. (Note.—Experiments No. 250 with serum 1 (*b*) and all experiments with serum mixture 0 and 00 have been excluded from this return as it was discovered that the serum did not possess the necessary preventive qualities, and, consequently, was not introduced into practice.)

Nos.	Dikkop appeared.	Mule died.
554	16th day	—
566	15th „	—
560	18th „	—
572	18th „	—

Nos.	Dikkop appeared.	Mule died.
577 ..	.. 14th „ ..	.. —
614 ..	.. 14th „ ..	.. —
564 ..	.. 15th „ ..	.. —
549 ..	.. 16th „ ..	.. —
619 ..	.. 16th „ ..	.. —
627 ..	.. 14th „ ..	.. —
620 ..	.. 13th „ ..	.. —
569 ..	.. 17th „ ..	.. —
695 ..	.. 17th „ ..	.. —
755 ..	.. 12th „ ..	.. 14th day.
739 ..	.. 14th „ ..	.. —
798 ..	.. 15th „ ..	.. —
816 ..	.. 15th „ ..	.. —
791 ..	.. 15th „ ..	.. —
795 ..	.. 15th „ ..	.. —
802 ..	.. 11th „ ..	.. 13th „

In all, 177 mules were inoculated in experiments 250 to 257, 2 of which contracted horse sickness and died.

Experiments 214-231. Year, 1904-5. (Experiment 214 was the first experiment made after the method of inoculating with 300 c.c. serum was finally decided upon.)

201 mules inoculated: 25 contracted dikkop, 8 died from horse sickness.

Table Shewing the Total Number of Mules Inoculated from the Years 1904-5 *and* 1905-6.

	Inoculated.	Contracted Dikkop.	Died.
1904-5 ..	201	25	8
1905-6 ..	138	23	5
„ ..	177	20	2
Total ..	516	68	15

Percentage of inoculated mules which contracted dikkop .. 13.1%.
Percentage of inoculated mules which died of horse sickness 2.9%.

Résumé.—The injection of virus into the jugular vein can be abolished with safety and substituted by a subcutaneous injection.

Virus obtained from a spontaneous case of horse sickness acted in the same way as virus which, on this station, had passed through several generations.

Mules, whilst undergoing horse sickness reaction, need not necessarily be stabled; there was no difference in the character of the reaction between stabled and non-stabled mules.

In no instance did the serum utilised prove to be hæmolytic for any of the injected mules.

TRANSMISSION OF HORSE SICKNESS INTO DOGS.

Experiment No. 1.

To note whether the injection of virulent horse sickness blood will produce a reaction in dogs.

1. *Dog 131.*
   Injected into the jugular vein with 2 c.c. virus of horse 785 on the 4th April, 1906.
   Reaction ensued; the temperature reached 105.6° F. on the 6th day after injection, but dropped and attained its normal course two days later.

2. *Dog 132.*
   Injected on the 4th April with 2 c.c. virulent blood of horse 785.
   Immediate reaction, the temperature became normal on the third day after inoculation—7th April, 1906.

3. *Dog 133.*
   Injected into the jugular vein on the 4th April, 1906, with 2 c.c. virulent blood of horse 785.
   Temperature rose on the evening of the 5th April to 105° F. but dropped the following day to normal, and to sub-normal the day after, when the dog died.
   Both apices of the lung were hepatised. The mucosa of the stomach was congested. All other organs were normal.

4. *Dog 134.*
   Injected on the 4th April, 1906, with 2 c.c. virus of horse 785 into the jugular vein.
   Temperature rose on the 6th day after inoculation to 104° F. in the evening, but dropped during the following day, and the dog died during the night of the 7-8th April, 1906.
   Autopsy revealed the lesions of œdematous lungs and of congested mucosa of the stomach.

5. *Dog 135.*
   Injected on the 4th April, 1906, with 2 c.c. virulent blood of horse 785 into the jugular vein.
   No reaction.

6. *Dog 139.*
   Injected on the 26th April, 1906, with 5 c.c. virulent blood of horse 857 (fresh blood).
   Temperature rose on the morning of the 28th April to 100° F., and in the evening to 103.8° F.; it dropped after this and the dog died in the morning of the 30th April, 1906.

Autopsy.—General condition good. There was a little clear fluid in the thorax and the lungs were but slightly oedematous. The heart was normal but the spleen was somewhat enlarged. The mucosa of the stomach was congested and there was also congestion throughout the whole length of the intestines, the fæces being blood-stained. Kidney and liver were also congested.

7. *Dog 141.*

Injected on the 26th April, 1906, with 5 c.c. virulent blood of horse 857 (fresh blood).

Temperature on the evening of the 28th April was 104.4° F. The following morning the dog collapsed; died on the morning of the 30th April.

Autopsy shewed the general condition good. The lungs were slightly œdematous with some petechiæ under the pleura. The spleen contained several hæmatomata. The mucous membrane of the stomach and intestines were congested. The fæces were blood-stained.

Experiment No. 2.

Transmission of Horse Sickness from a Dog to a Horse.

Horse 870.

Injected on the 11th April, 1906, intrajugularly, with 10 c.c. defibrinated blood of dog 131 taken on the previous day.

Typical horse sickness reaction ensued and the horse died from this disease on the 20th April, 1906.

On *post-mortem* the lesions of the lungs, heart and stomach were found to be strongly marked.

Experiment No. 3.

Transmission of Horse Sickness from a Dog to a Dog.

Dog 142. 26.IV.1906 Lee

Injected with blood of dog 131 taken on the 10th April. On the 28th April the evening temperature reached 104° F. It dropped to normal on the 30th April. After a lapse of seven days a second reaction ensued which culminated in death.

Autopsy revealed only a very œdematous condition of the lungs.

Note.—This dog (142) was tapped at the end of the first and second reaction—30th April and 11th May.

Dog 143.

Injected into the jugular vein with 5 c.c. blood of dog 131, tapped on the 10th April, 1906.

Date of injection, 26th April, 1906.

No immediate reaction. A rise of temperature was noticed for the three days 13th—16th May.

Dog 143 was tapped during this reaction on the 15th May, 1906.

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Dog 144.

Injected on the 26th April into the jugular vein with 20 c.c. blood of dog tapped on the 10th April.

No immediate reaction; a slight temperature disturbance was observed on the 12th day after injection and continued for a few days.

Dog 144 was bled on the 11th May, 1906—15 days after the injection.

Experiment No. 4.

Transmission of Second Generation of Horse Sickness in the Dog to the Horse and Mule.

Horse 940.

Injected on the 30th April, 1906, with 2 c.c. blood into the jugular vein from dog 142—bled on the 30th April.

Typical horse sickness reaction followed. The animal collapsed on the 9th day, the temperature on the previous day having reached 105.6° F. It had to be killed on the 14th day after injection—14th May, 1906—on account of collapse.

Post-mortem revealed lesions of horse sickness in the heart, stomach and intestines.

Mule 932.

Injected on the 15th May, 1906, intrajugularly with 5 c.c. blood taken from dog 143—19 days after the dog had been injected.

No reaction observed.

Result of the Previous Experiments.

The inoculation of fresh horse sickness virus caused a febrile reaction in two dogs out of six. Four animals died as the result of the inoculation, and on one it produced no effect. The reaction started almost immediately and death resulted within a few days. The *post-mortem* lesions found were œdema of the lungs and gastritis; these symptoms are those of horse sickness in equines.

The inoculation of blood taken from a dog which reacted into another dog caused, in this latter, a reaction, the blood of which proved to be virulent for a horse.

Conclusion.

It is possible to transmit horse sickness into dogs, and to transmit the virulency from dog to dog. Horse sickness in dogs has a very rapid course both in incubation and temperature reaction.

The *post-mortem* lesions found in dogs are identical with those of a horse.

THE IMMUNITY IN HORSE SICKNESS.

We have previously shewn that the mortality amongst inoculated mules exposed to natural infection amounted to about 0.6%. It was one of our instructions to the various Government Veterinary Surgeons and other men who took care of exposed immune animals to collect blood from any such animal which shewed symptoms or died from horse sickness and forward it to this Laboratory. In this way we obtained blood of recently immunised mules from Lydenburg, Piet Retief, Pretoria and Bulawayo. Of mules immunised about a year ago, blood was forwarded from Warmbaths, Lydenburg and Tzaneen, and also from a horse which, after hyperimmunisation, was exposed at the latter place, where it died from horse sickness. In the first instance, it was our object to prove the accuracy of the diagnosis of the disease of the animal, and, in the instances mentioned above, the experiments proved the presence of horse sickness. There was, therefore, no longer any reason to doubt that immunised mules may contract the disease and even die from it. The virus obtained from the districts mentioned was then used in connection with experiments to immunise horses, when it was noted that this virus was stronger than the one we have been experimenting with, or than any other derived from spontaneous cases in the various districts of Potchefstroom, Zoutpansberg, Barberton, Middelburg or Swaziland.

Of the new virus there was one which excelled in virulency over the remainder, and it was, therefore, found advisable to re-fortify a larger number of horses in order to obtain a more effective serum.

It is desirable to mention that in all our previous experience it was never noticed that a mule or a horse, which was immunised with the ordinary virus, shewed any reaction of horse sickness when it was fortified to the extent of 9 or more litres with either the blood of the strain which was utilised at the Laboratory for several years, or with any of the strains obtained from spontaneous cases.

A remarkable and noteworthy phenomenon ensued when we utilised the virus obtained from Tzaneen and Bulawayo with which the hyperimmunisations were made as set forth herewith:—

Hyperimmunisation of Immune Horses (Origin—Tzaneen Mule).

Horse 1381.—First hyperimmunisation on 28/11/05 with virus from horse 471.

Second hyperimmunisation on the 19/3/06 with virus horse 1398. (First generation; virus of a mule from Tzaneen.)

Slight reaction noted.

Third hyperimmunisation on the 22/6/06 with virus horses 2032 and 2027, Tzaneen. (Origin—horse 87.)
Immediate reaction with symptoms of dikkop.

Horse 1253.—First hyperimmunisation on the 26/8/06 with virus horse 1264.
Second hyperimmunisation on the 12/12/05 with virus horse 1529.
Third hyperimmunisation on the 22/3/06 with virus horse 1398. (First generation; virus mule Tzaneen.)
Reaction.
Fourth hyperimmunisation with virus horse 2027. (Origin—Tzaneen horse 87.)
Reaction, with symptoms of dikkop.

Horse 1263.—First hyperimmunisation on the 11/9/05 with virus horse 1284.
Second hyperimmunisation on the 19/12/05 with virus horse 1540.
Third hyperimmunisation on the 20/3/06 with virus horse 1398. (First generation; origin—Tzaneen mule.)
Slight reaction.

Hyperimmunisation of Two Immune Mules with Virus Mule 1964. (First Generation; Origin—Bulawayo.)

Mule 1028.—Immunised on the 10/4/05 against horse sickness; passed through a typical reaction, and shewed symptoms of dikkop.
Hyperimmunised on the 21/6/06.
Contracted horse sickness and died 27/6/06.

Mule 1031.—Immunised on the 10/4/05 against horse sickness; passed through a typical reaction.
Hyperimmunised on the 21/6/06 with virus horse 1964.
Contracted horse sickness and died on the sixth day after—27/6/06.

Hyperimmunisation of Immune and Hyperimmunised Horses with Virus; Origin—Tzaneen Horse 87.

I. Generation virus, Tzaneen—virus horse No. 869.
II. Generation virus horse No. 2001.

Horses hyperimmunised once before with virus; origin—Station.

Horse 1406.—Hyperimmunised on the 31/5/06 to the extent of 9 litres.
Reaction 24 hours later, lasting 9 days.

Horse 1453.—Hyperimmunised on the 2/6/06 to the extent of 8.5 litres.
Reaction 24 hours later, lasting 8 days.

Horse 1669.—Hyperimmunised on the 1/6/06 to the extent of 9 litres.
Reaction 48 hours later, lasting 5 days.

Horse 1580.—Hyperimmunised on the 5/6/06 to the extent of 6 litres.
Reaction 24 hours later, lasting 6 days.

Horse 1665.—Hyperimmunised on the 1/6/06 to the extent of 3 litres.
Reaction 24 hours later, lasting 7 days.

III. Generation virus horse 2027 and 2032.

(*a*) Horses hyperimmunised once before; virus, origin—Station.

Horse 1672.—Hyperimmunised on the 22/6/06 to the extent of 9 litres (from horse 2027).
Reaction four days later, and lasting 8 days; symptoms of dikkop present.

Horse 1781.—Hyperimmunised on the 23/6/06 to the extent of 3 litres (from horse 2027).
Reaction 24 hours later, lasted 8 days; died of piroplasmosis on the 6/7/06.

(*b*) Horses hyperimmunised twice before; virus, origin—Station.

Horse 1352.—Hyperimmunsed on the 22/6/06 to the extent of 9 litres (from horse 2027).
Reaction 48 hours later, lasted 8 days; dikkop present 6 days after infusion; died on the 9th day from horse sickness.

Horse 1381.—Hyperimmunised on the 22/6/06 to the extent of 1 litre from 2032, and 3 litres from horse 2027.
Immediate reaction, lasting 7 days; dikkop present on the 5th day.

(*c*) Horses hyperimmunised three times before with virus; origin—Station.

Horse 1238.—Hyperimmunised on the 22/6/06 to the extent of 9 litres (from horse 2027).
Reaction 48 hours later, lasted 7 days; dikkop present on the 8th day, and died on the 9th day after infusion—1/7/06.

Horse 1239.—Hyperimmunised on the 22/6/06 to the extent of 6 litres; virus horse 2032.
Immediate reaction, lasted 6 days.

Horse 1251.—Hyperimmunised on the 22/6/06 to the extent of 3 litres; virus horse 2032.
Immediate reaction, lasted 5 days; dikkop present on the 5th day 27/6/06.

Horse 1253.—Hyperimmunised to the extent of 6 litres on the 22/6/06.
Reaction 24 hours later, lasted 6 days; dikkop present on the 8th day—30/6/06.

IV. Generation. Virus horse 2040 and 2028.

(*a*) Horses not hyperimmunised previously but immunised with virus—Station.

Horse 1860.—Hyperimmunised on the 28/6/06 to the extent of 9 litres virus horse 2040.
Reaction 24 hours later, lasted 12 days, and complicated with piroplasmosis.

Horse 1958.—Hyperimmunised on the 28/6/06 to the extent of 9 litres virus horse 2040.
Immediate reaction lasted 10 days. Dikkop present on the 8th day—6/7/06.

Horse 1963.—Hyperimmunised on the 30/6/06 to the extent of 3 litres virus horse 2040, and with 6 litres virus horse 2056—fifth generation.
Immediate reaction lasting 6 days; dikkop present on the 5th day—5/7/06.

Horse 2003.—Hyperimmunised on the 26/6/06 to the extent of 9 litres virus horse 2028.
Reaction 24 hours later lasting 10 days; dikkop present on the 6th day—2/7/06.

Horse 2004.—Hyperimmunised on the 26/6/06 to the extent of 9 litres virus horse 2028.
Reaction 48 hours later lasting 7 days.

Horse 2011.—Hyperimmunised on the 28/6/06 to the extent of 9 litres virus horse 2040.
Reaction 2 days later lasting 7 days.

(*b*) Horses hyperimmunised once previously with virus—Station.

Horse 1775.—Hyperimmunised on the 28/6/06 to the extent of 3 litres virus horse 2028.
Immediate reaction lasting 5 days.

(*c*) Horses hyperimmunised twice previously with virus—Station.

Hyperimmunised on the 27/6/06 to the extent of 9 litres virus horse 2028.
Immediate reaction lasted 8 days; dikkop on the 4th day; died on the 17th day from rupture of stomach.

Result of Experiments.

Of three horses hyperimmunised previously and re-fortified with virus obtained from an immune mule which died in Tzaneen, all shewed horse sickness reaction as a result of this virus. Two of these horses shewed a second and even more distinct reaction with the symptoms of dikkop after a further hyperimmunisation with a virus obtained from the hyperimmunised horse 87 which had died in Tzaneen.

This latter virus gave typical reactions in all 22 horses which were hyperimmunised; nine cases shewed symptoms of dikkop, a noticeable characteristic of horse sickness—and two deaths occurred due to this infusion.

The virus obtained from a mule in Bulawayo which had recovered from horse sickness caused the death of two immune mules from horse sickness after hyperimmunisation.

Conclusion.

The breaking down of immunity in immune mules and horses is, in all probability, due not to the loss of immunity but to the presence of an extremely virulent strain of virus in certain localities of the country against which the immunity obtained by an ordinary virus does not protect the animals. The experiments prove that one and the same locality may possess various strains of virulency, in particular at Tzaneen, where three strains of different virulency were obtained.

The fact that only a very small number of immune mules exposed to horse sickness contracted this disease may be accounted for, either that the extreme virulency of virus does not exist to a very great extent, or that only a small minority of immune mules shew reactions due to the natural infection, which latter, as a rule, passes unnoticed.

The Boers have, for some considerable period, noticed that there are different degrees of immunity against horse sickness which they express by the words "a horse may be salted for one district and not for another." The above experiments prove the accuracy of their statement.

HORSE SICKNESS.

The Result of the Inoculation in Practice.

The inoculation of mules against horse sickness was started in the month of November, 1905, in the most exposed portions of the Northern and Eastern Districts, such as Barberton, Lydenburg, Middelburg, Zoutpansberg, Waterberg, Rustenburg, Marico, Pretoria and Piet Retief; and in January, 1906, at Heidelberg, Potchefstroom, Krugersdorp, Swazieland and Rhodesia.

The following is a return of the monthly inoculations in the various districts:—

1905.	On hand.	Inoculated.	Discharged.	Deaths.	Remaining.
November ..	—	37	—	—	37
December ..	37	533	99	10	461
1906.					
January ..	461	583	706	21	317
February ..	317	433	440	15	295
March ..	295	380	433	21	221
April ..	221	223	281	9	154
May	154	127	197	8	76
June ..	76	48	95	4	25

Statistics of mortality from inoculations during the various months:—

Month, 1905.	Discharged.	Deaths.	Percentage.
November	Nil	Nil	Nil
December	99	10	9.9
1906.			
January	706	21	2.9
February	440	15	3.4
March	433	21	4.8
April	281	9	3.2
May	197	8	4.0
June	95	4	4.2

These statistics require some explanation as the mortality in the month of December seems to be rather high, but it must be borne in mind that the inoculation began at the end of November and the mortality for that month fell into the month of December, thus bulking the average mortality of this month. It should be divided over the two months, November and December, when a right average will be obtained. It will be noticed that the mortality in March was higher from inoculation. This is probably due to the fact that, during that month, horse sickness was most rampant, and probably some of the mules inoculated had the disease in its incubation period, when our serum naturally would not have the desired result.

Percentage of deaths in the various districts from inoculation to June 30th, 1906:—

District.	No. Inoculated.	Deaths.	Per cent. from Inoculation.
Barberton	187	3	1.6
Krugersdorp	35	1	2.8
Lydenburg	106	4	3.7
Middelburg	71	2	2.8
Piet Retief	84	5	5.9
Potchefstroom	159	8	4.8
Pretoria	646	28	4.3
Rustenburg	493	18	3.9
Waterberg	174	10	5.7
Marico	48	1	2.0
Zoutpansberg	316	7	2.2
Heidelberg	6	0	0

Results of complication of the inoculation with biliary fever:—

District.	Number.
Barberton	1
Krugersdorp	2
Lydenburg	0
Middelburg	1
Piet Retief	2
Potchefstroom	3
Pretoria	4
Rustenburg	3
Waterberg	3
Marico	0
Zoutpansberg	0
Heidelberg	0
At Station	7

Complications with biliary fever .. .. 26 = 0.8%.
Deaths complicated with biliary fever .. 11 = 0.3 „
Recovered from biliary fever .. .. 15 = 0.4 „

Statistics of mortality after discharge:—

District.	Number Inoculated.	Deaths.	Per cent. of Deaths after Discharge.
Krugersdorp	35	0	—
Barberton	187	3	1.6
Lydenburg	106	6	5.6
Middelburg	71	0	—
Piet Retief	84	1	1.1
Potchefstroom ..	159	0	—
Pretoria	646	0	—
Rustenburg	493	5	1.0
Waterberg	174	0	—
Marico	48	0	—
Zoutpansberg ..	316	1	0.2
Heidelberg	6	0	—

Summary.

	Inoculated.		Deaths.		%
Inoculated at Station since February 28th, 1905, by all methods	522,	of which died	20	=	3.8
Inoculated by the G.V.S.'s ..	2,325,	„	87	=	3.9
Inoculated in Rhodesia ..	388,	„	9	=	2.3
	3,235,	„	116	=	3.7

In order to obtain some reliable information as to the mortality amongst inoculated mules after discharge, a circular was sent to the various proprietors whose mules had been inoculated by the Government Veterinary Surgeons. It was collected, worked up for statistical purposes, and brought up by them at a meeting afterwards held. It must be mentioned that, with a few exceptions, an account of the greater majority of the inoculated animals was given. The number of animals of which no account was given amounts to about 5%. In giving this return of mortality, it must be mentioned that such cases which were reported to have died of horse sickness, and which proved, on a subsequent test, not to be horse sickness, were excluded from the statistics. In such cases, however, where the diagnosis was not certain, and the information as to the cause of death was obtained from the proprietor, the benefit of the doubt of the diagnosis was given to the proprietor's statement, and the death was returned as a case of horse sickness.

In this way the statistics shew:—

Total number exposed in the following districts, 3,195.

District.	Deaths.
Barberton	3
Lydenburg	6
Piet Retief	1
Rustenburg	5
Tzaneen	4
Zoutpansberg	1
Warmbaths	1
	21

This represents a mortality of 0.6%.

The following statistics have been completed in a different way, leaving out the cases which, in all probability, were not horse sickness, and we arrive at these figures:—

District.	Deaths from Horse Sickness.	Deaths Doubtful.
Barberton	2	1
Lydenburg	4	2
Piet Retief	1	0
Rustenburg	1	4
Zoutpansberg	0	1
Tzaneen	4	0
Warmbaths	1	0
	13	8

This gives a percentage of 0.4 of probable deaths from horse sickness.

Included in these two sets of statistics is the number of animals inoculated and discharged from the Bacteriological Laboratory, Daspoort, which dates as far back as 1902, and which amounts to 651. The bulk of these were inoculated in 1905.

The following table gives a return of the relapses which occurred in animals after discharge. The diagnosis of some cases of relapses was confirmed by the Government Veterinary Surgeons, and, in several instances, it was confirmed by us in testing the blood from a relapsing animal; but, in my opinion, it is doubtful whether some of the diagnoses, which were returned as relapses, were really such. Although the symptoms were similar to those of horse sickness, yet they might have been due to other diseases. The following is a return of the relapses after discharge:—

District.	Relapses.
Barberton	4
Krugersdorp	0
Lydenburg	6
Middelburg	0
Piet Retief	0
Pretoria	11
Potchefstroom	0
Rustenburg	18
Waterberg	5
Zeerust	0
Zoutpansberg	0
Rhodesia	1
	45

In order to obtain some information as to the severity of the test to which inoculated mules have been exposed, information was asked for as to the severity of horse sickness in the various districts. Generally speaking, with the exception of Pretoria, south of the

Magaliesberg, Krugersdorp, and Potchefstroom, the disease was reported to have been very prevalent, and that the season in the Northern and Eastern Districts was said to have been of the worst. From the circulars received, the return of the mortality, which came under the immediate cognizance of the Government Veterinary Surgeons for the various districts, was as follows:—

District.	Mules.	Horses.	Total Mules & Horses.
Barberton	—	—	57
Krugersdorp	—	—	4
Lydenburg	12	150	162
Middelburg	8	10	18
Piet Retief	—	—	40
Pretoria	—	—	38
Rustenburg	—	—	91
Waterberg	—	—	50
Zoutpansberg	132	190	322
			782

All the Government Veterinary Surgeons state that the return of the mortality is not accurate and does not by any means give an exact statement of the death of mules and horses in their districts, but it shews, so far, the value of our inoculation. If we put down the number of mules which are stated to have died at a minimum of 300, just for comparison's sake, it means, with regard to our inoculation, that 289 animals died whose deaths could have been prevented if they had been inoculated against horse sickness. The effect of the mortality amongst non-inoculated mules is better shewn when singular occurrences are considered in which inoculated and non-inoculated mules in one and the same span were exposed to infection invariably with the result that the inoculated ones did not contract the disease, whereas the non-inoculated ones died or contracted the disease in an extraordinary proportion. In order to shew the seriousness of the disease in the various districts, it must be further mentioned that mules which were considered to be salted, having been running in those districts for several years and on that account immune, died of the disease. Moreover, one proprietor stated that a mule which he personally saw salting, contracted this disease this year and died of it.

Conclusion.—We may, therefore, consider that the inoculation of mules against horse sickness has stood a very severe test, and that the results must be considered very satisfactory.

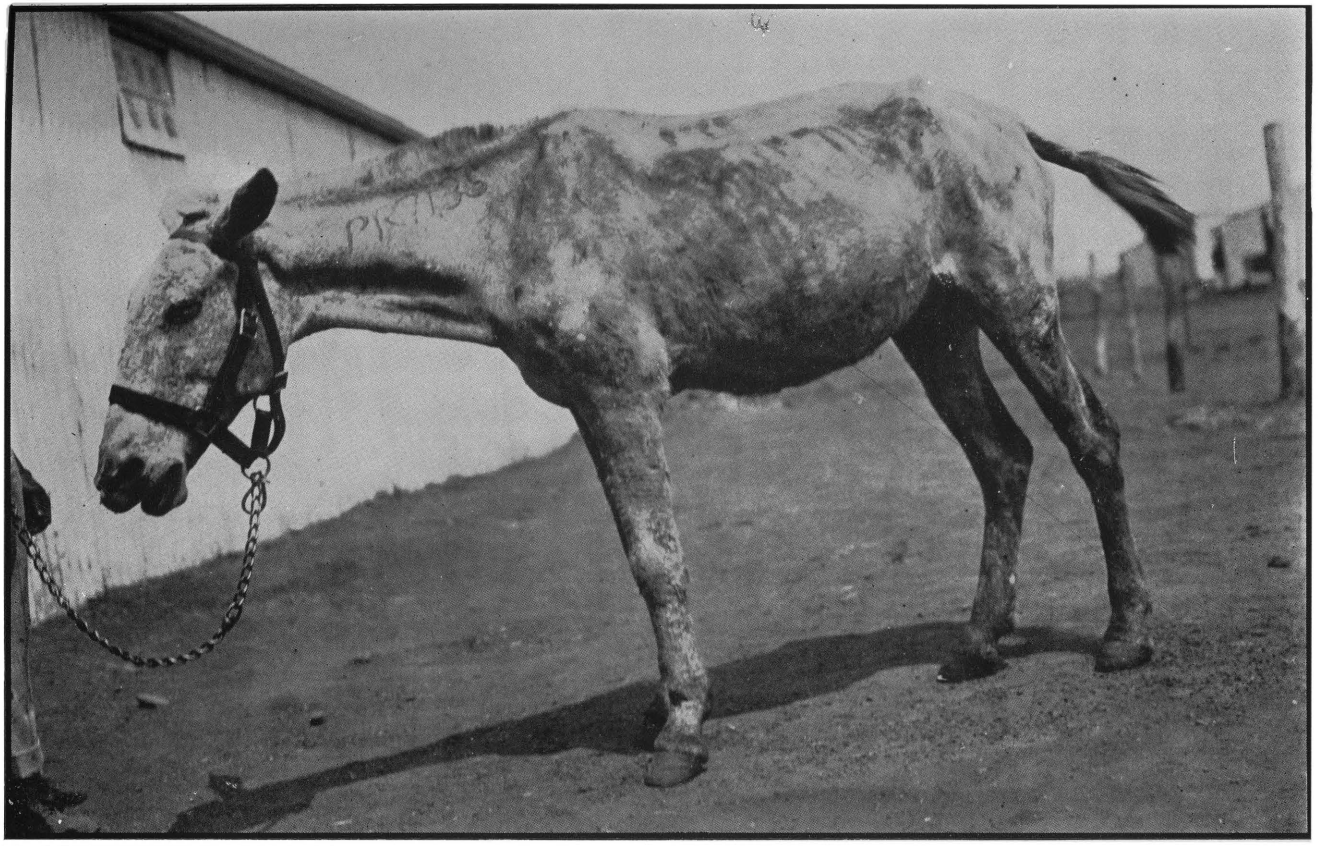

Plate 7.

Horse Sickness.

Dunkop. Just previous to death.

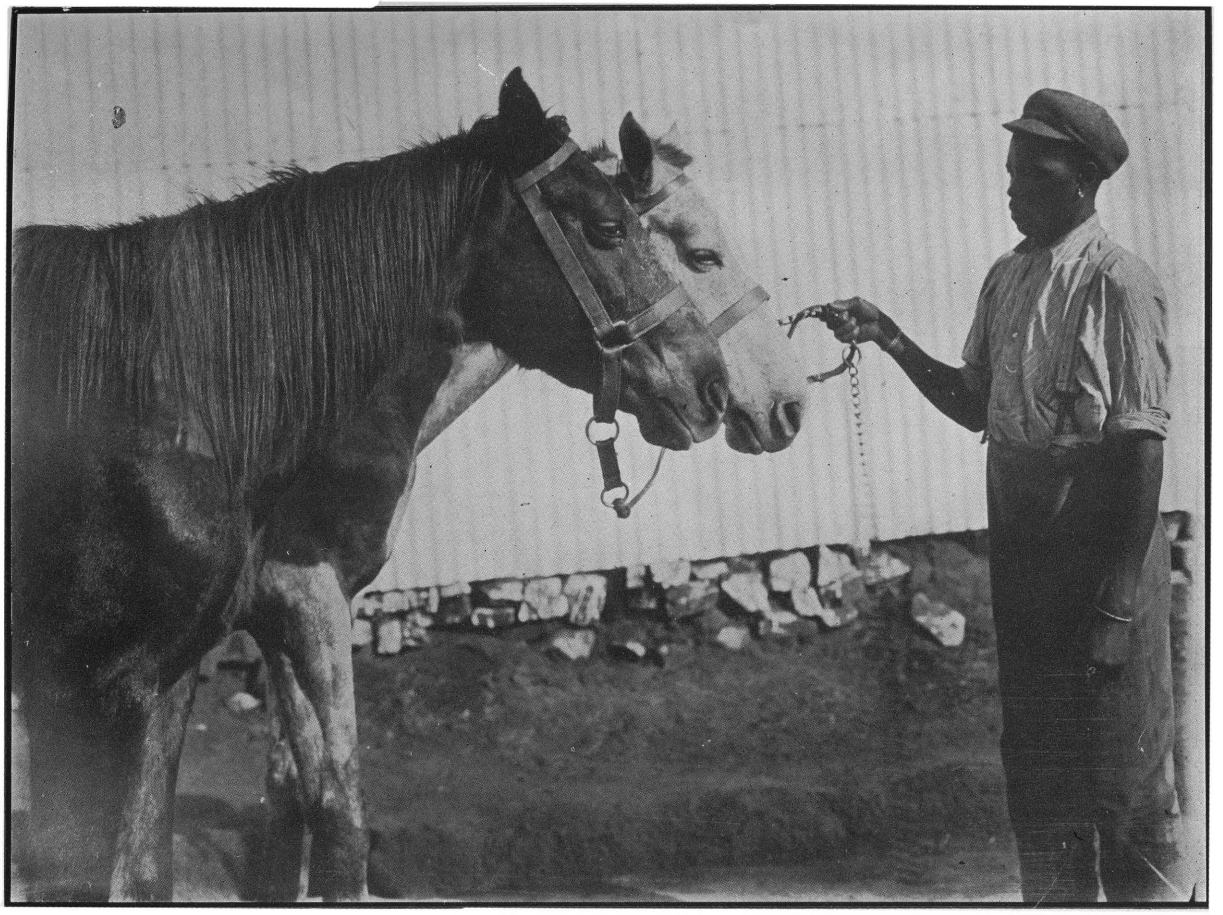

Plate 8.

Horse Sickness.

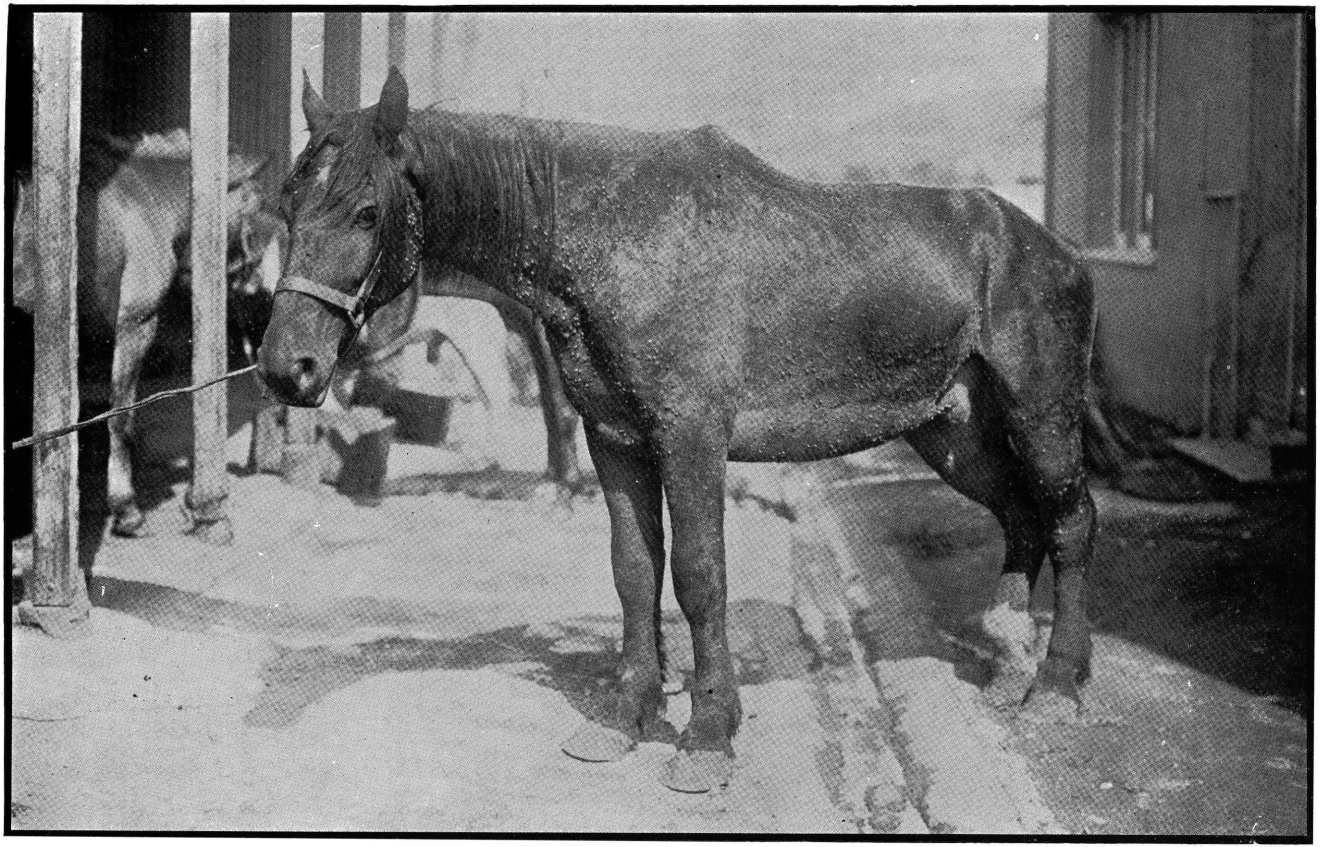

Plate 9.

Piroplasmosis.

Horse infested with Blue Ticks (*Rhipicephalus decoloratus*).

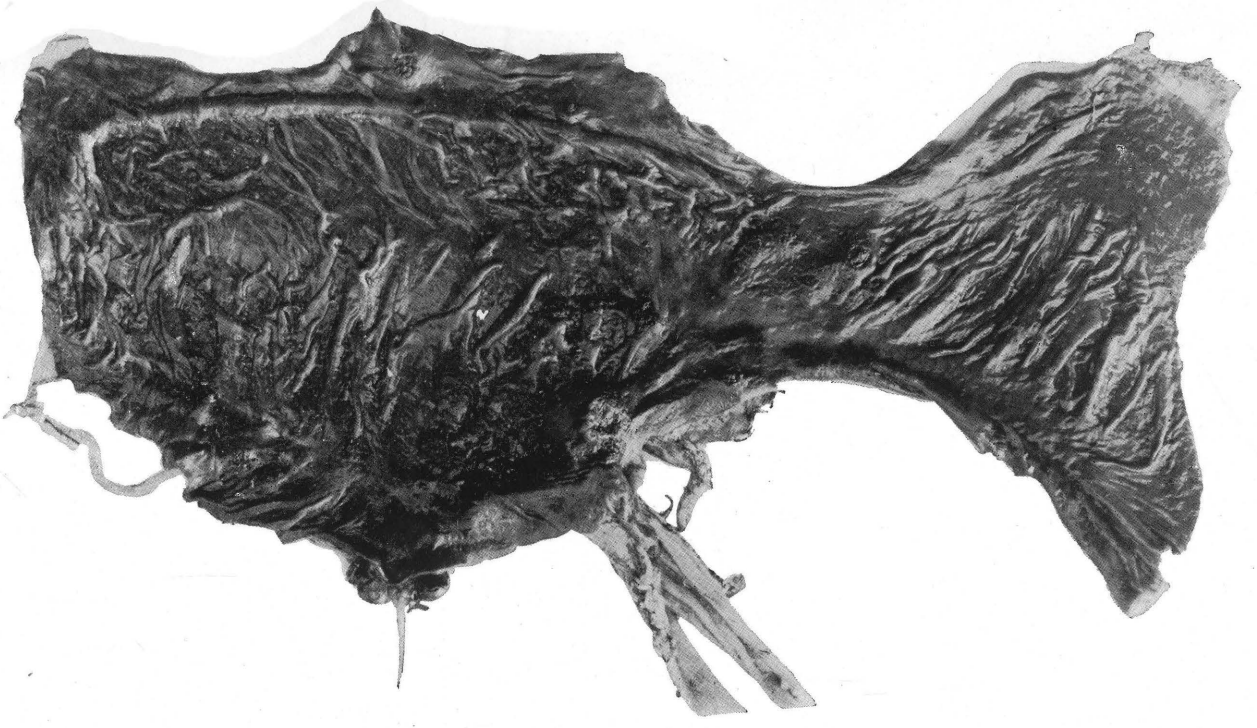

Plate 10.

Swine Fever.

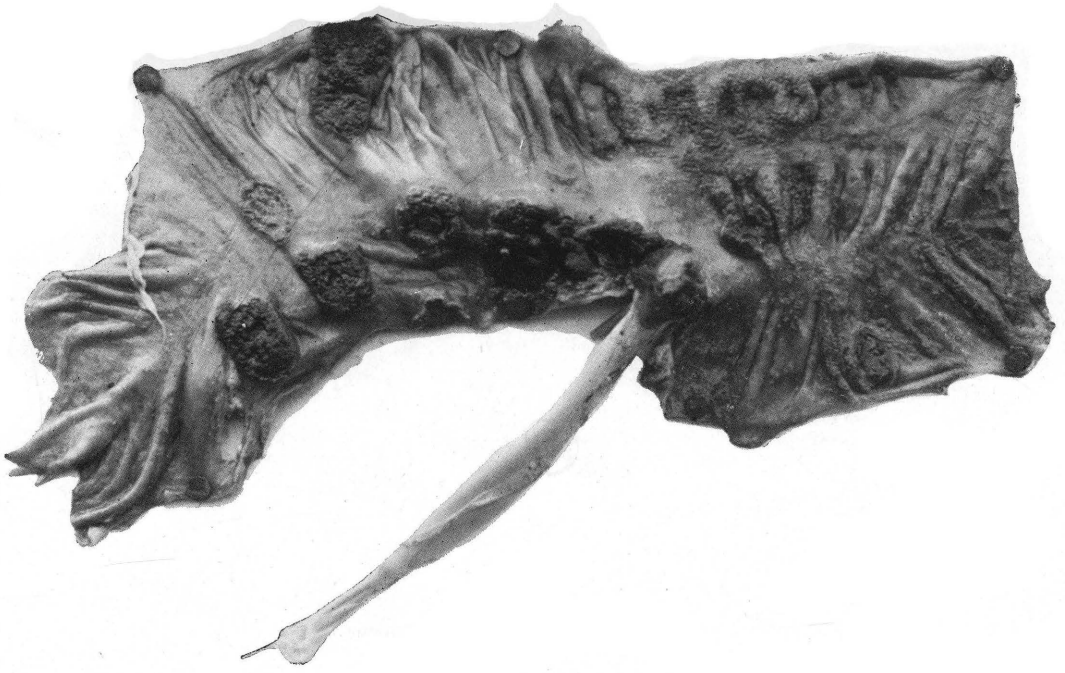

Plate 11. **Swine Fever.**