REPORT OF THE GOVERNMENT VETERINARY BACTERIOLOGIST.

Division of Veterinary Science,
Pretoria, 1st July, 1905.

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 1904-1905.

I regret to state that I was unable to carry out, *in extenso*, the programme which I had laid down for the year. This was chiefly due to the sad loss of two of my assistants, from enteric fever, when naturally the work had to suffer for some time. The main object of my work during the year, was the further study of horse sickness, especially to produce a serum such as would be free from haemolytic effect. You will notice in my last annual report that the inoculation against horse sickness was worked out for mules, but that, owing to the hæmolytic effect which such serum had, I did not recommend its application in practice before that fault had been overcome. You will also see in this year's report, on the same subject where I give the work in detail, that I have been able to remedy this defect.

During the year it was noticed that biliary fever played a most important role in connection with the simultaneous injection of serum and virus on freshly imported horses and mules, to which the treatment was applied. The inoculation resulted in many instances, in the development of biliary fever, after the animals had gone through a typical horse sickness reaction. This is due to the fact that horses and mules, born in this country, are immune against the disease and that their blood contains the microorganism of the disease in a latent form and causes the disease in a susceptible animal. I have in a special article in this report entered fully into this question and have proved that the piroplasmosis of the horse, mule, and donkey is one and the same disease. So far as our few experiments lead us to conclude there is a way out of the difficulty and I have every hope that our further experiments will point to some means to overcome this disease—a disease which is very frequently met with and which by laymen is often mistaken for horse sickness. It does not, however, occur only in imported horses and mules but in country bred ones as well which are treated by the simultaneous injection. Under the influence of the horse sickness reaction the immunity against piroplasmosis seems to break down. I do not yet know whether we shall be able to overcome this difficulty but there is only a small percentage of deaths—a percentage which might be considered as a negligible quantity.

The results of the horse sickness experiments are favourable. The last experiment resulted in the death of only 3 per cent. due to horse sickness. The remaining 97 per cent. went through a typical horse sickness reaction and must be considered immune. Since October last 156 salted mules and horses have been exposed in various parts of the country, especially in those parts which we know to be unhealthy for horses and mules. There is one report of a mule in Barberton which died of a disease which, from the description, points to a diagnosis of horse sickness. It may have been horse sickness but the mule was hyperimmunised to a very high degree and accordingly I have some difficulty in believing that this animal died of genuine horse sickness. Certainly, it is an old experience that salted horses may contract the disease a second and even a third time, and the object of our experiment in the first instance,

was to ascertain to what extent relapses would occur in the immunised animals. This year was an extremely mild one for horse sickness and, therefore, I do not consider that the trial of the immunity of the mules and horses, treated by me was sufficient. The same number of animals, or even more, should be exposed next year in order to complete the experiment of the immunity of inoculated animals. It is now my intention to hyperimmunise a sufficient number of animals for the purpose of producing serum, so that we shall be able to introduce the inoculation into practice with the coming horse sickness season and I feel confident that, at that time, we shall be ready with prepared serum to inoculate, at least, 10,000 animals. I have principally spoken of inoculation of mules against horse sickness. I have not yet had the opportunity, or rather I have not had a sufficient number of horses at my disposal, to push the experiment as regards horses. There is every reason to believe that the method applied to mules will, with a slight alteration, also prove successful for horses. The difficulty with horses is especially in connection with biliary fever. Mules do not suffer from biliary fever to the same extent as horses do, and I have repeatedly observed that the animal suffers from this disease after it has gone through horse sickness reaction.

East coast fever was another subject which took up my consideration during the year. Last year, in conjunction with Mr. Stewart Stockman, Principal Veterinary Surgeon, experiments were conducted to see how long an infected area remained infected. It must be taken as a firmly established fact that an east coast fever infected farm becomes purified after a certain lapse of time. A full description of the experiments undertaken for this purpose is given hereafter. A further and important point in connection with east coast fever was also settled this year. This was whether an ox which had recovered from east coast fever is still infective. This point has now been settled. Immune animals do not carry the disease. These experiments will form the basis of further legislation, inasmuch as they clearly indicate when a farm can be re-stocked. The experiments at Nelspruit also show that it is possible to save cattle on an infected farm provided certain measures are taken which I have referred to in my report on that subject.

I suggested to Mr. Woollatt, Principal Veterinary Surgeon of Natal, when he visited this Laboratory, that he should make a trial, as the distribution of the disease in that Colony allowed such to be undertaken. In a letter addressed to me and also in an article in the "Natal Agricultural Journal," Mr. Woollatt states that he has carried out the suggestion with very great success. What can be done on a large scale can also be done on a smaller scale provided that care and judgment be used. In the face of these experiments east coast fever loses much of its danger provided care is taken at the very start when the disease breaks out.

In connection with east coast fever certain dipping experiments were carried out on this station of which I give no detailed report as they have not yet come to a conclusion. The object of these experiments was to ascertain the effect of the dips which are in common use. It was noted that the dipping of animals does, as a rule, not destroy the female tick. It was thought that dipping would have little effect but the experiments proved that dipped ticks did die later. The majority of them may lay eggs but they do not hatch out; indeed in our experiments a very small percentage hatched. Thus dipping does destroy ticks but the effect is not immediate. I am therefore in a position to recommend dipping if it is undertaken as a measure of cleansing a farm from ticks; but it must be systematically carried out and stretched over a long period.

My experiments clearly demonstrated that there is still another disease produced by ticks, viz.—Spirillosis in cattle. It is true that this disease does not play such an important part as, for instance, redwater; but it is

another indication to what extent ticks are responsible in this country for the dissemination of diseases. It is a curious fact that the spirillosis is carried in the same way as ordinary redwater, and that the immune cattle retain the microorganisms in their blood. It would be of further interest to elucidate the influence such blood has on susceptible animals when used for inoculating purposes, as we know that the inoculation of susceptible animals against Redwater often results in death. It is possible that the two diseases have something to do in connection therewith. A full report of the experiments relative to the transmission of this by means of ticks is given later.

During the year opportunity was given me to begin certain investigations in connection with osteoporosis. Colonel Smith, Principal Veterinary Officer, South Africa, having kindly placed a number of mules and donkeys at my disposal. It is a common theory that it is a want of certain mineral substances in the food of the animal which causes this disease; a supposition which I have reason to doubt. Accordingly, I have placed myself in communication with Mr. Ingle, Chief of the Chemistry Division, who has courteously consented to undertake the analysis of bones of diseased and non-diseased animals with which I supplied him. As this study is principally a chemical one it does not enter, so much into my particular sphere as it does that of Mr. Ingle, who will no doubt give information on this subject in due season.

I have also had the opportunity of noting some new diseases in the country which I had not hitherto observed. One was bursattee, a disease which is known in India among horses. It is a wound disease caused by a hyphomycete. I have observed it in one horse resulting from a wound. The character of the disease is an ulcerative growth which when not treated surgically tends to grow and to form big ulcers. The other was also a wound disease, known as summer sores and was caused by filaria irritans. A specimen was handed to me by the Army Veterinary Department.

Another subject to which I gave close attention was the immunisation of sheep against blue tongue. Mr. Spreull, Veterinary Surgeon, Cape Colony, introduced inoculation against the disease. It was my object to repeat his experiment and I am in the position to state that I have met with similar success. My results differ in some points, but not materially. The immunisation could not be pushed as I should have wished, as the death of one of my assistants caused delay. I hope, however, to continue the experiment in order to introduce the inoculation into practice at an early period.

Heartwater experiments could not be carried on for the same reason, but the results obtained give some guarantee that it is possible to produce a serum which may be applied against the disease. The experiments in connection with this investigation are given later.

Some experiments were also carried out with poisonous plants, or plants supposed to be poisonous, which were supplied to me by Mr. Burtt-Davy, agrostologist and botanist. So far, I have only been able to cause death with one kind of tulip. I expect further details on this subject will be given by Mr. Burtt-Davy. I certainly admit the importance of the study of poisonous plants; but my work, in connection with the most rampant diseases of the country, do not give me sufficient time to enter into the details of less important investigations as I should wish.

For the first time during this year, a disease well known in Europe was discovered in pigs, viz., swine plague. We were able to isolate the microorganism from the organs of a pig, which had been sent by D. V. S. Edgar of Pietersburg, and the subsequent test made with it proved the correctness of our diagnosis. I do not know to what extent the disease exists in this country, but this experience should be a warning to us to be on the look-out for any obscure disease in pigs which may be similarly reported from various quarters.

We have also had swine fever, or a complication of swine fever with swine plague. This is also frequently met with in Europe and it seems to play an equally important role here as there.

The general health of the stock under my care was excellent. I have been able to eradicate completely epizootic lymphangitis from my stables, thanks to radical measures in the treatment of the cases. No contagious diseases occurred among the stock during the year.

In the tabulated record dealing with microscopical examinations, annexed to this report you will see that 1,124 examinations were made. This is principally due to the prevalence of east coast fever. It is gratifying to see that laymen rely on our diagnosis, inasmuch, as in several cases, when the services of the District Veterinary Surgeon were not obtainable, they made smears themselves and sent them directly to the Laboratory, and thus several outbreaks were recognised in their early stages and in others anxiety was allayed. This system of microscopical examinations is really a good one as it enables us, not only to detect east coast fever but ordinary redwater and anthrax, last named disease being as you will notice from the returns rather frequently met with.

The return of the vaccine statistics show a considerable increase in the production. This is mainly due to the demands of our neighbouring Colonies rather than to that of our own. We were able to cope with the demand although we were severely handicapped in many directions. 443,783 tubes were issued representing a value of £3648 3s. 10d. of which £1,869 14s. 4d. was recoverable. For the production of vaccine 54 calves were used and the total production amounted to 5,256 grammes. The result has met with uniform success. The Health Officer of Natal in his letter to the Medical Officer of Health for the Transvaal when asking us to discontinue the production of lymph on his account writes:—"I must again thank you for supplying us so promptly, as occasion demanded with lymph the quality of which was uniformly of a high character."

The production of pleuro-pneumonia virus was less than last year. This is a sure indication that the disease is generally on the decrease and it was with much pleasure that I was able to discontinue the manufacture of virus.

The following is the issue of pleuro-pneumonia virus during the year 1904-5, together with their relative values.

1904-5.	*Issued.*	*Value.*
	c.c.	
July	600	£15 0 0
October	25	0 12 6
March	300	7 10 0
May	60	1 10 0
June	130	3 5 0
Total ..	1,115	£27 17 6

At the beginning of the year we had 838,775 *c.c.* of rinderpest serum in stock. A demand was made from German West Africa for a supply of 5,000 doses. As we had sufficient serum in stock for our own purposes we complied with the request and delivered 500 litres.

Although it is not our intention to show profit out of our work, it is gratifying to see that we have been able to return £4,926 1s. 4d. to the revenue as the result of our various productions.

TRAVELLING.

The necessity of my constant presence at the Laboratory in connection with horse sickness did not allow me much travelling. I undertook, however

a visit to the the Agricultural Society at Barberton on two occasions to discuss with the members certain experiments in connection with east coast fever and horse sickness, which resulted in the exposure of a number of cattle in the town of Barberton and district, and of mules in the same district.

A journey was undertaken with Mr. Stockman to Delagoa Bay and various farmers were consulted in connection with east coast fever. In conjunction with Mr. Stockman I drew up an enquiry form which was distributed amongst farmers. As but a small number of these were returned, I have not been able to utilize them for the present report, but I hope to enter into the question at a later date.

A visit was also made to Nelspruit in connection with experiments there, and with the object of winding up that experimental station as the experiments had come to a conclusion with the end of the season.

At your request a visit was made to Heidelberg to meet the farmers of that district to explain to them the nature and propagation of east coast fever, which was introduced into that district. I certainly agree that visiting the farmers and explaining to them verbally the various diseases is very important. But on the other hand it is not always gratifying as one sometimes meets with obstinacy which even the soundest reasoning cannot overcome.

During the year Mr. Heron visited Carolina for the purpose of enquiring into a fowl disease which had made great ravages in that district. He prepared cultures on the spot and the investigation into that disease is still being made, but so far no definite result has been arrived at.

At the beginning of the year a course of lectures was given to certain non-commissioned officers and men of the South African Constabulary. A full programme was arranged by Mr. Stockman and myself. The course lasted three months. The men were taught to make smears, they were shown the principal diseases and they were instructed in the diagnosis of scab. After a lapse of three months they were examined on various matters and it is gratifying to report that they all passed successfully.

There is no doubt that such a course of lectures is decidedly advantageous as the men learn the necessary routine work in connection with our stock regulations; but on the other hand it does most certainly interfere with my ordinary work on the station. When a qualified assistant is at the station, I propose to continue similar lectures.

Mr. E. Esselen attended the work at this Laboratory for three months and obtained the necessary training which is required for a practical farmer to deal at the outset with the various diseases amongst stock. It is practically the beginning of a new era in South Africa when the sons of the country begin to realize that it is more necessary to know about diseases than to rely upon the teaching of ancient tradition.

VISITS.

Our Laboratory has been visited by a good many farmers, deputations of Agricultural Societies and Veterinary Surgeons, to whom, whenever possible, I explained the work which is conducted here and which might be useful in connection with their work.

ERECTION OF BUILDINGS.

The increase of our work necessitated the erection of several new buildings. The Laboratory for the production of serum was built, and for the purpose of hyperimmunising horses and mules a special building was put up. Horse sickness experiments having taken a good deal of attention, it was requisite for the ordinary work that there should be a newly equipped Laboratory, but as in the near future this special work will attain enormous dimensions, larger and better installations will be required than at present exist.

OFFICE WORK.

In connection with the office work on the station it may be stated that 2,257 letters were received and 1,857 letters and 581 wires were despatched. Naturally the general increase of our work also necessitates the increase of office routine. A considerable time was taken up in replying to people who ask information upon various diseases; and I am sorry to say that in order to oblige them a considerable amount of time is often wasted since much information could be gathered from our publications which I give from time to time in the Agricultural Journal.

STAFF.

As mentioned in the beginning of my report the station experienced the loss of two good assistants. Mr. B. Porta, first assistant, who was primarily engaged for the work of horse sickness in which he proved to be of great assistance to me, died from enteric fever on January 18th, 1905. Mr. Schneeberger, who principally carried out the work in connection with ticks, died on January 11th, 1905, from the same disease. Both men were most able and excellent officers and their death is a severe loss to the work of the station. I was in the fortunate position of being able to replace these gentlemen by assistants who had had previous work on the Laboratory, and who had the necessary training, so that the work could be successfully continued at an early period. At the end of the year the following assistants were engaged on the station; Messrs. C. Favre, E. Heron, D. Ferriera, A. von Bergen, V. Baerlocher W. B. Beeton and Neville Edwards. The clerical work is undertaken, by Mr. C. Stevenson Cameron, the Secretary. It gives me much pleasure to certify to the good work done by all during the year.

I have the honour to be,
Sir,
Your obedient servant,

ARNOLD THEILER,
Government Veterinary Bacteriologist.

VACCINE.

1904—05.	Transvaal.	O. R. C.	Natal.	Portuguese Territory.	Swaziland.	Mozambique.	No. of Lots.
July	8,188	1,000	37,500	2,000	—	100	58
August	12,187	2,000	71,000	4,000	50	—	100
September ...	12,951	5,000	41,995	2,000	—	—	87
October	16,457	5,000	—	2,000	150	—	96
November ...	7,865	8,600	—	2,000	—	20	64
December ...	9,149	—	—	2,000	—	20	78
January	9,658	1,500	400	2,000	200	20	66
February ...	12,195	500	2,550	2,500	—	40	67
March	14,774	2,000	650	4,000	—	—	70
April	8,635	1,000	600	3,000	6	—	50
May	50,828	800	200	7,500	—	—	103
June	56,530	1,300	—	7,000	50	115	109
	219,417	28,700	154,895	40,000	456	315	948

TOTAL ... 443,783 tubes at 2d. = £3,648 3s. 10d.

STATEMENT OF MICROSCOPICAL, PATHOLOGICAL, AND ANATOMIC EXAMINATIONS, GIVING MONTHLY SUMMARY, DISTRICT SUMMARY, AND SUMMARY.

Monthly Summary.

July, 1904.	
East Coast Fever	23
East Coast Fever and Ordinary Redwater	2
Anthrax	1
Saccharomy. Farcimi.	1
Acariasis	2
Equine Piroplasmosis	1
Nothing Seen	48
	78
August, 1904.	
East Coast Fever	33
East Coast Fever and Ordinary Redwater	3
Saccharomy. Farcimi.	2
Broncho Pneumonia	1
Traumatic Pericarditis	1
Chronic Interstitial Pneumonia	1
Anthrax	1
Nothing Seen	55
	97
September, 1904.	
East Coast Fever	26
Saccharomy. Farcimi.	7
Acariasis	1
Piroplasma Equi	1
Swine Plague	1
Peritonitis	1
Septic Pneumonia	1
Nothing Seen	46
	84
October, 1904.	
East Coast Fever	21
Saccharomy. Farcimi.	6
Acariasis	3
Piroplasma Canis	1
Tuberculosis Bovine	1
Black Quarter	1
Nothing Seen	33
	66
November, 1904.	
East Coast Fever	4
Saccharomy. Farcimi.	2
Piroplasma Equi.	1
Atelectasis of Lung	1
Nothing Seen	46
	54

December, 1904.	
East Coast Fever	13
Saccharomy. Farcimi.	4
Ordinary Abscess	4
Filaria Irritans	1
Broncho Pneumonia	1
Tuberculosis Bovine	1
Tuberculosis Avian	1
Dog Piroplasma Sequela	1
Coli Bacillosis	2
Nothing Seen	27
	55
January, 1905.	
East Coast Fever	27
Ordinary Redwater	6
Saccharomy. Farcimi.	2
Basic Cells	4
Swine, Bronchitis	2
Spirillosis	1
Distomatosis	
Necrosis of Liver	1
Ring Forms	
Black Quarter	1
Nothing Seen	63
	109
February, 1905.	
East Coast Fever	55
East Coast Fever and Ordinary Redwater	8
Ordinary Redwater	8
Ox, Pericarditis Traumatica	1
Pig, Pleuritis Fibrinosa, Pneumonia Crouposa	1
Milk, Streptococcus Infection	1
Saccharomy. Farcimi.	4
Basic Cells	2
Anthrax	3
Swine Fever	1
Nothing Seen	60
	145
March, 1905.	
East Coast Fever	45
East Coast Fever and Ordinary Redwater	3
Ordinary Redwater	8
Basic Cells	9
Saccharomy. Farcimi.	1
Tuberculosis	1
Gregarinosis of Turkey	1
Anthrax	4
Coccideum Oviforme	1
Doubtful, East Coast Fever	1
Nothing Seen	68
	142

April, 1905.

East Coast Fever	18
Ordinary Redwater	1
Basic Cells	4
Saccharomy. Farcimi.	1
Anthrax	2
Swine Fever	1
Inflammation of Stomach	1
Doubtful, East Coast Fever	1
Nothing Seen	50
	79

May, 1905.

East Coast Fever	33
Ordinary Redwater	5
Basic Cells	2
Saccharomy. Farcimi.	2
Anthrax	4
Swine Fever	2
Tuberculosis	2
Piroplasmosis of Dog	1
Polychromatic Cells in Dog	1
Strongylus Contortus	2
Septic Pneumonia	1
Filariasis	1
Doubtful, Anthrax	1
Nothing Seen	54
	111

June, 1905.

East Coast Fever	36
Ordinary Redwater	4
Saccharomy. Farcimi.	3
Piroplasma Equi.	2
Nucleated Cells	1
Taenia Infection	1
Fibrous Tumor	1
Abscess	2
Acariasis	1
Basic Cells	1
Pericarditis	1
Nothing Seen	51
	104

District Summary.

Barberton.

East Coast Fever	10
Ordinary Redwater	1
Saccharomy. Farcimi.	5
Dog, Piroplasma Sequela	1
Nothing Seen	12
	29

Ermelo and Carolina.

East Coast Fever	7
Saccharomy. Farcimi.	9
Abscess	1
Basic Cells	1
Acariasis	1
Swine Bronchitis and Bronchial Pneumonia	1
Distomatosis	1
Coccideum Oviforme	1
Nothing Seen	36
	58

Heidelberg.

East Coast Fever	3
Pericarditis	1
Acariasis	3
Basic Cells	1
Septic Pneumonia	1
Atelectasis of Lung	1
Nothing Seen	23
	33

Johannesburg.

East Coast Fever	6
Ordinary Redwater	4
Basic Cells	1
Anthrax	3
Tubercle	1
Nucleated Cells	1
Doubtful, Anthrax	1
Nothing Seen	20
	37

Krugersdorp.

East Coast Fever	13
Ordinary Redwater	3
Basic Cells	2
Necrosis of Liver	1
Swine Fever	1
Anthrax	2
Traumatic Pericarditis	1
Tuberculosis Bovine	1
Pig, Pleuritis Fibronosa, Pneumonia Crouposa	1
Milk, Streptococcus Infection	1
Abscess	1
Nothing Seen	22
	49

Lichtenburg.

Anthrax	1
Nothing Seen	3
	4

Lydenburg and Machadodorp.

East Coast Fever	17
Saccharomy. Farcimi.	3
Piroplasma Equi.	1
Swine Bronchitis and Broncho Pneumonia	1
Ox, Nephritis	1
Nothing Seen	18
	41

Middelburg.

East Coast Fever	26
East Coast Fever and Ordinary Redwater	1
Ordinary Redwater	4
Basic Cells	1
Broncho Pneumonia	1
Saccharomy. Farcimi.	1
Strongylus Contortus	2
Nothing Seen	79
	115

Nylstroom.

East Coast Fever	11
East Coast Fever and Ordinary Redwater	1
Ring Forms	1
Septic Pneumonia	1
Nothing Seen	34
	48

Pietersburg.

East Coast Fever	66
East Coast Fever and Ordinary Redwater	2
Ordinary Redwater	1
Basic Cells	1
Saccharomy. Farcimi.	7
Swine Plague	1
Acariasis	1
Piroplasma Equi.	3
Filariasis	1
Nothing Seen	60
	143

Piet Retief.

East Coast Fever	18
East Coast Fever and Ordinary Redwater	2
Ordinary Redwater	1
Basic Cells	1
Nothing Seen	14
	36

Potchefstroom.

Ordinary Redwater	2
Spirillosis	1
Acariasis	1
Basic Cells	3
Anthrax	4
Tuberculosis	1
Nothing Seen	15
	27

Pretoria.

East Coast Fever	86
East Coast Fever and Ordinary Redwater	8
Ordinary Redwater	14
Anthrax	5
Basic Cells	10
Saccharomy. Farcimi.	10
Acariasis	1
Taenia Infection	1
Fibrous Tumor	1
Tuberculosis Bovine	1
Ordinary Abscess	4
Tuberculosis	1
Filaria Irritans	1
Pericarditis	1
Tuberculosis, Avian	1
Coli Bacillosis	2
Ox, Pericarditis Traumatica	1
Inflammation of Stomach	1
Gregarinosis, Turkey	1
Swine Fever	3
Piroplasmosis of Dog	1
Polychromatic Cells in Dog	1
Nothing Seen	149
	304

Rustenburg.

East Coast Fever	54
East Coast Fever and Ordinary Redwater	2
Ordinary Redwater	1
Piroplasma Equi.	1
Piroplasma Canis	1
Black Quarter	2
Nothing Seen	60
	119

Standerton.

Ordinary Redwater	1
Broncho Pneumonia	1
Nothing Seen	15
	17

Swaziland.

East Coast Fever	12
Ordinary Redwater	1
Nothing Seen	8
	21

Wakkerstroom.

Nothing Seen	6

Zeerust.

East Coast Fever	8
Doubtful, East Coast Fever	1
Nothing Seen	14
	23

Bechuanaland.

Chronic Interstitial Pneumonia	1
Nothing Seen	12
	13

Rhodesia.

Basic Cells	1

Summary.

East Coast Fever	334
East Coast Fever and Ordinary Redwater	16
Ordinary Redwater	32
Basic Cells, Ox (Redwater)	22
Saccharomy. Farcimi.	35
Anthrax	15
Swine Fever	4
Gastritis, Pig	1
Tuberculosis, Avian	1
Dog, Piroplasma Sequela	1
Coli Bacillosis in pig	2
Swine Bronchitis and Broncho Pneumonia	2
Spirillosis in ox	1
Distomatosis, liver of ox	1
Necrosis of liver in ox	1
Ring Forms, ox (Redwater)	1
Piroplasma of Dog	1
Polychromatic Cells, Dog (Piroplasmosis)	1
Strongylus Contortus, sheep, stomach	2
Milk, Streptococcus Infection	1
Filaria Irritans, skin of horse	1
Ordinary Abscess, skin of horses and udder of cow	4
Broncho-pneumonia, Bovine	2
Tuberculosis Bovine	5
Septic Pneumonia, horse, lung	2
Filariasis, Ostrich	1
Gregarinosis of Turkey	1
Black Quarter	2
Piroplasma Equi.	5
Atelectasis, lungs, cow	1

Acariasis	7
Piroplasma Canis	1
Coccideum Oviforme in sheep	1
Ox, Pericarditis Traumatica	1
Pig, Pleuritis Fibrinosa, Pneumonia Crouposa	1
Peritonitis, ox	1
Nucleated Cells, ox (Redwater)	1
Taenia infection, sheep	1
Fibrous Tumour, fowl	1
Abscess, lung of horse	2
Pericarditis, ox	1
Chronic Interstitial Pneumonia, ox	1
Doubtful (East Coast Fever 1, Anthrax 2)	3
Nothing Seen	601
	1,124

FURTHER EXPERIMENTS TO NOTE HOW LONG AN AREA REMAINS INFECTED WITH EAST COAST FEVER.

In my last annual Report a certain experiment was described showing the possibility of introducing fresh cattle on pasture which about fourteen months previously was badly infected with East Coast Fever. This experiment was planned and conducted with Mr. Stewart Stockman, then Principal Veterinary Surgeon. The continuance of the experiment on more farms and under various conditions was contemplated and partially carried out. At the same time we drew up an enquiry form to be answered by farmers who had had experience with East Coast Fever in order to ascertain their observations on the subject, and to compare them with the result of our own experiments.

The information obtained was rather meagre, the returns of the forms being but a small percentage of those issued. There were only a few observations which corresponded with the conditions under which our experiments were started. It must be remembered that on December 15th, 1903, ten Texas heifers were exposed at Mr. Hall's farm, where for about a period of fourteen months no other cattle had been running, and which had been badly infected before that time. The result was that none of these heifers contracted East Coast Fever, although they were running there up to May 30th, 1904. On this date, the animals were removed to another place previously infected. Mr. Hall, encouraged by the result of our experiments, decided to bring eight Madagascar oxen to the farm on which our heifers had been grazing. After obtaining the necessary permission, and the assistance of the railway authorities to detruck the oxen directly on his farm, he did so a little later. These oxen have since been on what was at one time badly infected ground, and none of them contracted East Coast Fever. This observation may be considered as the continuance of the experiment started on the farm and now lasting over fourteen months.

The Texas heifers, which were running up to the 30th May on Mr. Hall's farm were, as already mentioned, placed on a new piece of ground belonging to Mrs. Bester. On this place 28 head of our experimental cattle had died during January and February, 1903, all within an area hardly larger than three acres. *Post-mortem* records and microscopical examination proved the correctness of the diagnosis. During the month

of November, 1903, this piece of ground was fenced in, firstly, in order to prevent its re-infection by cattle which might stray there. After the fence was put up fifteen sheep and goats were placed in the paddock, and, with the exception of four, died from heartwater. These four returned to Pretoria on April 13th. The Texas cattle were put into this paddock on the above-mentioned date. They were driven over a stretch of ground which about fifteen months previously had probably also been infected with East Coast Fever. Since that date up to March 31st one case of death was reported, namely, during November, 1904, which was not the result of East Coast Fever, the microscopical examination giving negative results. Thus the cattle were running for fully nine months on ground which fourteen months previously had been badly infected with East Coast Fever and with the Brown Tick.

This experiment supports the result of that made on Mr. Hall's farm. There is, however, one difference, namely, that in this latter case the pasture was also grazed by fifteen sheep and goats, which, being immune to East Coast Fever, might have picked up the infected ticks, and these would then have become harmless. The engorged ticks were picked off the sheep for breeding purposes. The most common ticks were the following species, given in the order of their collection: *Hyalomma ægyptium, Amblyomma hebræum, Rhipicephalus simus, Rhipicephalus evertsi.* It is noteworthy that but few brown ticks were collected from the sheep and goats, although on the cattle which had died there they were found in great numbers. This fact, however, does in no way exclude the possibility that a larger number of brown ticks had been picked up by the sheep and goats, but that number would not have been very large, otherwise they would have been noticed more frequently. Thus, we may conclude that the purification of this piece of ground was not so much due to the herding of the small flock of sheep and goats, but to the lapse of time which passed between the death of the sick animals and the introduction of fresh cattle.

A second experiment, which by this time may be considered to be successfully concluded, was undertaken in the neighbourhood of Barberton. This was started under the auspices of the Agricultural Society of Barberton, some members of which were very anxious to test the once infected area of that district with a view of re-opening it for the introduction of stock. A meeting was held of the Society on September 10th, 1904, when the object was explained, and a sub-committee was formed to advise as to the most suitable areas previously infected. A piece of ground was then selected on Mr. Niekerk's farm, and sufficient grazing was fenced in to carry eight oxen. Cattle had died on this particular ground about fourteen months previously.

Some delay in putting up the fence was the cause that the fresh cattle which had to be brought down were only placed on that farm on December 1st, 1904. Eight oxen were exposed in the first instance. On November 24th, 1904, nineteen oxen were temporarily placed on the same farm, which, however, had to be removed, owing to the scarcity of grass. As the fencing of the new place on which the infection had died out within twelve months was not forthcoming at a reasonable price, the nineteen oxen were brought up to Nelspruit, where, in the meantime, the conditions for a similar experiment were more favourable. It must be understood that the original eight oxen which were sent to Niekerk's farm were not kept back, but eight out of the second lot, so that the experiment was not really carried out as originally intended. Since no death due to

East Coast Fever occurred either amongst the cattle on Niekerk's farm or later amongst the lot sent to Nelspruit, we are entitled to conclude that the piece of ground which was infected sixteen months previously had become purified.

A third observation was made in Nelspruit in connection with our experiments carried out during the months of November and December, 1903, and January, 1904. The result of these experiments is given in the Annual Report of last year. Suffice it to say that in November, 1903, fifteen animals died from East Coast Fever, in December eight animals, and January, 1904, seven animals died which had all contracted the disease within a certain area known to have been infected.

The date of the last death of East Coast Fever amongst the experimental animals at Nelspruit was January 25th, 1904. Out of 46 animals exposed only one survived. The temperature of this animal was constantly taken, and whenever any disturbance took place a microscopical examination was made which in every case gave negative results. We had, therefore, to conclude that this was a case of rare exception when an ox was naturally immune against the disease. Since November, 1903, immune oxen—for convenience sake called Komati oxen, the survivors of a herd of 500 oxen which had perished in August, 1902, at Komati Poort—were running on the same pasture as the above cattle which had died. Five of this number were returned to Pretoria on January 13th, 1904, to be used for inoculation purposes. These immune animals—namely, the one survivor of the experiment and the remaining Komati oxen—were the only animals grazing from January 25th to April 13th on the infected ground. On this date three fresh heifers were exposed for experimental purposes, namely, Nos. 200, 222 and 234. Heifer 200 was returned to Pretoria on June 25th, 1904, being in high fever, the nature of which could not be ascertained, although East Coast Fever had to be excluded. The heifer recovered, and is still alive. Heifer 222 died on October 13th from heartwater. Microscopical examination excluded East Coast Fever. Out of this lot of three animals there remains at the time of writing one still alive on the Nelspruit Experimental Farm. On June 22nd, ten oxen were sent to the same place, seven of which had been inoculated according to Professor Koch's method, and were then ready to be exposed for a test. Three were never injected with Coast Fever blood, and were to serve as controls for the inoculated cattle. Out of this lot one ox died, namely, No. 239, on January 10th, 1905. Unfortunately, no smear was obtainable for microscopical examination, the assistant in charge being absent on duty on that date; but the record of the temperature, which was taken twice every day, indicated clearly that the animal did not die of Coast Fever, but met with a sudden death through some rapidly acting cause, such as vegetable poison. On December 8th, 1904, the five Komati oxen which on January 13th, 1904, were sent to Pretoria, were returned to Nelspruit, simply to get them out of the way.

On January 10th, 1905, nineteen oxen sent on November 24th, 1904, to Barberton were brought to Nelspruit. At that time 11½ months had passed without a single case of Coast Fever. It was considered that a larger number of oxen exposed would demonstrate more clearly whether the pasture had become purified within these eleven months, as there was the likelihood that more ticks would be picked up, and accordingly with them the disease if it still existed. No case of Coast Fever was, however, so far recorded. Thus we must

conclude that the once badly infected area of our Nelspruit Experimental Station had become purified, and—what is particularly striking—during the period in which susceptible cattle were still exposed and immune oxen had been permanently kept.

A glance at the record table of the microscopical examinations during the first few months of the year 1905 shows the presence of Coast Fever in the various infected districts. Most outbreaks occurred in the month of February. This observation may serve as a control to the experiment just related to show that the conditions in the country had not changed in the same way as they had altered on the Nelspruit farm, which at one time was notorious for bad infection. In order to understand this change of affairs in Nelspruit it will be necessary to explain how the experiments were conducted. The temperature of every exposed animal was taken in the morning and in the evening. As soon as a reaction was noticeable, the animal was stable kept, caps were drawn over the ears, and all engorged ticks collected. This was done with every animal. No sick animal was allowed to run on the farm, and death took place in the stable. It is evident in this way a re-infection of the ground is prevented, and the disease must die out. The experiment proved, moreover, that immune oxen do not give the infection to the pasture, as was thought by Professor Koch; otherwise this pasture would have been permanently infected, as immune oxen were constantly running on the place. Indeed, this fact may have helped to purify the ground, as infected ticks (larvæ or nymphæ) which suck on non-susceptible animals lose their virulency. The principal question arising out of our experiment is whether the purification of the ground was due to the disappearance of the Brown Tick, or to losing its virulency by the length of time which elapsed before the progeny could have become infected.

As already stated, a collection of all available ticks was made on sick animals, which were forwarded to Pretoria, where they served for further experiment, as will be referred to in another portion of this Report. The collection of ticks from susceptible animals and those immune animals which were exposed was continued. The result was that brown and black-pitted ticks, principally the former, were constantly found on the immune cattle, and also on the non-immune cattle which were exposed on April 13th. There was, however, a distinct decrease in their numbers during the month of April, and in May, June and July none were collected. The absence of brown ticks during these months may be explained by the winter season, and the burning of the grass which then took place. In August the ticks became more numerous, and principally nymphæ were collected both from the immune and the non-immune cattle. The same took place during September and October, and in November the adult ticks again appeared. They were, however, found in small numbers. In December none were met with, and only one specimen was obtained in January, and since then no more were seen, although other ticks, such as Bont, Bont-leg, Red-legs, were constantly found. It is now clear that the brown tick has almost completely vanished in an area which was once badly infected. The disappearance of the disease does not, however, coincide with the disappearance of the tick, brown nymphæ and adults being found on all susceptible animals, and at a time when infection could reasonably be expected, namely, within six months after the death of the last animal, on January 25th, 1904.

That the place did, however, not become re-infected was due to the fact that we did not allow a sick animal to graze. Hence the infected ticks which were not picked up by the cattle at that time probably were dead before the susceptible animals had arrived or had become purified. On the place in question, besides the immune oxen other non-susceptible animals were running, such as four camels and some mules. We also found the brown tick on a wild hare. All these conditions taken together will account for the disappearance of the disease.

This observation is a striking illustration that it is possible to clean an infected area even when there are susceptible cattle running on it, provided the cattle, as soon as their temperature rises, are so confined that they are unable to drop ticks on the pasture. In practice, use can be made of this observation to save cattle when the disease breaks out in a herd. Then all cattle should be confined in a small non-infected area. The temperature of every animal should at once be taken. This enables us to divide the herd into two lots, the sick ones and those not yet affected. The former are either killed or returned to the infected ground, and the latter remain where they are, being closely confined.

Now we know that the incubation time of Coast Fever at its longest lasts 20 days. We, therefore, take the temperatures at intervals during this time, and act in the manner indicated. There is no danger within this time of non-infected cattle becoming infected by ticks which may have dropped from sick cattle with which they were mixed, because the tick did not take that infection during the incubation time, and not even during the first few days of the disease, and the tick which drops to the ground must in the first instance undergo a moulting process before it is able to re-infect fresh cattle. This period lasts more than 20 days. After the lapse of this time, all the oxen which have never shown an elevation of temperature indicating Coast Fever can be removed to clean areas. This policy can even be simplified on an infected farm. The temperature need not necessarily be taken, as within 20 days most infected animals will show symptoms of the disease. The moving about of stock from infected to non-infected ground should be undertaken about every month. The sick cattle will then gradually die out and no more will sicken if not returned to infected areas. After the lapse of a year, the cattle could be put on to the land where the infection started, and which in the meantime had become clean. These are the practical deductions which can be made from the foregoing experiments.

II.

DO SALTED CATTLE CONTAIN THE PIROPLASMA PARVUM IN THEIR BLOOD?

At the Conference on Cattle Diseases held at Bloemfontein on December 3rd, 4th and 5th, 1903, Professor Koch stated that the blood of recovered animals still retained the micro-organisms of East Coast Fever, which can only be detected by an expert by means of microscopic examination, and that these recovered animals will maintain the infection. At that time I doubted the correctness of such a statement, because a striking observation in the field showed that immune and susceptible stock could graze together with impunity for over nine months. I have since shown that the forms of parasites which Professor Koch saw in oxen immune against Coast Fever had nothing to do with that disease, as they were found in susceptible cattle consequent to an injection of blood of an ox immune against ordinary redwater. In addition to this observation may be quoted our experiment at Nelspruit, where, notwithstanding the fact that salted oxen were constantly grazing,

the farm became purified. The question could readily be decided by experiments with brown ticks. I have shown how easily they disseminate Coast Fever, and that it is usually the adult brown tick which acts as the principal propagator.

The following tabulated form records the origin, the feeding of brown ticks on the immune ox, No. 101, which had been brought up from Nelspruit :—

No.	Origin.	Date of collection.	Egg laying starts.	Hatching into larvæ.	Feeding as larvæ.	Dropping as engorged larvæ.	Moulting into Nymphæ.	Feeding as Nymphæ.	Dropping engorged Nymphæ.	Moulting into adults.	Feeding as adults.
A.	Sheep, Nelspruit No. 8	21-12-03	10-1-04	9-2-04	23-2-04 on Texas ox 224	29-11-04	26-3-04	5-5-04 on Komatie ox 101	15-5-04	15-8-04	25-8-04 on B249
B.	Ox Nelspruit 28	22-12-03	12-1-04	9-2-04	do.	do.	do.	do.	do.	do.	do.
C.	Station ox 34 ...	27·12-03	12-1-04	15-2-04	do.	do.	do.	do.	do.	do.	do.
D.	Nelspruit ox 32	2-1-04	10-1-04	11-2-04	do.	do.	do.	do.	do.	do.	do.
E.	Station ox 207...	4-1-04	13-1-04	17-2-04	do.	do.	do.	do.	do.	do.	do.
F.	Station ox 190...	7-1-04	13-1-04	13-2 04	do.	do.	do.	do.	do.	do.	do.
G.	Oxen 29, 31, R.T.K.	18-1-04	24-2-04	7-3-04	11 3-04, on Somalie ox 186	—	7-4-04	12-4-04 on Komatie 101	16-4-04	24-6-04	25-8-04 on B249
H.	Oxen 39, 43, 45, Nelspruit	20-1-04	10-2-04	3-3-04	14-3-04, on Somalie 186	—	7-4 04	19-10-04 Komatie 101	22-4-04	24-6-04	25-8-04 on B249
I.	Oxen 38, 39, 41, 42, 43, 46	23-1-04	8-2-04	7-3-04	18-3-04 on Somalie 186	—	7-4-04	19-4-04 Komatie 101	22-4-04	4-7-04	25-8-04 on B249
K.	Ox 39, Nelspruit	25-1-04	6-2-04	7-3-04	14-3-04, on Ox 709	19-3-04	3-4-04	5-5-04 Komatie ox 101	10-5-04	11-8-04	25-8-04 on B249

From the tabulated record it can be seen that ten different ticks of various origin were utilised. The first feeding stage as larva was passed on cattle susceptible to Coast Fever. The second feeding stage as nympha was passed on the immune ox No. 101. The various adult ticks were collected and mixed, and on

1. August 25th, 1904, Ox B 249 was infested with part of this lot. Further infestations took place on 30/8/04, 3/9/04, 7/9/04, 13/9/04, 15/9/04 and 17/9/04. There were 61 engorged females collected, and numerous males remained attached for several weeks. Ox B 249 is still alive.

2. Ox B 149 was infested with part of the above lot of brown ticks on 7/9/04. The engorged females began to drop on 16/9/04. Eleven engorged females were collected. Numerous males remained on the animal for some time. Ox B 149 was still alive on 1/4/05.

3. Part of the lot of ticks referred to under letter K in the tabulated record was placed on Ox B 245 on September 19th, 20th and 21st, 1904. Only a few females were amongst these adults, which consisted principally of males. They were observed to be attached for a long time to B 245, which animal was still alive on 1/4/05.

4. The brown nymphæ which were collected from the immune oxen exposed at Nelspruit on 13/8/04, 24/8/04, 12/9/04, 19/9/04, 26/9/04, 1/10/04 and 25/10/04, moulted in the Laboratory, and the adults were placed from 2/11/05 to 21/11/05 on Bull 257. Forty engorged females dropped and were collected. The males were present up to 4/12/04. This bull was still alive on 1/4/05.

Conclusion.

Taking into consideration that in my experiments with pathogenic brown ticks I was able, out of nine cases, to produce the disease eight times, that only two ticks were necessary to cause the disease, the failure of the four experiments, two of which were carried out with a large number of ticks, has only one possible interpretation, namely, that the brown adults feeding as nymphæ on cattle immune against Coast Fever do not propagate the disease, hence the blood of immune oxen does not harbour the parasite.

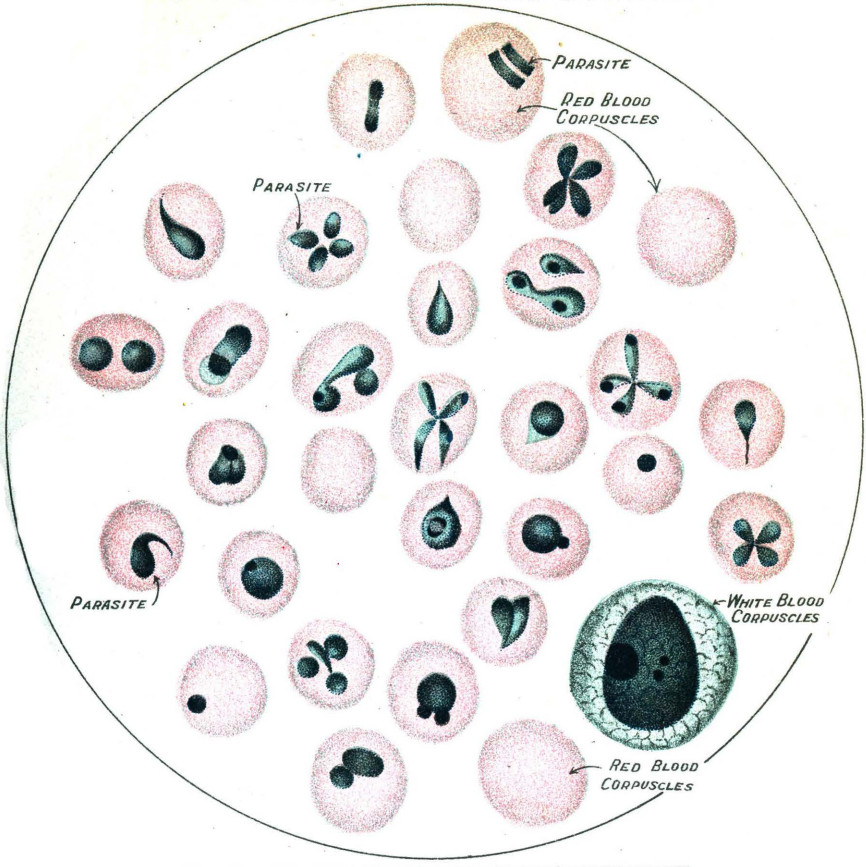
FURTHER NOTES ON PIROPLASMOSIS OF THE HORSE, MULE, AND DONKEY.

INOCULABILITY OF PIROPLASMOSIS.

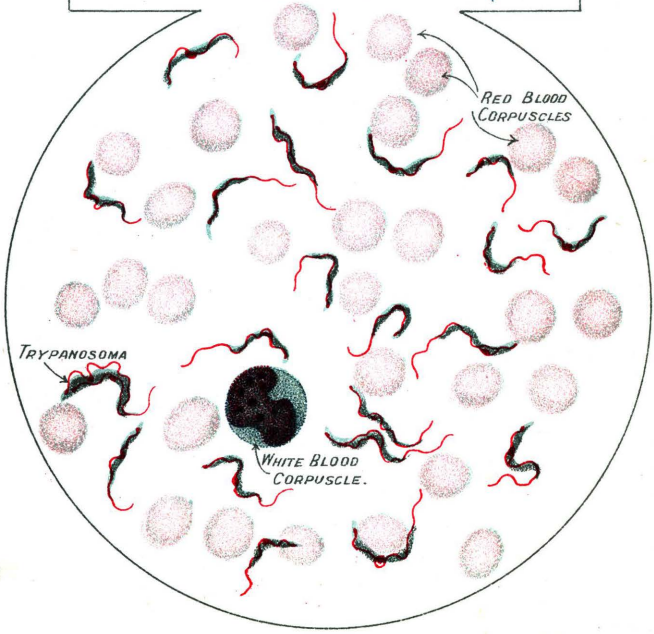
The description of the piroplasma found in horses, mules and donkeys was fully entered into in my previous communications on this subject. Symptoms and *post-mortem* lesions observed in the disease in mules and donkeys as encountered in the field were dealt with in my Annual Report for the year 1903-4. At that time I expressed the opinion that the piroplasmosis in the three above-mentioned equines was identical, due to the same species of piroplasma, namely, Piroplasma Equi (Laveran). The conclusive proof—viz., that of producing the disease in the horse with virulent mule or donkey blood, or *vice versa*, was at that time wanting. I had only one observation at my disposal, that showing the inoculability of equine piroplasmosis from an immune horse into a susceptible one by means of the defibrinated blood of the former. This observation indicated a close relationship of equine, bovine and canine piroplasmosis, where the blood of immune animals proves to be

PYROPLASMOSIS

OF THE HORSE, MULE AND DONKEY.



TRYPANOSOMIASIS



C. FAVRE

GOVT. PRINTING WORKS, PRETORIA.

infective for susceptible animals of the same species. Having failed in former experiments to transmit the piroplasmosis of the donkey into the horse, because only country-bred or well acclimatised horses were obtainable, resource was had this time to imported horses, mules and donkeys from the Argentine Republic—animals which, as we know from practical observations, are very susceptible to piroplasmosis. To exclude any possible natural infection they were kept stabled on the premises of the Laboratory. The infective material was derived in every instance from immune animals. The first experience with the blood of an immune horse having proved successful, the same might be expected from immune mules and donkeys. The object of the experiment was not solely directed to demonstrate the identity of the piroplasma in the various equines, but also to test the possibility of a preventive inoculation similar to the one in redwater. The experiment also gave us useful information respecting the incubation course of the disease and its immunity, and will prove so closely allied to redwater in cattle that more than a similarity must be expected, although an absolute identity is not probable.

A.—EXPERIMENTS TO PROVE THAT THE INOCULATION OF BLOOD OF A HORSE IMMUNE AGAINST EQUINE PIROPLASMOSIS PRODUCES PIROPLASMOSIS IN SUSCEPTIBLE ANIMALS.

1. Experiment, Argentine horse, No. 599, about four years old, brought directly from board ship to the premises, was injected on October 3rd, 1904, both intrajugularly and subcutaneously with 10 c.c. defibrinated blood of a horse, No. 523, which in July of the same year had recovered from a spontaneously acquired attack of biliary fever, during the fever reaction of which piroplasma equi was very frequently found in the blood. After an incubation period of six days, viz., to October 9th, 1904, the temperature rose, and on the seventh day (10/10/04) piroplasmas were present in the blood. These parasites had increased within 24 hours, after which time rosettes were especially numerous (11/10/04). Red urine was voided for the first time on 11/10/04, and it was noticed during the following few days. A spectroscopical examination both in my laboratory and in that of Mr. Ingle, Chief of the Chemical Division, proved the exclusive presence of oxyhæmoglobin, which was still present in traces after the urine had cleared up and taken a more brownish colour. On 15/10/05 a decrease of the number of parasites was distinctly noticeable, the parasites becoming rarer, and at the same time a slight poikylocytosis made its appearance. The decrease kept on, and on October 20th the parasites had disappeared. The symptoms which were observed during the course of the disease, besides red urine, were a strongly pronounced jaundice, a rapid loss of condition, accelerated hard breathing and rapid pulsation of the heart. The hard breathing kept on for some time after the temperature had returned to more or less normal elevation. The examination of blood was discontinued. A slight secondary reaction was noticed starting at 24 days after inoculation and lasting about ten days. Since no microscopical examination was made during that reaction, it is impossible to say to what it is due.

2. Argentine Horse No. 598, about four years old, injected on November 4th intrajugularly with a mixture of defibrinated blood of Horses 442, 523 and 599, all of which had passed through an attack of biliary fever (compare Nos. 523 and 599 to first experiment), No. 442 on September 19th to 27th, 1904. A reaction began four days later,

but did not reach a high elevation. The piroplasma equi could only be traced during the descending period of the fever, at which time rosettes were found. Then followed a period of six days when the temperature was normal, and during which no piroplasmata were seen, to be succeeded by a high elevation of the fever and the reappearance of piroplasma equi and an irregular temperature curve, which after seven days became normal. In this horse the peculiarity was noticed that during the first fever reaction the round piroplasma was principally noticed; in the second one quite an atypical form was seen, more oval, even willow leaf shaped, and twin parasites in one and the same red blood corpuscle, as in the case of piroplasma bigeminum.

3. Argentine Horse 603, about four years old, was injected on November 28th, 1904, with defibrinated blood of Horses 442 and 599, 15 c.c. under the skin and 15 c.c. into the vein. Piroplasma equi was noticed in this horse on 6/12/04, when rosettes were present, and at a time when fever reaction had not yet properly started. The parasites were present during the first seven days, when they disappeared completely. The animal had been treated with a subcutaneous injection of quinine. As is frequently the case in biliary fever, a local phlegmosis formed on the place of injection, which was responsible for the continuance of the fever. The following tabulated record of the count of red corpuscles during the disease shows their decrease and increase :—

Date.	No. of Red Corpuscles.	Presence or absence of Piroplasma.
29/11/04	7,200,000	
30/11/04	6,610,000	
1/12/04	6,260,000	
2/12/04	6,400,000	
3/12/04	6,000,000	
4/12/04	5,840,000	
5/12/04	5,800,000	
6/12/04	5,080,000	Piroplasma equi appears. Rosette.
7/12/04	4,792,000	Piroplasma fairly frequent. Rosettes round oval and flagellated forms.
8/12/04	3,420,000	Piroplasma rarer.
9/12/04	2,320,000	Piroplasma still rarer. Some bacillary-shaped forms are visible.
10/12/04	2,888,000	Examination remains negative.
11/12/04	2,764,000	Piroplasma equi very rarely met with.
12/12/04	3,552,000	Piroplasma absent, 13/12/04.
13/12/04	4,560,000	„ „
14/12/04	5,936,000	„ „
15/12/04	6,160,000	„ „
16/12/04	6,552,000	„ „
17/12/04	6,496,000	„ „

During this time the usual characteristic symptom of equine piroplasmosis—viz., jaundice—was present, and a rapid loss of condition took place. The horse had apparently recovered, when, on December 21st, a second attack of fever occurred lasting five days, to which the animal succumbed. During this reaction round and willow leaf shaped parasites were noticed, and twin forms were also met with.

Post-mortem revealed a slight jaundiced condition of the serous membranes of the body. There were petechiæ on the endocord of the left ventricle, and also on the epicard. The liver was enlarged and of

a normal colour. The spleen was but slightly enlarged. The remainder of the organs seemed to be normal.

4. Argentine Horse 597, about four years old, was injected on 28/11/04 with 10 c.c. blood of Horse 523 (compare Exp. A1). In this case piroplasmata were seen before there was an elevation of temperature, which elevation took place late and lasted for four days. The piroplasmata were but rarely met with; the symptoms of an anæmia were well pronounced by an extensive paleness of all mucous membranes. There was no jaundice present. The following tabulated record shows decrease and increase of the red corpuscles during the reaction.

Date.	No. of Red Corpuscles.	Presence or absence of Piroplasma.
29/11/04	6,080,000	
30/11/04	5,144,000	
1/12/04		
2/12/04	3,536,000	
3/12/04	3,948,000	
4/12/04	3,720,000	
5/12/04	3,984,000	Piroplasma equi present.
6/12/04	4,652,000	Piroplasma equi present, but rare.
7/12/04	4,800,000	" " "
8/12/04	4,884,000	" " "
9/12/04	4,954,000	Piroplasma equi very rare.
10/12/04	3,416,000	Piroplasma equi absent.
11/12/04	2,496,000	" "
12/12/04	3,052,000	
13/12/04	2,480,000	
14/12/04	3,408,000	
15/12/04	5,238,000	
16/12/04	5,392,000	
17/12/04	6,132,000	

On December 30th, 1904, this horse was subjected to the simultaneous horse sickness inoculation. It passed through an attack of dikkop and recovered, the temperature returning to normal, when on January 16th, 1905, the temperature rose suddenly again, piroplasma equi reappeared in the blood, and the animal died on January 23rd, 1905.

Post-mortem showed a fair condition. There was a yellow discolouration of the mucous membranes, a subcutaneous collection of yellow liquid. The lungs were in a state of oedema, and somewhat pale in colour. The epicard was diffusely reddened by petechiæ, and large echymosis were found on the endocard of both ventricles. The liver was much enlarged, yellow, and the central veins were filled with blood. The spleen was but slightly enlarged and rather firm. The kidneys were pale. The lymphatic glands of the various regions were enlarged.

5. Argentine Horse No. 606, about four years old, injected on 28/11/04 with 30 c.c. of a mixture of blood of Horses 442 and 599 (compare Exp. A 1 and A 2), 15 c.c. subcutaneously and 15 c.c. into the jugular vein. After an incubation period of five days the temperature rose. The following day symptoms of jaundice were noticeable in the eye. Piroplasmas were very frequent during the reaction, which lasted ten days and ended with death on 13/12/04. The *post-mortem* revealed the typical picture of equine piroplasmosis with general jaundice.

Conclusions.

1. Equine piroplasmosis is inoculable with blood of an immune horse into a susceptible horse, the Argentine horse proving very suitable for this purpose.

2. The piroplasmosis produced by an inoculation with immune blood has an incubation time ranging from five to six days.

3. In cases of recovery from the first attack of piroplasmosis, a second attack is usually observed, during which the piroplasma equi is less frequently found than in the first attack, and also changed in shape, usually adopting the leaf form. Death may result from either the first or the second attack, but is more frequent after the second.

4. The pathological change caused by the piroplasma equi is an acute anæmia, due to the destruction of red corpuscles.

5. The inoculation of blood from an immune horse into a susceptible one proves to be very dangerous when injected intrajugularly, and offers but little prospect for a practical inoculation.

B.—EXPERIMENTS TO SHOW THAT THE INOCULATION OF BLOOD OF A HORSE IMMUNE AGAINST EQUINE PIROPLASMOSIS PRODUCES THE DISEASE IN SUSCEPTIBLE MULES.

1. Argentine Mule No. 587, about three years old, directly imported from board ship, was injected on November 4th, 1904, with a mixture of defibrinated blood of Horses 523, 599, 442 (compare A1 and A2), of each 5 c.c., viz., 15 c.c. into the jugular vein and 15 c.c. under the skin. On the fifth day a first rise in temperature took place, but the examination of the blood remained negative. It was followed by a second reaction, during which piroplasma equi was present. After the lapse of about ten days, during which the examination of the blood gave negative results, another reaction took place, during which the piroplasma equi again made its appearance. Since then no further disturbance was noticed in the temperature curve. This animal showed during the first reaction a pronounced paleness of the mucous membranes, and lost considerably in condition.

Argentine Mule No. 589, about three and a half years old, same origin as above. Injected on 28/11/04 with a mixture of equal parts of blood of three immune horses Nos. 442, 523, 599 (compare Exp. A 1 and A 2). A first slight disturbance took place on 4/12/04, viz., six days after injection. Piroplasma equi was seen the next day; it increased in numbers, and during the 8-9/12/04 rosettes were fairly frequent. From the 10/12/04, when the temperature had reached its highest point, the parasites became rarer; at the same time some atypical bacillary shaped ones appeared. The next day, viz., 11/12/04, the parasites had almost vanished out of the blood. After a lapse of about seven days, a second reaction took place, starting 19/12/04, during which, unfortunately, no examination took place.

2. Mule 596, about three years old, similar origin as previous one, was injected on 28/11/04 intrajugularly with 10 c.c. of blood of Horse 523. On the eighth day after inoculation, a slight increase of temperature occurred, and the following day piroplasma equi appeared in the blood. The reaction was, however, light, and the parasites were very rarely seen. Also in this case a second reaction occurred a few days after the first one had abated, the examination of the blood during which gave

negative results. The following tabulated form gives the record of the number of red corpuscles during the first reaction :—

Date.	No of Red Corpuscles.	Presence or absence of Piroplasma.
5/12/04	5,792,000	
6/12/04	6,448,000	
7/12/04	6,600,000	Piroplasma present, but rare.
8/12/04	6,712,000	,, ,,
9/12/04	6,140,000	,, ,,
10/12/04	4,392,000	,, ,,
		Mucous membranes pale.
11/12/04	5,312,000	Piroplasma present.
12/12/04	4,948,000	
13/12/04	4,728,000	
14/12/04	6,500,000	
15/12/04	6,336,000	
16/12/04	6,720,000	
17/12/04	6,832,000	

3. Mule 592, about four years old, same origin as previous one, was injected on 28/11/04 with a mixture of blood of Horses 599 and 442 (compare Exp. A 1 and A 2), viz., 15 c.c. subcutaneously and 15 c.c. intrajugularly. After a lapse of five days a slight rise of temperature was noticeable, during which the piroplasma equi was fairly frequent; then a distinct high reaction set in, lasting from December 8th to 14th, 1904, and during which the piroplasma was but rarely noticed. Also in this case a second reaction, although not very characteristic, took place, but no parasites were seen. The following tabulated form gives a record of the number of red corpuscles during the first reaction :—

Date.	No. of Red Corpuscles.	Presence or absence of Piroplasma.
29/11/04	6,580,000	
3/12/04	7,012,000	
5/12/04	5,816,000	
6/12/04	6,120,000	
7/12/04	6,698,000	Piroplasma fairly frequent.
8/12/04	5,400,000	
9/12/04	4,528,000	Piroplasma rare.
10/12/04	3,064,000	,, ,,
11/12/04	3,928,000	,, ,,
12/12/04	4,376,000	
13/12/04	4,072,000	
14/12/04	5,048,000	
15/12/04	4,908,000	
16/12/04	5,000,000	

4. Mule 595, about four years old, same origin as previous one, was injected on January 16th, 1905, with 10 c.c. defibrinated blood of Horse 597 (compare Exp. A 4). A slight reaction took place, and on the ninth day piroplasma equi was noticed in rare numbers. In this instance no second reaction was noticeable.

Conclusions.

1. Equine piroplasmosis is inoculable with the blood of an immune horse into a susceptible mule, Argentine mules proving very susceptible for this purpose.

2. The incubation time of piroplasmosis in mules corresponds with that found in horses.

3. All inoculated cases ended in recovery. As in horses, two reactions are observed, the second one being characterised by the scarcity or complete absence of parasites.

4. The inoculated piroplasmosis is in mules not so fatal as in horses, and the anæmia is also less pronounced.

5. The inoculation of blood from an immune horse into a susceptible mule can be made use of as a means of preventive inoculation against piroplasmosis.

C.—EXPERIMENTS TO SHOW THAT THE INOCULATION OF BLOOD OF A HORSE IMMUNE AGAINST PIROPLASMOSIS PRODUCES THIS DISEASE IN SUSCEPTIBLE DONKEYS.

Donkey 736, about four years old, imported from the Argentine, and brought by rail from Capetown off the boat, was injected with blood of Horse 597 (compare A 4). There was a distinct primary, followed up shortly afterwards by a secondary reaction. By some mistake, the blood of this donkey was only examined after the secondary reaction had passed. Piroplasma equi was, therefore, not found in this case. The donkey lost rapidly in condition, went down and could not lift himself. He died on February 12th, 1905.

A *post-mortem* revealed a slightly enlarged spleen, with softened pulpa. There was a slight increase of liquid in the heart bag, and *ante-mortem* clots in both ventricles. These lesions indicate piroplasmosis, viz., the chronic form of this disease.

Donkey 739 and Donkey 740 were both injected on 21/3/05 with 10 c.c. blood of Horse 724 (compare Exp. D1) subcutaneously. Both donkeys showed a typical fever reaction, during which the piroplasma equi was found, namely, in Donkey 739 on 27/3/05 and 28/3/05, and in Donkey 740 on 29/3/05 and 30/3/05. The reaction in both cases was very slight, and could only be diagnosed as piroplasmosis by the microscope.

Conclusion.

The injection of blood of a horse immune against piroplasmosis into a susceptible donkey produces piroplasmosis in the donkey.

D.—EXPERIMENTS TO SHOW THAT THE BLOOD OF A MULE IMMUNE AGAINST EQUINE PIROPLASMOSIS REMAINS INFECTIVE FOR SUSCEPTIBLE HORSES.

1. Horse No. 724, about four years old, imported from Argentine and landed at Capetown; brought from aboard ship direct by rail to Pretoria. It was inoculated intrajugularly on January 16th with 10 c.c. def. blood of Mule 592 (compare B 3). After three days already a reaction started, which lasted five days, piroplasma equi was noted for the first time on January 22nd, and disappeared after the temperature had returned to normal. The animal showed all the symptoms of biliary fever, and had lost in condition, lying down, and being unable to stand for some days. From the 14th to the 23rd of February a second reaction took place, during which, however, no piroplasma was seen. This second reaction reduced the animal to a skeleton.

2. Horse 721, the same origin as above. It was injected subcutaneously on January 15th with 10 c.c blood of Mule 592 (compare B3). A reaction began already on the fifth day. The temperature did not go high: its culmination point was at 104° Fahr. Piroplasma equi

was present on February 23rd, and during the following days up to date of death, which occurred on February 26th. *Post-mortem* was made soon after death. There was a slightly jaundiced condition of the serous membranes. The spleen was about the double of its normal size, with softened pulpa. The liver was dark brown, strongly congested. The kidneys showed the colour of sepia.

Conclusions.

1. Equine piroplasmosis is inoculable from immune mules into susceptible horses.

2. The piroplasmosis of horses produced by immune blood of mules is of a very grave character, and there is little prospect that immune mule blood can be utilized for the inoculation of susceptible horses.

E.—EXPERIMENTS TO SHOW THAT THE BLOOD OF A MULE IMMUNE AGAINST EQUINE PIROPLASMOSIS REMAINS INFECTIVE FOR SUSCEPTIBLE MULES.

1. Mule 593, Argentine, about four years old, brought directly from board ship to the laboratory, was injected on January 16th, 1905, with 10 c.c. defibrinated blood of Mule 587 into the jugular vein (compare Exp. B 1). From the sixth day a disturbance of the temperature took place without showing any regular course. The examination of the blood gave negative results.

Conclusion.

Although the strict proof—viz., the demonstration of the piroplasma equi—is lacking, we are entitled to accept that the temperature reaction was due to the inoculation of the blood, since we know from previous experiments that mules support inoculations with immune blood easily.

F.—EXPERIMENTS TO SHOW THAT THE BLOOD OF A MULE IMMUNE AGAINST EQUINE PIROPLASMOSIS REMAINS INFECTIVE FOR SUSCEPTIBLE DONKEYS.

1. Donkey 737, imported from the Argentine, and from ship at Capetown brought directly to Pretoria, was inoculated on January 16th, 1905, with 10 c.c. def. blood into the jugular vein. A slight disturbance of the temperature only took place after fourteen days, and a further disturbance was noticeable after three weeks. There is, however, no proof that the reaction was due to the presence of piroplasma equi.

2. Donkey 741, same origin as above. It was injected on February 15th, 1905, with 10 c.c. blood of Mule 592 (compare Exp. B 3). On February 27th, viz., twelve days after inoculation, and during a very slight fever reaction, the piroplasma equi was found in the blood. They had appeared already the next day. A second more distinct reaction started about sixteen days after the inoculation. The examination of the blood gave, however, negative results.

Conclusion.

The inoculation of blood of a mule immune against equine piroplasmosis produces a mild disease in donkeys, with a primary and secondary reaction; the inoculation of such blood may, therefore, be made use of as a practical inoculation of donkeys against the disease.

G.—EXPERIMENTS TO SHOW THAT THE BLOOD OF A DONKEY IMMUNE AGAINST EQUINE PIROPLASMOSIS IS INFECTIVE FOR SUSCEPTIBLE HORSES.

1. Horse No. 600, Argentine, about four years old, brought from board ship in Durban to Pretoria, was, on January 16th, injected intrajugularly with 10 c.c. def. blood of a Donkey No. 306. This donkey was

a country-bred animal, and has been hyper-immunised with horse-sickness blood of horses. On January 22nd, 1905, the temperature began to rise, to reach 105° Fahr. on January 25th, on which day the piroplasma equi was frequent. A very severe attack of biliary fever had developed, the symptoms of jaundice being well pronounced on the 25th January. The horse recovered from the first reaction, when a slight secondary reaction took place, closely followed by a third distinct one, during which the piroplasma equi was seen again. The horse recovered, but had lost enormously in condition.

2. Horse 723, Argentine, about three years old, brought from board ship in Capetown direct to the laboratory. Inoculated on February 15th, 1905, with 10 c.c. def. blood of Donkey 306 (compare previous experiment) under the skin. From the fifth day after the injection a slight reaction began. On the ninth day piroplasma equi was present. The number increased during the next few days, rosettes being noticed freely. The reaction was not actually a heavy one ; notwithstanding this, all the clinical symptoms of jaundice had developed. A second slight reaction occurred on the 7th and 8th March, viz., three weeks after inoculation, during which, however, no piroplasma was seen. The horse contracted horse-sickness spontaneously, and died on March 15th.

Conclusions.

1. The inoculation of the blood of a donkey immune against equine piroplasmosis produces this disease when injected into susceptible horses.

2. In the course of the disease, two reactions were noticeable.

3. Although the clinical symptoms indicated a fully-developed disease, it was not so grave as to cause death.

There does exist some prospect of utilising the blood of immune donkeys for inoculation purposes against biliary fever of the horse.

H.—EXPERIMENTS TO NOTE WHETHER THE BLOOD OF A DONKEY IMMUNE AGAINST EQUINE PIROPLASMOSIS WILL PRODUCE THE DISEASE WHEN INJECTED INTO SUSCEPTIBLE MULES.

1. Mule 590, Argentine, about three and a half years old, brought from board ship at Durban to Pretoria, was inoculated in the jugular vein on January 16th, 1905, with 10 c.c. def. blood of Donkey No. 306 (compare G 1, 2). On the ninth, tenth and eleventh days a slight reaction was noticeable. The temperature on January 25th was 103.8° Fahr. Piroplasma equi was not seen. Nothing to identify with a second reaction occurred after this.

2. Mule 591, Argentine, about three years old, same origin as previous one. It was inoculated on February 15th. A slight reaction took place and a second, likewise a slight one, was noticeable about the third week. Piroplasma equi was not seen.

Conclusion.

The blood of a donkey immune against equine piroplasmosis produced a slight reaction in susceptible mules. This reaction corresponded in its character to those found in mules injected with immune blood of horses and mules. There exists every prospect of utilising the blood of an immune donkey for the inoculation of mules against biliary fever.

I.—EXPERIMENTS TO SHOW THAT THE BLOOD OF A DONKEY IMMUNE AGAINST EQUINE PIROPLASMOSIS WILL PRODUCE THE DISEASE WHEN INJECTED INTO SUSCEPTIBLE DONKEYS.

1. Donkey 738, Argentine, brought from board ship at Capetown to Pretoria, was injected into jugular vein with 10 c.c. def. blood of Donkey

306 on January 16th, 1905. The ninth day afterwards the reaction commenced, and the tenth day piroplasma equi was very frequent. It increased in numbers during the following days, and the donkey died on January 28th or 29th, 1905. *Post-mortem* was made in the morning. The flesh had a slight brownish hue. There was a big *ante-mortem* clot in the right, and a red clot in the left ventricle. There were no petechiæ in the heart. The liver was slightly jaundiced. The spleen was very much enlarged, about four to five times its normal size, the pulpa was very soft. The kidneys were pale, and the bladder was full of red urine.

2. Donkey 744, Argentine, similar origin as previous one, was injected subcutaneously on February 15th, 1905, with 10 c.c. def. blood of Donkey 306. A first reaction was noticeable after five days, and piroplasma equi was seen for the first time on January 22nd; it was still present on January 24th, and absent on the following day. Between the first and second reaction was an interval of four days. The second reaction lasted three days, but no piroplasma was traceable during that time.

Conclusions.

The blood of a donkey immune against equine piroplasmosis produced a deadly disease in a susceptible donkey and a distinct reaction in a second one. There is accordingly but little prospect of utilising the blood of an immune donkey for inoculation purposes against the piroplasmosis in donkeys.

Summary of Conclusions.

1. The piroplasma found in the horse, the mule, and the donkey represents the species of piroplasma equi, as first found in the horse.

2. The disease caused by this piroplasma is inoculable with blood of immune animals into susceptible ones belonging to the domesticated species equi.

3. The horse shows the greatest susceptibility for this piroplasma; the donkey is less and the mule the least susceptible.

4. The possibility of a practicable inoculation against piroplasmosis stands in the reversed order of the susceptibility. The mule may be safely inoculated with immune blood of any of the three respective equines. The immune horse blood produces the severest reaction. The immune mule blood causes little reaction, and so does the immune donkey blood.

For practical purposes the inoculation of mules with blood of immune donkeys may prove successful.

The donkey proves to be equally susceptible to immune blood taken from horses and donkeys, and shows the lightest reaction to the injection of immune mule blood.

For practical purposes the inoculation of donkeys with blood of immune mules may prove to be successful.

The horse, being extremely susceptible to piroplasmosis due to the injection of immune blood from the horse and mule, seems to suffer less from inoculation of immune donkey blood.

For practical purposes the inoculation of horses with blood of immune donkeys will have to be considered.

PIROPLASMA EQUI AS A COMPLICATION OF HORSE-SICKNESS.

In the notes on equine Piroplasmosis I have demonstrated the inoculability of this disease into susceptible animals with the blood of immune horses, mules and donkeys. It must accordingly be anticipated that a similar occurrence may be expected whenever the blood of a country-bred horse or mule has to be utilized for inoculation purposes. Such is the case with the inoculation method I have introduced against horse-sickness where serum and virulent blood are used for the simultaneous injection. Naturally, we expect that horses and mules born and bred in the country where the disease is existing do not contract it from the inoculation, because such animals have a high degree of immunity. We would expect it in freshly imported horses and mules, and less in such which have lived one or more years on the South African pasture. Again, the disease would only be inoculated with fresh blood which is used as virus, or with serum which contains a certain amount of red corpuscles. The danger of inoculating the disease with this material can be reduced to almost nil by using either absolute clear serum or sanguinolent serum which has been kept for some time, and in which the parasites have died and as virus preserved old blood which, as is known, retains the virulency for horse-sickness for many years. Notwithstanding these precautions, the disease is sometimes observed after inoculation, and in horses and mules which one could reasonably expect were immune against Biliary Fever. We can explain this fact by analogy to Redwater in cattle, which may appear in an ox accompanying another disease, as is often the case in Coast Fever. Indeed, we know that piroplasma equi must live in a horse immune against Biliary Fever, otherwise its blood would not be infective for susceptible animals. Under the influence of a severe febrile reaction, as is often the case with horses suffering from horse-sickness, the dormant parasite may actively revive and cause a disease against which the animal was immune before. The following notice will help to illustrate the remarks on this subject :—

I.

PIROPLASMOSIS OBSERVED AFTER THE SIMULTANEOUS HORSE-SICKNESS INOCULATION, DUE TO THE INJECTED BLOOD.

Mule 594, about four years old, was an Argentine mule and freshly imported. On December 18th, 1904, this animal was submitted to horse-sickness inoculation, as virus being used fresh bled from a horse 694, suffering the same day from horse-sickness. This horse was an aged, broken down animal, and utilized for the collection of virus. Mule 594 went through a typical horse-sickness reaction, which after the usual time did not, however, abate, but continued for some time, while on the twelfth day after inoculation red urine was voided and the clinical examination of the animal proved the diagnosis of Biliary Fever ; the piroplasma equi was noted in large numbers. The animal was very ill, but finally recovered from the attack.

Horse 614.—This was a cast, imported military horse, about nine years old. It was subjected, on December 18th, 1904, to the horse-sickness inoculation. As virus served the blood of a horse 658, five days previously

tapped. Typical horse-sickness reaction developed with symptoms of dikkop. At the same time clinical signs of Biliary Fever made their appearance, such as red urine, and piropluma equi was seen on the twelfth day after inoculation. The horse died in the night from 31/11/04 to 1/1/05. A *post-mortem* revealed a slight jaundiced condition of the flesh and the serous membranes, an enormously large spleen weighing about 12 lbs.

Conclusions.—Both animals, horse and mule, were susceptible to Biliary Fever, which they contracted from the injected blood. Both were very severe cases, red urine being as a rule a symptom not so frequently met with in spontaneous cases of equine piroplasmosis.

II.

PIROPLASMOSIS OBSERVED IN ANIMALS ACCLIMATISED TO THE COUNTRY.

The observations were made on mules which for at least three years were in the Transvaal and accordingly had to be looked upon as thoroughly acclimatised. Ninety-eight of these mules were subjected to the serum treatment for horse-sickness (in two lots). The first lot was inoculated with fresh serum and fresh blood, the second one with fresh serum and preserved virus. It must be understood that fresh serum as we used it always contained a certain amount of red corpuscles, thus it could not be doubted that the piroplasmosis which was observed in connection with this experiment was due to the inoculation of immune blood. But the experiments proved that by submitting mules well acclimatised to the Transvaal to horse-sickness inoculation a certain percentage will develop the disease when fresh blood or sanguinolent serum is used. Nine cases of piroplasmosis were observed in the total number of experiments, out of which three mules died. The very severe cases were clinically recognised and showed red urine, whereas the slight cases were traced microscopically and then when the temperature at the end of the horse-sickness reaction did not abate. On *post mortem*, lesions of horse-sickness were noticed in all cases.

III.

PIROPLASMOSIS OBSERVED IN ANIMALS KNOWN TO BE IMMUNE AGAINST THE DISEASE.

Horse 523 in July, 1904, contracted Biliary Fever and recovered from the disease. On October 13th of the same year 2,500 c.c. blood of horse 599, at that time suffering from piroplasmosis and containing numerous piroplasmata in the red corpuscles, was injected into horse 523. The horse stood this injection without ever showing any reaction. Thus it must have acquired a good amount of immunity by the previous attack. In December, 1904, the same animal was submitted to the horse-sickness inoculation, showing a typical reaction with the symptoms of dikkop and recovering from the disease. The symptoms of dikkop had not yet disappeared a second reaction started which lasted ten days, and during this all the symptoms of Biliary Fever were noticeable and the examination of the blood proved the presence of piroplasma equi. Although the temperature was lower after this reaction, yet it could not be called normal, taking an irregular course, and the horse died on January 26th from piroplasmosis. The *post-mortem* was made one hour after death. The condition was very poor. The flesh had a brownish hue. There was a jaundiced discolouration noticeable over the whole body; the heart seemed to be large and contained well coagulated blood. The lungs were but slightly oedematous; the liver was enlarged, hard

and of yellow colour. The spleen was but slightly enlarged, very hard, and on section showed a mottled appearance by the presence of yellow and red spots which turned out to be pigments. The kidneys were pale, otherwise no pathological lesions were noticed. This case represents, in my opinion the chronic piroplasmosis of the horse, showing an alteration of both liver and spleen, the two principal organs involved by the destruction of the red corpuscles.

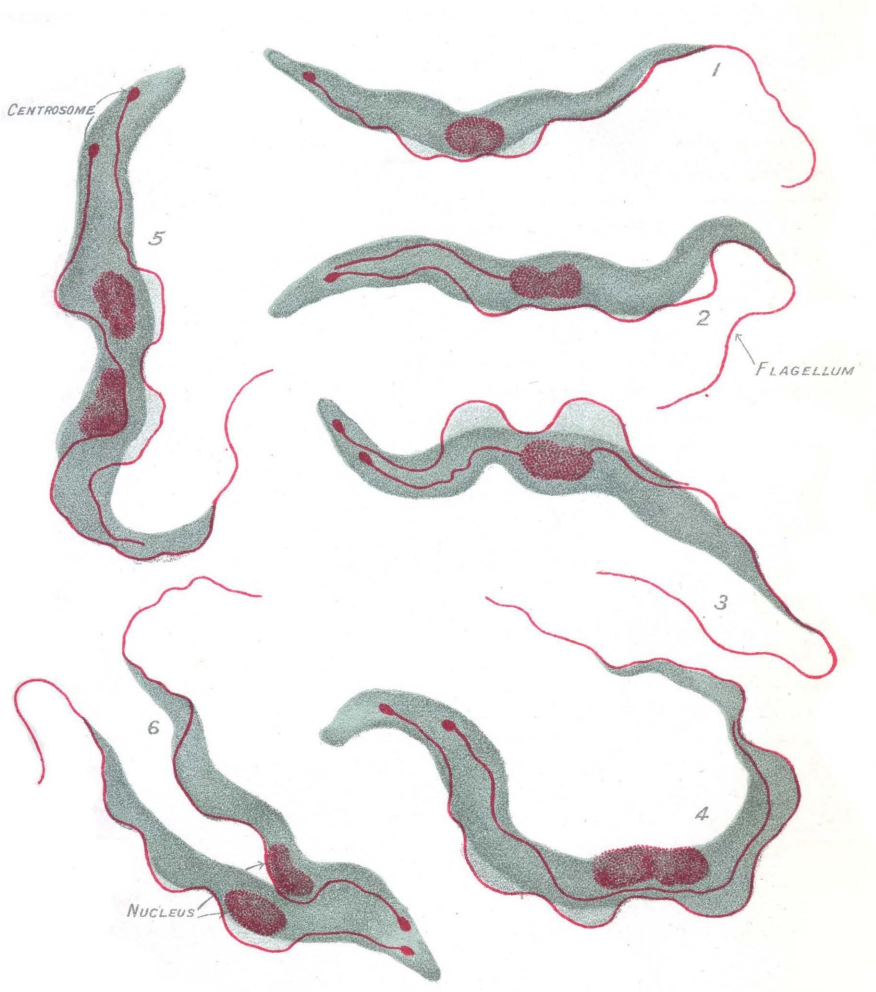
Conclusions.—This case clearly demonstrates that the strongest immunity against Biliary Fever may break down under the influence of some virulent fever such as horse-sickness. This observation and those made before indicate the importance Biliary Fever has in connection with the horse-sickness inoculation. In this respect it somewhat resembles the danger of the active immunisation by means of the simultaneous method against Rinderpest. Against horse-sickness active immunity is required ; serum alone would not be sufficient to bring a susceptible animal over a horse-sickness season. One has also to put up with a certain amount of complication and even death after inoculation not due to the horse-sickness, but to Biliary Fever brought on by horse-sickness. Since we shall be able to prevent Biliary Fever in susceptible animals by using preserved virus and serum, the danger of causing the disease is greater with immune animals—those which are born either in the country or have been here for several years. Accordingly, the process for imported animals would be to inoculate them in the first instance against horse-sickness and then against Biliary Fever. If carried out in this way a relapse to Biliary Fever need not be feared.

TRYPANOSOMIASIS IN CAMELS.

On December 3rd, 1904, a herd of 36 camels arrived in Pretoria. They were, according to the statement of the proprietor, imported from Somaliland, had a sea journey of 17 days behind them, during which time they had been kept in the hold of the ship. Shortly after landing in Lourenço Marques one of the animals died. The cause of death, as well as the poor condition of the animals, was considered to be due to the long sea journey and unsuitable treatment on board. On the day the camels arrived in Pretoria the proprietor invited me to inspect them. One of the camels was lying down and unable to rise, whilst two more had a sickly appearance, and several were in a rather poor and unthrifty condition. The sick animal died the same evening, and the proprietor was good enough to send the carcase to the Laboratory, where, on December 4th, a *post-mortem* was made. It was noted that the carcase was in a poor condition, the flesh and blood having a rather pale colour. A particularly striking lesion was the collection of clear liquid in the heart bag, which was somewhat distended, and of a similar collection of liquid in the peritonal cavity. There were no other lesions by which we could make a definite diagnosis. The symptoms described, however, indicated that some blood disease was present, the cause of which would probably have to be looked for in a trypanosoma.

Accordingly, a dog was inoculated subcutaneously with about 5 c.c. blood from the heart. A microscopical examination proved the absence of parasite, but knowing the character of the disease due to trypanosoma

SHOWING THE VARIOUS STAGES IN THE DIVISION OF A *TRYPANOSOMA*.



C. FAVRE

GOVT PRINTING WORKS, PRETORIA.

well, the diagnosis was postponed for the development of the disease in the dog; this animal being very susceptible to trypanosomiasis, and accordingly, the question of its existence or non-existence in the camels would promptly be solved. On December 7th, the morning temperature of the dog was higher than usual, a microscopical examination was at once made and the presence of a trypanosoma was noted. There was no doubt left that the dog contracted the infection through the injection of camel blood and that the camel was suffering from a trypanosomiasis. There was, however, the main question what kind of a trypanosomiasis was the one found in the camel; as from the nature of the trypanosomiasis all precautions against it are dependent. The following diseases are due to a trypanosoma which is inoculable into dogs and found in camels :—

1. Nagana, or tstese fly disease, due to Trypanosoma Brucei, still found in Zululand, but especially in the regions of East and Central Africa.
2. Surra, due to Trypanosoma Evansi, especially well known in India, and during the South African war imported into the Island of Mauritius, where it devastated almost all domesticated live stock, especially horses and mules, and to a lesser degree the bovines.
3. El Debab, a disease described by Sergent in Algeria, not yet identified with either Surra or Nagana. El Debab means fly, which is, however, not identical with the tsetse fly.
4. Mbori, a camel disease described by Cazalbou, who discovered it in camels in 1903 coming from the Sahara to the Soudan. This name also means a fly.

The latter malady was at that time not yet differentiated from any of those mentioned before, although it was probable that El Debab and Mbori are probably one and the same. Since then a communication from Vallée and Panisset of Paris, to the Academies des Sciences, demonstrated that Mbori and Surra are the same disease. Animals which were immunised against the former did not contract the infection when injected with blood of an animal suffering from the latter. In our case it had to be proved whether the disease in question was Surra or Nagana. It must be remembered that the differentiation of the two parasites is microscopically almost impossible, there being no definite marks which would under all circumstances indicate which is which. Then again, it must not be forgotten that such a great authority as Koch considers both diseases as identical, and admits only a variation of virulency of the various strains of the infection. All these points had to be taken into consideration, but especially the fact that the Island of Mauritius introduced Surra by the importation of Indian cattle, its stock became decimated by the disease, and further, knowing that Indian camels were, during the Somali war, imported into Somaliland they probably also introduced the disease there. I found it, therefore, advisable to consider the trypanosomiasis in the imported camels as the most dangerous one, and accordingly recommended the sequestration on to a place where no mixing with stock of any description was possible. This was promptly done, and the camels were kept under close supervision. On December 4th a second camel was found down and unable to rise. It was tapped and the blood injected into a dog which, however, only after some weeks developed the disease. On December 15th another camel was found

dead; a *post-mortem* was made and the symptoms as before described were present. A dog was injected with blood taken from the heart and the trypanosomiasis appeared in the dog on 20th December, 1904. On December 17th camel No. 4 was found dead, and again lesions identical with those found in the other camels were present. Also this time a dog was injected in whose blood the trypanosoma developed.

The mortality amongst the camels continued and by December 24th their number was reduced to 29 animals. The blood of the dead camels never showed trypanosomata and yet it remained infective for dogs, a phenomenon frequently met with in trypnosomiasis. Therefore a microscopical examination of the blood of the surviving animals would not have allowed us to separate with certainty the infected camels from the non-infected ones. On the other hand the dog being susceptible to the disease and showing the trypanosomata in rather large numbers could be utilised to trace the infected animals.

Accordingly, on December 24th, all the camels were tapped and a dog corresponding to the number of the camel was injected with blood. All precautions were taken to prevent any mistake, and all the syringes and needles used for the inoculation were boiled before the tapping of the camels was performed. The dog's temperature was taken daily, and as soon as a rise was noticeable a microscopical examination of the blood was made. Twenty-one dogs were found to be infected with trypanosoma, which, with the exception of a few which were kept for further observation, were destroyed on January 7th, 1905. The herd of camels was now separated into two lots, the infected and the non-infected ones, in which latter lot only eight animals remained. It was now thought advisable to test the remaining few again in order to be safeguarded against any possibilities of a latent infection not detected in the first instance. Accordingly, the camels were again tapped on January 27th, 1905, and a dog, with the corresponding number, was injected with 20 c.c. blood. It was now observed in two cases that the dog which was injected in the first instance on December 24th, and which did not show trypanosomata after that injection, showed them after the second injection. In three other cases the dog who had the first injection died without showing trypanosomiasis, whilst they were found in the dog of the second injection. There thus remained out of the eight animals only two which in the second test did not prove to be infected. The camels went down in condition considerably, and some of them had already died. It was considered advisable to kill the infected ones after the first test had been made, and this was carried out. When the second test showed that it was possible that the infection of some of the camels could not be detected even by an injection of blood into dogs, then it was thought advisable in order to be absolutely on the safe side, to kill the remaining apparently non-infected camels. This was promptly carried out.

As above mentioned in this stamping out policy, we looked at the disease as being Surra, although there was only circumstantial evidence to point to this diagnosis. It was, therefore, essential for our guidance in the future to give more attention to this disease, and especially to compare it with the facts known about Nagana. For this purpose a stable was made absolutely insect proof in which all infected experimental animals were kept. Various species of animals were injected with the blood of a dog, viz., horse, mule, donkey, ox, sheep, goat, rabbit and rat.

The tabulated form will show the difference in the duration of the disease as shown in Nagana and the camel disease :—

	Nagana.			Camel Disease.		
	Short.	Long.	Average.	Short.	Long.	Average.
Horse	9 days	18	13½	Died on 66th day.		
Mule	52 „	53	52½	Still alive on 100th day.		
Donkey	—	—	—	„ „ 100th day.		
Ox	—	—	—	„ „ 100th day.		
Goat	30 days	30	30	„ „ 100th day.		
Sheep	26 „	48	37	„ „ 100th day.		
Rabbit	25 „	35	30	Died on 108th day.		
Rat	—	—	9	Died on 30th day.		
Dogs	9 days	34	21½	12	90	51

A comparison of these figures with those given in Laveran & Mesnil's book on trypanosomiasis in the camel disease called Mbori demonstrates a striking similarity; according to the above-mentioned scientists inoculated horses lived respectively 136 days or were still alive on the 144th and 184th days. The goat was alive on the 97th day; a sheep's blood was still infective 70 days after being inoculated. Dogs died respectively 30, 48, 56, 65, and 75 days after the inoculation. One rabbit died 51 days after inoculation, and a second one was still alive after two months. The figures given on Nagana are those which I experienced in experiments made in the years 1897-99 with a South African strain of virus coming from Zululand. We must in the face of these experiments identify our camel disease with Mbori, and thus with Surra.

The appearance and the disappearance of the trypanosomata in the blood differs in the various species of animals, and even in one and the same animal, and there is much variation in their number. From the length of time animals can live with trypanosomata in their blood, it follows, as shown above, that the disease is of a very chronic character. It shows very few symptoms which would permit of a certain diagnosis.

The incubation time in dogs was noticed to be as short as three days when the temperature had risen and the parasites appeared; the elevation of temperature kept on for a few days, to drop again. This phenomenon repeated itself several times so that the temperature curve shows exacerbations and remissions which become typical for a trypanosomiasis. The parasites were most frequent during the exacerbations and less or absent during the remissions. Some of the dogs whose temperature showed the typical reaction had nevertheless no parasites in the blood, whilst in others the trypanosoma only appeared after a long time, in one instance on the 72nd day, and in another it was only noticed after 74 days after injection. These observations have to be taken into consideration when using the dog as a means to diagnose the disease. Towards the end of the disease the temperature curve becomes irregular, dropping either completely to sub-normal or rising very high in the evening and dropping in the morning. There are but few symptoms present during life. Notwithstanding a good appetite, the animal becomes poorer and poorer, is reduced to a skeleton, and is unable to rise. In some of the dogs a dark greenish diarrhœa was noticed during the disease. Some of the dogs had the symptoms of a purulent conjunctivitis and diffuse keratitis.

On *post-mortem* the most constant lesion found was a general anæmia and tumour of the spleen, reaching from three to four times its normal volume, with softened pulpa. Occasionally jaundice, accompanied with a hyperæmic liver and a full bile bag, were noticed. The blood usually

showed ante-mortem clots. The rise in temperature of the horse, mule and donkey began respectively on the 5th, 8th and 17th day. The temperature curve of these animals showed, at the beginning of the disease, exacerbations and remissions similar to those noticed in the dog. The trypanosomata were most frequently met with during a rise of the temperature and would absolutely disappear for several days. A complete disappearance of the parasites might take place within 24 hours to reappear almost suddenly. Except a continual loss of condition, which was especially marked in the horse, no other symptoms were noticed. The *post-mortem* of the horse revealed no typical lesions. Anæmia was marked by the excessive paleness of the serous and mucous membranes, there was some liquid in the heartbag, and the sulci transversales and longitudinales were infiltrated with yellow liquid and gave that part of the heart a jelly-like aspect. The sheep, goat and ox showed the peculiarity that, in every instance, when their blood was examined the trypanosomata were absent. Yet, from the temperature chart it had to be expected that at least sheep and goats were infected, as irregular exacerbations were noticeable, whereas the ox never showed any fever reaction at all. To test whether these animals were infected with trypanosoma, about the fifth week after the injection with virulent blood, they were tapped and the blood injected into dogs with the result that the trypanosomata made their appearance. It is a further remarkable fact that during the hundred days of trypanosomiasis, the bull lost nothing in condition, and there was no noticeable change in that of the sheep and the goat.

The rabbits, towards the end of the disease, showed, besides excessive anæmia, a blepharo-conjunctivitis, which in one case was very marked. The trypanosomata were but rarely met with in the blood. In the blood of rats the parasites were very numerous at the end of the disease.

With the exception of one trypanosomiasis, the Dourine, all the others are communicated by means of flies. With regard to the names Mbori and El Debab, just as tsetse indicates a fly, Mbori indicates a tabanus. It was, therefore, interesting to see whether our common bloodsucking flies, such as Hippobosca and Stomoxys, would transfer the disease. For this purpose a series of trials was undertaken. First the flies were fed on the infected horse or donkey and then, in turn, after intervals of 12 to 1 hours on donkeys. These experiments were carried out almost daily during three weeks, but in no instance was the disease transmitted by these flies.

I am not prepared to draw any conclusions from these negative results. It is possible that a certain species of fly is required to transmit the infection, and before we know what that species is and whether such species exist in this Colony all trypanosomiasis imported from abroad should be dealt with under the Contagious Diseases (Animals) Act.

BLUETONGUE IN SHEEP.

Bluetongue is an inoculable disease of sheep, principally affecting the mucous membranes of the mouth, nose, and intestines, very often accompanied with inflammation of the laminæ of the feet. The popular name, as given above, is derived from the bluish discolouration of the lining of the lips, the tongue, and the gums. A better name than the one men-

tioned is " Catarrhal fever of sheep," which is also applied on the Continent for similar diseases in the same species of animals, without, however, in my opinion, being identical with the one found in South Africa. Hutcheon, who was the first to give us a full description of this malady and of the nature of its occurrence, calls the disease " Malarial Catarrhal Fever," and thereby characterises the same as distinct from the one found in Europe. This disease has also been the subject of careful and painstaking investigations by Spreull, Assistant Government Veterinary Surgeon of the Cape Colony. His researches led me to carrying out further experiments in order to see whether the disease found in the Transvaal was identical with that found in the Cape, and more especially since the research in the Cape Colony resulted in the discovery of a practical method of inoculation. The following notes are principally based on experimental researches, observations, and on the communications of farmers. I am especially indebted to Mr. T. C. Rous, who gave me the opportunity of collecting virus, and who generously placed his herd at my disposal for observation. He has already contributed his own observations to the " Agricultural Journal " (No. 10, p. 225), which bear out his practical experience. In the course of this paper I shall have occasion to allude repeatedly to the observations and conclusions of my Cape colleagues, which I am able to substantiate in most points.

GEOGRAPHICAL DISTRIBUTION.

Bluetongue is known almost in all parts of the Transvaal ; the altitude does not seem to have any influence on its occurrence, since it is observed in the High veld as well as in the Middle and Low veld. There are, however, certain telluric conditions required which are favourable for its prevalence, such as a certain amount of humidity. Thus we find the disease alongside rivers, but more particularly in the vicinity of marshy ground, viz., around vleis and pans. Indeed, in the Transvaal, many farmers consider the local conditions are more or less identical with those which give rise to the disease of horse-sickness.

EPIDEMIOLOGY.

Bluetongue does not appear every year to the same extent ; in fact, in some years the disease is more prevalent than in others. The malady is not known to exist all the year round, but in certain seasons it is more rampant than in others. In the winter bluetongue is not known to exist, or only in a very slight degree. The real season are the months of March, April, also May ; it begins sometimes earlier—as early as December. This is due to the rainfall ; the sooner the wet season sets in and the longer it lasts, the more is bluetongue noticed. Similar observations have been made regarding horse-sickness. These facts and those mentioned above concerning the geographical distribution, and, in addition, the blueish discolouration of the mouth in one form of horse-sickness, have led many farmers to believe that the two diseases are identical. The experiments given hereafter will demonstrate that this opinion is erroneous, although the observations are right. Notwithstanding, the information collected by farmers is useful in affording us a basis for a theory as to how the disease is likely to spread—a theory which can be partially supported by experiments. It is common knowledge that the malady can be prevented by removing sheep out of the marshy area, and by placing them on high-lying ground ; and, more so, by putting the herd before sundown under shelter, or in elevated kraals, and in not letting them out before sunrise. In the Cape Colony the observation has frequently been made that by

dipping with parasiticid remedies, especially with those composed of tar derivations, an outbreak can be checked. These observations are of some theoretical, but of still more practical, value.

SYMPTOMS AND DEVELOPMENT OF THE DISEASE.

Bluetongue begins with an incubation period, which, under natural conditions, we have not been able to determine so far. The inoculated disease appears after an average incubation of four days, but it may be as short as two days, which likewise seems to be somewhat rare. The first symptom noticed is an elevation of temperature, which may begin suddenly or perhaps gradually, and reach 107° F.; again, the disease runs a slow and continual course or the attack may be rapid and irregular. In my experiments the temperature elevation lasted on an average six days, but it was also observed to be as short as two days and as long as eleven days.

Accordingly, we may distinguish between various clinical forms of the disease, which might be classed as abortive forms, acute and sub-acute forms. Clinically, one would be justified in admitting a chronic form; this is, however, in my opinion, nothing else but a sequela—a stage of debility of the animal—produced by the acute or sub-acute form, and usually accompanied by normal and sub-normal temperature. The temperature curve shows nothing typical during the course of the fever, and indicates an attack of bluetongue, as it is in the majority of cases quite irregular, high and low elevations being registered. In many instances, where other symptoms made an unmistakable diagnosis possible, barely any fever was noticed. Again, this irregular temperature curve continued, in several cases, for some time without any actual disturbance in the health or condition of the sheep. Taking the variability of a sheep's normal body-heat into consideration, it is sometimes almost impossible to state where the limit of normal and abnormal temperature exists.

In the abortive cases of bluetongue no outward symptoms are noticed; the fever is the only indication of a disturbance. These cases are in practice never seen, and prove the existence of a varying susceptibility in different animals. They are by no means rare. The fever starts in acute cases as early as indicated above; notwithstanding this, but little disturbance in the animal's health is observed before the lapse of a few days. One of the first symptoms to be noticed is a peculiar movement of the tongue; the animal seems to lick its lips at intervals. This licking is frequently repeated, when, at the same time, a want of appetite may be noticed, although chewing the cud is still performed. The examination of the mouth will now show a distinct reddening of its mucous lining, especially of the lips, the gums, and the tongue. These may be all the symptoms which are observed. In very acute cases at this period, and also in sub-acute cases still later, symptoms may be observed which give the disease its popular as well as its scientific name. The collection of a little froth around the lips, a slight watery discharge from the nose, less frequently from the eyes, mark the beginning of catarrh, which in due time may develop into a mucous discharge intermixed with blood, and accompanied with swelling of the lips and nostrils. In this stage the sick sheep feeds but little; it loses condition and is mostly found lying down, usually with its head doubled around to the side. An examination of the mouth now shows a purple to blueish discolouration of the mouth, swelling of the gums and lips, excoriation of the epithelium of the lips, usually beginning at the junction of the skin and mucous membrane.

Such lesions may recover at this stage. If this does not take place, then further and more serious changes are noticeable, namely, the appearance of sores on the upper jaw and on the tongue, the epithelial cover of which sloughs off and a hemorrhagic surface becomes visible. The animal does not feed, while a tough and often sanguinary saliva is retained in the mouth, producing an offensive smell, and hanging around the lips, which have a swollen appearance. The excoriations may start on the outside of the lips, more especially between the upper lip and the nostrils, and even on the nostrils; a copious purulent discharge may hang around the nose. Besides these symptoms in the head, a disturbance of the digestive tract may be present in some animals in the form of diarrhœa, which is usually followed by collapse. When the disease is at its height, symptoms of an inflammation of the feet become apparent. It will then be seen that the sick sheep walks but little, and if forced to do so, has its back curved. In bad cases the lameness increases gradually; the animal remains down and is unable to stand on its feet. This generally happens after the lesions in the mouth have reached their full development and are beginning to recover; that is, when the patient again begins to feed. In the pasture the sheep may then be seen to crawl about on its knees to feed. An examination of the feet shows a great tenderness and increased heat; also a swollen red band around the coronet. This discolouration is especially well marked in lambs, whose hoofs are thin, and the walls of which are transparent, so that the inflammation of the laminæ becomes easily recognisable by its uniform red colour. The red colour soon changes into blue, and for some time after recovery traces of the inflammation are noticeable in the hoofs. This lameness is usually most pronounced from the fifteenth day after the inoculation, or ten days after the onset of the fever. In the majority of the cases the lameness is not present, or not to such an extent as described. Some of the sheep are noticed to be lame on one leg or another, or then become slightly stiff.

As a general rule, Bluetongue ends fatally only in the minority of cases. I have seen sick sheep dying during the height of the disease, that is, about the seventh day after inoculation; but these cases were aggravated by diarrhœa. Death is more frequently observed from a fortnight to three weeks after inoculation, and is mostly due to exhaustion. The animals lose condition rapidly and become very poor and weak. This may happen whilst all the time the animal has been feeding; the last few days it lies prostrated, stretched out, and, having stopped feeding, will die of mere hunger; in other instances, the head is doubled back to the flank, in which position it may be found dead. An additional but rare symptom is casting of the wool; this is first seen on the inner side of the leg. Spreull noticed also that some sheep became very thirsty, and after having had free access to water, vomiting supervened—the ingesta passing through the nose. Personally, I have seen this symptom also, but only in one sheep. Shedding of the hoof is also reported to have been seen in the field, but I have never observed it.

PATHOLOGICAL LESIONS.

In describing the lesions found in Bluetongue, one can consider as typical only those found in such cases where death supervened in the acute stage. The mouth is then the principal part affected, showing the sores already described, viz., excoriations on the lips, on the gums of the upper jaws, and sloughing off of the epithelium of the tongue, whilst the base of this organ is normal. The mucous membrane of the nasal septum is

usually strongly congested. Sometimes lesions are present in the first stomach, showing red patches and stripes, and the top of the epithelial papillæ and trabeculæ are reddened. The contents of the third stomach are usually found soft. The fourth stomach may be the seat of an acute gastritis. In severe cases the mucous membrane is of a purple colour, swollen, either uniformly discoloured or perhaps in patches; the folds thickened and sprinkled with petechiæ. The small intestines may be in a stage of acute catarrh, the mucosa swollen throughout the whole length and covered with a viscid mucous. This is especially the case when the disease is complicated with diarrhœa. The cœcum and the colon may be the seat of red patches or slate-coloured patches, and their contents are thin and watery.

The lungs are, as a rule, normal, but symptoms of slight and even complete œdema, with froth in the trachea, are occasionally met with. Sometimes the pericardial sac contains a little liquid. Petechiæ are found in almost every acute case on the endocard of the left ventricle, and sometimes on the epicard. The blood is, as a rule, well coagulated. In acute cases tumefaction of the spleen is usually present, but does not reach large dimensions; the pulpa is slightly softened. The liver is usually congested, occasionally slightly icteric, and the gall bladder may be full of dark green or brown bile. The kidneys are often of a watery œdematous condition, the capsula easily stripping off; there is sometimes a well-marked congestion of the cortex. The urine was found clear in all cases. The following *post-mortem* report records the typical lesions of an acute case :—

Sheep 119 was injected on the 3rd of July, 1904, with blood from a sheep suffering from Bluetongue. On the 9th the symptom indicated a severe attack of the disease. There was foamy saliva around the lips and a profuse running from the nose; the epithelium had sloughed off the tip of the tongue, and copious diarrhœa was conspicuous. It died on the afternoon of the following day.

A *post-mortem* was made two hours after death. The condition of the cadaver was good. Rigor mortis was present. The flesh had a normal appearance. The lungs were in a stage of acute œdema. The heart was in diastyle, filled with dark blood, badly coagulated. There were petechiæ under the endocard of the left ventricle. The liver was slightly icteric; the gall bladder was contracted and contained but little bile, which was of a brown colour. The spleen was enlarged. The kidneys were normal. In the first, second, and third stomachs were red patches and stripes—visible under the epithelial layer—and the tips of the papillæ were reddened. The abomasum was in a stage of acute inflammation, of a blueish-red colour, and much swollen. The folds were thickened. The small intestines had a swollen appearance. The mucosa was mottled throughout the whole length with sugillations, and covered with a viscid mucous. The cœcum was uniformly reddened and the colon was of a slate colour.

The *post-mortem* lesions found in cases of a long duration leave nothing typical. Usually there are lesions of extreme emaciation; paleness of the flesh and organs. The lesions in the mouth have, as a rule, already healed out.

DIAGNOSIS.

The symptoms by which a diagnosis is made are those which give the disease its name; they are, however, not always pronounced, and there

are other diseases in which catarrhalic conditions are found. Sheep suffering from a severe cold and those badly infected with œstrus larvæ in the nasal cavities may show discharges from the nostrils and even sores; but the principal changes must be looked for in the mouth. Besides which, the fever and the rapid loss of condition have to be taken into consideration. The peculiar stretching movement of the tongue should always lead to an examination of the mouth, when some changes will be found. To diagnose Bluetongue after *post-mortem* at a time when the lesions in the mouth are healed out is almost impossible, since nothing typical is found for guidance. Again, in diagnosing Bluetongue one will naturally take into consideration the locality where the sheep are, and the season in which the disease is observed.

ETIOLOGY.

Inoculability of the Disease.—Modes of Infection.—Bluetongue is not a contagious disease. Observations in the field, as well as experiments, viz., placing sick and healthy susceptible animals in the same pen, feeding out of the same trough, and watering them out of the same bucket, tying them closely together—so that the closest contact is maintained for a time—have always given negative results. The blood contains the virus; since inoculation of defibrinated and non-defibrinated blood taken during the fever reaction or shortly afterwards will communicate the disease to susceptible sheep when injected, either subcutaneously or intrajugularly. The serum of diseased blood is likewise virulent, and even in the same degree as the blood itself. This observation is important, and leads us not to look exclusively to the red corpuscles for the discovery of the micro-organism, to which the disease is due. Quantities of 1 to 20 c.c. of virulent material produced the malady equally promptly, and there did not seem to be any difference in the severity of the disease produced by the smaller or larger amount of blood. When huge quantitties of virulent blood (about 50 c.c.) were injected, then the disease took a grave aspect, developing the severest symptoms and ending fatally. The disease caused by the injection of blood may vary from a slight febrile stage to one possessing all the severity indicated above, and the blood drawn from any of these stages has the same degree of virulency. It is probably due to the susceptibility of the animal, and not to the virulence of the sick blood that the variation in the severity of the disease is due. Although the virulent blood never fails to produce the disease when injected under the skin or into the jugular vein, it does so when given through the mouth. Two attempts were made. The freshly drawn defibrinated blood was diluted with physiological water and the sheep were carefully drenched. No reaction ensued.

CAUSE OF THE DISEASE.

That Bluetongue is due to a micro-organism is shown by its inoculability; that this organism has to be looked for in the blood is the natural conclusion; that it is not attached to the red corpuscles proves the virulency of the serum of the sick blood. So far all attempts to trace this organism microscopically or bacteriologically have failed. This fact may be readily understood, because the virus passes through a Berkefeld filter; thus the microbe must be of such small dimensions that it is beyond the power of our microscopes. To demonstrate this, some experiments were made with blood and serum of sick animals, after it had been proved that such serum was virulent.

EXPERIMENT 1.

On September 12th, 1904, sheep 141, then suffering from Bluetongue, was tapped, and the defibrinated blood was diluted with 50 c.c. of a 2 per cent. salt solution, and filtered through a Berkefeld candle. The non-filtered and filtered solution were injected into sheep, both of which developed typical Bluetongue, the control sheep succumbing to the disease.

EXPERIMENT 2.

On 22nd September, 1904, a sheep, No. 145, then suffering from a severe attack of Bluetongue, was tapped and the blood defibrinated. After a lapse of twenty-four hours the serum was carefully pipetted off and diluted with physiological water in the proportion of 10 c.c. serum to 90 c.c. physiological water. One-half of this mixture was filtered through a Berkefeld filter and injected into sheep 150; the other was not filtered; half served as control, to prove the virulency of the mixture, and was injected into sheep 149. Both sheep developed a grave attack of Bluetongue, to which No. 150 succumbed, and the control one was killed on the same date to obtain its blood, being not far from death.

By these experiments it is clearly shown that the micro-organism of Bluetongue belongs to the ultra-visible ones, to which class that of horse-sickness also belongs. Spreull mentions in his paper on the same disease that Robertson, Government Veterinary Surgeon, has discovered that this disease is caused by a small intra-corpuscular parasite or plasmodium, affecting the red corpuscles. There is no doubt that the disease described by Spreull is identical with the one above, and in which no visible micro-organism has been observed. Therefore, the plasmodium found by Robertson cannot be the cause; it may be an accidental occurrence or is perhaps, the piroplasma ovinum. Lately, Mr. Robertson, who has repeated the filtering experiments, informs me that he does not consider that this organism is connected with Bluetongue, since he also found that the filtered blood remains infective.

RESISTANCE OF THE VIRUS.

The blood of a sick sheep remains infective for a long period after its withdrawal. I have tested, at repeated intervals, blood of various dates up to four months, and the result of inoculation was with but little exception positive, producing fever reaction, and such clinical symptoms as are observed after injection of fresh blood. The blood can be preserved by addition of glycerine and carbolic acid, as is done with horse-sickness, and the mixture remains infective for a long period. The blood may become putrid and completely decomposed; notwithstanding this, when it is inoculated into a susceptible sheep, Bluetongue will result. It is, however, not advisable to inject such blood, since putrefactive bacteria may produce local swellings, high fever, and even death, so that the aspect of the disease may become masked by secondary infection. I found it, therefore, advisable to filter such bacteria off by means of a Berkefeld candle, when after injection of the filtrate the disease will appear just as if fresh virus was injected. Dried blood does not seem to lose is virulency. Four experiments were undertaken for the purpose of elucidating this point. The blood was air-dried by making a thin film in a sterile Petri-dish and exposing it in the shade at the temperature of the room.

Sheep No. 151 was injected on September 23rd, 1904, with an emulsion of 4.8 grammes dried blood, drawn from sheep 145 and dried the same day. A typical fever reaction followed, the temperature rising as high as 107.4° F., but the other clinical symptoms typical for Bluetongue were wanting.

On October 12th, 1904, three sheep, Nos. 156, 157, and 158, were injected with a 10 per cent. emulsion of dried blood in physiological water, respectively, with 1 c.c., 5 c.c., and 10 c.c. emulsion. The three sheep showed, after the typical inoculation time, a fever reaction which was but slight, indicating that the virus becomes attenuated, a noteworthy fact, since it may lead to a vaccination, the further study of which I have not yet carried out.

NATURAL INFECTION AND PROPAGATION OF BLUETONGUE.

We do not know yet how the disease is contracted and spread under natural conditions. The foregoing experimental facts and, above all, the observations of farmers give us some valuable hints to form a hypothesis, which will explain all that is known about the occurrence of this disease. Let us recapitulate the principal facts. Bluetongue only occurs at certain seasons of the year, characterised by much rain; it occurs principally in certain regions, characterised by moisture. It is not contracted during the day, but at night. Sheltering and removal of a herd stop the disease; dipping with antiseptics has the same effect. The disease can easily be communicated by inoculation under the skin, but it cannot be produced by drenching. All these facts point to some intermediate host, which must act as a carrier of the infection. I do not think that we are far wrong in accepting as such a host—a night insect. Everybody knows that during the rainy periods and in marshy places night insects are more frequent than at any other period and in any dry place. Indeed, the climatic and telluric conditions for Bluetongue are identical for the development of horse-sickness. For this latter disease it has been proved that horses exposed in the worst areas can be protected by keeping them in insect-proof houses, although exposed to night air. There is, therefore, a marked analogy. The actual proof has not yet been given, but will certainly be forthcoming. There are, however, still some points to be considered in connection with this theory. We are entitled to ask: "Where does the infection remain during the cold and dry season, when no Bluetongue is observed?" We know that the blood of an immune sheep does not remain infective as is the case in some of the diseases caused by piroplasmoses. It is, therefore, plain that the infection remains either latent in the intermediate host, viz., that it undergoes there a certain evolution closely connected with the life cycle of the host, or that a third animal, and in this case probably a water animal, is the actual host, in which it may live as a harmless parasite. Again, we need not go far for analogy. The tsetse disease is spread by a fly, which takes the infection from game which is immune against the same disease. Thus the night insect would act only as an inoculator.

SUSCEPTIBILITY OF SPECIES AND BREEDS.

It has already been pointed out that, in popular opinion, Bluetongue is identified with horse-sickness, to which, in the course of this paper, several allusions have been made. It, therefore, became interesting to know whether the blood of a sick animal which produces the disease in susceptible sheep would also produce horse-sickness.

For this purpose a horse and a mule recently imported from the Argentine, both animals being highly susceptible to horse-sickness, were injected on September 23rd, 1904, with 20 c.c. blood from sheep No. 144, showing all the symptoms of Bluetongue. No reaction ensued from this inoculation. The mule was later submitted to the horse-sickness inoculation, showed a typical reaction, and recovered. This proves that the

mule must have been susceptible to horse-sickness, and that the inoculation of Bluetongue virus did not produce the disease. Hence the experimental proof that the two diseases are not identical. Defibrinated blood of the same sheep was also injected into an ox freshly imported from the Cape; the result was also negative. I have likewise inoculated goats so far with no effect. Spreull shows, however, that goats may successfully be inoculated and their blood proved to be infective for sheep. In practice, one never hears of Bluetongue in goats. These facts, taken into consideration, point to a strong immunity of the species. All breeds of sheep are susceptible to Bluetongue. My inoculation experiments show that Merinos, Fat-tails, and Persians can be inoculated with equal success, but I am not able at present to draw any conclusions from these experiments as to which breed is the most susceptible. Generally speaking, Merinos are considered to be the most apt to contract Bluetongue under natural conditions. Spreull says that he successfully infected a rabbit, in which he found the plasmodium. Since Bluetongue is caused by an ultra-visible micro-organism, the parasite in the rabbit cannot have been that of Bluetongue.

MORTALITY IN BLUETONGUE.

Bluetongue is not a very deadly disease, as most of the sick sheep will recover, especially when they are cared for. In his communication to the "Agricultural Journal," Rous says that out of sixty cases he only lost two animals; however, he attended to his animals very carefully. My inoculation experiments show likewise that death only results in the minority of cases; barely 20 per cent. succumbed to the disease, although they were not treated. But one often hears of heavy mortality of sheep from Bluetongue. Apparently, there does exist a difference in the virulency of the infection in certain localities and at certain seasons.

IMMUNITY.

A sheep which has gone through an attack of the disease acquires a certain amount of immunity. This, at least, is the result of experiments. I do not know how strong the immunity is against a natural infection, and whether under such conditions a sheep may contract the disease several times. A single attack brought on by inoculation of virulent blood does by no means produce a highly active immunity, since subsequent inoculations with increased quantities of blood are usually followed by febrile reactions with symptoms of Bluetongue, and, in exceptional cases, with death. Some of the experiments will demonstrate those points.

Sheep No. 111 was injected on March 29th, 1904, with 20 c.c. defibrinated blood taken from a sick sheep at Mr. Rous' farm. There was a very distinct reaction, the temperature elevation as high as 107.2° F. on the sixth and seventh days. Thirty-three days afterwards, viz., 30th April, 1904, the sheep was tested for its immunity with 50 c.c. fresh blood of the sick sheep No. 117. A reaction ensued. On June 2nd, 1904, a third inoculation with virulent blood (100 c.c.) of sheep 130 was made: again followed by high fever and with catarrhal symptoms of the nose.

Lamb 118 was injected on April 22nd with the blood of a sick sheep, No. 113. All the typical symptoms encountered in the head and in the feet were noticeable; the lamb recovered. On July 2nd the lamb was injected intrajugularly with 60 c.c. fresh blood of the sick sheep 130. High fever reaction resulted; the lamb was very ill, and on the sixth day slight erosions on lips and tongue with catarrhal conditions of the mucous membrane were present. This time the lamb did not rally, but died seventeen days after from the effect of the inoculation.

Not all fever reactions observed after the injection of fresh Bluetongue blood must be put down to this disease. We know that a spirillum is encountered living in the blood of immune and healthy sheep, which, when injected into susceptible sheep will cause fever reaction, and which by the microscope is not always traceable, since it occurs in rare numbers, and thus escapes observation.

Further investigation has shown that, after a repetition of the virulent blood inoculation, complete immunity becomes established, and the sheep no longer react. This usually takes place after the second inoculation.

IMMUNISATION AND SERUM TREATMENT.

The repeated injection of virulent sheep blood into sheep which have passed through an attack of Bluetongue leads to the production of a strong preventive serum. This fact was first shown by Spreull. I have treated several immune sheep with blood injections, going to a total of 500 c.c. per sheep. They were then tapped, and the serum of various animals was mixed. The following preliminary experiments were made to test the value of such a serum mixture :—

Control Experiment.—Sheep No. 203 was injected on December 7th, 1904, with 5 c.c. virulent blood of sheep No. 191. This sheep had to act as a control. It contracted a typical reaction and developed Bluetongue, from which it recovered.

Sheep 199 was injected on the same date with 5 c.c. virus of sheep 191 and 10 c.c. serum mixtures ; both injections were made subcutaneously. No Bluetongue reaction ensued.

Sheep 200 was injected on the same date with 5 c.c. virus into the jugular vein and with 10 c.c. serum subcutaneously. No reaction resulted.

Sheep 201, injected on December 7th, 1904, subcutaneously with 10 c.c. serum and twenty-four hours later with 5 c.c. virus. From the seventh day a slight disturbance was noticeable, which, however, can hardly be called a typical Bluetongue reaction.

Sheep 202.—On December 7th, 1904, 5 c.c. virus was injected subcutaneously and twenty-four hours later 10 c.c. serum mixture. From the seventh day onward a distinct reaction took place, but the animal did not show any other clinical symptoms, and the microscopic examination was negative.

From these experiments it follows that the serum of immune sheep hyperimmunised to the extent of 500 c.c. virulent blood injected at different intervals has highly developed protective qualities, which should permit its use for a practicable inoculation. With the exception of sheep No. 202, in the above experiments, only a passive immunity may be expected, because no reaction took place, and since, according to Spreull, the simultaneous method promises the best result, a further experiment was undertaken to find out, on the one hand, the susceptibility of the different breeds of sheep, and, on the other, the dose of serum required to allow a slight reaction which would result in active immunity. In order to obtain a reliable control of the effect such an inoculation may have, not only the temperature of the sheep was taken, but also their weight, before and during the time when a reaction might be expected.

The following tabulated form gives the record of these conclusions :—

No. of Sheep.	Breed.	Weight before inoculation.	11-3-05	13-3-05	15-3-05	17-3-05	19-3-05	21-3-05	22-3-05	Remarks.	Weight. Loss.	Weight. Gain.
				A.—Simultaneous Inoculation of 5 c.c. Serum and 2 c.c. Virus.								
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.		lbs.	lbs.
182	Merino	61½	61.5	63.5	63¼	62.0	65.0	67.5	66.0	No reaction	—	4.5
183	Merino	60.0	57.5	63.5	60.0	60.0	60.5	63.5	61.5	No reaction	—	1.5
185	Persian	52.5	53.0	52.0	51.5	54.0	50.5	49.5	47.0	No reaction	5.0	—
189	Persian	58.5	59.0	58.0	55.0	59.0	61.5	58.5	56.0	No reaction	2.5	—
204	Merino	50.5	50.0	46.5	44.0	46.0	Dead.	—	—	Poverty ; no reaction ...	5.5	—
205	Bastard	31.0	30.5	29.0	30.5	32.0	33.5	33.0	31.5	No reaction	—	0.5
206	Persian	40.0	41.5	42.0	42.5	42.0	43.5	46.0	45.0	Indistinct reaction ...	—	5.0
207	Bastard	73.0	74.0	80.0	82.0	82.0	78.0	78.5	79.5	No reaction	—	6.5
218	Bastard	46.0	47.0	46.0	47.0	48.0	44.0	45.5	47.0	No reaction	—	1.0
219	Bastard	46.0	46.0	46.0	47.0	52.0	46.0	47.0	44.5	Reaction not typical ...	1.5	—
				B.—Simultaneous Inoculation of 10 c.c. Serum and 2 c.c. Virus.								
184	Persian	51.0	51.5	54.0	59.0	59.0	51.0	52.5	51.0	No reaction	—	—
213	Merino	37.0	35.5	34.0	37.0	37.5	36.5	31.5	28.0	Indistinct reaction ...	9.0	—
214	Merino	60.5	62.5	61.0	62.0	64.0	68.5	66.0	64.0	No reaction	—	3.5
215	Bastard	40.0	39.5	43.5	42.0	42.0	44.0	43.5	42.0	Reaction	—	2.0
217	Bastard	44.5	44.0	04.0	43.0	40.5	47.0	44.0	43.0	Reaction not typical for Bluetongue	1.5	—
220	Merino	69.0	72.5	72.5	69.0	73.5	70.0	66.0	65.0	No reaction	4.0	—
226	Persian	40.5	43.0	42.0	42.5	43.0	39.0	39.0	41.0	No reaction	—	0.5
227	Merino	48.0	49.0	51.0	49.0	52.0	51.0	54.0	52.0	No reaction	—	4.0
231	Bastard	43.0	43.5	46.5	41.0	42.0	44.0	44.0	44.0	No reaction	—	1.0
232	Persian	72.0	66.5	64.5	63.0	64.0	67.5	67.5	69.0	No reaction	3.0	—
				C.—Control sheep injected with 2 c.c. Virus.								
233	Merino	77.0	71.0	71.5	75.5	74.0	69.0	69.5	66.0	Typical reaction ; died ...	11.0	—
234	Bastard	36.0	38.0	38.0	38.0	37.0	37.0	33.5	32.0	Typical reaction ...	4.0	—
235	Persian	58.5	56.5	54.0	51.0	53.5	52.0	49.5	47.0	Indistinct reaction ...	11.5	—

The quantity of serum injected in this experiment was too large to allow a reaction, without which no active immunity can be expected. The serum has, undoubtedly, a very strong preventive action. In order to ensure a distinct reaction, the quantity of serum will have to be decreased.

PROPHYLACTIC TREATMENT OF THE DISEASE.

The observations of practical sheep-farmers, to which I have already alluded, and which form the base of the theory as to how the spread of the infection can be explained, comprise the essential hints as to what to do when an outbreak of Bluetongue occurs in a herd of sheep. Sheltering and removal are, however, not always possible. Under such conditions, and more particularly in noteworthy Bluetongue districts, the inoculation both with serum and virus, or with serum alone, should prove benecial. Thus far no field experiments are at our disposal. These will, however, be undertaken as soon as opportunity offers, when the serum will be put to a practical test and a method to meet all practical requirements will be worked out.

TREATMENT OF SICK ANIMALS.

The disease not being of a deadly character, much hope may be entertained respecting successful treatment. I am inclined to give preference to dietetic treatment alone. Mr. Rous has clearly shown that it is good nursing which gives the best results. Sick sheep should be picked out of the herd, kept alone, and well cared for; as long as they can feed, succulent food is essential; when they can no longer eat, gruels should be given. Sheep which become lame should be well fed. In case of bad sores in the mouth, some dressing with astringent drugs may be administered. A weak solution of alum has proved to have good effect both on the mucous membrane of the mouth and also the intestines in cases of diarrhœa.

IMMUNISATION AGAINST HEARTWATER.

In last year's annual report the pathology of Heartwater in cattle, sheep and goats was described as I had observed it, principally in experimentally produced cases. The main object of my research was to ascertain whether it would be possible to produce a serum against the disease which might be utilized for working out a method of inoculation for practical purposes. There was every reason to expect such a possibility by analogy to other blood diseases, such as Rinderpest, Horse-sickness and Blue Tongue—all diseases due to ultravisible micro-organisms, against which a serum has been found. There was another point to consider which was favourable to the prospect of producing such a serum. Heartwater is a disease of cattle, sheep and goats: thus of three different species of animals. The hyperimmunisation of one species with virus derived from sick animals of the same species would,

naturally, lead to the production of three different sera, all directed against the same micro-organisms. According to Ehrlich's literal chain theory, the activity of a serum depends, in the first instance, upon the presence of amboceptors, and as a multitude of amboceptors are considered to be present in a serum, and the activity of a serum increases with the number of various amboceptors, a polyvalent serum could be formed by mixing the three sera of the three different species of animals.

During last year a number of oxen and sheep were injected, at intervals, viz., Ox 187 with 7,950 c.c. blood of sick cattle, Ox 448 with 5,730 c.c. blood of sick cattle, Sheep 102 with 440 c.c. blood of sick sheep, and Sheep 103, 104, and 135, respectively, with 720, 780, 820 c.c. virulent sheep blood.

The animals were then bled and the following experiment was made on 3/12/04. The serum of the above animals was mixed in equal quantities. Twenty c.c. of serum were taken as a standard dose and 5 c.c. as a dose of virus.

1. Goat 192 was injected subcutaneously with serum and virus simultaneously.

Result.—There was no reaction noticeable. The goat was still alive on 1/4/05.

2. Goat 193 was injected subcutaneously with serum, and with virus intrajugularly.

Result.—The goat developed Heartwater and died on December 18th, 1904.

3. Goat 194 was injected with 20 c.c. serum, and, 24 hours later, with virus.

Result.—There was a reaction which, however, was not typical of the disease. The sheep was still alive on 1/4/05.

4. Goat 195 was injected with virus, and, 24 hours later, with serum.

Result.—There was some doubtful reaction. The sheep is still alive.

5. Control experiment for virus: Persian sheep was injected with virus alone.

Result.—Strong Heartwater reaction from which, as is always the case with Persian sheep, the animal recovered.

Conclusion.

The serum did not show protective properties when virus was injected into the jugular vein simultaneously with serum, but did show strong protective qualities when virus was injected under the skin, and even 24 hours before the serum. Since no definite reactions resulted, it cannot be expected that active immunity ensued. A method of a preventive inoculation with serum is thus possible.

TRANSMISSION AND INOCULABILITY OF SPIRILLOSIS IN CATTLE.

In 1903, Laveran described, under the name of Spirillum Theileri, a micro-organism which I had found in several instances in sick cattle. In 1904, I described six cases of Spirillosis in bovines. These cases, however, in my opinion, did not represent a pure infection of spirillum, since lesions were present in the red corpuscles, such as basophile granulations indicating a previous infection with Piroplasma bigeminum. Indeed, in three out of the six cases the typical Piroplasma bigeminum was still present, whilst in a fourth case endoglobular parasites were noticed, which at that time could not be distinguished from what I later described as Piroplasma parvum the cause of tropical piroplasmosis of cattle. At that time I expressed my uncertainty as to the true nature of this parasite in the following manner :—"I may state here that I have reason to believe that bacillary piroplasmas are also found in other diseases than East Coast Fever, but I cannot as yet substantiate this statement."

This has been done in the article "The Piroplasma bigeminum of the Immune Ox" which appeared in my last annual report. The piroplasma found in the fourth case in company with spirillum was that form, which I call the Piroplasma bigeminum of the Immune Ox. The two remaining cases showed only basophile red corpuscles, which I consider a sure indication of the ordinary Piroplasmosis of cattle, *alias* Redwater or Texas Fever. Thus all of the six cases quoted represented a mixed infection of piroplasma bigeminum with spirillum. The rôle which the spirillum plays as a cause of disease has, as yet not been ascertained. The subsequent notes will help to throw some light on the pathogenic action of this parasite.

In the communication alluded to I quoted experiments to inoculate spirillosis with defibrinated and non-defibrinated blood from sick into healthy calves and also full grown oxen. This trial failed in 11 animals, although the precaution was taken to utilize principally imported stock and the inoculations were made with quantities ranging from 5 to 100 c.c. per dose, which were injected subcutaneously, intrajugularly and intraperitoneally. The injections into three sheep, two goats, two horses, six rabbits, one guinea pig, and one rat also gave negative results. It was then concluded that spirillosis is not inoculable, a striking difference to similar diseases in men and fowls, just as the tropical Piroplasmosis is a striking exception to the inoculable Piroplasmoses. Since it had been proved that the Brazilian fowl spirillum is transmitted by a species of ticks, an argas, and as our spirillum being also found in the blood, and notwithstanding at that time believed to be un-inoculable, the opinion was expressed that it is also likely to be transmitted by ticks, in view of the conclusive proofs of past experiments in tropical piroplasmosis. If such was the case it followed therefore that the spirillum requires to go through the intermediate stages in the life cycle of the tick. Further, I may remark, as previously mentioned, that I had found the spirillum in a horse and a sheep, without being able to express a positive opinion as to their identity with that which I observed in cattle. The subsequent notes will help us to rectify some of the former conclusions concerning inoculability, whereas the opinion as previously expressed regarding the transmission of spirillosis will be demonstrated.

TRANSMISSION OF SPIRILLOSIS BY TICKS.

First Observation.

Bull 257 was about 18 months old and imported from the neighbourhood of Capetown, a district free of such tick diseases as piroplasmosis of cattle and heartwater. After arrival in Pretoria it was kept inside the premises attached to the Laboratory. From October 14th to October 22nd, 1904, this animal was daily infested with a certain number of larvae of Rhipicephalus decoloratus (Koch). These larvae had been hatched in a glass dish in the Laboratory and were the offspring of ticks which had sucked on a Transvaal ox, born and bred in the country and accordingly immune against the various indigenous diseases. The object of the tick infestation was to rear a sufficient number of female ticks for various experiments. The animal, being a tame bull whose scrotum offered a convenient place for rearing the various species of ticks, was hereafter used for this purpose. It was infested with adults of Rhipicephalus appendiculatus (Neumann) and the larvae of Amblyomma hebreum (Koch), on November 6th, 1904. On November 10th, 1904, adult ticks of Rhipicephalus evertsi (Neumann), Hyalomma aegyptium (Koch), and Rhipicephalus appendiculatus (Neumann) were again placed on the scrotum for feeding purposes. From October 2nd, 1904, the morning temperature was above the normal, rising gradually during the next few days when the curve became completely irregular, and the temperature rose as high as 106° Fahr. during the last few days of reaction. The following chart shows the temperature reaction of Bull 257 after infestation with ticks :—

Temperature record Bull 257.

CATTLE 257.

Oct. 15, 1904.		16		17		18		19	
M	E	M	E	M	E	M	E	M	E
	102^8	99^4	102^6	101	102	101	102^6	100^1	102^4
20		21		22		23		24	
M	E	M	E	M	E	M	E	M	E
101^4	102	100^8	102	100^8	102^4	102	102^8	100^4	102^8
25		26		27		28		29	
M	E	M	E	M	E	M	E	M	E
102^2	102^4	101^2	102^8	101	102^4	101^2	102^2	101^2	102^2
30		31		Nov. 1, 1904.		2		3	
M	E	M	E	M	E	M	E	M	E
100	102^6	100	102^2	100^4	102^6	101^4	102^4	101^4	102

CATTLE 257.—*Continued.*

Nov. 4, 1905.		5		6		7		8		9	
	M	E	M	E	M	E	M	E	M	E	M	E
	100^4	102	101^8	102^8	101^4	102	101^4	102^6	102^2	103^2	101^8	103^2
10	11			12		13		14		15		
	M	E	M	E	M	E	M	E	M	E	M	E
	101^8	102	102	103	102^2	103^8	102	105	103^2	103^6	100^6	104
16	17			18		19		20		21		
	M	E	M	E	M	E	M	E	M	E	M	E
	100^6	106^2	103^6	105^8	101	102^4	100^4	103	102^8	105	101^6	103^6
22	23			24								
	M	E	M	E	M	E						
	102^4	103^4	101^4	102^4	102^2	[104						

A microscopical examination of blood smears was made for the first time when the temperature reached 105° Fahr., viz., on 13/10/04, special stress being made to trace the Piroplasma bigeminum, which in this instance was suspected. The examination remained negative for the next four days. Spirillum was noticed for the first time on the 18/10/04, and again on the 20/10/04, and on the 27 and 28/10/04. The parasites were, however, never so frequent as I was accustomed to find them in the first cases of my observations.

Conclusion.—Personally, I had no doubt that in this instance, at least, the spirillosis was caused by some of the ticks which had been placed on the animal. The origin of the various ticks was known. All came from cattle which were healthy and in good condition. Accordingly, it was difficult to understand how those ticks could become infected with spirillum. Although, in this case, spirillosis was evidently induced by ticks, yet it was impossible to trace the species which was responsible, since nearly all known South African species had fed on the bull.

Second Observation.

The heifers 275 and 276 were imported from Aliwal North, a district of the Cape Colony known to be free from Redwater. The object of this

experiment was to ascertain whether larvæ of the blue ticks (Rhipicephalus decoleratus), whose mothers had been feeding on an ox known to be immune against Redwater, would contract this disease. For this purpose Ox 347, which two years previously had passed through an attack of Redwater, and whose blood, at different intervals injected into susceptible calves proved to be infective, was heavily infested with blue ticks' larvæ, which developed into full grown imagines. The larvæ of the females were placed on heifers 276 and 277, both about 18 months old, on 11/11/04. Heifer 276 began to rise in temperature on 26/11/04, viz., 15 days after the infestation of ticks. Spirillum was found on November 30th and the next four days. The following chart shows the temperature reaction of the heifer after infestation with blue tick larvæ :—

CATTLE 276.

Nov. 11, 1904.		12		13		14		15		16		17		18	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103²	103	102⁸	100⁶	103⁶	102²	102⁶	100⁶	103⁴	101⁴	103²	100⁶	102⁶	100²	102⁶
19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
100	103⁸	101⁴	103⁶	101⁴	103	102⁴	103	101⁶	103	101⁸	103²	101⁸	100⁶	101⁸	103⁸
27		28		29		30		Dec. 1, 1904.		2					
M	E	M	E	M	E	M	E	M	E	M	E				
105⁴	106²	103⁶	104⁶	101⁶	104⁸	101⁴	102⁴	103	101⁴	102⁶	102⁴				

Heifer 277 was treated exactly in the same way as Heifer 276. The temperature reaction began in this animal on 27/11/04, viz., 16 days after the infestation of ticks. Spirillum was noticed on 28/11/04, on 30/11/04, and on 1 and 2/12/04. The following chart shows the temperature reaction of Heifer 277 :—

CATTLE 277.

Nov. 12, 1904.		13		14		15		16		17		18	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
	102^{8}	102	102^{8}	102^{2}	102^{8}	101	102^{2}	100^{4}	102^{6}	100^{2}	101^{6}	100^{2}	102^{2}
19		20		21		22		23		24		25	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
100	101^{8}	100	102^{8}	100	103^{2}	100^{6}	101^{6}	100^{8}	102	101^{6}	101^{6}	102	102
26		27		28		29		30		Dec. 1, 1904.		2	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
100	103	101	101^{8}	102	103^{6}	102^{8}	105^{8}	103	106^{2}	101^{6}	103	101^{2}	102^{2}

Conclusion.—There is no doubt that both cases of spirillosis were produced by the ticks. There remains the remarkable fact that during the time the blue tick was developing on Ox 347, that is, a period of three weeks, no temperature reaction was noticed ; nor did a repeated microscopical examination of the blood of this ox show the presence of spirillum. I therefore concluded that similar to the ordinary piroplasmosis of cattle, this parasite must be present in the immune animal. Proof of this will be given in a subsequent chapter.

2.

EXPERIMENTS TO PROVE THAT SPIRILLUM IS TRANSMITTED BY RHIPICEPHALUS DECOLORATUS.

The blue ticks which developed on Bull 257 began to drop from 8/11/04 and kept on dropping for over a week. They were collected daily and kept in glass dishes, where they laid eggs and hatched. The larvæ of the females collected on November 13th and 14th, 1904, viz., at the beginning of the fever reaction, but before microscopic examination demonstrated the presence of the spirillum, were placed on Heifer 282, belonging to the same lot of cattle as 276 and 277, which were imported from Aliwal North. The infestation took place on 17/1/05. Sixteen days later the temperature reaction began. On 2/2/05 and 3 and 4/2/05 spirillum was noticed. The temperature reaction was but slight, as is shown in the following chart :—

CATTLE 282.

Jan. 17, 1905.		18		19		20		21		22		23	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
	102	101	103	101^3	101^6	101^4	103	102	104^4	103^4	104	101^8	103^5
24		25		26		27		28		29		30	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
102^3	104^6	103	103^3	103	105	101^8	104^6	103^4	104	102	103	102^2	103^6
31		Feb. 1, 1905.		2		3		4		5		6	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
104	102^6	**102**	102^6	101^6	104	104	105	106	105^4	105	105	104	105
7		8		9		10		11		12			
M	E	M	E	M	E	M	E	M	E	M	E		
104^4	105	102^8	105^8	104^2	105^6	104^4	105^6	104	105^4	103^2	104^6		

2.—Heifer 285. On 17/1/05 the larvae of the blue ticks collected on 16/11/04, viz., during fever reaction two days before the spirillum was observed, were placed on this animal. The reaction began on 3/2/05, viz., 17 days after the infestation of the larval ticks. Spirillum was noticed on 3 and 4/2/05. The following chart shows the temperature of Heifer 285 :—

CATTLE 285.

Jan. 17, 1905.		18		19		20		21		22		23		24	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
103	103	101	102[8]	102[4]	104	101[6]	102[4]	101[4]	103	102	102	102[8]	101[8]	102	104[6]
25		26		27		28		29		30		31		Feb. 1, 1905.	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
102	102	102	102	101[4]	102[4]	102	104[8]	101[8]	102	102[6]	104	102	102[4]	101[8]	102
2		3		4		5		6							
M	E	M	E	M	E	M	E	M	E						
103	105	104[6]	104	101[2]	102[4]	102	102[4]	103	103						

3.—Heifer 284. On 3/2/05 the larvae of the blue ticks which dropped from 13 to 15/11/04 from Bull 257, viz., at the beginning of the reaction, when spirillum was noticed on 17/11/04, were placed on this animal. The morning temperature on 19/2/05 was 102·8°F. An examination of the blood proved the presence of the spirillum, viz., 16 days after tick infection. The following chart shows the temperature reaction of Heifer 284 :—

HEIFER 284.

Feb. 3, 1905.		4		5		6		7		8		9		10		11	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
—	104	101^{2}	103^{8}	101^{8}	103^{6}	102	105	103^{8}	105	102^{4}	105^{2}	102^{4}	103^{2}	102	104	101^{8}	103^{8}
12		13		14		15		16		17		18		19		20	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{6}	102^{4}	102^{4}	103^{4}	102^{2}	104	102^{2}	102^{8}	102	102^{4}	101^{6}	103	101^{4}	103	102^{6}	103^{8}	102	106
21		22		23													
M	E	M	E	M	E												
103	104^{4}	101^{6}	103^{4}	102	102^{6}												

4.—Heifer 281. On 1/2/05 this animal was infested with the larvae of the blue ticks, which dropped from Heifer 277 on 3, 4 and 5/12/04, viz., the first few days after the reaction, during which spirillum was noticed. The temperature began to rise in Heifer 281 on 14/2/05, viz., 13 days after tick infestation, while the spirillum was noticed on 15/2/05. The following chart shows the temperature reaction of Heifer 281 :—

CATTLE 281.

Feb. 2, 1905		3		4		5		6		7		8		9		10	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103⁴	102	103	101⁶	104⁴	101⁸	102⁸	102⁴	103	102⁴	103²	101⁶	103⁴	101⁴	102⁶	101	103⁴
11		12		13		14		15		16		17		18			
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E		
101⁶	103⁸	103⁶	102	101	102⁴	103⁶	105	104²	106	104	102⁴	101⁴	102	101⁴	101⁸		

5.—Heifer 274. On 3/2/05 this animal was infested with the larvæ of blue ticks, which were collected from Heifer 277, on the dates indicated in Experiment 4. The temperature began to rise on 18/2/05, viz., 15 days after the tick infestation. Spirillum was noticed on 19, 20, 22/2/05. The chart of Heifer 274 is given below.

CATTLE 274.

Feb. 3, 1905		4		5		6		7		8		9		10		11	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103.4	101.4	102.4	102	102	101	102.8	101	102	101	103.2	101.6	102.6	101.4	103.6	101	102.4
12		13		14		15		16		17		18		19		20	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
100.6	101.2	100.8	102	101.4	102.8	101.4	101.4	100.4	101.4	102.4	102.8	101.6	103.4	103	104	102.4	104.6
21		22		23													
M	E	M	E	M	E												
103	103	101.6	103.6	101	103.6												

6.—Heifer 283. On 3/2/05 this animal was infested with ticks of Heifer 277 (compare the previous experiments). The temperature began to rise on 16/2/05, viz., 13 days after the tick infestation ; spirillum was noticed on 19/2/05, viz., on the 16th day.

HEIFER 283.

Feb. 3, 1905.		4		5		6		7		8		9	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
—	102[8]	101[6]	102[6]	100[8]	103[4]	100[8]	103	101	102[4]	101[6]	102[8]	101	102
10		11		12		13		14		15		16	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
102[6]	102[8]	101[4]	103	101[4]	102[4]	101[2]	102[8]	1008	103[4]	101[2]	103[2]	104	104[6]
17		18		19		20		21		22		23	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
104	105[4]	104[8]	106	101[8]	101	100	102[8]	99[8]	103[6]	101[4]	103[6]	100[8]	102[6]

Conclusion.—These six heifers which were of about the same age and which came from Aliwal North, and were stalled during their stay on the premises of the Laboratory, were successfully infected with spirillum. In these cases the infection was derived by the ticks during and shortly after a reaction, when the spirillum was noticed. The shortest incubation time was observed to be 13 days and the longest 17 days. Taking the experiments with Heifers 276 and 277 into consideration it is noticeable that the tick which transmits spirillosis into susceptible cattle will reinfect itself on the same animal, either during the fever reaction or shortly afterwards, when the animal is no longer in reaction, but has nevertheless the spirillum in its blood as was the case in Ox 247 and also in Heifer 277. The foregoing experiments also indicate the effect the spirillum produces, since all were pure cases of spirillosis. The pathogenic effect on the blood will be shown in another experiment.

INOCULABILITY OF SPIRILLOSIS INTO CATTLE AND SHEEP.

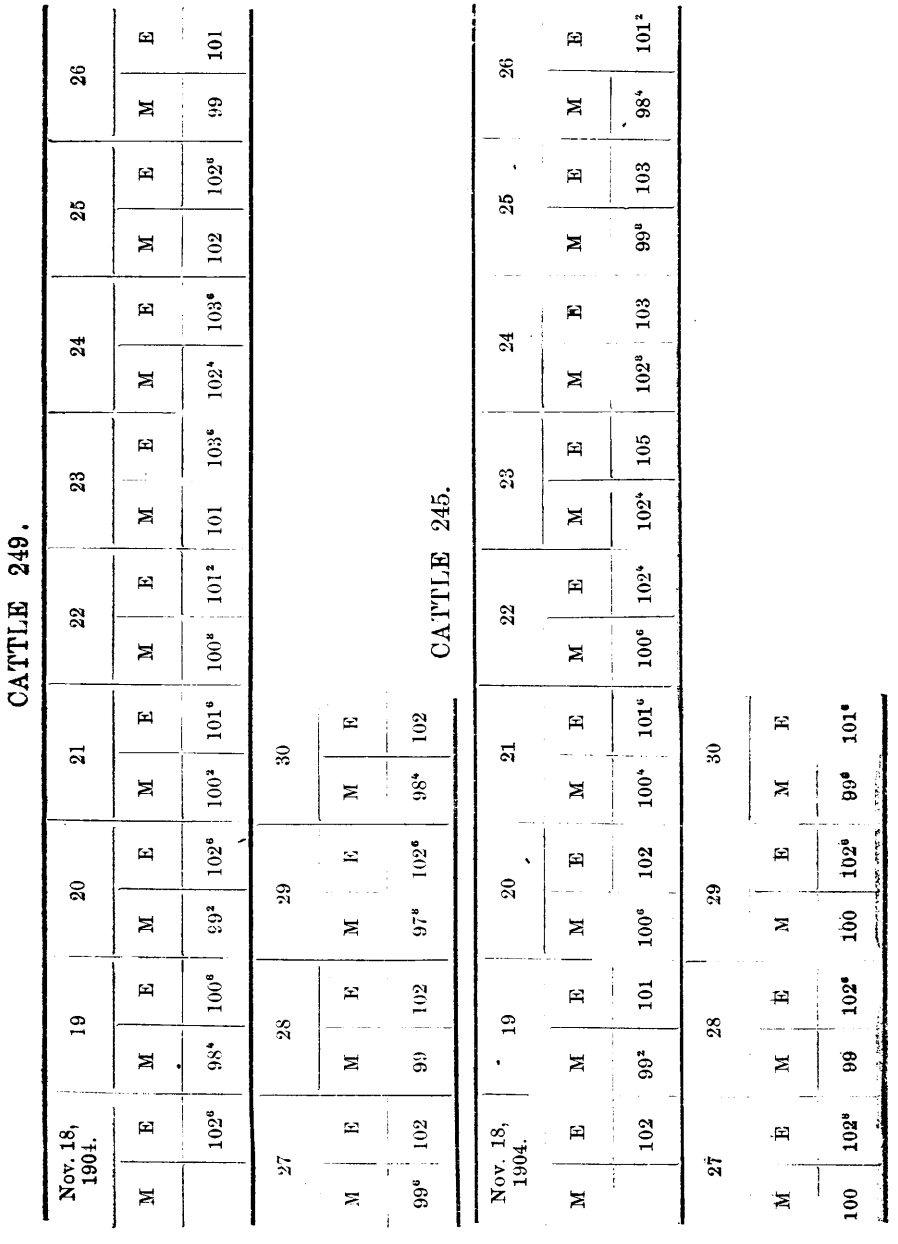
When the spirillum was noticed in Bull 257 on the 18/11/04, it was thought advisable to make another attempt to transmit the spirillosis by inoculation. The bull was accordingly bled and the following experiments were made :—

7.—Ox 229 was about three years old and imported a year previously from Aliwal North, and had been kept on the premises of the Laboratory and hyperimmunised for heartwater with blood of animals not immune against redwater. On 18/11/04, 10 c.c. defibrinated blood of Ox 257 were injected intrajugularly. A reaction began on the third day after the inoculation and the spirillum was noticed on the fifth day, viz., on 23/11/04 and again on 25/11/04. The following chart gives the temperature reaction of Ox 229 :—

OX 229.

Nov. 18, 1904.		19		20		21		22		23		24	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
100^{2}	102	98^{4}	101^{6}	99^{6}	102	99	104	100^{6}	103^{2}	103	104^{8}	103	103^{8}
25		26		27		28		29		30			
M	E	M	E	M	E	M	E	M	E	M	E		
100^{8}	107^{6}	102^{4}	102^{2}	98^{8}	103^{2}	100	103	100	102^{8}	100^{2}	103^{2}		

8 and 9.—A young Bull 245, about 18 months, and an ox, of about the same age, No. 249, were both injected with 10 c.c. of defibrinated blood of Bull 257, drawn on 18/11/04. Both animals were imported from the Cape. In both animals reaction was noticeable on the fifth day after the injection, but in no case could the spirillum be detected microscopically.



CATTLE 249.

Nov. 18, 1904.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	102[6]	98[4]	100[6]	99[2]	102[6]	100[2]	101[6]	100[8]	101[2]	101	103[6]	102[4]	103[6]	102	102[6]	99	101
27		28		29		30											
M	E	M	E	M	E	M	E										
99[6]	102	99	102	97[8]	102[6]	98[4]	102										

CATTLE 245.

Nov. 18, 1904.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	102	99[2]	101	100[6]	102	100[4]	101[6]	100[6]	102[4]	102[4]	105	102[8]	103	99[6]	103	98[4]	101[2]
27		28		29		30											
M	E	M	E	M	E	M	E										
100	102[8]	99	102[6]	100	102[6]	99[6]	101[6]										

10.—Sheep 184, belonging to the Persian breed and bred in Standerton, a district of the Transvaal Highveld, where ticks are rarely met with, was injected intrajugularly on 18/11/04 with 10 c.c. defibrinated blood of Bull 257. A reaction began two days later and the spirillum was noticed on the third and fourth days.

PERSIAN SHEEP 184.

Nov. 18, 1904.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	104	102	101[2]	100[4]	105[4]	104	105[2]	104	103[6]	101	103[4]	103[2]	103[6]	103[2]	103[8]	103	103[4]
	27		28		29		30										
M	E	M	E	M	E	M	E										
102[6]	104	102[6]	103[4]	101[6]	104[8]	103[6]	102[4]										

11.—Persian Sheep 185, from the same origin as the previous one, was injected with 10 c.c. defibrinated blood subcutaneously on 18/11/04. Reaction began on 21/11/04, viz., 3 days after the injection and the spirillum was noticed on 21, 22, and 25/11/04.

PERSIAN SHEEP 185.

Nov. 18, 1901.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103	101^{6}	103	102^{4}	103^{2}	104	105	104	105	102	103^{2}	100^{2}	103	101^{2}	103^{4}	101	104^{2}
27		28		29		30											
M	E	M	E	M	E	M	E										
100^{4}	103^{6}	101^{6}	103^{8}	102^{4}	104^{8}	102^{2}	104^{2}										

12, 13, 14 and 15.—Four Merino Sheep 182, 183, 102, and 104, all imported from the Cape Colony, were injected with 10 c.c. defibrinated blood of Bull 257, on 18/11/04, viz., 182 and 183 subcutaneously and 102 and 104 intrajugularly. A slight reaction was noticeable in all of them, but spirillum could not be traced.

SHEEP 182.

Nov. 18, 1904.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103⁸	102⁸	103⁶	103²	104	103⁸	105	103²	104⁶	103⁴	103	102⁴	104	102²	102⁸	104⁸	105²
27		28		29		30											
M	E	M	E	M	E	M	E										
102⁴	104⁴	102	104	104	103²	102⁶	103⁴										

SHEEP 183.

Nov. 18, 1904.		19		20		21		22		23		24		25		26	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	103⁸	102	102²	101⁶	102⁴	102⁴	103⁸	105²	104⁴	103	102⁸	101²	103⁴	101⁶	102⁸	102⁸	102²
27		28		29		30		Dec. 1, 1904.									
M	E	M	E	M	E	M	E	M	E								
103	104⁴	101⁸	103²	101⁴	104	102⁸	103⁴	103²	102⁴								

SHEEP 102.

Nov. 18, 1904.		19		20		21		22		23		24		25		26		27		28	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^4		102^6	102^6	103^8	104^4	103^4	104	103^6	103	101^4	103	102^6	102^6	103	104^6	103	103^2	103^8	104^2	103^4	103^2

29		30		Dec. 1, 1904	
M	E	M	E	M	E
102^2	104	101^6	102^4	101^8	101^4

SHEEP 104.

Nov. 18, 1904.		19		20		21		22		23		24		25		26		27		28	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101	103^4	101^2	101^8	101^4	102^6	102^8	103	103	104^6	104	102	101^6	102^8	102^8	102^6	101^6	102^6	101^2	103^8	102	103^6

29		30	
M	E	M	E
101^6	103^2	101^4	103

16 and 17.—Angora Goats 186 and 187 were injected on 18/11/04 with 10 c.c. blood of Bull 257 intrajugularly and subcutaneously respectively. A slight reaction was noticeable, but spirillum could not be traced.

GOAT 186.

Date	M	E
Nov. 18, 1904.	.	104^{2}
19	102^{6}	102^{4}
20	101^{8}	103^{4}
21	103^{8}	104^{4}
22	103^{6}	104^{1}
23	103^{2}	105^{2}
24	202	102^{8}
25	102^{4}	104^{8}
26	102^{8}	103^{2}
27	101^{8}	103^{6}
28	101	103^{6}
29	101^{6}	103^{6}
30	102	102^{4}

GOAT 187.

Date	M	E
November 18, 1904.		103^{2}
19	102	103^{4}
20	101^{6}	104
21	101	103^{4}
22	102^{6}	103^{6}
23	101^{4}	103^{4}
24	103^{8}	105^{6}
25	104	104
26	101^{4}	102^{8}
27	100^{4}	103^{8}
28	101^{4}	103^{6}
29	101^{6}	104
30	101^{8}	102^{2}

18.—Horse 604, shortly imported from the Argentine Republic and continuously kept in the stable, was injected, subcutaneously, on 18/11/04 with 10 c.c. blood of Bull 257. No reaction took place.

HORSE 604.

Nov. 18, 1904.		19		20		21		22		23		24	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
99[8]	100[6]	99[6]	100[4]	99[8]	100	99[8]	100	100[8]	100[6]	100	100[4]	98[8]	100
25		26		27		28		29		30			
M	E	M	E	M	E	M	E	M	E	M	E		
98[4]	101	98[8]	100[4]	99[6]	100	99[8]	100[6]	98[4]	100	99[8]	100[4]		

19.—Heifer 286, from Aliwal North, was injected intrajugularly on 2/1/05 with 10 c.c. defibrinated blood of a Sheep 215, which at that time showed spirillum in its blood (compare Experiment 24). A reaction set in on the third day and the spirillum was noticed on 9, 10, 11, 12/1/05. A count of the red corpuscles was made in this animal, which is shown in the following table :—

3/1/05	...	...	6,600,000	per cub.	mm.
4/1/05	...	...	5,893,000	,, ,,	,,
5/1/05	...	...	5,416,000	,, ,,	,,
6/1/05	...	...	5,568,000	,, ,,	,,
7/1/05	...	...	5,080,000	,, ,,	,,
8/1/05	...	...	—		
9/1/05	...	...	4,536,000	,, ,,	,,

19. Heifer 286, from Aliwal North, etc.—*(Continued).*

10/1/05	...	...	4,430,000	per cub.	mm.
11/1/05	...	...	4,040,000	,,	,,
12/1/05	...	...	3,462,000	,,	,,
13/1/05	...	...	4,160,000	,,	,,
14/1/05	...	...	5,184,000	,,	,,
15/1/05	...	...	5,832,000	,,	,,
16/1/05	...	...	6,320,000	,,	,,
17/1/05	...	...	5,728,000	,,	,,
18/1/05	...	...	5,978,000	,,	,,
20/1/05	...	...	6,376,000	,,	,,

CATTLE 286.

Jan. 3, 1905.		4		5		6		7		8		9		10	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{2}	102^{2}	101^{2}	102^{6}	101^{6}	104^{2}	101	103	102^{2}	105^{4}	101^{2}	104^{6}	102^{4}	104^{2}	102^{2}	105^{2}
11		12		13		14		15		16		17			
M	E	M	E	M	E	M	E	M	E	M	E	M	E		
103^{6}	105	102^{2}	103^{4}	103^{4}	103	10 2	102	100^{6}	101^{8}	102	103	101^{4}	102^{4}		

20.—Sheep 219, fat-tail bastard from Standerton, was injected intrajugularly on 2/1/05 with 10 c.c. defibrinated blood of Sheep 215 (compare Experiment 24). Reaction was noticeable on 5/1/06 and the spirillum was seen on 5, 8 and 10/1/05.

SHEEP 219.

Date	M	E
Jan. 3, 1905.	102^4	104^8
4	104^8	104^4
5	103^4	105^6
6	104^2	104^6
7	105	105^4
8	102^4	107
9	104^8	105^4
10	104	105
11	102^4	106
12	103^4	102^4
13	106^4	104
14	105	105^6
15	102^4	104^4
16	102^8	102^6
17	101^6	104^4
18	102^2	103^2
19	103	103^2
20	101^8	104^8
21	102^4	102^8
22	101^8	103^4
23	102^4	103^8
24	103	104^2
25	105^8	104^6
26	104	103^6
27	102^4	103
28	102^4	102^8
29	102^4	102^6
30	102^4	103^4
31	104^6	104

21.—Sheep 220, Merino, Cape Colony, was injected intrajugularly on 2/1/05 with 10 c.c. defibrinated blood of Sheep 215 (compare Experiment 24). The spirillum was noticed on 5/1/05 and again on 9, 10, 11/1/05.

SHEEP 220.

Jan. 3, 1905.		4		5		6		7		8		9		10	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{6}	103^{6}	102	102^{4}	104^{2}	104^{2}	103^{6}	103^{2}	102^{2}	103^{4}	103^{8}	104^{6}	106	105^{2}	103^{8}	104^{6}
11		12		13		14		15		16		17		18	
101^{8}	103^{4}	103^{6}	102^{8}	103^{2}	103^{4}	102	104	102^{2}	105	101^{6}	103^{2}	103^{8}	102^{4}	101^{8}	102
25		26		27		28		29		30		31		19	
101^{8}	104^{8}	102	103^{8}	102	103^{4}	102	103	101^{6}	103	101^{6}	102^{2}	102	104	101^{8}	103^{4}
20		21		22		23		24							
101^{4}	102^{6}	101^{8}	103	102^{4}	102^{4}	102	103^{8}	102^{4}	103						

Conclusion.—The spirillosis of cattle is inoculable from ox into ox, from ox into sheep, from sheep into sheep, and from sheep into ox. It is likely that it is also inoculable into goats, since but a slight reaction was noticeable in the two instances ; the demonstration of the spirillum has not yet been made in this species of ruminants. The horse proved again to be refractory to spirillosis, as in former experiments. The incubation time of the inoculated spirillosis was as short as two and three days, a striking difference to the one noted after tick infection. It is not probable that the larval tick transmits the spirillum in the same form as is done by blood inoculation, hence a certain development is required before its appearance. There is also another possible explanation. The development of the blue

tick on the host embraces the three stages, larva, nympha and imago. The larvæ moult into nymphæ, and these into imagines at intervals of about seven days. It is therefore possible, taking the short incubation time after inoculation into account, that the spirillum is transmitted by the nympha or the adult tick.

In no instance did spirillosis in any of the animals end fatally. Hardly any symptoms of ill-health were noticeable. The count of the red corpuscles of Heifer 286 shows the pathogenic effect the spirillum has on the blood. It produces slight anæmia, not sufficient however to cause death. In some of the animals a loss of condition was noticeable, others showed a slight indisposition by not feeding properly.

4.

EXPERIMENTS TO SHOW THAT THE BLOOD OF OX 347, ON WHICH BLUE TICKS WERE REARED, WHICH TRANSMITTED SPIRILLOSIS TO HEIFERS 276 AND 277, IS INFECTIVE.

22.—Sheep 213, Merino, Cape Colony, was injected intrajugularly on December 17th, 1904, with 10 c.c. defibrinated blood of Ox 347. A reaction started 4 days later and spirillum was noticed on 10/1/05

SHEEP 213.

Dec. 28, 1904.		29		30		31		Jan. 1, 1905.		2		3	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{6}	101^{8}	100	103	100^{2}	102^{8}	101^{8}	105	103^{8}	104	102^{8}	—	102	104

4		5		6		7		8		9		10	
M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{6}	104	102^{6}	104	101^{8}	104^{6}	102^{2}	104^{2}	100	104^{2}	100^{8}	103^{8}	101^{6}	

23:—Sheep 214, Merino, Cape Colony, was treated in the same way as Sheep 213. Reaction began after 4 days. Spirillum was observed on 2, 5, 6, 7/1/05.

SHEEP 214.

Dec. 27, 1904.		28		29		30		31		Jan. 1, 1905.		2		3		4	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
	101^{6}	102	102^{8}	103^{6}	102^{4}	102	103^{6}	102^{2}	105^{2}	104^{2}	103^{6}	105		104	103^{8}	103	105

5		6		7		8		9		10		11		12		13	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
102^{8}	103^{6}	102^{2}	104	102	103^{8}	101	103^{4}	102	102^{6}	102^{2}	103^{6}	101^{6}	103^{2}	101^{2}	103^{4}	103^{2}	104

14		15		16	
M	E	M	E	M	E
104	102^{8}	103	102^{4}	102	102^{8}

24 :—Sheep 215, Fat-tailed bastard, treated in the same way as Sheep 213 and 214. Fever reaction began after four days. Spirillum was noticed on 8, 9, 10, 11/1/05.

SHEEP 215.

Dec. 28, 1904.		29		30		31		Jan. 1, 1905.		2		3		4		5		6	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
103^2	103	102^2	102^2	102^2	103	101^2	104^8	104	104^6	104	—	101^6	106	104^8	104^4	104^6	105^2	103^4	105
7		8		9		10		11		12		13		14		15		16	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
104^2	105	103^2	104^6	104^2	103	104	105^4	102^6	103^8	102	104	102	104^2	103	104	102^6	103	103^4	103

25 :—Sheep 216, Fat-tail bastard, treated as Sheep 213, 214 and 215. Reaction began after four days. Spirillum was noticed on 2, 6, 8/1/05.

SHEEP 216.

Dec. 28, 1904.		29		30		31		Jan. 1, 1905.		2		3		4		5		6	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
103	103[8]	102[2]	103[4]	103[6]	102[8]	104[2]	105[4]	102[2]	105[6]	102[4]		102[4]	106	104	105	103[2]	105	105	104[8]
7		8		9		10		11		12		13		14		15		16	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
103[8]	104[4]	103[2]	104[8]	102	103[6]	101[6]	103[2]	104[2]	103	102[2]	104[2]	103[2]	103	102[4]	102[6]	102[4]	102[2]	102[4]	

26:—Sheep 217, Fat-tail bastard, treated in the same way as Sheep 213 to 216. Reaction began after two days. Spirillum was seen on 5, 7, 8, 11/1/05.

SHEEP 217.

Dec. 28, 1904.		29		30		31		Jan. 1, 1905.		2		3		4		5		6	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^4	102^6	102	103^4	102^8	105	103	104^4	102^6	105^6	102^4	104^4		104^4	103^4	103^6	103^6	104	104^4	104^2
7		8		9		10		11		12		13		14		15		16	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
105	104^2	104^6	103^6	102^6	103^4	102	103^6	103^6	103^8	101^4	102^8	101^2	103^8	101^4	103^4	102^8	102^4	101	103^4

27 :—Sheep 218, Fat-tail bastard, treated in the same way as Sheep 213 to 217. Reaction began after four days. Spirillum was noticed on 8, 9, 10/1/05.

SHEEP 218.

Dec. 28, 1904.		29		30		31		Jan. 1, 1905.		2		3		4	
M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
101^{6}	103^{4}	102^{2}	103^{4}	102^{2}	102^{8}	103^{2}	105	104^{6}	105^{8}	103^{4}		105	105	103^{2}	106
5		6		7		8		9		10		11		12	
103^{2}	102^{8}	103^{8}	103^{8}	103^{8}	104^{2}	102^{4}	104	102^{2}	104	104^{6}	106	104^{4}	103	104^{2}	103^{4}
13		14		15		16									
104	104	103^{8}	102^{6}	104	104^{2}	102^{4}	103^{8}								

Conclusion.—All five sheep, which were injected intrajugularly with defibrinated blood of Ox 347, contracted the spirillosis. Thus blood of the healthy Ox 347 contained the spirillum. So far it was impossible to trace this parasite microscopically in this ox. Ox 347 is immune against Redwater. Its blood has, in many instances, been utilised for inoculation purposes, but, so far, only in one instance was spirillum noticed in the inoculated animal. It will be of the utmost importance to ascertain the effect of the inoculation of the blood of this ox into animals susceptible to spirillosis and redwater.

SUMMARY OF CONCLUSIONS.

1.—The natural transmission of spirillum is caused by the progeny of Rhipicephalus Decoloratus, which have developed on cattle suffering or immune from spirillosis.

2.—It is possible to inoculate spirillosis into susceptible cattle and sheep with blood of sick and immune animals. The proof that immune sheep blood is infective is yet wanting.

3.—The pathogenic effect of spirillum is a slight anaemia accompanied by fever. No death was observed due to the presence of the spirillum.

4.—The cases of death, in which spirillum was found, were complicated with Piroplasmosis (Redwater). These cases can be readily explained, when we consider the fact that immune animals retain the spirillum in their blood, which, under the influence of a virulent fever such as is caused by Redwater, may become active and multiply. It may also be conceived that both parasites—Piroplasma bigeminum and Spirillum—can be inoculated at the same time and by the same tick—Rhipicephalus Decoloratus—viz., the Redwater tick of South Africa.*

In our experiments, however, this was not the case. Hence it will be necessary to investigate carefully whether the presence of spirillum in the tick prevents the piroplasma from developing in the same host.

5.—In its evolution spirillosis in cattle presents the same characteristics as the piroplasmosis of the type caused by Piroplasma bigeminum, a fact which leads us to infer that the Spirillum is also a protozoon.

HORSE SICKNESS EXPERIMENTS.

1.—HYPERIMMUNISATION with Virulent Serum.

A.—Mules hyperimmunised with mule serum.

No. 368	12,200 c.c.
No. 464	12,000 c.c.
No. 445	14,200 c.c.
No. 482	18,400 c.c.

B.—Donkeys hyperimmunised with mule serum.

No. 379	5,900 c.c.
No. 515	6,000 c.c.
No. 516	6,400 c.c.

C.—Mules hyperimmunised with virulent horse serum.

No. 413	18,400 c.c.
No. 415	17,800 c.c.
No. 479	18,100 c.c.

D.—Donkeys hyperimmunised with horse serum.

No. 381	7,900 c.c.
No. 514	8,700 c.c.

To note whether the serum of the above animals, hyperimmunised with virulent serum, proves to be hæmolytic in vitro.

*Laveran and Vallée, of Paris, to whom I sent larval ticks infected with Spirillum and Piroplasma bigeminum, produced a successful infection of both Spirillum and Piroplasma in one and the same cow at the premises of the Veterinary College at Alfort. The animal showed first Spirillum and then Piroplasma bigeminum. It died of Redwater.

HÆMOLYSIS TEST A. and B.—25/8/04.

Blood	Mule 368	Mule 464	Mule 445	Mule 482	Donkey 379	Donkey 515	Donkey 516
Horses—							
290	traces	traces	dist.	traces	—	—	—
536	clear	clear	dist.	clear	clear	slight	clear
470	clear	clear	dist.	clear	clear	slight	clear
476	clear	clear	dist.	clear	clear	slight	clear
524	clear	clear	dist.	clear	traces	slight	traces
Mules—							
547	clear	clear	dist.	clear	clear	slight	clear
548	clear	clear	dist.	slight	clear	clear	traces
555	clear	clear	dist.	traces	clear	traces	clear
558	clear	clear	clear	clear	traces	slight	clear
560	clear	clear	clear	traces	slight	slight	traces

Result.—Serum mule 445 proves to be very hæmolytic, then that of donkey 515. Serum of donkey 516 and mule 482 are less hæmolytic.

Conclusion.—The injection of large quantities of virulent mule serum can still produce a hæmolytic serum in mules and donkeys.

HÆMOLYSIS TEST C. and D.—25/8/04.

Blood	Mule 413	Mule 415	Mule 479	Donkey 381	Donkey 514
Horses—					
290	traces	slight	traces	—	—
536	clear	clear	clear	dist.	slight
470	clear	clear	traces	dist.	slight
476	clear	clear	clear	dist.	slight
524	clear	clear	clear	dist.	slight
Mules—					
547	clear	clear	clear	dist.	slight
548	clear	clear	clear	dist.	traces
555	clear	clear	clear	dist.	traces
558	clear	clear	clear	dist.	clear
560	clear	clear	clear	dist.	traces

Result.—Both donkeys hyperimmunised with virulent horse serum produced a serum hæmolytic for horse blood and mule blood.

TO NOTE the Hæmolytic effect of different Serum Mixtures.—26/8/04.

Mixture	A	...	...	Serum mules	368, 413, 415, 464.
„	B	...	...	„ „	479, 482.
„	C	...	...	„ „	368, 413, 415, 464, 479.
„	D	...	...	„ „	368, 413, 415, 464, 482.
„	E	...	...	„ „	368, 413, 415, 464, 479, 482.
„	F	...	...	„ „	368, 464.
„	G	...	...	„ „	368, 464, 482.
„	H	...	...	„ „	413, 415.
„	I	...	...	„ „	413, 415, 479.
„	K	...	...	„ „	368, 464, 482, and donkeys 379, 516.
„	L	...	...	„ „	413, 415, 479, and donkeys 379, 516.

HÆMOLYSIS TEST with various Serum Mixtures.—26/8/04.

Blood	Mixt. A	Mixt. B	Mixt. C	Mixt. D	Mixt. E	Mixt. F	Mixt. G	Mixt. H	Mixt. I	Mixt. K	Mixt. L
Horses.—											
290	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
536	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
470	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
476	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
524	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
Mules.—											
547	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
548	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
555	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
558	—	—	—	—	—	—	—	clear	clear	clear	clear
560	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear

Result.—The various mixtures of various sera did not prove to be hæmolytic for horse and mule blood.

Experiment No. 174.—To note—1. Whether the mixture of various serum of mules and donkeys hyperimmunised with virulent serum of horses and mules, which in vitro did not produce hæmolysis, will not produce hæmolysis in vivo.

2. Whether such a serum mixture passes an animal through a horse sickness reaction.

Horse 476 injected subcutaneously with 400 c.c. of a mixture of serum of mules 368, 413, 415, 464, and donkeys 379, 516, and intrajugularly with 5 c.c. virulent serum mule 534 simultaneously.

Mule 558 injected subcutaneously with 300 c.c. of the above mixture and intrajugularly 5 c.c. of above virus simultaneously.

Result.—Horse 476. After an incubation time of 3 days, reaction set in, lasted 5 days, and the horse died of horse sickness on 8,9/9/04.

Mule 558 had no reaction. It was tested later and proved to be immune.

Conclusion.—The above serum mixture did not produce hæmolysis. The serum had no effect whatever on the course of the horse sickness reaction.

HÆMOLYSIS TEST with Serum of 2 Horses hyperimmunised with Horse Blood.—30/8/04.

Blood	Horse 160	Horse 172	Remarks.
Horses—			
290	clear	traces	Horse 160 has been injected with blood of 11 different horses up to the amount of 12,010 c.c.
536	clear	traces	
470	clear	traces	
476	clear	clear	
524	clear	clear	
Mules—			
547	traces	traces	Horse 172 has been injected with blood of 11 horses up to the amount of 10,650 c.c.
548	clear	traces	
555	traces	traces	
558	traces	traces	
560	traces	traces	

HÆMOLYSIS TEST with Mixture of 8 Sera, viz., Horses, 160, 172; Mules, 368, 413, 415, 464; and Donkeys, 379, 516.

Blood	Serum Mixture.
Horses—	
290	clear
536	clear
470	clear
476	clear
524	traces
Mules—	
547	clear
548	clear
555	clear
558	clear
560	clear

Result.—The serum of horses 160 and 172, hyperimmunised with horse blood, had a noticeable hæmolytic effect in vitro on blood of mules and horses. The mixture of the same sera with mule sera, not hæmolytic, proved to have a non-hæmolytic effect in vitro on horse and mule blood.

Experiment 175.—To note 1. Whether the serum mixture, which had a minimal hæmolytic effect in vitro, will produce hæmolysis in vivo (*vide* hæmolytic effect on No. 524).

2. Whether such serum will pass an animal through horse sickness reaction.

Horse 524 injected subcutaneously, on 8/9/04, with 500 c. c. serum mixture of horses 160, 172, mules 368, 413, 415, 464, and donkeys 379 and 516, and intrajugularly with 5 c.c. virulent serum horse 536. Mule 560 treated as above with 300 c.c.

Result.—

Count of Red Corpuscles.	Horse 524.	Mule 560.	
9/9/04	6,572,000	4,955,000	per c.m.
10/9/04	6,494,000	6,818,800	,,
11/9/04	7,256,000	3,844,000	,,
12/9/04	7,714,000	4,845,000	,,
13/9/04	7,601,000	3,801,000	,,
14/9/04	5,407,000	5,760,000	,,
15/9/04	8,400,000	7,670,000	,,

There was no clinical symptoms of hæmolysis. Horse 524 had an incubation time of 3 days, the disease lasted 4 days and it died on 15/9/04. Mule 560 had an incubation time of 3 days, the disease lasted 5 days and died on 15/9/04.

Conclusion.—The serum mixture had no hæmolytic effect on the horse and caused a reduction in the number of red corpuscles in the mule, without producing any clinical symptoms. The mixture did not influence the course of horse sickness.

HÆMOLYSIS TEST of Serum of Various Horses, Mules, and Donkeys. —20/9/04.

Blood	Horse 160	Horse 172	Horse 264	Horse 301	Mule 368	Mule 413	Mule 415	Mule 464	Donkey 379	Donkey 516
Horses—										
618	traces	clear	clear	clear	clear	clear	traces	clear	clear	clear
619	traces	traces	slight	traces	clear	clear	traces	clear	clear	clear
621	traces	clear	clear	clear	clear	clear	traces	clear	clear	clear
622	traces	clear	dist.	clear	clear	clear	slight	clear	clear	clear
623	slight	clear	traces	clear	clear	clear	slight	traces	clear	clear
624	slight	traces	traces	clear	traces	clear	traces	traces	clear	clear
Mules—										
559	slight	traces	traces	traces	clear	traces	traces	clear	clear	clear
571	traces	clear	clear	traces	clear	clear	traces	traces	clear	clear
576	traces	traces	traces	slight	clear	clear	traces	clear	clear	clear
577	clear	traces	traces	traces	clear	clear	traces	traces	clear	clear
616	traces	clear	slight	traces	clear	traces	clear	clear	clear	clear
617	clear	traces	slight	clear	clear	traces	traces	clear	clear	clear

Remark.—Horse 160 hyperimmunised with defibrinated virulent horse blood.

Horse 172 hyperimmunised with defibrinated virulent horse blood.

Horse 264 hyperimmunised with 5,000 c.c. virulent mule blood.

Horse 301 hyperimmunised with 5,000 c.c. virulent mule blood from 4 animals.

Mule 368 hyperimmunised with 12,300 c.c. virulent mule serum from 9 animals.

Mule 413 hyperimmunised with 18,400 c.c. virulent horse serum from 11 animals.

Mule 415 hyperimmunised with 17,800 c.c. virulent horse serum from 10 animals.

Mule 464 hyperimmunised with 12,000 c.c. virulent mule serum from 5 animals.

Donkey 379 hyperimmunised with 5,900 c.c. virulent mule serum from 5 animals.

Donkey 516 hyperimmunised with 6,400 c.c. virulent mule serum from 6 animals.

HÆMOLYSIS TEST with Serum Mixtures.

Mixture A.	...	Serum horses 160, 172.
„ B.	...	Serum horses 264, 301.
„ C.	...	Serum horses 160, 172, 264, 301.
„ D.	...	Mules 368, 413, 415, 464.
„ E.	...	Donkeys 379, 516.

HÆMOLYSIS TEST of Different Mixtures.—20/9/04.

Blood.	A.	B.	C.	D.	E.
Horses—					
618	clear	clear	clear	clear	clear
619	traces	slight	slight	clear	clear
621	clear	clear	clear	clear	clear
622	clear	slight	traces	clear	clear
623	traces	traces	traces	traces	clear
624	traces	traces	traces	traces	clear

HÆMOLYSIS TEST, ETC.—*Continued.*

Blood.	A.	B.	C.	D.	E.
Mules—					
529	traces	traces	traces	traces	clear
571	clear	traces	clear	clear	clear
576	clear	slight	traces	traces	clear
577	traces	slight	clear	traces	clear
616	traces	traces	traces	clear	clear
617	traces	traces	traces	clear	clear

Result.—The serum mixtures A, B, C, D, in the descending order quoted proved to be hæmolytic. Mixture E was not hæmolytic in vitro on blood of horses and mules.

Experiment 176.—To note whether the mixture of serum of horses, serum of mules, and serum of donkeys, which had some hæmolytic effect in vitro, has no effect in vivo on the same animals, and whether such serum has a preventive value against horse sickness.

A. Horse 621 was injected on 25/9/04, with 500 c.c. mixture serum horses 160, 172, 264, 301 (mixture C) subcutaneously and 1 c.c. virus mule 526 intrajugularly simultaneously.

Result.—Incubation time 3 days. Reaction lasted 6 days. Died on 4.5/10/04, of Dikkop.

Mule 577 was injected on 25/9/04 with 400 c.c. mixture serum horses 160, 172, 264, 301 (mixture E) subcutaneously, and 1 c.c. virus mule 526 intrajugularly simultaneously.

Result.—Incubation time 3 days. On the fifth day hæmoglobinuria was noticed. Died on 4.5/10/04, from horse sickness (pulmonary form).

Conclusion.—The mixture of the different horse sera, which in vitro had no hæmolytic effect on horse 621 and a very slight one on mule 577, proved to be hæmolytic for the latter in vivo.

The serum had no preventive effect against horse sickness.

B. Horse 619 was injected on 25/9/04 with 500 c.c. serum mixture of mules 368, 413, 415, 464 (mixture D), subcutaneously and 1 c.c. virus mule 426 intrajuglarly simultaneously.

Result.—Incubation time 3 days. The horse developed Dikkop and died on 3.4/10/04.

Mule 616 was injected on 25/9/04, with 400 c.c. serum mixture of mules 368, 413, 415, 464 (mixture D) and 1 c.c. virus mule 426 intrajugularly simultaneously.

Result.—Incubation time 2 days. Died on 4.5/10/04 of Dunkop.

Conclusion.—The mixture of mule serum, which in vitro had no hæmolytic effect on the blood of the horse and of the mule, had no hæmolytic effect in vivo.

The serum had no preventive effect against horse sickness.

C. Horse 622 was injected on 25/9/04 with 500 c.c. serum mixture donkeys 379, 516 (mixture E), and 1 c.c. virus mule 526 intrajugularly simultaneously.

Result.—Incubation time 3 days. Died on 2/10/04 from Dunkop.

Mule 617 was injected on 25/9/04 with 400 c.c. with serum mixture donkeys 379, 516 (mixture E), and 1 c.c. virus mule 526 intrajugularly simultaneously.

Result.—Incubation time 2 days. Died on 2.3/10/04 from Dunkop.

Conclusion.—The mixture of the donkey serum, which in vitro had no hæmolytic effect, had no hæmolytic effect in vivo.

The mixture had no preventive power against horse sickness.

HÆMOLYSIS TEST with serum of Mules Hyperimmunised with Virulent Mule Blood.—27/9/04.

Blood.	Mule 462.	Mule 463.	Mule 468.
Horses—			
623	clear	clear	clear
625	clear	clear	clear
Mules—			
456	clear	clear	clear
570	clear	clear	clear

30/9/04.

	Mixture Mule 462.	Mixture Mule 463.	Mixture Mule 468.
Horse—			
614	clear	clear	clear
Mules—			
539	clear	clear	clear
550	clear	clear	clear
552	clear	clear	clear
565	clear	clear	clear
572	clear	clear	clear

Remark.—Mule 462 hyperimmunised with 12,000 c.c. serum from 11 animals.

Mule 463 hyperimmunised with 12,000 c.c. serum from 12 animals.

Mule 468 hyperimmunised with 18,500 c.c. serum from 9 animals.

Result.—The serum of mules hyperimmunised with virulent mule blood did not prove to be hæmolytic for horse and mule blood.

Experiment 177.—27/9/04.—To note whether the serum of mules hyperimmunised with blood, which in vitro did not prove to be hæmolytic, is hæmolytic in vivo and whether such a serum has protective power.

Horse 612 was injected on 1/10/04 with 400 c.c. serum mixture of mules 462, 463, 468 subcutaneously, and 1 c.c. virus mule 561 intrajugularly and simultaneously. On 5/10/04 it was injected with 150 c.c. of the above mixture.

Result.—No hæmolysis observed. Incubation time 7 days. Reaction lasted 8 days. Symptoms of Dikkop. Recovered. This horse was hyperimmunised.

Mule 570 was injected, on 1/10/04 with 300 c.c. serum mixture of mules 462, 463, 468 subcutaneously, and 1 c.c. virus mule 561 intrajugularly and simultaneously. On 5/10/04 it was injected subcutaneously with 100 c.c. of above mixture.

Result.—Slight reaction. Tested for immunity on 12/10/04. It was hyperimmunised later.

Conclusion.—The serum mixture of the three mules had no hæmolytic effect in vivo. It proved to have strong protective power against horse sickness.

Experiment 178.—With mixture of old carbolized serum of Horses SH 1, 160 172 (Compare Annual Report 1903/1904).

4/10/04. The hæmolysis test in vitro was positive, owing to the presence of carbolic acid. The carbolic solution of 6 per cent. added to normal saline solution had the same hæmolytic effect. The object was to ascertain whether such old serum could be utilised as preventive serum.

Horse 618 was injected on 4/10/04 with 150 c.c. carbolized serum mixture horses SH 1, 160, 172. On 5/10/04 it was injected with 250 c.c. carbolized serum mixture subcutaneously, and 1 c.c. virus mule 561 intrajugularly and simultaneously.

Result.—Incubation time 4 days. Died on 13/10/04 from horse sickness.

Mule 570 was injected on 4/10/04 with 100 c.c. carbolised serum mixture horses SH 1, 160, 172 subcutaneously. On 5/10/04 it was injected with 200 c.c. carbolised serum mixture, and 1 c.c. virus mule 561 intrajugularly and simultaneously.

Result.—Long reaction followed. Tested for immunity on 2/11/04. Proved to be immune. It was hyperimmunised later.

Conclusion.—Serum mixture of old carbolised serum did not prove to be a preventive for the horse and produced a modified attack of horse sickness in the mule.

Experiment 179.—To note whether mixture of old carbolized serum, two of which had previously had hæmolytic effect, can be utilised as preventive inoculation against horse sickness.

Mules 549 and 556 were injected on 3/10/04 with 300 c.c. carbolized serum mixture of horses SH 1, 160, 172, 264, 301 subcutaneously, and 1 c.c. virus mule 561 intrajugularly and simultaneously. On 6/10/04 both mules were injected with 100 c.c. each carbolized serum mixture of horses SH 1, 160, 172.

Result.—Both mules contracted hæmolysis and died, mule 549 on 10.11/10/04, and mule 556 on 12.13/10/04.

Conclusion.—The old serum retained its hæmolytic action.

HÆMOLYSIS TEST with serum of mules which were hyperimmunised with boullion made virulent by intraperitonial injection into sick animals.

Blood.	Mule 469	Mule 492	Mule 496	Mule 501	Mule 509
Horses—					
290	clear	traces	clear	traces	clear
536	clear	traces	clear	slight	clear
470	clear	traces	clear	clear	clear
476	clear	slight	clear	traces	clear
524	clear	traces	clear	traces	clear
Mules—					
547	clear	clear	clear	clear	clear
548	clear	clear	clear	traces	clear
555	clear	clear	clear	traces	clear
558	clear	traces	clear	clear	clear
560	clear	clear	clear	clear	clear

Mixture A ... ... Serum 469, 492, 496, 509.
Mixture B. ... ... Serum 469, 492, 496, 501, 509.

Blood.	Mixture A.	Mixture B.
Horses—		
290	clear	clear
536	clear	clear
470	clear	clear
470	clear	clear
476	clear	clear
524	clear	traces

HÆMOLYSIS TEST, ETC.—*Continued.*

Blood.	Mixture A.	Mixture B.
Mules—		
547	clear	clear
548	clear	clear
555	clear	clear
558	clear	clear
560	clear	clear

Result.—The serum of mules 492 and 501 proved to have a slight hæmolytic effect in vitro. The mixture had no noticeable hæmolytic effect in vitro on blood of horses and mules.

HÆMOLYTIC TEST with mixture of 3 mule sera hyperimmunised with intraperitoneal boullion which in a previous experiment proved to be non-hæmolytic in vitro.—11/10/04.

Blood.	Mixture 469, 496, 509.
Horse—	
625	clear
Mule—	
562	clear

Result.—The mixture of mule sera which individually were not hæmolytic proved to be non-hæmolytic when mixed.

Experiment 180.—To note whether the serum mixture of mules hyperimmunised with virulent boullion has hæmolytic effect in vivo, and whether such serum has protective properties.

Horse 625 was injected subcutaneously on 10/10/04 with 400 c.c. mixed serum of mules 469, 496, and 509, and with 1 c.c. virus horse 623 intrajugularly and simultaneously. On 15/10/04 it was injected with 100 c.c. of the above mixture.

Result.—Incubation time lasted two days. Horse died on 15.16/10/04 from Dunkop.

Mule 562 was injected subcutaneously on 10/10/04 with 300 c.c. serum mixture of mules 469, 496, and 509, and with 1 c.c. virus horse 623 intrajugularly and simultaneously.

Result.—No reaction. It was hyperimmunised later.

Conclusion.—The serum mixture had no hæmolytic effect in vivo. It had no protective properties for the horse. Mule 562 was probably immune prior to injection.

HÆMOLYSIS TEST with mixture of serum of mules 462, 463, and 468. —11/10/04.

Blood.	Mixture.
Horses—	
611	clear
627	clear
Mules—	
530	clear
559	clear

Result.—The mixture of four mule sera hyperimmunised with mule blood showed but a minimal hæmolytic effect in vitro.

Experiment 181.—To note whether the serum mixture of the three mule sera, which in vitro had a minimal hæmolytic effect, has a hæmolytic effect in vivo and whether it has preventive properties.

Horse 611 was injected on 11/10/04 with 400 c.c. serum mixture of mules 462, 463, and 468 subcutaneously, and 1 c.c. virus horse 623 intrajugularly and simultaneously. On 16/10/04 it was injected with 100 c.c. of above mixture.

Result.—Long reaction. Dikkop. Recovery. Hyperimmunised later.

Mule 559 was injected on 11/10/04 with 400 c.c. serum mixture of mules 462, 463, and 468 subcutaneously, and 1 c.c. virus horse 623 intrajugularly and simultaneously. On 16/10/04 it was injected with 100 c.c. of same mixture.

Result.—Reaction doubtful. It was hyperimmunised later.

Conclusion.—Serum of above mixture has no hæmolytic effect in vivo and has strong protective properties.

HÆMOLYSIS TEST with Mixture of Serum of Mules Hyperimmunised with Mule Blood.—17/10/04.

Blood.	Serum Mixture.
Horses—	
615	clear
627	clear
Mules—	
450	clear
566	clear

Result.—The mixture had no hæmolytic effect on horse and mule blood in vitro.

Experiment 182.—To note whether the serum mixture of the three mule sera, which in vitro had no hæmolytic effect, has a hæmolytic effect in vivo and whether it has preventive properties.

Horse 627 was injected on 18/10/04 with 150 c.c. serum mixture subcutaneously. On 19/10/04 it was injected subcutaneously with 250 c.c. serum mixture, and intrajugularly with 1 c.c. virus horse 623 simultaneously. On 23/10/04 it was injected subcutaneously with 100 c.c.

Result.—Reaction with much prostration. Dikkop. Recovery. Tested on 15/11/04. It was hyperimmunised later.

Mule 550 was injected subcutaneously on 18/10/04 with 100 c.c. serum mixture as above. On 19/10/04 it was injected subcutaneously with 200 c.c. serum mixture, and intrajugularly with 1 c.c. virus horse 623 simultaneously. On 23/10/04 it was injected subcutaneously with 100 c.c.

Result.—Slight reaction. It was hyperimmunised later.

Conclusion.—The mixture of the three sera had no hæmolytic effect in vivo and showed strong preventive qualities.

HÆMOLYSIS TEST with Mixture of Serum of Mules 462, 463, and 468.—19/10/04.

Blood.	Serum Mixture.
Horses—	
600	clear
606	clear
613	clear
614	clear
615	clear

HÆMOLYSIS TEST, ETC.—*Continued.*

Blood.	Serum Mixture.
Mules—	
530	clear
556	clear
570	clear
573	clear
578	clear
579	clear
589	clear
593	clear

Result.—The mixture of the three mule sera had no hæmolytic effect in vitro on blood of horses and mules.

Experiment 183.—To note whether the serum mixture of Mules 462, 463, and 468, which proved to be non-hæmolytic in vitro does not produce hæmolysis in vivo and whether such serum has preventive properties.

Horse 615 (military horse, imported) was injected subcutaneously on 20/10/04 with 300 carbolised serum mixture, and intrajugularly with 5 c.c. virulent serum mule 547 simultaneously. On 25/10/04 it was injected subcutaneously with 200 c.c. of the above serum.

Result.—Reaction. No clinical symptons. Recovery. Hyperimmunised later.

Horse 606 (Argentine, freshly imported) was injected subcutaneously on 20/10/04 with 350 c.c. carbolised serum mixture, and intrajugularly with 5 c.c. virulent serum mule 547 simultaneously. On 27/10/04 it was injected subcutaneously with 200 c.c. serum mixture.

Result.—Strong reaction. No clinical symptons. Recovery. Died of biliary fever 28/11/04.

Horse 616 (military horse, imported) was injected subcutaneously on 20/10/04 with 400 c.c. carbolised serum mixture and intrajugularly with 5 c.c. virulent serum mule 547 simultaneously. On 25/10/04 it was injected subcutaneously with 200 c.c. of the above serum.

Result.—Horse died on 3/11/04 from dikkop, 14 days after injection.

Mule 579 was injected subcutaneously on 20/10/04 with 200 c.c. serum mixture and intrajugularly with 5 c.c. virulent serum mule 547. On 25/10/04 it was injected with 100 c.c. serum mixture.

Result.—Slight reaction. It was hyperimmunised later.

Mule 578 was injected subcutaneously on 20/10/04, with 250 c.c. carbolised serum mixture, and intrajugularly with 5 c.c. virulent serum mule 547 simultaneously. On 25/10/04 it was injected with 100 c.c. serum mixture.

Result.—Slight reaction. It was hyperimmunised later.

Mule 589 (Argentine, freshly imported) was injected on 20/10/04 with 300 c.c. carbolised serum mixture, and intrajuglarly with 5 c.c. virulent serum mule 547 simultaneously. On 25/10/04 it was injected subcutaneously with 100 c.c. serum mixture. On 1/11/04 it was injected subcutaneously with 200 c.c. serum mixture.

Result.—Distinct reaction started later than usual. It was inoculated against biliary fever on 6/11/04. It was hyperimmunised later.

Conclusion.—The mixture did not prove to be hæmolytic. It passed, with the exception of horse 613, all the animals through a horse sickness reaction.

HÆMOLYSIS TEST with Mixture of Serum Mules 462, 463 and 468.—2/11/04.

Blood.	Serum Mixture.
Horses—	
398	clear
407	clear
470	clear
473	clear
614	slight
Mules—	
530	clear
552	clear
554	clear
555	traces
565	distinct
566	traces
572	clear
573	clear
574	slight

Result.—The mixture proved to have a hæmolytic effect in vitro on the blood of some of the animals.

Experiment 184.—To note whether serum mixture of mules 462, 463, and 468 which had no hæmolytic effect in vitro on the blood of some animals proves to be non-hæmolytic in vivo and whether such serum has protective properties.

Horse 398 was injected subcutaneously with 300 c.c. serum mixture, and intrajugularly with 1 c.c. virus mule 547 simultaneously. On 10/11/04 it was injected subcutaneously with 200 c.c. serum mixture. On 15/11/04 it was injected subcutaneously with 200 c.c. serum mixture.

Result.—Distinct reaction. Dikkop. Recovered. It was hyperimmunised later.

Horse 473 was injected as above on 10/11/04.

Result.—Distinct reaction. No clinical symptoms. Recovered. It was hyperimmunised later.

Mule 530 was injected subcutaneously on 5/11/04 with 50 c.c. serum mixture, and intrajugularly with 1 c.c. virus mule 547 simultaneously. On 10/11/04 it was injected subcutaneously with 200 c.c. serum mixture.

Result.—Reaction. Dikkop. Recovered. It was hyperimmunised later.

Mule 552 was injected subcutaneously on 5/11/04 with 100 c.c. serum mixture, and with 1 c.c. virus intrajugularly and simultaneously. On 10/11/04 it was injected with 200 c.c. serum mixture.

Result.—Reaction. No clinical symptoms. Recovered. It was hyperimmunised later.

Mule 554 on 5/11/04 was treated as mule 552.

Result.—Reaction. No clinical symptoms. Recovered. It was hyperimmunised later.

Mule 572 was injected on 5/11/04 subcutaneously with 100 c.c. serum mixture, and intrajugularly with 100 c.c. virus mule 547 simultaneously.

Result.—Reaction. No clinical symptoms. Recovered. It was hyperimmunised later.

Conclusion.—The serum mixture, which had no hæmolytic effect on the blood of some animals, proved to be non-hæmolytic for the same animals in vivo and had a strong preventive effect on Horse Sickness.

Experiment 185.—To note whether the serum mixture, which had a very slight hæmolytic effect in vitro, loses this quality in vivo when previously heated to 56 degrees C., and whether such serum has protective properties.

Horse 407 was injected subcutaneously on 5/11/04 with 300 c.c. heated serum mixture, and intrajugularly with 1 c.c. virus mule 547 simultaneously. On 10/11/04 and 13/11/04 it was injected with 200 c.c. heated serum mixture.

Result.—Heavy reaction. Dikkop. It was hyperimmunised later.

Horse 470 was injected subcutaneously on 5/11/04 with 300 c.c. heated serum mixture, and 1 c.c. intrajugularly virus simultaneously. On 10/11/04 it was injected with 200 c.c. heated serum mixture.

Result.—Died of Horse Sickness on 14/11/04.

Conclusion.—The mixture proved not to be hæmolytic in vivo, whether due to heating it is impossible to decide. Protective powers were less noticeable than in previous experiments.

Experiment 186.—To note the effect of a non-hæmolytic mixture injected 12 hours after virus.

(Compare hæmolysis test 2/11/04.)

Mule 473 was injected on 4/11/04 intrajugularly with virus of mule 547. On 5/11/04 it was injected subcutaneously with 300 c.c. serum mixture.

Result.—Slight reaction. It was hyperimmunised later.

Conclusion.—The serum mixture had a strong preventive action even when virus was injected 12 hours previous to serum.

Experiment 187.—To find the minimum quantity of serum required to pass a mule through Horse Sickness, and whether one or two injections are required.

HÆMOLYSIS TEST with Mixture of Serum Mules 462, 463, and 468

The mixture of serum used was the same as in Experiment 184, page—

Blood of Mules.	Mixture.	Mule 462.	Mule 463.	Mule 468.
551 ...	slight	slight	slight	slight
553 ...	clear	clear	clear	clear
555 ...	clear	clear	clear	clear
565 ...	clear	clear	clear	clear
566 ...	clear	clear	clear	clear
567 ...	traces	traces	slight	slight
568 ...	clear	clear	clear	clear
569 ...	traces	traces	traces	traces
574 ...	clear	clear	clear	clear
575 ...	clear	clear	clear	clear
576 ...	clear	traces	traces	clear
583 ...	clear	clear	clear	clear
584 ...	clear	clear	traces	clear
629 ...	slight	clear	distinct	distinct
630 ...	clear	clear	clear	clear
631 ...	clear	clear	traces	traces
632 ...	traces	clear	clear	clear

HÆMOLYSIS TEST, ETC.—*Continued.*

Blood of Mules.	Mixture.	Mule 462.	Mule 463.	Mule 468.
644 ...	clear	clear	clear	clear
645 ...	traces	traces	traces	traces
646 ...	clear	clear	clear	clear
647 ...	clear	clear	clear	clear
648 ...	clear	clear	clear	clear
649 ...	clear	clear	clear	clear
650 ...	clear	clear	clear	clear
651 ...	clear	clear	clear	clear
652 ...	clear	clear	clear	clear
653 ...	clear	clear	clear	clear
654 ...	clear	clear	clear	clear

Result.—The mixture proved to be hæmolytic for the blood of some of the mules.

Experiment 188.—Simultaneous injection of various doses of serum mixture of mules 462, 463, and 460, and 1 c.c. virus horse 640 intra-jugularly, on 16/11/04, and second injection on 21/11/04.

Mules.	1st Injection.	2nd Injection.	Remarks.
630	50 c.c.	—	Died 28/11/04.
631	50 c.c.	50 c.c.	Died 22/11/04.
652	50 c.c.	100 c.c.	Died 22/11/04.
646	50 c.c.	150 c.c.	Died 25/11/04.
565	50 c.c.	200 c.c.	Slight reaction. Dikkop. Hyper-immunised.
651	100 c.c.	—	Reaction. Hyperimmunised.
467	100 c.c.	50 c.c.	Died 22/11/04.
644	100 c.c.	100 c.c.	Died 25/11/04.
566	100 c.c.	150 c.c.	Reaction. Hyperimmunised.
555	100 c.c.	200 c.c.	Reaction. Hyperimmunised.
649	150 c.c.	—	Died 27/11/04.
650	150 c.c.	50 c.c.	Reaction. Hyperimmunised.
574	150 c.c.	100 c.c.	Reaction. Hyperimmunised.
553	150 c.c.	150 c.c.	Reaction. Hyperimmunised.
648	200 c.c.	—	Died 27/11/04.
568	200 c.c.	50 c.c.	Reaction. Hyperimmunised.
575	200 c.c.	100 c.c.	Died 21/11/04.
654	250 c.c.	—	Reaction. Hyperimmunised.
583	300 c.c.	—	Slight reaction. Hyper-immunised.

Conclusion.—The dose of 50 c.c. serum did not prove to have any preventive action, unless followed by a second injection of 200 c.c. serum.

The dose of 100 c.c. serum had distinct preventive reaction when followed by a second injection of 150 c.c. and more serum.

The dose of 150 c.c. serum had distinct preventive action when followed by a second injection of 50 c.c. and more serum.

The dose of 200 c.c. serum had no decisive reaction.

Doses of 250 and 300 c.c. proved to be sufficient injected in one dose.

Experiment 189.—To test whether the serum of mules 459, 483, 485, 558, 448, and 570 has a hæmolytic effect on mule blood and whether such serum, which has no hæmolytic effect, has preventive properties.

Remark.—Mule 459 was hyperimmunised to the extent of 8,750 c.c.
Mule 483 was hyperimmunised to the extent of 8,500 c.c.
Mule 485 was hyperimmunised to the extent of 10,000 c.c.
Mule 558 was hyperimmunised to the extent of 8,500 c.c.
Mule 448 was hyperimmunised to the extent of 8,000 c.c.
Mule 570 was hyperimmunised to the extent of 9,400 c.c.

HÆMOLYSIS TEST with Serum of Mules 459, 483, 485, 558, 448, and 570.—15/11/04.

Blood Mules	Mule 459	Mule 483	Mule 485	Mule 558	Mule 448	Mule 570
551	slight	slight	slight	slight	slight	slight
553	clear	slight	clear	traces	clear	clear
555	clear	slight	clear	slight	clear	clear
565	clear	clear	clear	clear	clear	clear
566	clear	dist.	slight	dist.	clear	clear
567	slight	dist.	slight	slight	slight	slight
568	clear	slight	clear	traces	clear	clear
569	traces	dist.	clear	dist.	clear	clear
574	clear	clear	clear	clear	clear	clear
575	traces	dist.	clear	slight	traces	clear
576	slight	dist.	clear	dist.	traces	clear
583	clear	clear	clear	clear	clear	clear
584	clear	dist.	traces	dist.	traces	traces
629	slight	dist.	slight	slight	dist.	dist.
630	clear	slight	clear	slight	clear	clear
631	traces	dist.	slight	dist.	traces	clear
632	traces	slight	clear	slight	traces	clear
644	clear	traces	traces	traces	clear	clear
645	slight	slight	slight	traces	traces	traces
646	clear	clear	clear	clear	clear	clear
647	clear	clear	slight	clear	clear	clear
648	clear	clear	clear	clear	clear	clear
649	clear	clear	clear	clear	clear	clear
650	clear	clear	clear	clear	clear	clear
651	clear	clear	clear	clear	clear	clear
652	clear	clear	clear	clear	clear	clear
653	clear	clear	clear	clear	clear	clear
654	clear	clear	clear	traces	clear	clear

Conclusion.—The serum of mule 483 proved, out of 28 cases, to be hæmolytic in 16 cases, serum of mules 558, 485, and 448 in 9 cases and serum of mule 570 in 5 cases.

Experiment 190.—To note what effect mixture 459, 483, 485, and 558 has on a mule whose blood was not hæmolytic in the test.

Mule 569 was injected on 19/11/04 with 100 c.c. serum mixture 483, 485, 558, and 459, and intrajugularly with 1 c.c. virus horse 640. On 22/11/04 it was injected subcutaneously with 200 c.c. serum mixture.

Result.—Reaction. Hyperimmunised.

Experiment 191.—To note effect of serum 448 on a mule injected subcutaneously and intrajugularly virus simultaneously.

Mule 653 was injected subcutaneously on 19/11/04 with 100 c.c. serum 448, and intrajugularly 1 c.c. virus horse 640. On 22/11/04 it was injected subcutaneously with 200 c.c. serum 448.

Result.—Reaction. Died on 26/11/04 of Horse Sickness.

Conclusion.—The serum of one hyperimmunised mule in the initial dose of 100 c.c., followed by 200 c.c. serum, was not sufficient to pass a mule through the horse sickness reaction.

Experiment 192 with serum of horses hyperimmunised with horse blood.

Horse 611 was hyperimmunised to the extent of 4,500 c.c.
Horse 612 was hyperimmunised to the extent of 4,500 c.c.
Horse 627 was hyperimmunised to the extent of 4,500 c.c.

HÆMOLYSIS TEST with Serum Horses 611, 612, and 627, and mixture of these three Sera (Serum B).—29/11/04—1/12/04.

Blood		Horse 611	Horse 612	Horse 627	Mixture (Serum B)
Mules—					
551	...	slight	slight	slight	—
567	...	clear	clear	clear	clear
576	...	slight	traces	slight	clear
587	...	clear	clear	clear	clear
588	...	clear	slight	clear	clear
590	...	clear	traces	clear	clear
591	...	clear	clear	clear	slight
593	...	traces	clear	clear	clear
594	...	clear	clear	clear	traces
595	...	clear	clear	clear	clear
598	...	clear	clear	clear	clear
Horses—					
599	...	clear	clear	slight	clear
600	...	clear	clear	clear	traces
601	...	clear	clear	clear	slight
602	...	clear	slight	clear	clear
604	...	clear	traces	clear	clear
605	...	clear	clear	clear	clear
Mules—					
629	...	clear	clear	clear	clear
645	...	clear	traces	clear	—
659	...	traces	clear	traces	—
660	...	traces	traces	clear	clear
661	...	slight	slight	clear	clear
662	...	clear	clear	clear	clear
663	...	traces	traces	clear	slight
664	...	clear	traces	traces	clear
665	...	traces	clear	clear	clear
666	...	clear	clear	clear	clear
667	...	clear	clear	clear	clear
632	...	clear	clear	clear	clear

Conclusion.—The serum of the horses 611, 612, and 627 proved to be but little hæmolytic.

Remark.—Mules 602 and 662 are selected for experiment.

Experiment 193.—To note hæmolytic and preventive effect of serum horses 611, 612, and 627 on 1 horse and 1 mule and virulent mule serum, intrajugularly and simultaneously.

Horse 602 was injected subcutaneously on 1/12/04 with 300 c.c. mixture of serum horses 611, 612, and 627, and intrajugularly with 5 c.c. virulent serum mule 659.

Result.—Reaction. Slight Dikkop.

Mule 662 was injected subcutaneously with 200 c.c. mixture of serum horses 611, 612, and 627, and intrajugularly with 5 c.c. of virulent mule serum mule 659.

Result.—Reaction. Hyperimmunised.

Remark.—Horse 602 was an Argentine horse. Virulent serum was used to prevent infection with Piroplasma Equi.

Experiment 194.—To note effect of mixture of serum of mules 448, 462, 463, on a horse and a mule simultaneously injected with 5 c.c. virulent mule serum mule 659.

HÆMOLYSIS TEST Serum Mules 448, 462, 463, and 468 (Serum A.)

Blood Mules.	Mule 448	Mule 462	Mule 463	Mule 468	Mixture A.
551	slight	slight	slight	slight	—
567	clear	clear	clear	clear	clear
576	clear	clear	clear	traces	clear
587	clear	clear	clear	clear	clear
588	clear	clear	traces	clear	clear
590	clear	traces	clear	clear	clear
591	—	clear	clear	clear	traces
593	—	traces	traces	clear	clear
594	—	clear	clear	clear	clear
595	—	clear	clear	clear	clear
598	—	clear	clear	clear	clear
Horses—					
599	—	traces	clear	clear	clear
600	—	clear	clear	clear	clear
601	—	clear	clear	clear	clear
602	—	traces	traces	traces	clear
604	—	traces	clear	clear	clear
605	—	clear	clear	clear	clear
Mules—					
629	—	traces	clear	traces	clear
645	—	traces	traces	traces	—
659	—	clear	traces	clear	—
660	—	traces	traces	traces	clear
661	—	clear	clear	traces	clear
662	—	clear	clear	clear	clear
663	—	clear	clear	clear	clear
664	—	clear	clear	clear	clear
665	—	clear	traces	traces	clear
666	—	clear	clear	clear	clear
667	—	clear	clear	clear	clear
632	—	traces	clear	clear	clear

Conclusion.—The serum of mules 462, 463 and 468, which, individually tested, proved to be but little hæmolytic, was less so when mixed.

Horse 599 was injected subcutaneously on the 2/12/04 with 300 c.c. serum mixture of mules 448, 462, 463, and 468, and simultaneously with 5 c.c. virulent serum mule 659. On 6/12/04 it was injected with 200 c.c. serum mixture.

Result.—Died of Horse Sickness on 4/12/04.

Mule 663 was injected subcutaneously on 2/12/04 with 200 c.c. serum mixture and simultaneously with 5 c.c. virulent serum. On 6/12/04 it was injected subcutaneously with 100 c.c. serum mixture.

Result.—Reaction. Hyperimmunised.

Remark—Horse 599 had been inoculated with Equine Piroplasmosis on 10/10/04. It was an imported Argentine horse. Virulent serum was used to prevent infection with Piroplasma Equi.

Conclusion—The serum of the four mules had no effect on the Horse Sickness reaction in the horse, but passed one mule through the reaction

Experiment 195.—With serum mixture 448, 462, 463, and 468, on ten mules injected in quantities of 200 c.c. for first injection ; and 100 c.c. after 5 days for second injection, simultaneously with virus as indicated hereunder :—

	1st Inj. 1/12/04	2nd Inj. 6/12/04	Virus.	Result.
588	200	100	5 c.c. Virulent serum, Mule 659.	Reaction. Dikkop.
590	200	100		Reaction.
593	200	100		Reaction.
595	200	100		Reaction.
Mules—				
567	200	100	1 c.c. Virulent blood, Horse 640.	Reaction. Dikkop. Hyperimmunised.
576	200	100		Reaction. Dikkop. Hyperimmunised.
629	200	100		Reaction. Hyperimmunised.
632	200	100	1c.c. vir. blood	Reaction. Hyperimmunised.
664	200	100		Reaction. Hyperimmunised.
665	200	100	mule 659	Reaction. Hyperimmunised.

Remark.—The Argentine mules were injected with virulent serum in order to prevent a possible infection with Piroplasma Equi. which must always be expected in defibrinated blood.

Conclusion.—The mixture of four mules proved to have strong protective properties.

Experiment 196.—To test the hæmolytic effect of mixture of serum of mules 448, 462, 463, and 468, and horses 611, 612, and 627 (Serum C).

HÆMOLYSIS TEST.—Mixture 448, 462, 463, 611, 612 and 627.

	Mixture.
Mules—	
567	clear
576	clear
587	clear
588	clear
590	clear
591	traces
593	clear
594	clear
595	clear
598	clear

HÆMOLYSIS TEST, ETC.—*Continued.*

	Mixture.
Horses—	
599	clear
600	clear
601	clear
602	clear
604	clear
605	clear
Mules—	
629	clear
632	clear
660	clear
661	clear
662	clear
663	clear
664	traces
665	clear
666	clear
667	clear

Horse 605 (Argentine horse) was injected subcutaneously on 1/12/04 with 300 c.c. serum mixture 448, 462, 463 and 468 (mules); 611, 612 and 627 (horses); and with 5 c.c. virulent serum mule 659. It was again injected on 6/12/04 with 200 c.c. serum.

Result.—Reaction. Dikkop. Died of sequelae.

Mule 660 was first injected on 1/12/04 subcutaneously with 200 c.c. serum mixture of mules 448, 462, 463, and 468, and horses 611, 612, and 627, and 5 c.c. virulent serum mule 659. The second injection was on 6/12/04 with 100 c.c. serum mixture.

Result.—Reaction. Dikkop. Hyperimmunised.

Conclusion.—The mixture of the various sera proved to be non-hæmolytic and passed both animals through the disease. Horse 605 succumbed to a sequela, a diagnose of which could not be made with certainty.

Experiment 197.—With serum of mules 462, 463 and 468, which mules were for the last time hyperimmunised respectively on 13/9/04, 30/0/04 and 14/9/04.

To note whether it still has preventive qualities (Serum A).

HÆMOLYSIS TEST with Mixture of Serum (Serum A).—13/12/04.

Blood.	Mixture.
Mules—	
673	clear
675	clear
682	clear
686	clear

Conclusion.—The mixture proved to be non-hæmolytic for four mules.

Experiment 198.—Mule 673 was injected on 18/12/04 subcutaneously with 200 c.c. serum mixture of mules 462, 463, and 468 and with 1 c.c. virulent blood Horse 658 intrajugularly. The second injection was on 22/12/04 with 100 c.c. serum mixture.

Result.—Reaction. Dikkop. Exposed.

Experiment 199.—Mule 675 was injected subcutaneously on 18/12/04 with 200 c.c. serum mixture and with 5 c.c. virus intrajugularly. The second injection was on 22/12/04 as above.

Result.—Reaction. Dikkop. Symptoms of Biliary Fever. Exposed.

Conclusion.—The mixture C proved to have strong protective powers.

Experiment 200.—To note the effect of the four sera of mules 462, 463, 468, and 448, hyperimmunised to the extent of 8,000 c.c. on 1 and 2/11/04.

HÆMOLYSIS TEST with Mixture of Four Mule Sera.

(448, 462, 463, 468) Mixture B.
13/12/04.

Blood	Mixture B.
Horses—	
290	clear
376	clear
439	clear
441	clear
521	clear
522	clear
523	clear
541	clear
545	clear
546	clear
600	clear
601	clear
604	clear
614	clear
655	clear
656	clear
Mules—	
674	clear
676	clear
678	clear
681	clear
683	clear
684	clear
685	clear
687	clear
688	clear
689	clear
690	clear
691	clear
677	clear
682	clear
686	clear

Conclusion.—The mixture proved to be but little hæmolytic.

Remark.—The animals 681, 682, 686, 687 and 680 were selected for experiments.

Experiment 201.—First injection of 200 c.c. serum mixture 448, 462, 468 and 463 subcutaneously, and with virulent blood horse 658 intrajugularly. Second injection of 100 c.c. serum mixture.

	1st Injection.	2nd Injection.	Result.
Mules—			
681	18/12/04 1 c.c. vir.	22/12/04	Reaction. Dikkop. Hyperimmunised.
682	18/12/04 1 c.c. vir.	22/12/04	Reaction.
686	18/12/04 1 c.c. vir.	22/12/04	Reaction.
687	18/12/04 5 c.c. vir.	22/12/04	Reaction. Exposed.
688	18/12/04 5 c.c. vir.	22/12/04	Died 22/12/04 of Horse Sickness.

Conclusion.—Out of 5 mules injected 1 died. 200 c.c. does not seem to be sufficient as the initial dose.

Experiment 202.—With mixture of mule sera 448, 462, 463, and 468 and horse sera 611, 612 and 627 (Serum C.)

HÆMOLYSIS TEST with Mixture of Mule and Horse Sera, Mules 448, 462, 463, and 468, and Horses 611, 612, and 627 (Mixture C.)

Blood.	Mixture C.
Horses—	
290	slight
376	traces
439	clear
441	traces
521	clear
522	clear
523	clear
541	clear
545	clear
546	clear
600	clear
601	traces
604	slight
614	clear
655	clear
656	clear
Mules—	
674	traces
676	clear
677	clear
678	traces
681	traces
683	clear
684	clear
685	clear
687	clear
688	clear
690	clear
691	traces

Experiment 203.—First injection of 200 c.c. serum mixture of mules 448, 462, 463 and 468, and horses 611, 612 and 627 (Serum C.) subcut-

taneously and simultaneously with virulent blood Horse 658 intrajugularly. Second injection with 100 c.c. serum mixture.

Blood.	1st Inject.	2nd Inject.	Result.
Horses—			
376	18/12/04 1 c.c. vir.	22/12/04	Died on 29/12/04 of Horse Sickness.
439	18/12/04 1 c.c. vir.	22/12/04	Died on 25/12/04 of Horse Sickness.
523	18/12/04 1 c.c. vir.	22/12/04	Reaction. Dikkop.
545	18/12/04 5 c.c. vir.	22/12/04	Reaction. Hyperimmunised.
614	18/12/04 5 c.c. vir.	22/12/04	Reaction. Died on 31/12/04 of Piroplasmosis.
Mules—			
674	18/12/04 1 c.c. vir.	22/12/04	Reaction.
676	18/12/04 1 c.c. vir.	22/12/04	Reaction. Exposed.
677	18/12/04 1 c.c. vir.	22/12/04	Reaction. Died on 1/1/05 of Piroplasmosis.
678	18/12/04 1 c.c. vir.	22/12/04	Reaction. Dikkop. Exposed.
683	18/12/04 1 c.c. vir.	22/12/04	Reaction. Dikkop. Symptoms of Biliary Fever.
684	18/12/04 5 c.c. vir.	22/12/04	Reaction. Died on 31/12/04 of Biliary Fever.
685	18/12/04 5 c.c. vir.	22/12/04	Reaction. Dikkop. Symptoms of Billiary Fever.
689	18/12/04 5 c.c. vir.	22/12/04	Reaction. Exposed.
690	18/12/04 5 c.c. vir.	22/12/04	Reaction.

Remark.—Count of red corpuscles.

Date.	614.	674.	677.	376.	678.
19/12/04	7,112,000	5,784,000	6,200,000	6,627,000	6,008,000
20/12/04	6,552,000	5,200,000	5,720,000	7,000,000	5,848,000
21/12/04	7,164,000	4,780,000	5,760,000	6,862,000	6,048,000
22/12/04	6,852,000	4,096,000	5,230,000	6,140,000	6,392,000
23/12/04	6,640,000	5,560,000	5,560,000	6,380,000	5,927,000
24/12/04	...	5,985,000	...	...	...

Conclusion.—The Mixture C. proved to be but slightly hæmolytic for mule 674 without producing clinical symptoms. Two horses died of horse sickness and three mules contracted biliary fever, probably owing to the fresh serum. (Compare article on Piroplasmosis.)

Experiment 204.—With serum of mules 530, 539, 550, 552, 554, 559, 562, 570 and 573, which were recently hyperimmunised with mule blood with the exception of mule 562, which was hyperimmunised with horse blood (Mixture D.).

HÆMOLYSIS TEST to find a Mule whose Blood is suitable for Hyperimmunisation.

Blood.	Mule 530	Mule 539	Mule 550	Mule 552	Mule 554	Mule 559	Mule 570	Mule 572	Mule 573
Mules—									
551	clear	traces	traces	clear	traces	clear	traces	slight	traces
567	clear	clear	clear	clear	clear	clear	clear	clear	clear
576	clear	slight	clear	clear	clear	clear	clear	traces	dist.
584	clear	clear	clear	traces	clear	clear	clear	clear	slight
587	clear	clear	clear	clear	clear	clear	clear	clear	clear

HÆMOLYSIS TEST, ETC.—*Continued.*

Blood.	Mule 530	Mule 539	Mule 550	Mule 552	Mule 554	Mule 559	Mule 570	Mule 572	Mule 573
588	clear	traces	traces	clear	clear	clear	clear	clear	slight
590	clear	clear	clear	clear	clear	clear	clear	clear	slight
591	clear	clear	clear	clear	clear	clear	clear	clear	dist.
593	traces	clear	clear	clear	clear	clear	clear	traces	clear
594	clear	clear	clear	clear	clear	clear	clear	clear	clear
595	clear	clear	clear	clear	clear	clear	clear	clear	clear
596	clear	clear	clear	clear	clear	clear	clear	clear	traces
629	clear	clear	slight	clear	clear	clear	clear	traces	clear
632	clear	clear	clear	clear	clear	clear	clear	clear	clear
645	clear	clear	clear	clear	clear	clear	clear	clear	clear
659	clear	clear	clear	clear	clear	clear	clear	clear	clear
660	clear	dist.	clear	clear	clear	clear	clear	clear	clear
661	clear	clear	clear	clear	clear	clear	clear	clear	slight
662	clear	traces	clear	clear	clear	clear	clear	clear	dist.
663	clear	traces	clear	clear	clear	clear	clear	clear	traces

Conclusion.—Mule 573 proved to be hæmolytic.

HYPERIMMUNISATION OF MULES.

Mule 530 was hyperimmunised to the extent of 5,950 c.c.
Mule 539 was hyperimmunised to the extent of 5,350 c.c.
Mule 550 was hyperimmunised to the extent of 5,850 c.c.
Mule 552 was hyperimmunised to the extent of 4,900 c.c.
Mule 554 was hyperimmunised to the extent of 7,250 c.c.
Mule 559 was hyperimmunised to the extent of 6,000 c.c.
Mule 570 was hyperimmunised to the extent of 5,300 c.c.
Mule 573 was hyperimmunised to the extent of 5,750 c.c.
Mule 562 was hyperimmunised to the extent of 3,500 c.c.

HÆMOLYSIS TEST with Serum of Mules 530, 539, 550, 552, 554, 559, 562, 570 and 573.

Mixture	Mule	Mule 530	Mule 539	Mule 550	Mule 552	Mule 554	Mule 559	Mule 562	Mule 570	Mule 573
clear	661	clear	clear	dist.	clear	clear	clear	clear	clear	clear
clear	667	clear	clear	clear	clear	clear	clear	clear	clear	clear
clear	669	clear	clear	dist.	clear	clear	clear	clear	clear	clear
clear	670	—	clear	clear	clear	clear	clear	clear	clear	clear
clear	671	clear	clear	dist.	clear	clear	clear	clear	clear	clear
clear	672	clear	clear	dist.	clear	clear	clear	clear	clear	clear
clear	679	clear	clear	dist.	clear	clear	clear	clear	clear	clear
clear	680	clear	clear	clear	clear	clear	clear	clear	clear	clear

Conclusion.—Mule 550 proved to be hæmolytic.

Note.—Serum of mule 550 was included into the mixture for inoculation because the mixture of the various sera did not prove hæmolytic. Accordingly mule 661 was selected, on whose blood it had a distinct hæmolytic effect, in order to see whether, in vivo, such mixture produces hæmolysis.

Experiment 205.—With mixture of various mule sera, mules 530, 539, 552, 554, 559, 562, 570 and 573, together with one hæmolytic serum mule 550 (Serum D.).

First injection subcutaneously with 200 c.c. serum mixture and virulent horse blood intrajugularly (horse 658). Second injection of 100 c.c. serum mixture.

Mules—	1st Injection.	2nd Injection.	Result.
661	18/12/04 1 c.c. vir.	22/12/04.	Reaction. Dikkop.
669	18/12/04 5 c.c. vir.	22/12/04.	Died on 27/12/04 of horse sickness.

Remark.—Count of red corpuscles.

Date.	Mule 661.	Mule 669.
19/12/04 ...	6,127,000 ...	4,956,000
20/12/04 ...	5,968,000 ...	6,128,000
21/12/04 ...	6,804,000 ...	5,484,000
22/12/04 ...	6,392,000 ...	5,068,000
23/12/04 ...	5,952,000 ...	5,848,000

Conclusion.—There was a slight hæmolytic effect in vivo noticeable on the count of the corpuscles.

Experiment 206.—With serum mixture of mules 530, 539, 552, 554, 559, 562, 570 and 573.

First injection with 200 c.c. serum mixture and virus intrajugularly (horse 658). Second injection with 100 c.c. serum subcutaneously.

Mules—	1st Injection.	2nd Injection.	Result.
667	18/12/04. 1 c.c. vir.	22/12/04.	Reaction. Dikkop.
672	18/12/04. 1 c.c. vir.	22/12/04.	Reaction. Dikkop.
679	18/12/04. 5 c.c. vir.	22/12/04.	Reaction.
680	18/12/04. 5 c.c. vir.	22/12/04.	Died on 26/12/04.

Conclusion.—The mixture of the various mule sera did not pass every animal through the disease.

HÆMOLYSIS TEST with Mixtures of various sera of Mules and Horses. —29/12/04.

(1.) 462, 463, 468, 448, 530, 539, 552, 554, 559, 570, 573.
(2.) 459, 483, 485, 558.
(3.) First and second mixtures together.
(4.) 473, 615.
(5.) 474, 615, 611, 612, 627.
(6.) First, second, third, fourth and fifth together.

Remark.—(1) Mule 462 was hyperimmunised to the extent of 12,000 c.c. blood of mules. Last injection 13/9/04.

Mule 463 was hyperimmunised to the extent of 12,000 c.c. blood of mules. Last injection 30/9/04.

Mule 468 was hyperimmunised to the extent of 8,500 c.c. blood of mules. Last injection 14/9/04.

Mule 448 was hyperimmunised to the extent of 8,000 c.c. blood of mules. Last injection 2/11/04.

Mule 530 was hyperimmunised to the extent of 5,950 c.c. blood of mules. Last injection 3/12/04.

Mule 539 was hyperimmunised to the extent of 5,350 c.c. blood of mules. Last injection 4/12/04.

Mule 552 was hyperimmunised to the extent of 4,900 c.c. blood of mules. Last injection 3/12/04.

Mule 554 was hyperimmunised to the extent of 7,250 c.c. blood of mules. Last injection 3/12/04.

Mule 559 was hyperimmunised to the extent of 6,000 c.c. blood of mules. Last injection 3/12/04.

Mule 570 was hyperimmunised to the extent of 9,400 c.c. blood of mules. Last injection 3/12/04.

Mule 573 was hyperimmunised to the extent of 5750 c.c. blood of mules. Last injection 13/12/04.

(2) Mule 459 was hyperimmunised to the extent of 9,750 c.c. blood of mules. Last injection 21/10/04.

Mule 483 was hyperimmunised to the extent of 8,500 c.c. blood of mules. Last injection 21/10/04.

Mule 485 was hyperimmunised to the extent of 10,000 c.c. blood of mules. Last injection 21/10/04.

Mule 558 was hyperimmunised to the extent of 8,500 c.c. blood of mules. Last injection 21/10/04.

(4) Horse 473 was hyperimmunised with horse blood to the extent of 6,000 c.c. Last injection 13/12/04.

Horse 615 was hyperimmunised with horse blood to the extent of 5,000 c.c. Last injection 13/12/04.

(5) Horse 611 was hyperimmunised with horse blood to the extent of 4,500 c.c. Last injection 17/11/04.

Horse 612 was hyperimmunised with horse blood to the extent of 4,500 c.c. Last injection 17/11/04.

Horse 627 was hyperimmunised with horse blood to the extent of 4,000 c.c. Last injection 17/11/04.

	Mixt. 1.	Mixt. 2.	Mixt. 3.	Mixt. 4.	Mixt. 5.	Mixt. 6.
Mules—						
596 ...	traces	dist.	traces	slight	clear	slight
592 ...	slight	dist.	slight	slight	slight	traces
594 ...	clear	traces	clear	clear	traces	clear
587 ...	clear	dist.	traces	traces	traces	clear
551 ...	clear	dist.	slight	slight	clear	clear
584 ...	clear	traces	traces	clear	traces	clear
691 ...	traces	slight	slight	traces	slight	traces
696 ...	clear	slight	slight	slight	clear	traces
695 ...	clear	dist.	slight	slight	slight	traces
692 ...	clear	slight	traces	slight	clear	traces
Horses—						
655 ...	clear	clear	clear	clear	clear	clear
656 ...	clear	dist.	traces	slight	traces	clear
597 ...	clear	dist.	traces	traces	clear	traces
600 ...	clear	slight	clear	slight	clear	slight
601 ...	clear	dist.	traces	slight	traces	clear
604 ...	clear	slight	slight	slight	clear	clear
521 ...	clear	clear	clear	clear	clear	clear
522 ...	clear	clear	clear	clear	clear	clear
441 ...	traces	traces	traces	clear	clear	clear

Conclusion.—Mixture 2 proved to be strongly hæmolytic.

Experiment 207.—The hæmolysis test having shown that the mixtures 2 and 6 were hæmolytic, the following mixtures of non-hæmolytic sera were utilized for the experiment:—

Serum F:—Mules 462, 463, 468, 448, 530, 539, 552, 554, 559, 570, 573.

Serum G:—Horses 611, 615, 627.

Serum H:—Serum F and G.

Experiment 208.—With serum mixture F injected subcutaneously in two injections: horses 300 c.c. and mules 200 c.c. for the first injection and 200 c.c. and 100 c.c. respectively for the second injection. In case of horses a third injection with 50 c.c. serum intrajugularly diluted with 50 c.c. aq. phy.

	1st Injection	2nd Injection	3rd Injection	Result.
Horse—624	30/12/04	3/1/05	5/1/05	Distinct reaction. Dikkop. Hyperimmunised.
Mule—592	30/12/04	3/1/05	—	Distinct reaction.

Experiment 209.—With Serum G.

	1st Injection	2nd Injection	3rd Injection	Result.
Horse—597	30/12/04	3/1/05	5/1/05	Distinct reaction. Dikkop. Died on 23/1/05 from piroplasmosis.
Mule—596	30/12/04	3/1/05	—	Reaction. Dikkop.

Experiment 210.—With Serum H.

	1st Injection	2nd Injection	3rd Injection	Result.
Horses—				
656	30/12/04	3/1/05	5/1/05	Died on 5/1/05 from horse sickness.
441	30/12/04	3/1/05	5/1/05	Reaction. Dikkop complicated with piroplasmosis. Died on 10/2/05 from piroplasmosis.
Mules—				
594	30/12/04	3/1/05	...	Reaction and piroplasmosis.
587	30/12/04	3/1/05	...	Died from Dikkop.

Note.—For complication with piroplasmosis compare article on this subject.

Conclusion.—The mixture of sera non-hæmolytic in vitro proved to be non-hæmolytic in vivo. Piroplasmosis complicated the reaction.

HÆMOLYSIS TEST.—8/1/05.

Serum I.—448, 462, 463, 468, 530, 539, 552, 554, 559, 570, 573.
Serum K.—485, 459.
Serum L.—611, 615, 627.
Serum M.—Serum I, K, and L.

Mules—	Mixture I.	Mixture K.	Mixture L.	Mixture M.
551	traces	slight	slight	traces
584	clear	clear	clear	clear
591	traces	clear	slight	clear
692	traces	traces	clear	clear
691	clear	clear	clear	clear
695	traces	clear	clear	clear
698	clear	traces	clear	clear
702	clear	clear	clear	clear
699	traces	traces	clear	clear
700	traces	clear	clear	clear

Conclusion.—Note the slight hæmolytic effect of some of the mixtures.

Experiment 211.—With serum mixture M. First injection to be 250 c.c. subcutaneously, and the second 50 c.c. intrajugularly, diluted with

50 c.c. aq. phys. For virus to be used blood of horse 694 for one half and blood of mule 666 for the other half.

Mules—	1st Injection	2nd Injection	Result.
		Virus Horse 694.	
584 ...	8/1/05	12/1/05	Reaction.
551 ...	8/1/05	12/1/05	Reaction.
702 ...	8/1/05	12/1/05	Died on 16/1/05, pulmonary and gastric form.
591 ...	8/1/05	12/1/05	Reaction.
695 ...	8/1/05	12/1/05	Reaction.
		Virus Mule 666.	
692 ...	8/1/05	12/1/05	Reaction.
691 ...	8/1/05	12/1/05	Reaction.
698 ...	8/1/05	12/1/05	Reaction.
699 ...	8/1/05	12/1/05	Reaction.
700 ...	8/1/05	12/1/05	Reaction.

Conclusion.—Out of 10 mules injected one died of horse sickness.

HÆMOLYSIS TEST with Serum Mixture M. (Compare 8/1/05.)—25/1/05.

Mules—	Serum Mixture.	Mules.	Serum Mixture.	Mules.	Serum Mixture.	Mules.	Serum Mixture.
755	clear ...	756	clear ...	757	clear ...	758	clear
759	clear ...	760	clear ...	761	clear ...	762	clear
763	clear ...	764	clear ...	765	clear ...	766	clear
767	clear ...	768	clear ...	769	clear ...	770	clear
771	clear ...	772	clear ...	773	clear ...	774	clear
775	clear ...	776	clear ...	777	clear ...	778	clear
779	clear ...	780	clear ...	781	clear ...	782	traces
783	clear ...	784	clear ...	785	clear ...	786	traces
787	clear ...	788	clear ...	789	clear ...	790	clear
791	traces ...	792	clear ...	793	clear ...	794	clear
795	clear ...	796	clear ...	797	clear ...	798	clear
799	clear ...	800	clear ...	801	traces ...	802	clear
803	traces ...	804	traces				

Conclusion.—Mixture M proved to be but little hæmolytic.

Experiment 212.—With serum mixture M. Injected subcutaneously in quantities of 250 c.c. for the first injection, and with 100 c.c. for the second injection. Virus horse 726 (Argentine horse). Date of first injection 26/1/05 and second injection 30/1/05.

Mules—	Result.
755 ...	Distinct reaction.
756 ...	Reaction. Dikkop.
757 ...	Distinct reaction.
758 ...	Died on 5/2/05.
759 ...	Reaction.
760 ...	Reaction.
761 ...	Distinct reaction.
762 ...	Died of Dikkop on 3/2/05.
763 ...	Distinct reaction.
764 ...	Died of Dikkop on 5/2/05.
765 ...	Distinct reaction.
766 ...	Distinct reaction.
767 ...	Died of Horse Sickness on 2/2/05.

Experiment 212.—Continued.

Mules—

	Result.
768 ...	Reaction.
769 ...	Died of Dikkop on 5/2/05.
770 ...	Reaction.
771 ...	Reaction.
772 ...	Reaction. Piroplasma Equi noted on 8/2/05.
773 ...	Reaction.
774 ...	Distinct reaction.
775 ...	Reaction.
776 ...	Reaction.
777 ...	Died of Dikkop on 5/2/05.
778 ...	Distinct reaction.
779 ...	Distinct reaction.
780 ...	Reaction.
781 ...	Reaction. Dikkop.
782 ...	Reaction.
783 ...	Died on 2/2/05.
784 ...	Distinct reaction.
785 ...	Reaction.
786 ...	Reaction.
787 ...	Reaction. Died of piroplasmosis on 8/2/05.
788 ...	Distinct reaction.
789 ...	Distinct reaction.
790 ...	Reaction.
791 ...	Reaction.
792 ...	Reaction.
793 ...	Reaction.
794 ...	Reaction.
795 ...	Reaction.
796 ...	Distinct reaction.
797 ...	Reaction.
798 ...	Reaction.
799 ...	Reaction.
800 ...	Reaction.
801 ...	Reaction.
802 ...	Reaction.
803 ...	Reaction. Piroplasmosis 8/2/05.
804 ...	Reaction.

Conclusion.—Mule 758 died on 10th day after inoculation.
Mule 762 died on 8th day after inoculation.
Mule 764 died on 10th day after inoculation.
Mule 767 died on 7th day after inoculation.
Mule 769 died on 10th day after inoculation.
Mule 777 died on 10th day after inoculation.
Mule 783 died on 8th day after inoculation.
Out of 50 mules injected 7 died from Horse Sickness.

HÆMOLYSIS TEST with Mixture N of serum mules 651, 566, 650, 572, and 567, and serum mule 567.

Mule—	Mixture N.	Mule 567.
696	clear	traces
Horses—		
735	clear	clear

HÆMODYSIS TEST, ETC.—*Continued.*

Horses—

	Mxture N.	Mule 567.
750	clear	clear
752	clear	slight
746	clear	clear
749	clear	clear
754	clear	clear
748	clear	clear
751	traces	traces
731	traces	slight
729	clear	clear
730	clear	clear
734	clear	slight

Conclusion.—The serum of mule 567 proved to be hæmolytic. The mixture was less so.

Experiment 213.—To note the hæmolytic effect of serum mixture N. and serum mule 567, which in vitro proved to cause slight hæmolysis.

Note that mule 696 was salted. It was injected with serum alone to prove hæmolytic effect.

Horses—	1st Injection.	2nd Injection.	Result.
732 ...	4/2/05 ...	8/2/05 ...	Died on 12/2/05.
730 ...	4/2/05 ...	8/2/05 ...	Died on 15/2/05.
Mule—			
696 ...	4/2/05 ...	8/2/05.	

URINE TEST.

Date.	Horse 732	Horse 730	Mule 696
7/2/05	clear	clear	clear
8/2/05	clear	clear	clear
9/2/05	clear	clear	clear
10/2/05	clear	clear	clear
11/2/05	clear	clear	clear
12/2/05	clear	clear	Albuminous precipitates
13/2/05	—	Alb. prec.	Albuminous precipitates
14/2/05	—	No prec.	Albuminous precipitates
15/2/05	clear	clear	clear

Conclusion.—The hæmolytic serum mixture in vitro proved to have a slight hæmolytic effect in vivo noticeable by the albumin reaction in the urine. It had no preventive qualities.

HÆMOLYSIS TEST with Serum of Horses.

Horses 611, 672, and 165 hyperimmunised for the second time.
Horse 611 hyperimmunised on 6·7/2/05 with 4,500 c.c. blood horse 712.
Horse 612 hyperimmunised on 6·7/2/05 with 4,500 c.c blood horse 712.
Horse 615 hyperimmunised on 6·7/2/05 with 3,000 c.c. blood horse 753.
Horse 545 hyperimmunised on 6·7/2/05 with 7,000 c.c. blood horse 712.
Horse 545 hyperimmunised on 9/2/05 with 1,500 c.c. blood horse 747.
Horse 624 hyperimmunised on 6·7/2/05 with 4,500 c.c. blood horse 753.
Horse 624 hyperimmunised on 8/2/05 with 3,500 c.c. blood horse 747.
Horse 398 hyperimmunised on 28-29-30/12/05 with 4,000 c.c. blood mule 666.

HÆMOLYSIS TEST, ETC.—*Continued.*

Mules—	Horse 611	Horse 612	Horse 615	Horse 624	Horse 545	Horse 398	Serum X —
834	clear	clear	clear	clear	clear	clear	clear
835	slight	clear	slight	traces	clear	traces	slight
836	clear	clear	clear	clear	clear	clear	clear
837	clear	clear	clear	clear	clear	clear	clear
838	clear	clear	slight	traces	clear	clear	clear
839	clear	clear	clear	clear	clear	clear	clear
840	clear	clear	clear	clear	clear	clear	clear
841	clear	clear	clear	clear	clear	clear	clear
842	clear	clear	clear	clear	clear	clear	clear
843	clear	clear	traces	clear	clear	clear	clear
Horses—							
872	clear	clear	clear	clear	clear	clear	clear
873	clear	clear	clear	clear	clear	clear	clear
875	clear	clear	clear	clear	clear	clear	clear
877	clear	clear	clear	clear	clear	clear	clear
878	clear	clear	clear	clear	clear	clear	clear
864	clear	clear	clear	clear	clear	clear	clear
865	clear	clear	clear	clear	clear	clear	clear
867	clear	clear	clear	clear	clear	clear	clear
868	clear	clear	clear	clear	clear	clear	clear
874	clear	clear	clear	clear	clear	clear	clear

Conclusion.—The serum of the hyperimmunised horses had but a slight hæmolytic effect on mule and horse blood.

Experiment 214.—With serum which in vitro proved to be non-haemolytic, and of mules which for more than three months had not been hyperimmunised.

Mixture S.—Serum of mules 448, 462, 463, 468, 459, and 485.

Simultaneous injection of 300 c.c. serum S and 2 c.c. preserved virus horse 726.

Mules—		1st injection.	2nd injection.	Result.
813	...	28/2/05	4/3/05	Reaction.
814	...	28/2/05	4/3/05	Reaction.
815	...	28/2/05	5/3/05	Died on 10/3/05 from horse sickness.
816	...	28/2/05	5/3/05	Reaction.
817	...	28/2/05	5/3/05	Reaction.

Conclusion.—The mixture of serum of mules hyperimmunised more than three months ago passed four mules out of five through the reaction.

Experiment 215.—With serum of mules not hæmolytic, and recently hyperimmunised with mule blood.

Serum mixture TW.—Mules 570, 530, 554, 559, 583, 565, 568, 574, 654, 578, 589, 576, 567, 660, 662, 663.

Simultaneous injection of 300 c.c. serum TW subcutaneously and 2 c.c. virus horse 726 intrajugularly.

	1st Injection.	2nd Injection.	Result.
Mules—			
818	28/2/05	4/3/05	Reaction.
819	28/2/05	4/3/05	Died on 14/3/05 of piroplasmosis.
820	28/2/05	5/3/05	Died on 10/3/05 of horse sickness.
821	28/2/05	4/3/05	Reaction.
822	28/2/05	4/3/05	Reaction.

Conclusion.—Mule 819 developed piroplasmosis and died. The serum mixture passed four mules out of five through the reaction. One mule contracted piroplasmosis.

Experiment 216.—With serum mixture TUW.

Simultaneous injection of 300 c.c. serum mixture and 2 c.c. preserved virus, horse 726.

	1st Injection.	2nd Injection.	Result.
Mules—			
828	28/2/05	5/3/05	Reaction.
829	28/2/05	5/3/05	Reaction.
830	28/2/05	4/3/05	Died on 17/3/05 of piroplasmosis.
831	28/2/05	5/3/05	Reaction.
833	28/2/05	5/3/05	Reaction. Piroplasma present.

Conclusion.—All the mules passed through the reaction of horse sickness. One mule contracted piroplasmosis.

Experiment 217.—With serum mixture X. (Compare Hæmolysis test Serum X.)

Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

	1st Injection.	2nd Injection.	Result.
Mules—			
834	29/2/05	5/3/05	Reaction. Piroplasmosis.
835	28/2/05	5/3/05	Reaction.
836	28/2/05	5/3/05	Reaction.
837	28/2/05	5/3/05	Reaction.
838	28/2/05	5/3/05	Reaction.

Conclusion. All mules passed through horse sickness reaction.

Experiment 218.—With serum mixture V which in vitro proved to be slightly hæmolytic.

Serum of mules 651, 566, 650, 572, 571. (Compare hæmolysis test 3/2/05.)

Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

	1st Injection.	2nd Injection.	Result.
Mules—			
832	28/2/05	5/3/05	Reaction.
853	28/2/05	5/3/05	Reaction. Piroplasmosis.

Conclusion.—The serum mixture which had a hæmolytic effect in vitro had none in vivo. One mule contracted piroplasmosis.

Experiment 219.—With serum mixture Y, consisting of Serum Mixture X and serum mixture U, not hæmolytic.

Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

Mules—	1st Injection.	2nd Injection.	Result.
839	28/2/05	5/3/05	Reaction.
840	28/2/05	4/3/05	Reaction.
841	28/2/05	5/3/05	Reaction.
842	28/2/05	5/3/04	Reaction.
843	28/2/05	5/3/05	Reaction.

Conclusion.—All the mules passed through horse sickness reaction.

Experiment 220.—With serum mixture AA, consisting of serum mixtures X, Y, and of mules 663, 662, 660, 576, 567, 570, 569, 579, 578 and 572, not hæmolytic.

Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

Mules—	1st Injection.	2nd Injection.	Result.
844	28/2/05	4/3/05	Reaction.
845	28/2/05	5/3/05	Reaction.
846	28/2/05	5/3/05	Reaction.
847	28/2/05	5/3/05	Reaction.
848	28/2/05	5/3/05	Reaction.

Conclusion.—All the mules passed through horse sickness reaction.

Experiment 221.—With serum mixture AB, consisting of serum mixture X, Y, and of Mules 660, 662, 663 and 699, not hæmolytic, and of mules 573, 539 and 552, supposed to be hæmolytic.

Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

Mules—	1st Injection.	2nd Injection.	Result.	
849	28/2/05	4/3/05	Reaction.	
850	28/2/05	4/3/05	Reaction.	
851	28/2/05	5/3/05	Reaction.	
852	28/2/05	5/3/05	Reaction.	Piroplasmosis.
854	28/2/05	5/3/05	Reaction.	

Conclusion.—All the mules passed through horse sickness reaction. One mule contracted piroplasmosis.

Experiment 222.—With serum mixture AC which in vitro showed distinct hæmolysis. Serum of mules 573, 539 and 552 recently hyperimmunised for a second time.

Mule 573 was hyperimmunised on 11/2/05 with 4,750 c.c. blood of mule 705.

Mule 552 was hyperimmunised on 10·11/2/05 with 5,250 c.c. blood of mule 705.

Mule 539 was hyperimmunised on 10·11-12/2 05 with 7,000 c.c. blood of mule 705.

HÆMOLYSIS TEST.—29/2/05.

Mules—	Mule 573	Mule 539	Mule 552
844	clear	clear	clear
845	clear	clear	clear
846	clear	clear	clear
847	slight	clear	traces

HÆMOLYSIS TEST, ETC.—*Continued.*

Horses—	Mule 573	Mule 539	Mule 552
848	traces	clear	clear
855	traces	traces	clear
856	traces	clear	traces
857	dist.	dist.	traces
858	clear	clear	clear
859	traces	slight	clear
866	clear	clear	clear
869	clear	clear	clear
870	slight	dist.	slight
876	clear	clear	clear
731	dist.	clear	traces

Experiment 223.—Simultaneous injection of 300 c.c. serum mixture subcutaneously, and 2 c.c. preserved virus horse 726 intrajugularly.

Mules—	1st Injection.	2nd Injection.	Result.
855	28/2/05	4/3/05	Died on 13/3/05 of Hæmolysis
858	28/2/05	4/3/05	Reaction.
859	28/2/05	4/3/05	Died on 14/3/05 of Hæmolysis

Conclusion.—The serum mixture proved to be hæmolytic for two mules.

Experiment 224.—With serum mixture A D of mules 462, 463 and 468, dated 12/10/04 and mule 448, dated 19/11/04.

Simultaneous injection of 300 c.c. serum mixture, and 2 c.c. preserved virus horse 726 intrajugularly.

Mules—	1st Injection.	2nd Injection.	Result.
860	28/2/05	4/3/05	Reaction.
861	28/2/05	4/3/05	Reaction.
862	28/2/05	5/3/05	Reaction.

Conclusion.—Old serum proved to have conserved its protective properties.

Experiment 225.—With serum mixtures A and A G. A G: Serum of mules 448, 485, 530, 554, 570 and 663.

Simultaneous injection of 300 c.c. serum mixture and 2 c.c. preserved virus.

Mules—	1st Injection.	2nd Injection.	Result.
		Virus Horse 726.	
856	20/3/05	24/3/05	Reaction.
857	20/3/05	24/3/05	Reaction.
883	20/3/05	24/3/05	Reaction.
884	20/3/05	24/3/05	Reaction.
885	20/3/05	24/3/05	Reaction. Dikkop.
886	20/3/05	24/3/05	Reaction.
887	20/3/05	24/3/05	Reaction.
		Virus Horse 727.	
888	20/3/05	24/3/05	Reaction.
889	20/3/05	24/3/05	Reaction.
890	20/3/05	—	Died on 1/4/05 of horse sickness.

Conclusion.—No hæmolytic effect. The animal which had only the first injection of serum died of horse sickness.

Experiment 226.—With serum mixture A G.

Simultaneous injection of 300 c.c. serum mixture and 2 c.c. preserved virus.

	1st Injection.	2nd Injection.	Result.
Mules—			
891	20/3/05	24/3/05	Reaction.
898	20/3/05	24/3/05	Reaction.
894	20/3/05	24/3/05	Dikkop.
895	20/3/05	24/3/05	Reaction.
896	20/3/05	24/3/05	Reaction.
896	20/3/05	24/3/05	Reaction.
897	20/3/05	24/3/05	Reaction.
898	20/3/05	24/3/05	Reaction.
		Virus Horse 727.	
899	20/3/05	24/3/05	Reaction.
900	20/3/05	24/3/05	Died on 31/3/05 of horse sickness.
		Virus Horse 726.	
901	20/3/05	24/3/05	Reaction.

Conclusion.—One animal died of horse sickness.

Experiment 227.—With serum mixture A G.

Simultaneous injection of 300 c.c. serum mixture and 2 c.c. preserved virus.

	1st Injection.	2nd Injection.	Result.
Mules—			
		Virus Horse 726.	
902	20/3/05	24/3/05	Reaction.
903	20/3/05	24/3/05	Reaction.
904	20/3/05	24/3/05	Reaction.
905	20/3/05	24/3/05	Dikkop.
906	20/3/05	24/3/05	Reaction.
907	20/3/05	24/3/05	Reaction.
908	20/3/05	24/3/05	Reaction.
		Virus Horse 727.	
909	20/3/05	24/3/05	Reaction.
910	20/3/05	24/3/05	Died on 28/3/05 of horse sickness.
911	20/3/05	—	Reaction. Dikkop.

Conclusion.—One out of 10 animals died of horse sickness.

Experiment 228.—With serum mixture A H.

Serum A H.:—462, 463, 468, 651, 566, 650, 660, 662, 665, 572, 654, 574, 578, 579, 589, 567, 576 and 699.

	1st Injection.	2nd Injection.	Result.
Mules—			
		Virus Horse 726.	
912	20/3/05	24/3/05	Reaction.
913	20/3/05	24/3/05	Reaction.
914	20/3/05	24/3/05	Reaction.
915	20/3/05	24/3/05	Reaction.
916	20/3/05	24/3/05	Reaction.
917	20/3/05	24/3/05	Dikkop.
918	20/3/05	24/3/05	Reaction.

Experiment 228.—Continued.

	1st Injection.	2nd Injection.	Result.
		Virus Horse 727.	
919	20/3/05	24/3/05	Reaction.
920	20/3/05	24/3/05	Reaction.
921	20/3/05	—	Dikkop. Few Piroplasma Equi.

Conclusion.—None of the injected animals died.

Experiment 229.—With serum mixture A H.

	1st Injection.	2nd Injection.	Result.
Mules—			
		Virus Horse 726.	
922	20/3/05	24/3/05	Reaction.
923	20/3/05	24/3/05	Reaction.
924	20/3/05	24/3/05	Reaction.
925	20/3/05	24/3/05	Dikkop.
926	20/3/05	24/3/05	Dikkop.
927	20/3/05	24/3/05	Reaction.
928	20/3/05	24/3/05	Reaction.
		Virus Horse 727.	
929	20/3/05	24/3/05	Reaction.
930	20/3/05	24/3/05	Reaction.
931	20/3/05	—	Reaction.

Conclusion.—None of the injected animals died of horse sickness.

Experiment 230.—With serum mixture A H.

Virus Horse 726.

	1st Injection.	2nd Injection.	Result.
Mules—			
932	20/3/05	24/3/05	Reaction.
933	20/3/05	24/3/05	Reaction.
934	20/3/05	24/3/05	Dikkop.
935	20/3/05	24/3/05	Reaction.
936	20/3/05	24/3/05	Dikkop.
937	20/3/05	24/3/05	Reaction
938	20/3/05	24/3/05	Reaction.
		Virus Horse 727.	
939	20/3/05	24/3/05	Reaction.
940	20/3/05	24/3/05	Reaction.
941	20/3/05	24/3/05	Reaction.

Conclusion.—None of the injected animals died.

Experiment 231.—With serum mixture A.G. Serum mixture A.G.: horses 611, 612, 615, 545, 624, and 398, and mules 448, 485, 530, 563, 589, 572, 654, 578, 567, 574, 576, 568, 650, 579, 662, 566, 462, 660, 463, 554, 570, 699, 468, and 651, and 2 c.c. preserved virus of horse 726 intrajugularly.

Two injections: first on 11/4/05 with 350 c.c. serum mixture, and second injection on 15/4/05 with 100 c.c. serum mixture.

		Results.
Mules—		
942	...	Reaction.
945	...	Reaction.
946	...	Reaction.
947	...	Reaction.

Experiment 231.—Continued.

		Results.
948	...	Reaction.
949	...	Reaction.
950	...	Reaction.
951	...	Reaction.
952	...	Reaction.
953	...	Reaction. Dikkop.
954	...	Died on 23/4/05 of traumatic rupture of spleen.
955	...	Reaction.
956	...	Reaction. Dikkop.
958	...	Reaction.
959	...	Reaction.
960	...	Reaction.
961	...	Died on 22/4/05, of horse sickness. Dikkop.
962	...	Reaction.
963	...	Reaction.
964	...	Reaction.
965	...	Reaction.
967	...	Reaction.
968	...	Reaction.
969	...	Reaction.
971	...	Reaction. Dikkop.
972	...	Reaction.
973	...	Reaction. Dikkop.
974	...	Reaction.
975	...	Reaction.
976	...	Reaction.
977	...	Reaction.
978	...	Reaction.
979	...	Dikkop.
980	...	Dikkop.
981	...	No reaction.
982	...	Reaction.
983	...	Reaction.
984	...	Reaction.
985	...	Reaction.
986	...	Reaction.
987	...	Reaction.
988	...	Reaction. Dikkop.
989	...	Reaction.
990	...	Reaction.
991	...	Reaction. Dikkop.
992	...	Reaction.
994	...	Reaction.
995	...	Reaction.
996	...	Reaction.
998	...	Reaction.
999	...	Reaction.
1000	...	Rectaion.
A1	...	Reaction.
A2	...	Reaction.
A3	...	Reaction.
A4	...	Reaction
A5	...	Reaction. Dikkop.

Experiment 231.—Continued.

		Results.
A6	...	Reaction.
A7	...	Impossible to take record.
A8	...	Reaction. Dikkop.
A9	...	Died on 21/4/05. Rupture of Aneurisma of Mesentric Artery.
A10	...	Reaction. Dikkop.
A11	...	Reaction.
A12	...	Reaction. Dikkop.
A13	...	Reaction.
A14	...	Reaction.
A15	...	Reaction. Dikkop.
A16	...	Reaction.
A17	...	Reaction.
A18	...	Reaction.
A19	...	Reaction.
A20	...	Reaction. Dikkop.
A21	...	Reaction.
A22	...	Reaction.
A23	...	Reaction.
A24	...	Reaction.
A25	...	Reaction.
A26	...	Reaction.
A27	...	Reaction.
A28	...	Reaction. Dikkop.
A29	...	Reaction.
A30	...	Reaction.
A31	...	Reaction.
A32	...	Died on 22/4/05 of Dikkop.
A33	...	Reaction. Dikkop. Died 15/4/05.
A34	...	Reaction.
A35	...	Reaction.
A36	...	Reaction.
A37	...	Reaction.
A38	...	Reaction.
A39	...	Reaction.
A40	...	Reaction.
A41	...	Reaction.
A42	...	Reaction.
A43	...	Reaction.
A44	...	Reaction. Dikkop.
A45	...	Reaction.
A46	...	Reaction.
A47	...	Reaction.

Conclusion.—Out of 100 mules inoculated 3 died from horse sickness.

REPORT OF THE CONSERVATOR OF FORESTS.

Division of Forestry,
25th August, 1905.

TO THE DIRECTOR OF AGRICULTURE.

Sir,

I have the honour to forward herewith my report on the work of this Division for the year ending June 30th, 1905.

During the period referred to, substantial progress has been made at each of the Nurseries and Plantations that were started last year.

The examination and subsequent Reservation of most of the Indigenous High Forest belonging to the Government has been completed.

The Scrub Forests of the Bushveld and Low Country have not so far been closely investigated, but in August of last year, Mr. D. E. Hutchins, Conservator of Forests, Capetown, to complete his Forest Report on the Transvaal, spent some days in the Low Country in the neighbourhood of Barberton and Komati Poort. As a result, he submitted a most helpful and interesting note on the method of dealing with these Forests, which, on account of its general interest, I have put in as *Appendix A* to this Report.

It may be as well to enumerate here the different centres at which work has been going on during the year, and then proceed to give particulars of what has been done at each. They are as follows :—

(1)—Government Nursery, Irene.
(2)—Government Nursery and Plantation, Lichtenburg.
(3)— „ „ „ Ermelo.
(4)— „ „ „ Gemsbokfontein, Pan.
(5)— „ „ „ Potchefstroom.
(6)— „ „ „ Belfast.
(7)— „ Plantation, Groenkloof.
(8)—The Forest Station at Woodbush, near Haenertsburg.
(9)— „ „ Barberton.

Government Nursery, Irene.—It will be remembered, operations were started at this Nursery in October, 1903, on the closing down of Skinner's Court, Pretoria, which was taken over by the Botanical Division. During the following July and August, the trees remaining unsold at Skinner's Court were transferred to Irene. They consisted chiefly of Oaks, Planes, Robinias and Box Elders, and were planted out in Nursery lines to develop for this season's use.

During the year, the main work at Irene has been the raising of trees for sale to the public and for supplying Government Institutions, though concurrently with that, good progress has to be recorded in the making of various permanent improvements—calculated to increase the efficiency and attractiveness of the Nursery.

The season was a very favourable one for Nursery work, as there was an absence of continuous heavy rain, and there were more cool days than usual.

The rainfall during the year was below the average, and only amounted to 21·96 inches, as compared with 25·90 inches last year from 4th November to 30th June. The rains set in late in the season. The highest reading of

the maximum thermometer in the tropical shelter, was 91·8° on the 29th December, and the lowest of the minimum in the Stevenson's screen, was 15 degrees on the 12th June, the lowest reading on the grass was 8·8° on the 12th June.

The following trees were transplanted or "pricked out" during the year:—

Into trays...	511,915
„ single tins	18,194
„ quarter tins...	41,349
Deciduous trees planted into open ground ...	11,962
Cuttings planted into open ground	45,617
Total	629,037

The demand for trees has been good throughout the year. In the early spring it was even greater than the supply, but later in the season, most requirements could be met. A large proportion of the trees have been disposed of to farmers in small lots.

The total number of orders executed for the Public was 361, and Free and Departmental Issues, 100 ; making a total of 461.

Below is a summary of the trees issued during the year. Fuller particulars will be found in the Nursery Return in *Appendix D.*

Sold to the Public:—

Trees in trays	143,506
„ quarter tins	9,950
„ single tins	11,599
Deciduous trees	3,931
Seedlings...	66,353
Cuttings	5,650
Total	240,989

Free and Departmental Issues:—

Trees in Trays	37,199
„ quarter tins	8,386
„ single tins	1,321
Deciduous trees	4,671
Seedlings	85,806
Cuttings	12,000
Total	149,383

Grand Total ... 390,372

The seedlings sold consisted mainly of varieties of Eucalyptus and hedge plants.

The revenue collected by sales of trees at Irene during 1904—1905 amounted to £1,654 13s. 5d.. and the value of the trees issued free to other Forest stations was £119 4s. 4d., making a total of £1,773 17s. 9d.

The expenditure on the Nursery for the year 1904—1905 was £2,351 17s. 9d., of which no less than £316 5s. 7d. was for tins.

At one time tins could not be had for less than 5d. each, but for the coming season arrangements have heen made to secure a supply at more reasonable rates.

The details of expenditure will be found on reference to *Appendix B.* If outlays which are of a capital nature are excluded and credit is taken for trees issued free and to other Departments, and for the value of the stock on hand, it will be seen the Nursery is really more than self-supporting.

The following was the stock of trees on hand on the 30th June, 1905 :

Trees in trays	265,053
Trees in single tins	15,238
Trees in quarter tins	25,939
Deciduous trees	17,848
Seedlings	236,756

Its value reckoned at tariff rates was £2,735.

As regards the permanent improvements referred to above, these have been effected by the planting of hedges of different kinds round the compartments into which the Nursery is divided, by the gravelling of the roads and by the erection of lath shelters to shield delicate young trees from the effects of radiation frosts.

The hedging already planted has proved most beneficial to the young nursery stock. The small trees protected by its means from dry winds need less watering, and in winter they endure without hurt frosts that would shrivel them up if they remained exposed.

During the coming season the large compartments will be subdivided with hedges so as to intensify the good results already obtained.

Overhead shelters of two patterns were erected for the purpose of shielding tender trees during winter from the intense frosts at night and excessive sunshine during the day.

The one is on the American principle with lath screens about 18 inches above the ground, which are capable of being raised and lowered as required.

The other consists simply of a fixed framework supporting a roof of Eucalyptus saplings by increasing or diminishing the number of which the amount of shade can be altered to suit requirements. The roof is 6 ft. 6 in. from the ground.

The latter screen has been found the most effective, and even very tender trees like Euc. pilularis have endured severe frosts while under its shelter. The former is not so satisfactory in this respect, though the ease with which shade is applied or removed by its means is a very strong point in its favour.

Some portable grass screens were made to fit on low frames to protect young plants requiring a little temporary extra attention. They have proved most useful.

The system of pipes laid down to facilitate the watering of the Nursery, which was referred to in my last report, has been very satisfactory and has quite fulfilled expectations.

A house for the Superintendent was put up by the P.W.D., and was occupied by him in September of last year. It is perhaps rather on the small side. It contains two sitting-rooms, two bedrooms and a kitchen.

During the year the correspondence at Irene has largely increased. Over 1,000 letters were written, and in addition 700 invoices and receipts were despatched.

Considerable numbers of farmers and of the general public visit the Nursery and take a keen interest in what is being done there. They are encouraged as much as possible to do so. for they can discuss the details of any planting they are undertaking with the Superintendent, who is thus enabled to give them many useful hints which could not be so satisfactorily

afforded by correspondence. Moreover, many people, though they know the trees they want by sight, are not quite sure of their names. By paying the Nursery a visit they learn the correct ones and avoid the disappointment they would experience if they wrote for the wrong trees.

Government Nursery and Plantation, Lichtenburg.—During the past season good progress has been made at this station.

The Nursery is now properly equipped and in good working order and fifty acres of land have been permanently planted with trees.

The hedges that were put in last year round the compartments of the Nursery have made fair growth and the development of the shelter belts of Eucalypts and Cypresses growing round the outside is remarkable.

Some individual specimens in favourable situations are as much as 10 feet high, and the average height of the trees is between 4 and 5 feet.

The good effects of the shelter are now beginning to be apparent and the trying conditions under which the first lot of young trees were raised in the Nursery are being rapidly improved. In consequence, when I visited Lichtenburg in April last, the appearance of the young Nursery stock left little to be desired.

Pending the establishment of these hedges and shelter belts, temporary protection was provided by the erection round the compartments of reed screens 4 feet high. They answered their purpose well and rendered successful nursery work possible in this windswept locality.

Two lath sheds similar to the ones at Irene were put up towards the end of the year and will prove of great value in protecting the more delicate trees from the effects of heavy frosts and excessive sunshine.

The number of trees issued to farmers and the public generally has not been very large, only 9,241.

It is probable the demand will increase as the Nursery becomes better known and as the trees planted out in the Plantation begin to attract attention. At present many of the farmers are very poor and can only afford to buy a few trees, and others have been so fully occupied in getting resettled on their farms that they have not had time to devote attention to tree planting.

The revenue collected for the sale of trees during 1904-1905 was £38 14s.

Nearly 200,000 transplants were raised during the year and will be ready for distribution and for planting out next season.

The plough was kept constantly at work whenever the ground was soft enough, and altogether 132 acres of land were broken up. Fifty-one of these were sown and planted.

The balance are lying fallow and will be afforested next season.

The following table gives the details of the planting operations :—

Name of Species used.	No. of Acres.
Euc. Rostrata	13
„ Hemiphloia	12
„ Polyanthemos	13
„ Tereticornis	3
„ Leucoxylon	2
P. Halepensis	4
P. „ sown	4
Total ...	51

All these trees promise well with, perhaps, the exception of the Pine sowings. These came up thinly and irregularly. I attribute their partial failure to too light seeding and the lateness of the rains.

A sudden and severe frost, accompanied by a bitter wind, that occurred at the commencement of May, was exceedingly trying for the newly planted trees, as they were sappy and full of growth at the time. However, beyond cutting back a small patch of Euc. rostrata planted in the vlei at the back of the Nursery, no permanent damage was done.

The sowing of Acacia saligna that was made last year along one side of the main Klerksdorp road bids fair to prove a success During the winter the frost cut back all the young seedlings which were only an inch or two above the ground, but early in spring they shot forth again from the root and some are now quite 5 feet high. So far this winter—and the frosts and cold winds have been very severe—they have come through well, only the soft shoots of the smallest plants being affected. The result is satisfactory, for only the very crudest treatment has been accorded the trees. The ground was roughly ploughed and harrowed before the seed was put in and since then the trees have had no cultivation at all. They are unfenced and have been exposed to the tender mercies of sheep and goats. Acacia saligna may ultimately prove a useful fuel and shelter tree in the Western Transvaal.

Though this Wattle has so far proved successful, others will be tried so as to determine which is the best.

An arboretum, 10 acres in extent, has been fenced and laid out into plots one-tenth of an acre in size. Sixteen of these plots have been planted with different varieties of trees to test their adaptability to the district.

It has been established that Euc. bicolor and Euc. microtheca are very hardy against frost, while Euc. salmonophloia and Euc. salubris are much less so.

Euc. bicolor and Euc. microtheca are natives of the most arid parts of Australia. As proved frost and drought resisters, they should turn out most valuable introductions to this district.

A house has been built for the Forester by the Public Works Department and its erection completes the equipment of the station.

The plant put down last year to water the Nursery has worked well and has much reduced the labour bill.

Three-quarters of a mile of fencing were erected round the Nursery and paddock.

The total expenditure for the year, including the Forester's salary, amounted to £1,221 13s. 11d., the details of which can be seen on reference to *Appendix B*.

Government Nursery and Plantation, Pan.—As will be seen on reference to my Report for last year, work was started at this station in November, 1903, and arrangements were then made for planting about 120 acres the following season. However, in September of last year a proposal was made by the Railway Department through the Railway Committee, that the Government should plant 400 acres of trees suitable for the production of sleepers, on the understanding that the Central South African Railways should provide the necessary funds for the purpose, that the Government should maintain the plantation—recouping themselves for any outlay by the sale of thinnings—and that the mature crop of timber should be the property of the Railway Department.

This proposal the Government agreed to and operations were started about the middle of October—somewhat late in the season, it is true—to carry through the undertaking.

Arrangements were made with the Stock Branch of the Agricultural Department to do the necessary ploughing, as they at that time had a large mob of mules grazing on the farm.

After the land had been broken up, it was harrowed and cultivated till it was brought into good trim for planting.

Meanwhile, a large supply of young trees were being raised in the Nursery. They consisted of the following species :—

Euc. sideroxylon (Red Ironbark).
Euc. tereticornis (Forest Red Gum).
Euc. resinifera (Resinifera Gum).
Euc. paniculata (Grey Ironbark).
Euc. polyanthemos (Red Box).
Euc. hemiphloia (Grey Box).

All these species are known to produce timber of high quality.

Young trees of the first four named varieties had been planted out on the veld the preceding February, and had stood the winter frosts well, thus practically demonstrating their hardiness. There was no doubt about the ability of Euc. polyanthemos and Euc. hemiphloia to resist any frost the others did.

Actual planting operations were started in January, and the 400 acres were completed early in March. At the end of that month the Railway Committee visited the plantation, and since then it has been decided to lay down a further 500 acres of trees next year.

The following is a statement of the acreage under each species :—

Euc. sideroxylon	140 acres.
Euc. tereticornis	112 acres.
Euc. resinifera	49 acres.
Euc. hemiphloia	41 acres.
Euc. paniculata	30 acres.
Euc. polyanthemos	28 acres.

In addition to the above, 20 acres were sown down with Pines (Pinus pinaster).

Altogether 550 acres of land were ploughed, but owing to the lateness of the season it was not considered advisable to complete the planting, as there was a risk that the young trees would not be sufficiently established by the winter time.

The trees put out have done very well, and many of them are now 2 ft.—3 ft. high. Euc. resinifera and Euc. tereticornis have proved the fastest growers, while Euc. sideroxylon, Euc. hemiphloia, Euc. paniculata, and Euc. polyanthemos are slower, in the order they are named.

At the beginning of June the Fowler's steam ploughing tackle was sent over to Pan from the Experimental Farm, Potchefstroom, and on the 30th of that month had completed 80 acres for next season's planting. The total area ploughed at Pan during 1904-1905 was therefore 630 acres. 120 acres of this area were ploughed during 1903-1904, but had to be cross-ploughed this year before planting operations could be started.

The whole of the farm has been fenced with 5 strands of barbed wire strung on iron standards spaced 7 yards apart.

The Nursery and Orchard have also been enclosed. The total length of of fencing completed is 10½ miles, of which six miles were erected this year.

The Orchard contains 100 assorted fruit trees, which were selected by the Horticulturist as being varieties worth testing in the district. They are doing well.

To facilitate the watering of the large nursery stock, a windmill and tanks were put up, and from the latter a system of pipes distributes the

water by gravitation all over the Nursery. A great saving of labour has resulted from this, and the watering is also done much more expeditiously and efficiently than formerly.

A large stone building 60 ft. x 22 ft. was erected to provide stable store, and office accommodation. It cost £250 to complete.

Substantial quarters for the natives were also provided. They were urgently required, as the natives during most of the year had had to live in tents.

The Public Works Department have built a house for the use of the Forester.

In the Nursery two lath houses, similar to those at Irene, were put up to shelter tender plants and freshly transplanted seedlings. Hedges have been planted all round the Nursery and along both sides of the dividing paths. As they grow up they will afford protection against the strong winds that are experienced here towards the end of winter and in early spring.

The demand by the public for trees was not large, but as the Nursery becomes better known it will surely increase.

The total number of trees issued was as follows :—

	Number.
Sold to the Public	13,801
Free Issues	3,400
Supplied to other Stations	2,045
Total	19,246

The number of transplants raised during 1904-1905, was as follows :—

Trays	592,213
Single Tins, ½ Tins	3,288
Quarter Tins	1,524
Deciduous Trees	8,768

of these nearly 600,000 were permanently planted out. The balance of transplants and seedlings on hand at the end of the year was 638,602.

Fuller particulars can be found on reference to the Nursery Returns in *Appendix D.*

The total expenditure amounted to £3,988 12s. 10d., the details of which will be seen on reference to *Appendix C.*

A piece of ground, 15 acres in extent, has been set aside, for an arboretum, in which every tree, from any part of the world, that is at all likely to succeed on the High Veld, will receive a thorough test. The results will be carefully recorded and published, so that it will be unnecessary for the same experiments to be repeated again. The Arboretum is laid out in plots one tenth of an acre in extent, and it is intended to keep trees belonging to the same groups as far as possible together. A good number of different varieties of trees have been put aside in the Nursery, ready for planting out in the arboretum in spring.

The rainfall for the year has been very small, only 20.99 inches on 87 days were registered.

The maximum temperature recorded in the tropical shelter was 91.2 and the minimum 20.2.

THE GOVERNMENT NURSERY AND PLANTATION, ERMELO.

It will be recollected, that the Plantation ground at this Station, 967 acres in extent, is situated on the Ermelo Town Lands, and adjoins the Government Experimental Farm. The Nursery is on the Government Farm; as no sufficiently strong permanent water supply could be obtained on the Town Lands themselves.

The Nursery is about 5½ acres in extent, and has been securely fenced and laid out in blocks of 60 ft. square. Around these blocks Privet Cuttings were planted, but they have not done well, owing to the late frosts and eel worms. A great many will have to be replaced.

During the year 329,000 seedlings were pricked out, composed chiefly of the following species:—Euc. resinifera, Euc. sideroxylon, Euc. viminalis, Euc. amygdalina, Euc. coriacea, Cupressus torulosa, Pinus halepensis, P. canariensis, Juniperus virginiana, Callitris calcarata.

To afford protection for young trees against wind and frost till such time as the shelter belts round the Nursery have grown up two of the 60 ft. blocks were walled round with bricks up to a height of 2 ft. 6 in., and were again further subdivided into smaller compartments with similar walls. In this way very effective shelter was obtained, and the young trees quickly responded to the improved conditions.

Two lath houses similar to the ones in use at the other Stations, were put up and have proved very useful in protecting the more delicate plants from the severity of the climate.

A belt of Silver Wattle (Ac. dealbata) has been planted round the Nursery on three sides while on the fourth a breakwind of Eucalyptus viminalis and Pines have been put in.

The number of transplants at present in the Nursery is 146,430 and of seedlings 91,100.

10,423 trees were sold to the public, 10,968 were issued to the Stud Farm, Standerton, and Experimental Farm, Ermelo, and 20,691 were supplied to the New Scotland Settlers. The Revenue collected during the year was £117 11s. 9d.

84 acres of the plantation area have been permantly afforested. Of these 40 were sown "*in situ*" with Pinus pinaster, the Cluster Pine, at the rate of 25 lbs. per acre. The germination was excellent, indeed it was so good that the "stand" of young trees was rather too thick. In future 15 or 20 lbs. per acre will be a heavy enough seeding. The young Pines though still small look very healthy and promising. A small area of about 5 acres planted with Pinus halepensis and Pinus canariensis have not done nearly so well. This I attribute to the trees being too small when they were planted out. I am very doubtful, too, whether one year old transplants of P. canariensis will prove sufficiently frost hardy in this district. Larger sized trees will have to be employed.

The rest of the planting consists of strips of Euc. resinifera, Euc. rostrata, Euc. tereticornis, Euc. sideroxylon, and Euc. crebra. These have been placed on the outside of the plantation so as to surround the Pines and serve to protect them against fire. They have made excellent growth and some of them are now 3 ft. 6 in. high, though they are yet not six months planted. They have not so far suffered from the very cold weather that has been experienced. They have purposely been left unweeded, as the grass and other herbage is a good protection for them.

As soon as the frosts are over they will be cleaned and cultivated.

The season has on the whole been a favourable one for trees, though the rains kept off till very late.

The rainfall for the twelve months was 28.82 inches, a little over the average.

3 miles of fencing were erected during the year and 254 acres of ploughing have been completed for next season's planting.

42 lbs. of Wattle (Ac. dealbata) and 9 lbs. of Juniper (Juniperus virginiana) seed were collected and the most of it utilised on the Plantation and Experimental Farm.

At the commencement of the year the work was in charge of Mr. J. Grant. In August his duties were taken over by Mr. T. S. Watkinson, and on that gentleman's resignation in April he was succeeded by Mr. G. J. Imrie from Potchefstroom.

Labour was scarce and difficult to procure probably on account of the demand for Natives for Railway Construction purposes. However, the Resident Magistrate kindly supplied some gangs of convicts during the busy season and these did most excellent work. In this way what threatened to be a serious hindrance to progress was overcome.

Owing to the difficulty in procuring seed of Cedrus deodara, it has only been possible to raise a few hundred plants of that tree, but as soon as adequate supplies can be obtained this will be one of the principal species grown at Ermelo where it thrives exceedingly well.

14 acres of ground have been set apart for arboretum purposes. As at Pan the plots will be one-tenth of an acre in extent, and every kind of tree likely to thrive in the district will be tested there.

As the Public Works Department had no funds to build a Forester's house, this necessary work has had to remain in abeyance. Meanwhile the Forester is living in an outhouse belonging to the Experimental Farm.

Expenditure and nursery returns will be found on reference to the *Appendices B.* and *D.*

GOVERNMENT NURSERY AND PLANTATION, MACHAVIE, NEAR POTCHEFSTROOM.

The progress made at this station during 1904-5 was less satisfactory than at any of the other plantations, and this was due mainly to the drought that affected the district. The total rainfall for the year was only 13·69 inches on 54 days. The most rain fell in January, when 4·57 inches were registered. In November, 2·83 inches, and in February, 3·42 inches of rain fell. The remainder of the total was made up by passing showers.

Arrangements had been made to plant 100 acres at this station during the season, but in view of the drought it was decided to suspend operations when 47 acres had been completed, and to keep the balance of the trees that were to have been planted out, in the Nursery till this year.

Planting operations are at present confined to the vlei ground adjoining the nursery.

The following list shows the acreage of each species that has been put out :

Euc. tereticornis	...	...	...	10 acres
Euc. sideroxylon	...	...	...	15 „
Euc. polyanthemos	...	...	...	20 „
Euc. crebra ...	...	...	...	2 „

These trees have done fairly well, but in May some got badly touched by frost. Whether the damage is permanent it is impossible to say yet. If so, the hardier varieties of trees will have to be used in future. As soon as their roots get down to the water level, which is not much more than 10 or 12 feet below the surface, the trees should make rapid progress.

The 53 acres that were ploughed and cultivated for the past season's operations, but not planted, will be completed the first rains this season.

During the year 150 acres of land have been broken up and 43 acres cross-ploughed.

The major portion of the ploughing was done by the steam tackle from the Experimental Farm.

In the Nursery good progress has been made. Pending the growing up of the hedges and shelter belts, reed screens have been erected round the various compartments to protect the young trees from the strong winds. A potting shed and two lath houses have also been put up for pricking out in, and for sheltering young tender trees.

The germination of seeds has been very good, but cuttings have not been successful owing to the ravages of white ants. The nests of these have now been dug out and it is anticipated they will cause no further bother.

369,495 transplants were raised during 1904-5.

The following is a return of the trees issued from the Nursery during the year :—

To the public	9,123
To the Irrigation Department (for planting Canal Banks)	1,410
To the Experimental Farm	12,585
To the Lands Settlement (Seedlings) ...	45,080
To other Forest Stations	500
Total	68,698

The Revenue derived from the sale of trees was £147 12s. 7d. The fencing of the plantation area could not be taken in hand as the survey was only completed just recently, in spite of many applications that were made to have it put through sooner. This work will be done during the present winter.

The water supply laid on to the Nursery last year, has proved quite satisfactory, except that two more storage tanks are required to tide over periods when there is not enough wind to work the mill.

A house has been erected by the Public Works Department for the accommodation of the Forester, who has hitherto had to live in tents.

A stable for the accommodation of two mules and one horse has also been provided.

Mr. G. Imrie was in charge of the station till March, when he was transferred to Ermelo to secceed Mr. Watkinson, who left us to start fruit growing on his own account. Mr. V. Glaeser, who was assistant at Pan and had acted for Mr. Imrie while the latter was away on sick leave, was appointed in Mr. Imrie's place.

GOVERNMENT NURSERY AND PLANTATION, BELFAST.

This reserve consists of 1,500 acres of land on the Town Commonage of Belfast. It is separated into two portions by a right-of-way which serves to give the townspeople access to the remote north-east section of the Town Lands.

During the past year both these portions have been surrounded by a fence consisting of five barb wires stretched on iron standards placed eight yards apart. The straining posts are at intervals of a little over 200 yards on an average. The total length of fencing erected was 11¼ miles.

Work was started at Belfast in May, 1904, and since then the Nursery has been laid out and planted with windbreaks and hedging to protect the young trees from the effects of the bitter winds that are common there. A potting shed and two lath houses have also been erected.

At present the water for the Nursery is being raised from the sluit by a horizontal double-acting pump worked by hand, but arrangements are being made for a gravitation supply to be installed shortly.

A good stock of trees have been raised at the Nursery, as will be noted on reference to Nursery Return in *Appendix D.*

21,020 trees were disposed of and the revenue collected amounted to £16 12s. 0d.

A very severe frost at the beginning of May did some damage in the Nursery. Some Pinus insignis, which are generally considered hardy, were killed (they had not been protected in any way,) and Pinus canariensis, even though covered with canvas, were to some extent affected.

A few Widdringtonia Whytei (M'lanji Cedar), which had been raised for trial, were killed outright.

Some seedling Deodars also suffered ; in fact there were few species that altogether escaped.

Unfortunately, there are no thermometers at this station yet, so it is not possible to say how many degrees of frost there actually were.

Pinus pinaster and pinus halepensis proved quite hardy.

50 acres of plantation land were broken up and sown with pinus pinaster.

It germinated well and is making good progress, though spring hares have been doing a little harm amongst them.

There are, however, more plants on the ground than are really required so that their depredations should not ultimately prejudicially affect the work.

Between the Pine sowings and the boundary of the Plantation a wattle fire line half-chain wide was put in. As this plantation is to consist almost entirely of Coniferæ, every care will be taken by planting fire lines at frequent intervals to ensure the safety of the crop.

Up to the 30th June the total area of land broken-up at this plantation amount to 110 acres.

An iron stable was erected for the forester's horse and the plantation mules, and a two roomed, wood-lined, galvanised iron cottage for the accommodation of an overseer.

The Public Works Department built a house for the forester on a commanding site overlooking the Nursery and the plantation.

The rainfall for the year amounted to 29·73 inches, which fell on 106 days.

GOVERNMENT PLANTATION, GROENKLOOF (NEAR PRETORIA).

This plantation, which was started by the late Government in 1896, is situated on the farm Groenkloof, which lies just south of Pretoria. Only half of the area, or rather more than 300 acres, has been planted. The species grown consist mainly of Eucalyptus tereticornis, E. globulus, E. robusta. There are also a few acres of Casuarinas which do not, however, give much promise of success.

During 1900 and 1901 the Military Authorities cut over the greater portion of the plantation for fuel.

The re-growth from the stools was very good, and would have been better had some care been exercised in the felling. Many of the trees have been cut over a foot or a couple of feet above the ground or have been otherwise badly handled.

During the past year it was found necessary to thin the plantation by reducing the number of coppice shoots to one on each stool—unless the canopy was too light when two were allowed to remain—and by removing dead, suppressed, and badly shaped stems. The large quantity of thinnings thus obtained have been utilized for making lath houses at the various Nurseries, and some have been sold as firewood, scaffolding poles, and poles for hut building.

There is perhaps no branch of silviculture in this country more neglected and about which information is more urgently required than thinning and the thinning of Eucalyptus Plantations particularly. It is quite a common thing to see Plantations 10 or 12 years old with trees in them 50—60 feet high standing 5ft. or 6ft. apart the same distance as that at which they were originally planted. The trees may be mere whipsticks and bending over, but still they are allowed to remain to encumber the ground and hinder the better stems from developing properly.

Probably this may be ascribed to the fact that many of the original plantations were laid down by Foresters who brought over with them from Europe ideas regarding the management of woods which they failed to modify to meet the requirements of the new species and the different climate they were called upon to deal with.

Eucalyptus are a very rapid growing, light demanding and naturally straight stemmed genus and on that account require earlier and more frequent thinnings than most European species. They have, however, but thin crowns and it may have been from fear of interrupting the cover overhead and letting in too much light that European Foresters neglected thinning.

Be that as it may, much information is required about thinning, and as it is information which will take a long time to collect, it is advisable a start should be made in gathering it now.

It is desirable to ascertain by experiment at what age thinnings should start, when they should be repeated, and how heavy they should be in order to produce the greatest quantity or the best quality of timber per acre.

Such investigations might be usefully taken up in the Frankenwald, but meantime some small areas are being set apart at Groenkloof to show the difference in growth in thinned and unthinned woods. Accurate measurements will from time to time be taken and the results made known.

It was intended at the beginning of the year to push on with the completion of the planting at Groenkloof ; but unfortunately just at the time active operations should have started it was found that some camels which had been sent there temporarily were afflicted with a dangerous disease. They were thereupon all slaughtered and the Plantation was placed in strict quarantine. Under these circumstances the cross-ploughing and harrowing required to get the land into order could not be done and most of the planting for the season had reluctantly to be abandoned.

However some unploughable gravelly soil was pitted and planted up with Euc. sideroxylon and some rows of Euc. tereticornis were put in at the East end of the Plantation to fill in the space between the fence and the existing Planting.

The total acreage actually completed was 11. The young trees put out are doing well.

A very marked improvement has taken place in the younger growth of the old Plantation since it was fenced. Many small trees that had been kept back by cattle are now shooting up and will presently form a dense plantation. To encourage some of the more backward plants a little cultivation and cleaning will be given them next spring.

A tariff for the sale of poles and firewood from the Plantation was drawn up, and adopted at the end of the year.

P

The following table shows at a glance the Trees on hand and the work done at the various Stations during 1904—1905 :—

STATION.	No. Seedlings raised.	No. Transplants raised.	Trees Sold.	Trees issued free and to other Depts.	Revenue collected from sale of trees.	No. of acres planted and sown.	No. of acres ploughed.	No. of Miles of Fencing completed.
Irene Nursery	296,329	316,217	240,989	37,194	£1,654 13 5	...	...	...
Lichtenburg „ ...	30,450	196,128	9,241	1,260	38 14 0	51	132	¾
Potchefstroom „ ...	542,090	369,495	9,123	59,075	147 12 7	47	150	300 yds.
Ermelo „ ...	110,141	64,583	10,423	31,659	117 11 9	84	254	3
Pan „ ...	179,156	605,793	13,801	3,400	55 14 6	420	*630	6
Belfast „ ...	108,065	198,580	20,850	170	16 12 0	58	110	11¼
Groenkloof Plantation...	...	...	...	...	42 17 4 (for sale of thinnings).	9½	11 (pitted).	...
Woodbush Nursery ...	53,318	2,217	...	...	...	...	3	350 yds.
TOTAL	1,319,549	1,753,013	304,427	132,758	2,073 15 7	669½	1,290	21 miles 650 yds.

The Revenue dirived from the sale of poles and firewood was £42 7s. 4d.

During the year a descriptive Catalogue of the trees and seeds, to be obtained from the above-mentioned Nurseries, was published for the information of the public. Copies of it were sent to Agricultural Societies and also to all Railway Stations, Post Offices, and Public Libraries throughout the country.

Small consignments of the different varieties of trees in the Nurseries were sent to the principal Agricultural Shows, and an Officer of the Department attended to give information on tree-planting to anyone who wished it.

These exhibitions attracted a good deal of attention, and they will be continued next year. Farmers who cannot manage to visit our Nurseries are there able to see the various varieties of trees that are supplied, and learn about their qualities and requirements. They can then better decide which trees are best for planting on their particular farms, and are prevented from suffering the disappointment that arises from injudicious selection of species.

THE FOREST STATION AT WOODBUSH.

By Executive Council Resolution, dated 10/5/1905, the following farms in the Zoutpansberg District were set aside as Forest Reserves and were duly gazetted as such :—

	No.	Area.
Helpmakaar	1934	285 M. 207 sq. R.
Graskop	661	209 M. 206 sq. R.
Baccarat	2094	462 M. 332 sq. R.
Grootbosch	2049	5700 M. approx.
		6656 M. 745 sq. R.

The larger portion of all these farms is heavily wooded. The forest at Hangklip, near Louis Trichard and the farms Potatahoek (412) and Goedgeluk (433) in extent respectively 809m. 173 sq. rds., 37 m. 173 sq. rds. and 99 m. 327 sq. rds. were also reserved.

The total area of Forest Reserves in the Zoutpansberg District up to date is 11,898 m.

Proposals have been made to the Native Affairs Department to reserve the Forests on Modjadjies Location and work them for the benefit of the Natives themselves.

If this is not done, it is only a question of time that the Forests be obliterated.

The Forests situated on the Headwaters of the Letsitele and Thabini Rivers have been demarcated, and will be surveyed in the course of the formation of the general Plan of the District by the Surveyor-General's Department.

Very little wood-cutting has gone on in the district during the year, probably owing to the fact that transport has been almost impossible to procure, and because several of the mines have closed down. On that account, it has not been considered an urgent matter to open any regular sections in the forest.

The species of timber cut has been mostly Waterwood and Yellowwood. The former is still abundant, but good Yellowwood is very scarce.

The Revenue derived from the sale of wood during the year was £97 17s. 10d.

There have been seven prosecutions for contravening Forest Regulations. In six, judgment was obtained against the offenders. In

the other case the accused got off owing to some doubt as to the position of certain beacons.

To prevent the destructions of the forests by the Natives, they are constantly patrolled, with the result that now little or no damage is done.

A small Nursery was started at Potatahoek, near Woodbush, to raise trees for planting up blanks in the Forest, and to afforest such grass lands as are included in the reserves.

It is proposed to go in mainly for growing Pines and other soft-woods, though Eucalyps and Wattles will be utilised to make fire belts with.

There is no doubt that the Woodbush District is about the best in the Transvaal for tree growth on account of the regular heavy rainfall and misty weather it enjoys. It is true it is somewhat remote from the railway at present, but it is inconceivable that in the development of agriculture in the Transvaal, this part of it, which is the one most favoured with rain, should be allowed to remain neglected and unutilised for the want of better means of communication.

I would recommend that expenditure on afforestation in this district be regular and liberal.

Particulars of the stock in hand in the Nursery on the 30th June, 1905, will be found on reference to *Appendix D.*

THE FOREST STATION, BARBERTON.

The Forest Ranger has under his care the Scrub Forests in the neighbourhood of Barberton and Komatie Poort and the High Forests, in the vicinity of Pilgrim's Rest and Sabie.

Timber from the former, wherever it is reasonably close to the railway, is cut under licence to provide Mining poles for the Rand. A small quantity is felled for firewood for local consumption.

As mentioned at the beginning of this report, these Scrub Forests were visited by Mr. D. E. Hutchins, Conservator of Forests, Capetown, in August last, and his note on the same is given in full in *Appendix A.*

The recommendations made by Mr. Hutchins are being carried out as far as possible.

The High Forests at Pilgrim's Rest fall naturally into two groups; the first on the East escarpment of the Drakensberg, where these mountains fall sharply to the low country, and on the right bank of the Blyde River. The second group is on the crest of a parallel chain on the left bank of the same river.

The first group comprises the following Government farms. Their area is given whenever known :—

Name.	No.	Area in Morgen.	Roods.
Graskop	27	3930	568
Sunlight, unnumbered, unsurveyed, probably about 1000 morgen	...	...	...
Quartz Kop	641	499	552
Houtbosch	293	1621	175
Kimberley	643	499	289
The Peak	638	499	208
Crystal	642	499	519
Diamond	651	499	512
Clear Stream	644	499	582
Belvedere Creak	78	349	341
Op-de-Berg	42	1183	572

Government Farms.—*Continued.*

Name.	No.	Area in Morgen.	Roods.
A strip of Government ground joining S. of Op-de-Berg to E. of Diamond, Clear Stream and Crystal, called Jerperg	71	318	50
The second group comprises—			
Bendigo Heights... ...	564	840	450
Concordia	646	1000	...
Grootfonteinberg ...	549	1000	...
Morgenzon	1487	1004	368
Van der Merwe Reef ...	565	1000	...
Kruger's Hoop	562	1000	...
Black Hill	647	488	546

The Forests lie at an elevation of between 4,000 and 5,000 feet. The rainfall is probably about 50 to 60 inches per annum.

The Forests are similar to those of Cape Colony and Natal.

Yellow Wood (both species), White Pear, Ironwood, Assegai, Saffraan and Red Pear are fairly plentiful, while Stinkwood, Bitter Almond, and Sneezewood are scarce.

Lemonwood is very abundant and is apt to choke out more valuable species.

All the Forests have been cut over for mining poles, timber, wagon wood, and in some cases, for fuel.

They are very foul, full of weeds, and in a bad state. Some have passed beyond possibility of repair at reasonable cost, and others it will be difficult and expensive to put in good order.

The Forests are called on to supply poles for the mines and small quantities of timber both to the mines and wagonmakers.

Most of the building timber used is imported. Last year no less than 37,754 poles and 67,267 pieces of lagging were required for the mines, most of which came from the Government Forests.

The area of Government Forests in groups 1 and 2, which is probably not more than 3,000 acres in extent, cannot continue to supply that amount of poles permanently. It will be necessary to make plantations for that purpose, and proposals have been put before you to reserve portions of Graskop with that object in view.

The only species that at present are not being cut for mining poles are the two Yellowwoods, White Pear, Assegai, and Stinkwood.

This, to a certain extent, retards the destruction of the Forests, but, to ensure their safety, it will be advisable to put the better ones under a regular system of management like Coppice with Standards, to execute cleanings and improvement fellings in the interior, and to close the worst ones altogether.

In addition to the Forests at Pilgrim's Rest the Government owns about 1500 acres of Forest at Sabie Hoek on the pieces of land known as lots C, D, and E.

With the exception of the lower portion of lot E the Forests have not been heavily worked of recent years. At present they are only worked

for mining poles, for Glynn's Lydenburg Mine, and for fuel for lime burning. The lower portion of lot E has been badly hacked about, and is very foul, but otherwise the Forests are clean.

These Forests are more than able to meet the present demand, and it would be desirable to work them departmentally rather than under the license system, provided that experience shows this can be done economically.

These Forests and those at Pilgrim's Rest were carefully examined by Mr. Grenfell, the Assistant Conservator. I have made use of his report in several of the above notes.

During the year there were 47 prosecutions for Forest offences, and all but three of the offenders were found guilty and punished. The culprits were all natives.

The revenue collected at Barberton and Pilgrim's Rest was as follows :—

Outstanding from last year ...	£235	16 6
Collected during present year :—		
Cutters' Licenses	19	10 0
Timber Dues	1360	17 6
Mining Poles		
Firewood	95	8 0
	£1475	15 6

The expenditure was £520 15s. 7d., particulars of which will be found in the Appendix B.

SEED DISTRIBUTION.

In pursuance of the policy of this division in regard to this matter, tree seeds were purchased from reliable sources in various parts of the world and were resold to the public at a price just sufficient to cover the actual cost and the expenses of handling and storing.

Considerable advantage was taken of the facilities thus afforded for getting good seed at reasonable rates.

Altogether 2,385 lbs. of seed, having a value of £250, were supplied to the public, and 440 lbs. were issued free to other divisions, to Government institutions, and to settlers. 4,775 lbs. were utilised at the Government Nurseries and Plantations.

The stock of seed on hand on 30th June, 1904, was 5,746 lbs., 4181 lbs. were taken on charge during the year under report, and 7,600 were disposed of as noted above.

The stock on hand on the 30th June, 1905, was 2,327 lbs.

The quantity of seed collected locally amounted to 173 lbs.

Small consignments of seeds of the rubber producing trees, Manihot, Glaziovii, and Hevea Brasiliensis, were received during the year, and were distributed gratis among farmers in the hottest and wettest parts of the country on condition that they would supply from time to time full in-

formation as to the results obtained. Most of them have been successful in raising some trees of each species, and the latest accounts state that the young trees promise well. It is far too early, however to say whether profitable rubber culture will be feasible in the Transvaal. That can only be determined by actual experiment. Further small lots of rubber seed have been ordered for trial next season.

Considerable difficulty has been experienced in getting supplies of seed of some of the species of trees which it is desirable to grow extensively in the Transvaal, and this has somewhat hindered progress.

For example, Cedrus deodara was only obtainable in comparatively small lots. Pinus longifolia, too, was scarce and dear, and the consignment of camphor seed ordered from France arrived in such bad order as to be useless.

Endeavours to obtain supplies of Mexican pine seed, so strongly recommended by Mr. Hutchins in his Transvaal Forest Report for trial in this country, have so far proved fruitless, though many letters have been addressed to firms in Mexico and America with regard to them. It is hoped that the Mexican Department of Agriculture, with whom we are at present in correspondence, will be able to assist us in this matter.

Through the co-operation and courtesy of the Inspector-General of Forests, India, it is probable good supplies of Deodar and Pinus longifolia will be forthcoming next season, and Camphor seed is expected from Japan and from Cape Colony.

GRANTS-IN-AID OF TREE PLANTING.

During the past year applications were received from eight Municipalities and one Agricultural Society for refunds on the £1 for £1 principle on expenditure incurred by them on tree planting. During the previous twelve months only three applications were received altogether.

The refunds granted vary in amount from £15 11s. 10d. to £100.

The following are the particulars :—

Name of Municipality.	Amount of Expenditure.	Amount of Refund.
	£ s. d.	£ s. d.
Ermelo	36 14 0	18 7 0
Pretoria	41 2 6	20 11 3
Machadodorp	110 17 6	55 8 9
Klerksdorp Agricultural Society	67 1 2	33 10 7
Carolina	124 17 1	62 8 6
Klerksdorp	272 7 10	100 0 0
Schweizer Reneke	67 2 3	33 7 10
Belfast	58 6 7	29 3 3
Volksrust	31 3 8	15 11 10
Total	£809 12 7	£368 9 0

The work done by these public bodies has been well and carefully executed, it will serve in a few years' time to greatly improve the appearance of the towns and villages throughout the country.

Grants-in-aid of tree planting were made out of this vote to the Government Settlers in the New Scotland District, to the Education and to other Government Departments, to the different Divisions of this Department and to the South African Constabulary.

The following are the particulars of these payments :—

Free Issues, other Divisons of the Agric. Dept. ...	£218	19	2
Grants-in-Aid to Agric. Socys. and Municipalities as per particulars above	368	9	0
Free Issues to Lake Chrissie Settlers	126	10	4
Free Issues Govt. Depts. and Institutions ...	148	9	6
Total	£862	8	0

The total expenditure for the year was £862 8s. 0d., and the unappropriated balance was £137 12s. 0d.

ROADSIDE ARBORICULTURE.

In the Transvaal and indeed in South Africa generally there has been very little done in the way of what may be called Roadside Arboriculture, and as it is a very effective permanent improvement to the country—and one likely to be popular with the whole community—an arrangement has been made with the Public Works Department to push the matter next season.

The scheme as at present outlined is to plant one or two rows of trees along each side of such roads as are definitely laid out and are being made and repaired by the Public Works Department.

The work is to be started on the main roads leading out of towns and is to be extended out into the country as opportunity offers. It is desired to have road-making and road-repairing accompanied whenever possible by tree planting, and if this policy is persisted in, it would not be long before there would be avenues running in all directions through the country connecting the various towns.

Apart from the pleasure and comfort they would afford to many, these avenues would serve to stimulate the interest of the public in tree planting.

It is proposed that this Division should supply all the trees for the roadside planting, while the Public Works Department would dig the holes and provide guards.

The chief dangers Roadside Planting will suffer from, will be fire and theft. To overcome the former fire resisting quick growing species will be employed, and the suppression of the latter may be left to the Police Forces of the country.

GENERAL.

Office work increased very greatly during the year and necessitated the employment of a shorthand and typewriter. Previously the typewriting was done by the Head Office Staff.

Mr. Ingle in addition to his clerical work took charge of the seed store and managed it most efficiently.

In the staff of the Division there is little change to record. Mr. Watkinson left Ermelo, to take up farming on his own account, and Mr. Imrie, from Potchefstroom, took his place. He, in turn, was succeeded at Potchefstroom, by Mr. Glaeser, the Assistant at Pan.

Mr. A. P. Grenfell, late of the Indian Forest Service, was appointnd Assistant Conservator in November.

All the members of the staff have worked well, and to them for their good services, I wish gratefully to express my thanks.

I have the honour to be,

Sir,

Your obedient Servant,

C. E. LEGAT,

Conservator of Forests.

Appendix A.

NOTE ON LOW COUNTRY SCRUB FORESTS, BY D. E. HUTCHINS, CONSERVATOR OF FORESTS, CAPE TOWN.

To form remunerative forests from the Low Country scrub would entail long and expensive treatment, with doubtful results on account of an insufficient rainfall. I do not recommend that any attempt should be made to retain as forest the whole of these scrub-covered areas. It would be preferable to convert them, instead, into park lands, interspersed with clumps of trees and with fringes of trees bordering the streams. It is probable that clearing the scrub will improve the sanitary condition of the country in lessening the number of mosquitoes, and consequently malarial fever; and by leaving clumps and fringes of trees a bare treeless aspect of the country will be avoided. It must be remembered, also, that even with this treatment there will be a considerable re-growth of the scrub forest.

When inspecting the poles being cut at the Lebombo Siding I noticed that the wood chiefly used for mining-props is known as Knoppies Doorn (Acacia nigrescens var. pallens). This species re-shoots freely from the stool when cut. Indeed, it was stated that Knobwood shoots grow up so quickly and readily that in one instance a seven years' growth produced small but quite serviceable mine props about four inches in diameter and nine feet long. This Knobwood must not be confounded with the *Knobwood Zanthoxylon capense* of the dense evergreen forest.

For the scrub forest I recommend the following treatment :—

(1) Enforce the rule which enjoins that forest-fringing streams should not be cut within a distance of 50 yards from the stream.
(2) Demarcate out any patches of good forests such as occur here and there on the banks of streams and on islands, showing these sketched on a large-scale plan.
(3) Demarcate also forests containing a notable proportion of Kajatenhout or Sneezewood or Leadwood or Inchlomu, and prohibit the cutting of these trees anywhere unless they are specially marked for felling by the Forest Department. Only dead or over-mature trees would be marked for felling.

There are also three other woods that it may be desirable to reserve—"Aapies Doorn," "Mingerhout," and "Tambootie." Of these trees Sneezewood requires no description. It is the well-known peerless, imperishable timber of Cape Colony and Natal. It is fairly abundant in parts of the Low Country, but very rare in the dense evergreen forests about Pilgrim's Rest. Trees up to 20 and 30 feet and stems of two feet thick are mentioned.

"*Leadwood or Elephant's Tooth*"* (*Kafre: Umpondo indhlovu*).—This wood is very hard. strong, and durable, and I conjectured it might be the "Red Ivory" of Natal; but Mr. Piers, the Pit-prop Contractor at Lebombo Siding, assured me that in the Natal forests, which he knew well, it was unknown. It is, no doubt, a very valuable timber—the "Sneezewood" of the Transvaal Low Country. Like Sneezewood, it is very liable to rottenness at the heart when growing, but heartwood, if sound when cut, is said to be imperishable.

Leadwood is slow-growing; it is thought to grow at only half the rate of Knobwood. It re-shoots badly or not all.

* Combretum Sp.

"*Inchlomu*" *or* "*Mango Pear.*"*—Leaves like a mango, but arranged in verticles like a Beukenhout. Timber is a "pear," and box-like in its close character. This tree is a good timber, *i.e.*, durable in the ground and ant-resistant. It is evergreen, and always found near streams. It grows to a tree of 18 inches and two feet in diameter and 40 feet high, but the trunk is short, like all the trees in the Low Country shrub. Photographs were taken of it near the Lomati at Komatipoort and near the Limpopo at Nelspruit. According to Mr. Piers it is rarely seen except near streams, and is then protected sufficiently by the rule requiring that no trees be felled within 50 yards of a stream.

"*Intomboti*" *(Excœcaria Africanca).*—This is, perhaps, the scented wood of the Transkei. It is said to occur to a small extent in the Low Country of the Transvaal, and to be occasionally used for mining props. This, if true, should be interdicted, for it is far too valuable a timber to be used for such a purpose.

Kajatenhout.—This appears to be a Pterocarpus, but I was unable to obtain any means of identifying the Low Country Kajatenhout. It is said to form nearly pure forests near Origstad, and to be particularly abundant in the sandy soils along the course of the Zand River, below the Belvidere Mountains. It is usually found in sandy soil. It reaches a total height of about 40 feet, with trunks yielding planks 12 or 14 feet long. Occasionally planks 20 feet long are obtained. Though this tree, like all the other trees of the Low Country, is short, the stem of Kajatenhout is not wanting in thickness. Trees up to five feet in diameter are cited. Some years back, when cattle were more abundant, it was generally sawn into planks and brought back from the Low Country with the cattle in Spring. It furnishes one of the most beautiful of furniture woods, with an appearance somewhat between that of Teak and Walnut. It warps little, cuts up well, and is sufficiently soft to be easily worked. It is said that only young trees coppice well when cut. Kajatenhout is used also for wagon wood.

AAPIESDOORN.

This is described as a big straight tree ranging up to 40 feet high, useful for wagon wood, also durable in the ground.

MINGERHOUT.

This is described as a straight tree attaining a height of about 40 feet and slender. It furnishes a useful wagon wood.

The adoption of these proposals, while preventing the country being denuded of trees, will interfere but little with the important industry of cutting poles for the mines. At Lebombo Siding we had the advantage of discussing the matter fully with Mr. Piers, the agent of the largest Johannesburg mine prop firm. Nine-tenths of the poles he is now cutting seem to be Knobwood and this species, as mentioned above, it is not proposed to reserve. It is little used except for mine props and re-shoots freely when cut. Another abundant wood in the Low Country about Komati Poort is the Plum, "Marula" (Selerocarya Caffra) from which the Kafirs make a drink. This tree is perfectly worthless for any purposes except for pit-props and they will now scarcely take it even for that.

* Adina Galpini.

Appendix B. EXPENDITURE FOR THE YEAR ENDING JUNE 30TH, 1905.

	Head-quarters.	Seed Store	Irene.	Potchef-stroom.	Pan.	Lichten-burg.	Ermelo.	Groen-kloof.	Belfast.	Woodbush.	Barberton	Louis Trichard.
	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.	£ s. d.
Purchase of Animals	—	—	—	130 0 0	—	—	181 6 0	—	336 6 8	252 10 0	—	—
Forage	—	—	46 13 6	0 16 6	13 3 6	22 8 10	—	—	70 19 9	22 7 9	19 5 0	—
Tins	—	22 16 8	316 5 7	48 12 3	300 7 4	74 12 7	163 16 5	—	63 6 5	—	—	—
Water Supply ...	—	—	50 15 6	38 18 4	412 4 4	19 14 2	—	—	—	—	—	
Cuttings & Seedlings	—	—	22 0 5	1 16 0	16 12 3	9 5 6	11 10 8	—	21 18 0	1 10 0	6 2 11	—
Transport	467 4 6	11 14 3	103 7 8	47 2 5	121 19 11	57 8 11	148 12 3	0 13 0	124 19 8	30 14 4	13 16 5	—
Harness	—	—	2 0 0	8 11 6	47 2 6	1 16 6	6 8 6	—	24 15 6	7 10 6	13 3 6	—
Tools & Implements	—	—	6 15 9	10 0 6	64 1 6	1 1 0	12 0 0	13 14 0	19 1 3	5 3 3	—	—
Fencing Material ...	—	—	102 17 2	20 4 5	20 13 3	22 16 4	20 3 11	—	205 17 0	5 4 6	—	—
Hessian	—	—	—	2 16 2	11 10 1	—	11 13 2	—	5 11 2	—	—	—
Ploughing	—	—	—	221 4 1	223 19 5	49 16 0	132 17 0	—	156 4 3	—	—	—
Seed Oats	—	—	—	—	8 5 0	—	—	—	—	—	—	—
Bldg's & Bldg. Material	—	—	5 18 1	29 14 1	169 6 4	49 3 11	73 7 3	3 0 0	57 16 5	11 12 3	—	—
Rent	—	—	102 0 0	—	—	—	—	—	—	—	—	—
Manure	—	—	4 10 0	—	—	—	3 10 0	—	1 4 0	—	—	—
Vehicles	—	—	3 12 6	7 0 0	50 6 0	7 10 0	22 15 0	15 4 0	5 10 6	12 5 0	23 17 0	—
Sundries	4 6 6	2 18 1	10 10 7	8 13 11	20 6 7	12 12 2	14 0 5	0 3 6	28 1 6	39 14 5	8 4 2	—
Salaries	1372 5 4	—	497 4 6	450 6 5	841 16 6	307 12 0	293 9 11	79 8 6	560 10 0	547 18 0	442 1 11	—
Native Pay	33 12 0	—	1058 11 3	422 10 8	1636 10 5	571 1 0	429 4 6	225 19 1	671 5 5	245 17 8	29 10 0	72 0 0
Travelling Expenses	221 11 8	—	18 15 3	8 8 6	23 8 5	—	20 9 7	—	2 5 6	47 7 6	66 4 6	—
Coal	—	—	—	—	6 5 0	—	0 12 0	—	10 1 0	—	—	—
Salt	—	—	—	—	0 14 6	1 0 0	—	—	2 0 0	0 4 3	0 18 9	—
Firewood	—	—	—	—	—	13 15 0	—	—	—	—	—	—
Seeds...	—	577 1 1	—	—	—	—	—	—	—	—	—	—
Surveying Instruments	—	—	—	—	—	—	—	—	—	17 9 6	—	—
Bags	—	5 0 8	—	—	—	—	—	—	—	—	—	—
Labels	—	51 12 0	—	—	—	—	—	—	—	—	—	—
Total	2099 0 0	671 2 9	2351 17 9	1456 15 9	3988 12 10	1221 13 11	1545 16 7	338 2 1	2367 14 0	1247 8 11	623 4 2	72 0 0
Grants... ...	862 8 0	—	—	—	—	—	—	—	—	—	—	—
£	2961 8 0	671 2 9	2351 17 9	1456 15 9	3988 12 10	1221 13 11	1545 16 7	338 2 1	2367 14 0	1247 8 11	623 4 2	72 0 0

Total ... ... £18,845 16s. 9d.

Appendix C.

REVENUE COLLECTED FOR THE YEAR ENDING JUNE 30TH, 1905.

Irene Nursery	£1654	13	5
Ermelo Nursery	117	11	9
Potchefstroom Nursery	147	12	7
Belfast Nursery	16	12	0
Lichtenburg Nursery	38	14	0
Pan Nursery	55	14	6
Groenkloof Plantation	71	4	0
Barberton	1475	15	6
Woodbush	97	17	10
Louis Trichard	14	13	10
Wolmaransstad	0	5	0
Waterberg	7	18	9
Lydenburg	0	10	6
Seed Store	312	18	2
Total ...	£4012	1	4

SPECIES	Stock on hand, 30/6/04.	Raised during Year.		TOTAL	Planted out in Plantation	Sold to Public.	Free Issues.	Otherwise disposed of.	Total on hand 30/6/05.
		Seedlings	Transplants.						
Acacia dealbata	200	—	506	706	—	500	46	—	160
„ melanoxylon	—	4,000	500	4,500	—	—	—	—	4,500
„ mollissima	28,072	—	—	28,072	—	16	243	27,813	—
Casuarina tenuissima	13,000	—	—	13,000	—	150	1,195	11,317	338
Callitris calcarata	35,000	—	—	35,000	—	—	—	21,500	13,500
„ rhomboidea	—	—	4,400	4,400	—	—	—	—	4,400
„ verrucosa	—	—	3,000	3,000	—	—	—	—	3,000
Cupressus lawsoniana	2,500	—	—	2,500	—	—	—	2,334	166
„ lusitanica	12,775	—	8,350	21,125	—	620	158	—	20,347
„ macrocarpa	500	1,558	—	2,058	—	—	8	—	2,050
„ sempervirens	25,000	—	—	25,000	—	25	80	18,395	6,500
„ torulosa	—	—	2,200	2,200	—	—	—	—	2,200
Cedrus deodara	—	700	—	700	—	—	—	—	700
Catalpa bignonioides	—	300	—	300	—	—	—	—	300
„ speciosa	—	600	—	600	—	—	—	—	600
Cytisus Laburnum	—	50	—	50	—	—	—	—	50
Eucalyptus coriacea	7,000	—	—	7,000	—	679	583	4,200	1,538
„ globulus	5,000	—	—	5,000	—	—	1,275	2,455	1,270
„ crebra	—	—	3,197	3,197	3,122	75	—	—	—
„ amygdalina ...	—	—	3,400	3,400	—	—	200	—	3,200
„ paniculata ...	26,000	—	—	26,000	—	1,000	1,150	23,850	—
„ pilularis	4,000	—	—	4,000	—	—	—	4,000	—
„ resinifera	32,350	—	—	32,350	29,245	150	860	—	2,095
„ rostrata	11,675	—	—	11,675	11,534	112	10	—	19
„ saligna	7,300	—	—	7,300	—	—	245	6,995	60
„ sideroxylon ...	66,600	40,000	—	106,600	50,482	130	850	—	55,138
„ siderophloia ...	7,000	—	—	7,000	—	1,000	1,000	4,977	23
„ Stuartiana ...	—	30	—	30	—	—	—	—	30

Eucalyptus tereticornis ...	10,000		—	10,000	9,590	100	150	—	160
,, melliodora .	—	20	—	20				—	20
,, polyanthema ...	—	8,000	6,800	14,800	—	—	—	—	14,800
,, hemiphloia ...	—	17,000	6,800	23,800	—	—	—	—	23,800
,, viminalis	4,000	14,975	—	18,975	—	1,350	9,135	—	8,490
Gleditschia triacanthos... ...	—	30	—	30	—	—		—	30
Juglans nigra	—	40	—	40	—	—	—	—	40
,, regia	—	80	—	80	—	—	—	—	80
Juniperus virginiana	—	—	2,200	2,200	—	—	—	—	2,200
Ligustrum japonicum	—	10,800	—	10,800	—	—	5,400	—	5,400
Pinus canariensis	11,950	—	—	11,950	6,790	75	—	5,085	—
,, contorta	—	1,700	—	1,700	—	—	—	—	1,700
,, Australis	—	30	—	30	—	—	—	—	30
,, divaricata	—	200	—	200	—	—	—	—	200
,, edulis	—	8	—	8	—	—	—	—	8
,, halepensis	83,300	—	—	83,300	6,790	2,465	4,109	66,574	3,362
,, Hamiltoni	—	—	10,800	10,800	—	—	—	—	10,800
,, insignis	2,000	—	6,450	8,450	—	672	3,582	—	4,196
,, Jeffreyi	—	120	—	120	—	—	—	—	120
,, mitis	—	700	—	700	—	—	—	—	700
,, monophylla	—	60	—	60	—	—	—	—	60
,, muricata	—	800	—	800	—	—	—	—	800
,, longifolia	2,210	—	—	2,210	1,947	213	—	—	50
,, pinaster	24,026	7,860	—	31,886	—	686	200	—	31,000
,, ponderosa	—	—	500	500	—	—	—	—	500
,, sylvestris	—	—	2,700	2,700	—	—	—	—	2,700
,, taeda	—	—	80	80	—	—	—	—	80
,, thunbergii	—	—	2,700	2,700	—	—	—	—	2,700
,, laricio	—	300	—	300	—	—	—	—	300
Schinus molle	700	—	—	700	—	—	—	700	—
Syringa vulgaris	—	150	—	150	—	—	—	—	150
Taxodium distichum	—	30	—	30	—	—	—	—	30
Thuya orientalis	3,500	—	—	3,500	—	405	1,180	1,075	840
TOTAL ...	425,658	110,141	64,583	600,382	119,500	10,423	31,659	201,270	237,530

FOREST NURSERY, BELFAST.

SPECIES.	Stock on hand, 30.6.04.	RAISED DURING YEAR.		Received from other Forest Stations.	TOTAL.	Planted out in Plantations.	Sold to Public.	Free Issue.	Total on hand, 30.6.05.
		Seedlings.	Transplants.						
Acer negundo	—	—	5,000	26	5,026	—	—	—	5,026
Acacia melanoxylon	—	—	5,200	—	5,200	—	—	—	5,200
Cupressus macrocarpa	—	1,700	13,050	—	14,750	300	250	—	14,200
„ torulosa	—	—	27,990	—	27,990	390	—	—	27,600
„ lusitanica	—	—	12,310	1,000	13,310	760	250	—	12,300
„ sempervirens	—	1,500	7,000	700	9,200	—	—	—	9,200
„ lindleya	—	100	—	—	100	—	—	—	100
Casuarina quadrivalvis	—	4,600	2,300	—	6,900	—	1,000	—	5,900
Catalpa bignonioides	—	650	—	12	662	—	—	—	662
„ speciosa	—	500	—	—	500	—	—	—	500
Cedrus deodara	—	11,375	—	100	11,475	—	—	—	11,475
„ libani	—	1,120	—	100	1,220	—	—	—	1,220
„ Atlantica	—	—	—	100	100	—	—	—	100
Callitris calcarata	—	—	2,100	—	2,100	—	—	—	2,100
„ cupressiformis	—	—	5,100	—	5,100	—	—	—	5,100
Eucalyptus viminalis	—	46,000	22,250	—	68,250	500	19,350	—	48,400
„ coriacea	—	—	4,500	—	4,500	—	—	—	4,500
„ amygdalina	—	—	500	—	500	—	—	—	500
„ sideroxylon	—	—	1,000	—	1,000	—	—	—	1,000
Gleditschia triacanthos	—	—	36	—	36	—	—	—	36
Hibiscus	—	—	1,000	—	1,000	—	—	—	1,000
Juglans nigra	—	50	—	12	62	—	—	—	62
„ regia	—	70	—	12	82	—	—	—	82
Leptospermum lævigatum	—	300	—	—	300	—	—	—	300
Ligustrum ovalifolium	—	—	7,000	—	7,000	7,000	—	—	—
Morus alba	—	—	100	—	100	—	—	—	100
„ nigra	—	—	100	—	100	—	—	—	100
Pinus australis	—	—	8	—	8	—	—	—	8