3. The three animals immune against redwater and heartwater showed slight fever reactions when exposed, accompanied in two instances with *Piroplasma mutans.*

4. The five animals immune against redwater, *Piroplasma mutans*, and heartwater all showed slight reactions when exposed, and only one animal (337), of which there exists a doubt as to whether it was immune to *Piroplasma mutans*, showed this parasite. In the other animals the reaction did not cause the reappearance of *Piroplasma mutans* ; in 337 it is probable that the reaction was either due to a relapse of heartwater or to some other agency.

5. Both control animals died, showing lesions of heartwater ; both showed *Piroplasma mutans* in the blood, and one also showed an infection of *Piroplasma bigeminum* at post-mortem.

6. All the exposed animals showed reactions a certain time after exposure ; these reactions cannot be determined with absolute certainty, although inoculation of blood collected during the reactions were made into various sheep. Only one goat contracted a typical heartwater reaction and died ; the remainder —all sheep—showed reactions, but since none died, and heartwater is usually fatal for sheep, we must conclude that not all the reactions given by the exposed cattle were due to heartwater, but that there was some other agency responsible, of which we have no knowledge at the present time.

7. With regard to the reaction, which cannot be definitely determined, it must be remembered that the animals had been for a considerable length of time away from tick infection, or at least were exposed to a minimum tick infection, and running on veld in which the blue and red ticks were present, and the brown was hardly ever noticed. In the bushveld the bont and the brown tick preponderate, and it is possible that these are responsible for this fever.

Conclusions.

1. The exposure of animals immune against redwater in the low veld proved that this immunity protected against the redwater of that veld.

2. Animals immune against heartwater were protected against that disease in the low veld.

3. Animals which were only immune against redwater contracted a *Piroplasma mutans* infection when exposed in the low veld.

4. All the animals which were not immune against *Piroplasma mutans* contracted this infection when exposed in the low veld, but none died.

5. Of the two control animals which were not immune against any of the diseases both died ; *Piroplasma mutans* was present in both cases, but the deaths were due to heartwater, and in one case complicated with redwater.

6. All the exposed animals showed reactions, due either to heartwater or to some other agency, and this reaction, in the majority of cases, caused an increase in the number of *Piroplasma mutans* present in the blood.

7. The animals which were immune against heartwater, *Piroplasma mutans*, and *Piroplasma bigeminum* showed a slight *Piroplasma mutans* infection, and also a slight reaction.

B.—THE INFLUENCE OF COLD ON TICKS AND PIROPLASMA PARVUM.

Shortly after the introduction of East Coast fever into the low veld of the Elands River Valley in the Eastern Transvaal, and before legislation prohibited the movement of cattle, in several instances infected herds were brought up from that district to the high veld. One particular case came under my

observation in the neighbourhood of Carolina. It was generally noted that directly after the introduction of sick cattle to the high veld, the infected cattle died out, and the remainder of the herd did not contract the disease, neither did the cattle which were grazing on areas over which the infected cattle must have dropped ticks. At that time it was not known that *Rhipicephalus evertsi* (the red tick) was a carrier of East Coast fever, and the observation was apparently explained since *Rhipicephalus appendiculatus* (the brown tick), which is the principal carrier of the disease, was not found amongst the surviving cattle at the time the examination was made and it was, therefore, concluded that *Rhipicephalus appendiculatus* could not live in the high veld.

Later investigations, however, proved that *Rhipicephalus appendiculatus* may live in the protected places in very high altitudes, such as dongas, but it is not present in large numbers. In 1906, Mr. Lounsbury, Government Entomologist of the Cape Colony, stated that *Rhipicephalus evertsi* was also a carrier of this disease, and in my previous Annual Report I was able to corroborate this statement, with the reservation that *Rhipicephalus evertsi* is not such a certain carrier of *Piroplasma parvum* as the brown tick, as in several instances I failed to infect *Rhipicephalus evertsi.*

Considering that *Rhipicephalus evertsi* is one of the most common ticks of the high veld, although it is not found in such large numbers as in the low veld, it became apparent that the disappearance of East Coast fever from the high veld had to be interpreted in a different way.

The influence of cold as a possible factor in the destruction of the virus within the tick suggested itself, and for this purpose, experiments were undertaken with *Rhipicephalus appendiculatus.*

In the first instance, it was surmised that on account of the considerable amount of liquid contained in engorged nymphae, this would be the stage more easily affected by cold, and should it not prove to be the case, it was thought that as a sexual development of the parasite probably ensues within the tick, the cold might retard that sexual development, or else completely inhibit it, in the same way as cold retards the development of the malarial parasite within the mosquito.

At the same time, it was decided to note the influence of cold on larvae of the blue tick, which are the sole survivors of the winter months.

Experiment No. 1.

To infect a beast with East Coast fever for the purpose of collecting engorged nymphae of Rhipicephalus appendiculatus.

Cow 455.—Infested on the 23rd May, 1907, with adults of *Rhipicephalus evertsi*, collected from ox 358, which at the time was suffering from East Coast fever.

Cow 455 died on the 17th June, 1907, from East Coast fever.

Engorged *Rhipicephalus appendiculatus* nymphae were collected from cow 455 three days before death (14th June, 1907).

Experiment No. 2.

To prove that these engorged nymphae of Rhipicephalus appendiculatus *will transmit East Coast fever in their adult stage.*

The nymphae of *Rhipicephalus appendiculatus* which were collected on the 14th June, hatched on the 16th July, thirty-two days after they were collected.

Ox 467.—Infested on the 22nd October, 1907, with adults of *Rhipicephalus appendiculatus* from cow 455.

Piroplasma parvum was noted from the 18th to 21st days, on which latter date the beast was killed.

Post-mortem examination showed all the lesions of East Coast fever.

Conclusion.—The adults of *Rhipicephalus appendiculatus* which, as engorged nymphae, were collected from cow 455, were capable of transmitting East Coast fever.

EXPERIMENT No. 3.

To note the effect of exposing engorged nymphae of Rhipicephalus appendiculatus *infected with* Piroplasma parvum, *to a temperature of* 0 *C.*

Some of the engorged nymphae of *Rhipicephalus appendieulatus*, which had been collected from cow 455 on the 14th June, were exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July, 1907. Twelve days later—31st July—they hatched.

(*a*) *Ox* 471.—Infested on the 23rd September with four of these adult *Rhipicephalus appendiculatus* (fifty-three days old). *Piroplasma parvum* appeared from the 23rd to 33rd days, on which latter date the beast died.

Post-mortem examination revealed all the typical lesions of East Coast fever.

(*b*) *Calf* 441.—Infested on the 22nd October, 1907, with eight *Rhipicephalus appendiculatus* adults of the same brood which had been exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July. (At the date of infestation these adults were ninety-five days old.)

Piroplasma parvum appeared from the 10th to 19th days, and the beast died on the 11th November.

Post-mortem examination revealed all the typical lesions of East Coast fever.

Conclusions.—(1) Engorged nymphae of *Rhipicephalus appendiculatus*, when exposed to a temperature of 0 C., do not hatch as quickly as under normal conditions.

Of the engorged nymphae collected on the 14th June, those exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July —twenty-one days—hatched on the 31st July, or forty-six days after collection, whilst those kept in the ordinary Petri dishes at a normal temperature hatched on the 16th July, or thirty-two days after collection.

(2) Engorged nymphae of *Rhipicephalus appendiculatus*, collected from a beast suffering from East Coast fever, and exposed to a temperature of 0 C. for half an hour daily for twenty-one days, were capable of transmitting *Piroplasma parvum* as adults; in one case these adults were fifty-three days old, and in the other ninety-five days old.

EXPERIMENT No. 4.

With larvae of Rhipicephalus decoloratus (*the blue tick*).

(*a*) Larval ticks exposed to a temperature of —18 C.

Date.	Time kept at this Temperature.	Result.
July 3, 1908	15 minutes ..	None died.
,, 3, ,, ...	25 ,, ..	Majority died.
,, 3, ,, ...	30 ,, ..	All died.
,, 2, ,, ...	60 ,, ..	,,

(*b*) Larval ticks of *Rhipicephalus decoloratus* exposed to a temperature of—5 C.

Date.	Time kept at this Temperature.	Result.
July 5, 1908	5 hours ..	None died.
„ 10, „	10 „ ..	„
„ 11, „	24 „ ..	„
„ 12, „	48 „ ..	Majority died.

Conclusions.—(1) Larval ticks of *Rhipicephalus decoloratus* die when exposed for thirty minutes to a temperature of — 18 C.

(2) Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of — 5 C. for twenty-four hours.

RÉSUMÉ.

1. A temperature of 0 C. retards the hatching of *Rhipicephalus appendiculatus* nymphae into adults.

2. A temperature of 0 C. does not interfere with the development of the parasite within the engorged nymphae.

3. A temperature of 0 C. does not kill the virus contained in engorged nymphae of *Rhipicephalus appendiculatus*.

4. Larval ticks of *Rhipicephalus decoloratus* die within thirty minutes when exposed to a temperature of —18 C.

5. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of —18 C. for fifteen minutes.

6. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of —5 C. for twenty-four hours.

7. The majority of larval ticks of *Rhipicephalus decoloratus* die when exposed to a temperature of —5 C. for forty-eight hours.

C.—FURTHER EXPERIMENTS WITH BILIARY FEVER IN EQUINES.

Continuing on the lines mentioned in my previous report, numbers of horses and mules were inoculated during the past year against piroplasmosis, and in the majority of cases I utilised donkey foal blood of the fourth, fifth, and sixth generations.

EXPERIMENT No. 12—(*continued*).

Fourth Generation.

Argentine horses injected with blood of a Transvaal donkey foal (third generation).

67. *Horse* 2845.—Injected on the 3rd July, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.
    Typical reaction from the 7th day.
    Piroplasms noted from the 14th day.

68. *Horse* 2840.—Injected on the 3rd July, 1907, subcutaneously with 5 c.c. blood of donkey foal 2564.
    Reaction from the 8th day.
    Piroplasms noted from the 11th day.

69. *Horse* 2975.—Injected on the 15th August, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.
    Reaction from the 7th day.
    Piroplasms noted from the 10th day.

Results.—Of three Argentine horses injected with blood of a Transvaal donkey foal (third generation) all reacted and recovered.

EXPERIMENT No. 13—(*continued*).

Fifth Generation.

Argentine horses injected with blood of Transvaal donkey foal immune against Piroplasma equi.