

Progress Report on the Possibility of Vaccinating Cattle against East Coast Fever.

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THE following progress report is in continuation of the experiments enumerated in my previous report*, when I demonstrated that it was possible to immunize a certain number of cattle against East Coast fever.

As it appeared from my previous investigations that the animal which supplied the material for inoculation had some connection with the results, I am giving the new experiments in chronological order, sub-divided according (1) to the animal from which the material was obtained, and (2) to the method of injection.

In all these experiments the lymphatic glands or spleen were either (1) ground in a Latapie apparatus (medium and fine grain), or (2) ground in an ordinary mincing machine (coarse or large grain), or (3) chopped with a knife into pieces of about $\frac{1}{2}$ c.c. (chopped or lumps).

It appeared from previous experiments that a successful transmission was only possible when pieces of organs or tissues were introduced into the animal, and naturally the next step was to find out the smallest size of pieces of tissue that could be used to convey the disease; finally, a practical method had to be adopted to introduce such pulp into the system. Hence the four sizes of grain used in the experiments, and the different ways of application.

In previous investigations it was found that a piece of tissue containing the plasma bodies could start the infection, hence it was concluded that embolism produced in some internal organs would have the same effect. In order to realize that object, a number of experiments were made with different material added to the pulp taken from the sick body, or with foreign material of which it was thought that it would cement the pulp together. At the same time it was also considered desirable to produce some irritation on the place where the pulp would be retained, and thus cause an influx of leucocytes and ensure a speedy resorption of the material. It was particularly with this object in view that peptone and aleuronat were used.

I was well aware that in conducting such experiments, embolism would lead to a number of accidents, but in order to see to what extent these accidents would occur, experiments were made with pulp which to the naked eye appeared clean and not contaminated, but which on microscopic examination contained either bacilli or cocci.

Pulp from animals killed at different stages of the disease was used in order to ascertain the day when such material would be most suitable, and would confer the disease or immunity on the largest number of animals.

For the purpose of thoroughly testing the surviving cattle on their immunity, recourse was taken to experimental tick infestation at the Laboratory, and later exposure on a farm in Natal, near Dundee, known as "Burnside". The following extract is from a letter sent to me by Mr. Wm. Power, Acting

* Annual Report Govt. Vet. Bacteriologist, 1909-1910.

Assistant Principal Veterinary Surgeon, Natal, and it gives a good idea of the degree of infection present:—"The disease first broke out on the farm Burnside on the 13th May, 1910, and cattle continued to die until the following November, about 150 head of cattle having died during that time. The farm is on the slopes of a large hill, and deep valleys, and is grossly tick-infected. At the time your cattle were sent down, you could not, I think, have obtained more grossly infected veld anywhere."

In order to make quite certain that the farm was infected at the time the cattle were exposed, seven control animals were purchased and placed on the farm on the 13th January, 1911; all showed *Theileria parva* in their blood by the 15th day, and had died of East Coast fever on the 6th March (24th day).

A further batch of four animals were exposed on the 10th April, 1911, and these had all died of East Coast fever by the 28th day.

EXPERIMENT No. 1.

TO NOTE THE EFFECT OF THE INJECTION OF MATERIAL TAKEN FROM COW 1011.

NOTE.—Cow 1011 was infested on the 27th June, 1910, with twenty brown nymphae off cattle Nos. 923, 917, and 700 (Reference Nos. 268, 335, and 309).

Temperature.—After an incubation time of thirteen days the temperature rose, to reach 105·8° F. twenty-four hours later. Three days afterwards the maximum of 106·8° F. was recorded, immediately followed by a remission in the temperature to 101·4° F. in the morning of the 21st day, when the animal was killed. (18th July, 1910.)

Microscopical Examination.—The lymphatic glands were punctured on the 15th and 18th day; microscopical examination showed the presence of free agamogonous forms of *Theileria parva* in rare numbers on the former date, and both gamogonous and agamogonous forms were noted on the latter date.

The examination of the blood proved the presence of small piroplasms on the 17th and 18th days in rare numbers. On the date of slaughter piroplasms and plasma bodies were both noted to be present in fairly large numbers.

The examination of the mesenteric glands and of the spleen on *post-mortem* proved both gamogonous and agamogonous forms to be very frequent.

NOTE.—The temperature reaction from the 13th to 21st days represent the first part of the East Coast fever reaction, and the animal was killed when the remission occurred.

(A).—INTRATHORACAL INJECTIONS.

(a) *Injections on the 18th July, 1910, with 30 c.c. spleen pulp (medium grain) of Cow 1011.*

(A).—*Heifer 831, about four years old, a Cape Province animal, which arrived at the Laboratory in April, 1909.*

NOTE.—This animal had been used previously on three occasions without contracting the disease, namely: On the 15th December, 1909, for an intraperitoneal injection of spleen pulp of cow 594 (Annual Report, 1909–10, p. 44); on the 14th April, 1910, for an intralymphal injection of gland juice of calf 917 (Annual Report, 1909–10, p. 47); and on the 3rd June, 1910, for an intrajugular injection of 10 c.c. gland juice of heifer 897 (Annual Report, 1909–10, p. 49).

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A reaction set in two days after inoculation of an irregular nature without any high records. From the 17th day onwards the temperature rose slightly, but there were no indications of a definite fever reaction.
- (b) Microscopical examination of blood: Leucocytes and lymphocytes were noted on the 18th to 23rd days. Puncture of the lymphatic glands on the 15th, 19th, 21st, and 22nd days gave negative results.

Immunity Test.—Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); heifer 1082 did not contract East Coast fever from the infestation of twenty-three ticks of the same batch.]

Remarks.—All ticks were fast the following day. The animal died on the 7th day from gangrenous pneumonia.

(b) *Injections on the 18th July, 1910, with 20 c.c. spleen pulp (coarse grain) of Cow 1011.*

(B).—Heifer 833, about three years old, a Cape Province animal, which arrived at the Laboratory in April, 1909.

NOTE.—This animal had been used previously on three occasions without contracting the disease, namely: On the 3rd February, 1910, for an intraperitoneal injection of glands of bull 458 (Annual Report, 1909–10, p. 45); on the 14th April, 1910, for an intralymphal injection of gland juice of calf 917 (Annual Report, 1909–10, p. 47); and on the 3rd June, 1910, for an intralymphal injection of gland juice of heifer 897 (Annual Report, 1909–10, p. 48).

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A reaction started a few days after inoculation with a maximum temperature of 104° F., but it could not be considered typical of East Coast fever.
- (b) Microscopical examination of blood: Polynuclear neutrophile leucocytosis, and eosinophilia were noted on the 20th day. Puncture of the lymphatic glands on the 16th and 24th days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); heifer 1082 did not contract East Coast fever from the infestation of twenty-three ticks of the same batch.]

Remarks.—Forty-five ticks were fast the following day. No reaction followed, and the microscopical examinations of the blood on the 6th day gave negative results.

- (2) Infested on the 15th September, 1910, with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment 25 (b). It is doubtful if these ticks were infective.]

Remarks.—All twenty ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 30th September, 1910, with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356.) [*Note.*—See Experiment No. 25 (h); heifer 1107 did not contract East Coast fever from the infestation of fifteen ticks of the same batch.]

Remarks.—Twelve ticks were fast the following day. No temperature reaction followed; microscopical examinations of the blood on the 11th day showed *Babesia bigemina*; examination of the gland juice on the same day proved negative.

- (4) Infested on the 20th October, 1910, with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—All six ticks were fast the following day; from the 19th to the 32nd day there was a definite reaction, commencing with a maximum temperature of 105° F., but later oscillating between 102° and 104° F. All microscopical examinations gave negative results, and examination of gland juice obtained by puncture on the 23rd, 25th, and 26th days also proved negative.

- (5) Infested on the 16th November with four brown adults, origin Natal (Reference No. 349). (*Note.*—See above.)

Remarks.—No ticks were found the following day, and no temperature reaction followed.

- (6) Infested on the 8th December with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Five ticks were fast the following day; no temperature reaction followed.

- (7) Infested on the 14th December with brown adults off heifers 1053 and 1111 (Reference Nos. 411 and 426). [*Note.*—See Experiments No. 25 (d) and 25 (e); heifers 1157 and 1158 contracted East Coast fever from the infestation of ticks of the same batches.]

Remarks.—Two ticks were fast the following day; no temperature reaction followed.

- (8) Exposed on the farm Burnside, in Natal, on the 7th January, 1911, and was still alive on the 31st August, 1911.

(c) *Injections on the 18th July, 1910, with 15 grammes spleen pulp, chopped, of Cow 1011.*

(C).—*Heifer 896, about three years old, purchased in the Transvaal, history unknown.*

NOTE.—This animal had been previously used on three occasions without contracting the disease, namely: On the 13th December, 1909, for an intralymphal insertion of glands of cow 677 (Annual Report, 1909–10, p. 49); on the 14th April, 1910, for an intralymphal injection of gland juice of calf 917 (Annual Report, 1909–10, p. 47); and on the 3rd June, 1910, for an intralymphal injection of gland juice of heifer 897 (Annual Report, 1909–10, p. 48).

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction followed three days after injection, reaching 103·8° F. in the evening, and returning to normal on the 11th day.

(b) Microscopical examination of blood: Anisocytosis was noted on the 15th and 23rd days. Eosinophile cells were seen on the 23rd day. Puncture of the lymphatic glands on the 15th and 23rd days gave negative results.

Immunity Tests.—

- (1) Infested on the 20th October, 1910, with brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b). It is doubtful whether these ticks were infected.]

Remarks.—Ten ticks were fast the following day. No reaction followed.

- (2) Infested on the 16th November with four brown adults from Natal. (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Four ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 8th December with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—One tick was fast the following day. No temperature reaction followed.

- (4) Infested on the 14th December with two brown adults off heifer 1053 and two off heifer 1111 (Reference Nos. 411 and 426). [*Note.*—See Experiment Nos. 25 (d) above and 25 (i); heifer 1158 contracted East Coast fever from the infestation of ten ticks of Ref. No. 426.]

Remarks.—Two ticks were fast the following day. No temperature reaction followed.

- (5) Exposed on the 7th January, 1911, on the farm Burnside, near Dundee, Natal.

Remarks.—

- (a) Temperature: Soon after exposure the temperature began to rise, reaching 105° F. on the 6th day; on this particular date the animal was noted to be badly infested with ticks, the head and ears of the heifer being very much swollen. Up to the 17th day, the temperature oscillated between 101° in the morning and 105° in the evening.

The animal died on the 17th day of East Coast fever.

- (b) Microscopical examination of the blood: Revealed the presence of parasites, and in the glands and spleen, plasma bodies were detected.

- (d) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (medium grain) of Cow 1011.*

D).—Heifer 1014, about three years old, a Cape Province animal, which arrived at the Laboratory in March, 1910.

NOTE.—This animal had been used previously without contracting the disease, i.e. on the 3rd June, 1910, for an intrajugular injection of gland juice of heifer 897 (Annual Report, 1909–10, p. 49).

Treatment.—As above.

Remarks.—

- (a) Temperature: An irregular reaction followed immediately after injection, with one exacerbation on the 7th day to 104° F.; a second reaction followed from the 14th to 21st days.

- (b) Microscopical examination of blood: *Babesia bigemina* and leucocytosis were noted on the 7th and 18th days respectively. Lymphocytosis and eosinophilia were seen on the 19th and 24th days respectively. Puncture of the lymphatic glands on the 15th, 18th, and 24th days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [Note.—See Experiment No. 25 (a); heifer 1082 did not contract East Coast fever from the infestation of twenty-three ticks of the same batch.]

Remarks.—Twenty-four ticks were fast the following day. No reaction followed. All microscopical examination gave negative results.

- (2) Infested on the 15th September, with twenty brown nymphae off heifer 908 (Reference No. 364). [Note.—See Experiment 25 (b); it is doubtful if these ticks were infective.]

- (3) Infested on the 30th September with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [Note.—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—

(a) Temperature: Ten ticks were fast the following day; no definite temperature reaction followed.

(b) Microscopical examination of the blood gave negative results, and examination of the gland juice on the 11th day also proved negative.

- (4) Infested on the 20th October, with six brown adults from Natal (Reference No. 349). [Note.—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Five ticks were fast the following day. Temperature reaction from the 17th day, typical for East Coast fever. *Theileria parva* appeared in the blood for the first time on the 23rd day; plasma bodies (both forms) were noted in the glands on the 21st day. The animal died of East Coast fever on the 32nd day.

- (e) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (coarse grain) of Cow 1011.*

- (E).—Ox 1066, aged; purchased in the Transvaal; history unknown.

NOTE.—Previously used on the 3rd June, 1910, without result (*vide* Annual Report, 1909–10, p. 48), for an intralymphal injection of 5 c.c. gland juice of heifer 897.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A slight irregular reaction followed the injection with a maximum record of 103.4° F. on the 5th day. After the 20th day, the temperature became very irregular, with evening readings of 105° F., but the reaction was not typical for East Coast fever.

(b) Microscopical examination of blood: Eosinophilia was noted on the 20th and 23rd day; polynuclear leucocytosis was also seen on the 23rd day. Puncture of the lymphatic glands on the 15th, 23rd, and 26th days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—About seventy ticks were fast the following day. No reaction followed.

- (2) Infested on the 15th September with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Twenty ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 30th September with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—Eighteen ticks were fast the following day. No temperature reaction followed. All microscopical examinations of the blood gave negative results, and examination of the gland juice on the 11th day also proved negative.

- (4) Infested on the 20th October, 1910, with six brown adults from Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—All six ticks were fast the following day. The temperature reaction started on the 14th day with evening exacerbations and morning remissions for some days after; normal limits were reached on the 23rd day.

Microscopical examination of gland juice obtained by puncture on the 15th and 21st days showed plasma bodies (agamogonous forms).

- (5) Infested on the 16th November with four brown adults from Natal (Reference No. 349). (*Note.*—See above.)

Remarks.—Two ticks were fast the following day. No temperature reaction followed.

- (6) Infested on the 8th December with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—All six ticks were fast the following day. No temperature reactions followed.

- (7) Infested on the 14th December with two brown adults off heifer 1053 (Reference No. 411), and two off heifer 1111 (Reference No. 426). [*Note.*—See Experiment Nos. 25 (d) above and 25 (e); heifer 1158 contracted East Coast fever from the infestation of ten ticks of batch No. 426.]

Remarks.—All ticks were dead the next day. No temperature reaction followed.

- (8) Exposed on the farm Burnside, Natal, on the 7th January, 1911, and died on the 2nd April, 1911 (85th day) of heartwater. Microscopical examination of the spleen and glands gave negative results.

(f) *Injections on the 18th July, 1910, with 20 grammes mesenteric lymphatic glands (chopped) of Cow 1011.*

(F).—*Bull 1071, aged; purchased in the Transvaal; history unknown.*

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An irregular reaction followed, lasting for about a week; another irregular reaction followed from the 14th day, but without any definite characteristics.
- (b) Microscopical examination of blood: Strong lymphocytosis was noted on the 20th day; eosinophilia was seen on the 24th day. Puncture of the lymphatic glands on the 16th and 24th days gave negative results. The animal was killed on the 16th August on account of poverty (29th day).

(B).—INTRALYMPHAL INJECTIONS.

(g) *Injections on the 18th July, 1910, with 20 c.c. spleen pulp (medium grain) of Cow 1011.*

(G).—*Bull 1039, aged; purchased in the Transvaal; history unknown.*

NOTE.—This animal was used previously on the 4th May, 1910 (*vide Annual Report, 1909–10, p. 48*), for an intralymphal injection of 3 c.c. gland juice of cow 596, without results.

Treatment.—As above.

Remarks.—

- (a) Temperature: A sharp rise was noted for a few days soon after and a swelling formed at the seat of injection.
- (b) Microscopical examination of blood: Lymphocytosis and leucocytosis were noted on the 15th day. Puncture of the lymphatic glands on the 15th and 24th days gave negative results.

NOTE.—Used on the 22nd August, 1910, in Experiment No. 5 (f) for an intrajugular injection of 20 c.c. spleen and glands of heifer 908; later used on the 15th November, 1910, for an intrajugular injection of spleen pulp of heifer 1107 [*vide Experiment 7 (b)*]; tested with ticks on two occasions, and finally exposed at Burnside on the 7th January, 1911, and died of East Coast fever twenty-seven days later.

(h) *Injections on the 18th July, 1910, with 20 c.c. spleen pulp (coarse grain) of Cow 1011.*

(H).—*Ox 1037, aged; purchased in the Transvaal; history unknown.*

NOTE.—Previously used on the 25th May, 1910, for an intralymphal injection of gland juice of heifer 928 without result (*vide Annual Report, 1909–10, p. 48*).

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight swelling was noticed at the seat of injection accompanied with a slight temperature disturbance.
- (b) Microscopical examination of blood: Eosinophilia was noted on the 15th day. Puncture of the lymphatic glands on the 15th and 22nd days gave negative results.

Immunity Test.—Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty-seven ticks were fast the following day. No reaction followed.

NOTE.—Used on the 27th October, 1910 [*vide* Experiment No. 6 (g)], for grafting of supramammary gland of heifer 1053, when small piroplasms were noted. Used again on the 3rd January, 1911 [*vide* Experiment No. 8 (u)] for intrathoracal injection of 20 c.c. spleen pulp of ox 179 without result. Finally tested on the 30th January, 1911, with brown nymphae off heifer 909; contracted East Coast fever and died on the 23rd day.

(i) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (medium grain) of Cow 1011.*

(I).—*Bull 1045, about three years old; purchased in the Transvaal; history unknown.*

Treatment.—Injected as above.

Remarks.—

(a) Temperature: Slight reaction for a few days after, probably the result of the swelling which formed at the seat of injection.

(b) Microscopical examination of blood: Eosinophilia and polynuclear neutrophile leucocytosis were noted on the 15th day. Puncture of the lymphatic glands on the 15th, 21st, and 24th days gave negative results.

Immunity Test.—Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty-five ticks were fast the following day. No reaction followed.

NOTE.—Used later on two occasions without contracting the disease, namely: On the 27th October, 1910, for an intrasplenic injection of spleen pulp of heifer 1053 [Experiment No. 6 (p)]; and on the 3rd January, 1911, for an intrathoracal injection of 20 c.c. spleen pulp of ox 179 [*vide* Experiment No. 8 (v)], and died fifteen days later of toxaemia.

(j) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (coarse grain) of Cow 1011.*

(J).—*Ox 1024, aged; purchased in the Transvaal; history unknown.*

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction followed almost immediately after injection, but of an irregular character, reaching normal limits again on the 14th day.

(b) Microscopical examination of blood: Negative. Puncture of the lymphatic glands on the 11th, 17th, and 24th days also gave negative results.

Immunity Test.—Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Forty-one ticks were fast the following day. No reaction followed. Microscopical examinations gave negative results, and examination of gland juice obtained by puncture on the 17th day also proved negative. The animal died on the 2nd October of poverty and enteritis.

(C).—INTRAJUGULAR INJECTIONS.

(k) *Injections on the 18th July, 1910, with 20 c.c. spleen pulp (medium grain) of Cow 1011.*

(K).—*Cow 1068, aged; purchased in the Transvaal; history unknown.*

NOTE.—Used on the 3rd June, 1910 (*vide* Annual Report, 1909–10, p. 49), for an intralymphal injection of 5 c.c. gland juice of heifer 897 without result.

Remarks.—

(a) Temperature: An immediate reaction followed, lasting until the 9th day, but the evening temperatures did not exceed 103° F.

(b) Microscopical examination of blood: Negative. Puncture of the lymphatic glands on the 19th and 24th days also gave negative results.

Immunity Tests.—

(1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See experiment No. 25 (a); these ticks were not infective.]

Remarks.—About seventy ticks were fast the following day.

(2) Infested on the 15th September with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Nineteen ticks were fast. No temperature reaction followed.

(3) Infested on the 30th September with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—Fourteen ticks were fast the following day. No temperature reaction followed. Microscopical examinations of the blood gave negative results, and examination of the gland juice on the 11th day also proved negative.

(4) Infested on the 20th October with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Five ticks were fast the following day. A temperature reaction followed from the 16th day typical for East Coast fever. *Theileria parva* appeared in the blood for the first time on the 18th day; plasma bodies (agamogenous forms) were noted in the glands on the same day. The animal died of East Coast fever on the 31st day.

(l) *Injections on the 18th July, 1910, with 20 c.c. spleen pulp (coarse grain) of Cow 1011.*

(L).—*Ox 1020, about two and a half years old; purchased in the Cape Province, and arrived at the Laboratory in March, 1910.*

NOTE.—Previously used on the 25th May, 1910 (*vide* Annual Report, 1909–10, p. 48), for an intralymphal injection of 10 c.c. gland juice of heifer 928 without result.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An immediate reaction followed the injection, reaching 105° F. on the evening of the 2nd and 5th days; the temperature was very irregular up to the 30th day.
- (b) Microscopical examination of blood: *Babesia bigemina* was noted on the 11th and 15th days. Eosinophilia was present on the 11th day. Puncture of the lymphatic glands on the 11th, 15th, 18th, 19th, and 24th days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty ticks were fast the following day. No reaction followed.

- (2) Infested on the 15th September with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Twenty ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 30th September, with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); it is doubtful if these ticks were infected.]

Remarks.—Seventeen ticks were fast the following day. An irregular reaction followed, but in no way typical for East Coast fever. Microscopical examinations of the blood showed *Anaplasma marginale* on the 11th day, and examination of the gland juice on the same day proved negative.

- (4) Infested on the 20th October with six brown adults, origin Natal. (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Four ticks were fast the following day. Again a slight irregular temperature reaction followed. Small piroplasms appeared in the blood on the 18th day, but examination of the gland juice on the same day proved negative.

- (5) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). (*Note.*—See above.)

Remarks.—Four ticks were fast the following day. No temperature reaction followed.

- (6) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Five ticks were fast the following day. No temperature reaction followed.

- (7) Infested on the 14th December with two brown adults off heifer 1053 and two off heifer 1111 (Reference Nos. 411 and 426). [*Note.*—See Experiments Nos. 25 (d) and 24 (i); heifer 1158 contracted East Coast fever from the infestation of ten ticks of batch No. 426.]

Remarks.—One tick was fast the following day. No temperature reactions followed.

- (8) Exposed on the 7th January, 1911, on the farm Burnside, Natal, and died on the 29th day, the cause of death being put down to extreme tick irritation and resulting poverty. This diagnosis was supported by the examination of the blood, which showed anisocytosis and poikilocytosis. Plasma bodies could not be found in the glands or spleen.

(m) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (medium grain) of Cow 1011.*

(M).—*Heifer 911, about three years old, purchased in the Cape Province, and arrived at the Laboratory in November, 1909.*

NOTE.—Previously used on the 25th May, 1910 (*vide* Annual Report, 1909–10, p. 49), for an intrajugular injection of 10 cc. gland juice of heifer 928 without result.

Treatment.—Injected as before.

Remarks.—

- (a) Temperature: A slight temperature reaction followed immediately after injection, regaining normal limits on the 10th day; the temperature oscillated from the 14th to 20th days, but never exceeded 103° F.
- (b) Microscopical examination of blood: Negative results on the 13th, 17th, and 24th days. Puncture of the lymphatic glands on the 18th and 25th days also proved negative.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—About seventy ticks were fast the following day. No reaction followed.

- (2) Infested on the 15th September, with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Twenty ticks were fast the following day. No temperature reaction followed. All microscopical examinations gave negative results.

- (3) Infested on the 30th September with brown nymphae off heifers Nos. 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—Twenty ticks were fast the following day. No temperature reaction followed. All microscopical examinations of the blood gave negative results, with the exception of a slight leucocytosis on the 11th day. Examination of the gland juice on the 11th and 13th days proved negative.

- (4) Infested on the 20th October with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Four adults were fast the following day. Temperature reaction occurred from the 4th day. *Theileria parva* appeared in the blood for the first time on the 9th day; plasma bodies were noted in the glands on the same day. The animal died of East Coast fever on the 17th day.

(n) *Injections on the 18th July, 1910, with 20 c.c. gland pulp (coarse grain) of Cow 1011.*

(N).—*Heifer 627, about three years old; purchased in the Cape Province, and arrived at the Laboratory in July, 1908.*

NOTE.—Used on the 25th May, 1910 (*vide* Annual Report, 1909–10, p. 49), for an intrajugular injection of gland juice of heifer 928 without result.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Reaction started immediately after injection with a maximum temperature of 105° F. on the 2nd day, and gradually descending until normal limits were reached on the 10th day.
- (b) Microscopical examination of blood: Lymphocytosis and eosinophilia were noted on the 21st day. Puncture of the lymphatic glands on the 15th and 21st days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty ticks were fast the following day. No reaction followed.

- (2) Infested on the 15th September with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment 25 (b); it is doubtful if these ticks were infective.]

Remarks.—All ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 30th September with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—Fourteen ticks were fast the following day. A slight irregular reaction followed, with a maximum record of 102·2° F. All microscopical examinations of the blood and examinations of the gland juice on the 11th day proved negative.

- (4) Infested on the 20th October with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Temperature reaction from the 16th day. *Theileria parva* appeared in the blood for the first time on the 25th day; plasma bodies were not noted in the glands on the 18th day. The animal died on the 38th day, and from the *post-mortem* examination, East Coast fever had to be considered responsible.

SUMMARY OF EXPERIMENT NO. 1, With Material from Cow 1011.

NUMBER OF DAYS WHICH ELAPSED BETWEEN INFESTATION OF ANIMAL WITH TICKS AND DEATH: 21.

NUMBER OF DAYS WHICH ELAPSED BETWEEN THE DATE OF THE FIRST RISE OF TEMPERATURE AND DEATH: 8.

DETAILS OF INJECTIONS AND RESULTS.

DETAILS OF TESTS ON IMMUNITY.

Ref. No.	Animal injected.	No. of times the animal was injected previously or subsequently.	References to these injections.	Method of injection.	Quantity injected.	Material injected.		Result.	No. of times tested with ticks.	Result.		Result of exposure at Burnside.	REMARKS.
						Pulp of	Grain.			No. of tick infestation.			
A.	Heifer 831	3	A. R. 1909-10	Intrathoracal	30 c.c.	Spleen	Medium	I.R.	1	—	† 7th day G.P.	—	
B.	Heifer 833	3	" "	"	20 c.c.	"	Coarse	I.R.	7	4	R.R.	Still alive.	
C.	Heifer 896	3	" "	"	15 grs.	"	Chopped	I.R.	4	—	N.R.	R.P. † 17th day.	
D.	Heifer 1014	1	" "	"	20 c.c.	Gland	Medium	I.R.	4	4	R.P. †	—	
E.	Ox 1066	1	" "	"	"	"	Coarse	I.R.	7	4	R.P.R.	† O.C. 85th day	Cause of death attributed to heartwater.
F.	Bull 1071	—	—	"	20 grs.	"	Chopped	I.R.	—	—	—	—	This bull was killed on account of poverty on the 29th day.
G.	Bull 1039	3	A. R. 1909-10; Expts. 5 P & 7 B	Intralymphal	20 c.c.	Spleen	Medium	N.R.	—	—	—	—	See Experiment 7 B for final history (R.P. † at Burnside).
H.	Ox 1037	3	A. R. 1909-10; Expts. 6 Q & 8 U	"	"	"	Coarse	I.R.	—	—	—	—	See Experiment 8 U for final history (R.P. † to ticks).
I.	Bull 1045	2	Expts. 6 P & 8 V	"	"	Gland	Medium	N.R.	—	—	—	—	See Experiment 8 V for final history († toxæmia).
J.	Ox 1024	—	—	"	"	"	Coarse	I.R.	1	—	N.R.	—	Died on 27th day of poverty and enteritis.
K.	Cow 1068	1	A. R. 1909-10	Intrajugular	"	Spleen	Medium	I.R.	4	4	R.P. †	—	
L.	Heifer 1020	1	" "	"	"	"	Coarse	I.R.	7	3 & 4	I.R.	† 29th day of poverty	
M.	Heifer 911	1	" "	"	"	Gland	Medium	I.R.	4	4	R.P. †	—	
N.	Heifer 627	1	" "	"	"	"	Coarse	I.R.	4	4	R. †	—	No plasma bodies seen, but from <i>post-mortem</i> examination death was due to East Coast fever.

EXPLANATION OF SYMBOLS.

R.P. †.—Indicates that the animal had a reaction, accompanied with the presence of plasma bodies, and died of East Coast fever

R.P.R.—Indicates that the animal had a reaction, accompanied with the presence of plasma bodies, and recovered.

P. †.—Indicates that the animal died before the disease had run its course, but that plasma bodies were found in the spleen or glands after death.

R.R.—Indicates that the animal had a reaction and recovered, but that plasma bodies were not detected.

I.R.—Indicates that the animal had an irregular reaction and recovered.

N.R.—Indicates that the animal did not react to the injection.

† O.C.—Indicates that the animal died later of other causes, and that East Coast fever could not be considered to be in any way responsible for death.

† G.P.—Indicates that the animal died of gangrenous pneumonia.

RESULTS.

Of fourteen animals injected with material taken from cow 1011 (which was killed twenty-one days after tick infestation, or eight days after the first rise of temperature), none showed a definite reaction, and plasma bodies could not be traced in the lymphatic glands in a single case. The majority showed irregular reactions, and of the thirteen animals exposed to tick infestation or natural infection, eight contracted the disease, and seven of these died of East Coast fever. The surviving animal had been injected intrathoracally with spleen pulp of cow 1011, but as it had been used on three other occasions, it is difficult to say which injection conferred immunity.

Attention must be drawn to the fact that of four animals injected intralymphally, two died of East Coast fever when tested, and two from other causes; of six animals injected intrathoracally, three died of East Coast fever when tested, one died of poverty, one died of gangrenous pneumonia, and one survived; of four animals injected intrajugularly, three died of East Coast fever when tested, and one died of poverty on the 29th day after exposure.

EXPERIMENT No. 2.

TO NOTE THE EFFECT OF THE INJECTION OF MATERIAL OBTAINED FROM HEIFER 913.

NOTE.—Heifer 913 had been infested on the 29th June, 1910, with twenty brown nymphae off cattle 923, 917, and 700 (Reference Nos. 268, 335, and 309). On the 13th day the temperature rose to 105·4° F. in the evening, remaining high for the next six days, from which date a gradual descent occurred, and 100·6° F. was recorded in the morning of the 24th day. The heifer was killed on this date.

The examination of the lymphatic glands on the 14th day proved the presence of free agamonts in rare numbers, and two days later small piroplasms were noted in the blood, increasing in numbers daily and reaching their maximum frequency on the date of slaughter.

This temperature curve represents the first half of the East Coast fever reaction, and is a typical one in every respect; the animal was killed at the time of the remission of the temperature.

(A).—INTRATHORACAL INJECTIONS.

(a) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (fine grain) of Heifer 913.*

(A).—*Bull 1051, aged; purchased in the Transvaal; history unknown.*

NOTE.—Had been previously used on the 17th May, 1910, for an intralymphal injection of gland juice of heifer 1018, without result (Annual Report, 1909-10, p. 48).

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction ensued the day after injection, reaching 105° F. on the 5th day, with very low morning records at the commencement; from the 10th day the temperature gradually descended, reaching normal limits on the 24th day.

(b) Microscopical examination of blood; Polynuclear leucocytosis was noted on the 6th day, and at frequent intervals afterwards. *Babesia bigemina* were noted on the 9th and 10th days. Anisocytosis was seen on the 17th day. Puncture of the lymphatic glands on the 11th and 21st days gave negative results. The animal died on the 32nd day of purulent pneumonia.

(b) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (coarse grain) of Heifer 913.*

(B).—*Ox 1046, aged; purchased in the Transvaal; history unknown.*

NOTE.—This heifer had been previously used on the 17th May, 1910 (*vide Annual Report, 1909-10, p. 48*), for an intralymphal injection of gland juice of heifer 1018, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A reaction set in on the 2nd day reaching 104·6 F., but returning to normal limits on the 8th day; a second reaction began on the 10th day, reaching its maximum record of 105·4 on the 18th day, the temperature oscillating between 102 and 103·5 for the next few days and returning to normal on the 23rd day.
- (b) Microscopical examination of blood; Anisocytosis was noted on the 6th and 19th day, eosinophilia and lymphocytosis were seen on the 19th day. Puncture of the lymphatic glands on the 13th and 18th days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty-eight ticks were fast the following day; no reaction followed.

- (2) Infested on the 20th October, 1910, with brown nymphae off heifer 908. (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Eight ticks were fast the following day; no temperature reaction followed.

- (3) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Four ticks were fast the following day; no temperature reaction followed.

- (4) Infested on the 14th December, 1910, with two brown adults off heifer 1053 and two off heifer 1111 (Reference Nos. 411 and 426). [*Note.*—See Experiment No. 25 (d) above and 25 (i); both lots of ticks were infective.]

Remarks.—Temperature reaction from the 16th day after infestation No. 3, or ten days after infestation No. 4. *Theileria parva* appeared in the blood for the first time on the 21st day; plasma bodies were noted in the glands on the 21st day after last infestation. The animal died of East Coast fever on the 8th January, thirty-one days after 3rd infestation, and twenty-five days after last infestation.

(c) *Injections on the 22nd July, 1910, with 15 grs. spleen pulp (chopped) of Heifer 913.*

(C).—*Cow 1030, aged; purchased in the Transvaal; history unknown.*

NOTE.—This animal had been previously used on the 4th May, 1910, for an intralymphal injection of gland juice of cow 596 (*Annual Report, 1909-10, p. 47*), without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Slight reaction from the 16th to 25th days, with an average evening record of 102° F.
- (b) Microscopical examination of blood: Negative results on the 18th day. Puncture of the lymphatic glands on the same day also gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Thirty ticks were fast the following day; a slight reaction followed from the 9th to 18th days, with a maximum evening record of 104° F., the morning temperature remaining almost constantly at 101° F. Microscopical examination of the blood on the 17th day, and of gland juice obtained by puncture on the same day proved negative.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Eight ticks were fast the following day; no temperature reaction followed; microscopical examinations showed the presence of *Babesia mutans* on the 14th day, and the animal died the following day, the cause of death being attributed to poverty.

(d) *Injections on the 22nd July, 1910, with 20 c.c. gland pulp (fine grain) of Heifer 913.*

(D).—*Bull 1049, aged; purchased in the Transvaal; history unknown.*

NOTE.—This animal had been used previously on the 4th May, 1910, for an intralymphal injection of gland juice of cow 596 (*vide* Annual Report, 1909–10, p. 47), without contracting the disease.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight reaction followed immediately, reaching 103·4° F. on the 2nd day and returning to normal on the 6th day. Irregular temperatures now followed until the 28th day, with occasional evening records of 104° F.
- (b) Microscopical examination of the blood: Lymphocytosis, leucocytosis, and eosinophilia were registered on different occasions between the 8th and 31st days. Puncture of the lymphatic glands on the 11th and 21st days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Twenty-one ticks were fast the following day; an irregular temperature reaction followed.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). *Note.*—See Experiment No. 25 (c); it is doubtful if these ticks were infective.]

Remarks.—Eight ticks were fast the following day; some irregular temperature reactions followed, but nothing typical; microscopical examination on the 13th day showed the presence of anisocytosis.

- (3) Infested on the 16th November, 1910, with ten red leg adults off heifer 923 (Reference No. 253). [*Note.*—See Experiment No. 25 (*f*); these ticks were not infective.]

Remarks.—Eight ticks were fast the following day; an irregular temperature reaction followed, but nothing typical.

- (4) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (*d*); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—All six ticks were fast the following day; no temperature reaction followed.

- (5) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two off heifer 111 (Reference No. 426). [*Note.*—See Experiment No. 25 (*d*) and 25 (*i*); both lots of ticks were infective.]

Remarks.—No temperature reaction followed.

- (6) Exposed at Burnside on the 7th January, 1911, and was still alive on the 31st August, 1911.

(*e*) *Injections on the 22nd July, 1910, with 5 c.c. gland pulp (coarse grain) of Heifer 913.*

(*E*).—Ox 1042, aged; purchased in the Transvaal; history unknown.

NOTE.—Used previously on the 4th May, 1910, for an intrajugular injection of spleen pulp of cow 596, without results (*vide* Annual Report, 1909-10, p. 49).

Treatment.—Injected as above.

Remarks.—

(*a*) Temperature; A reaction followed two days after injection reaching 104° F., with a remission to normal on the 6th day; a slight oscillating record ensued for the next twelve days, but not exceeding 103° F. in the evenings.

(*b*) Microscopical examination of blood: Eosinophilia was noted on the 8th day. Puncture of the lymphatic glands on the 21st and 32nd days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (*a*); these ticks were not infective.]

Remarks.—Twenty-two ticks were fast the following day; no reaction followed.

- (2) Infested on the 20th October, 1910, and 21st October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (*b*); it is doubtful if these ticks were infective.]

Remarks.—Eleven ticks were fast the following day; irregular temperature reactions followed.

- (3) Infested on the 16th November, 1910, with ten red leg adults off heifer 923 (Reference No. 253). [*Note.*—See Experiment No. 25 (*f*); these ticks were not infective.]

Remarks.—Five ticks were fast the following day; no temperature reactions followed.

- (4) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Five ticks were fast the following day; no reaction followed.

- (5) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two off heifer 1111 (Reference No. 426). [*Note.*—See Experiment No. 25 (d), above, and 25 (i); both lots of ticks were infective.]

Remarks.—Three ticks were fast the following day; no temperature reaction followed.

- (6) Exposed on the farm Burnside on the 7th January, 1911, and was still alive on the 31st August, 1911.

(f) *Injections on the 22nd July, 1910, with 15 grs. mesenteric gland pulp (chopped) of Heifer 913.*

(F).—Ox 1043, aged; purchased in the Transvaal; history unknown.

NOTE.—Used previously on the 4th May, 1910 (*vide* Annual Report, 1909–10, p. 47), for an intralymphal injection of gland juice of cow 596, without contracting the disease.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An irregular reaction followed, lasting until the 16th day.
- (b) Microscopical examination of blood: Lymphocytosis and leucocytosis were noted on the 11th day. Puncture of the lymphatic glands on the 11th day gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Thirty-three ticks were fast the following day; no reaction followed.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); these ticks were not infective in several instances.]

Remarks.—Six ticks were fast the following day; temperature reaction from the 12th day going over into a typical East Coast fever curve. *Theileria parva* appeared in the blood for the first time on the 21st day; plasma bodies (both forms) were noted in the glands on the same day. The animal died of East Coast fever on the 29th day.

(B).—INTRALYMPHAL INJECTIONS.

(g) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (fine grain) of Heifer 913.*

(G).—Ox 1038, aged; purchased in the Transvaal; history unknown.

NOTE.—This animal had been used previously on the 4th May, 1910 (*vide* Annual Report, 1909–10, p. 47), for an intralymphal injection of gland juice of cow 596, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Irregular records followed, but nothing typical for East Coast fever.
- (b) Microscopical examination of blood: Eosinophilia was noted on the 19th day. Puncture of the lymphatic glands on the 13th and 19th days gave negative results.

NOTE.—This animal was used subsequently on the 22nd August, 1910 [*vide* Experiment No. 5 (e)], for an intrajugular injection of spleen and gland pulp of heifer 908, and also on the 5th November, 1910 [*vide* Experiment No. 7 (c)], for an intrajugular injection of spleen pulp of heifer 1107, without contracting East Coast fever. Finally infested on the 14th December, 1910, with brown adults, and died on the 22nd day of East Coast fever.

(h) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (coarse grain) of Heifer 913.*

(H).—Ox 1048, aged; purchased in the Transvaal; history unknown.

NOTE.—This animal had been used previously on the 4th May, 1900 (*vide* Annual Report, 1909-10, p. 47), for an intralymphal injection of gland juice of cow 596, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Some irregular records were noted soon after injection.
- (b) Microscopical examination of blood: *B. mutans* in rare numbers were noted on the 17th day. Eosinophilia and slight lymphocytosis were seen on the 18th day. Puncture of the lymphatic glands on the 13th and 18th days gave negative results.

NOTE.—This animal was used subsequently on the 22nd August, 1910 [*vide* Experiment No. 5 (g)], for an intrajugular injection of spleen and gland pulp of heifer 908, and also on the 15th November, 1910 [*vide* Experiment No. 7 (a)], for an intrajugular injection of spleen pulp of heifer 1107, without contracting East Coast fever. Finally tested on the 14th December, 1910, with brown adults [*vide* Experiment No. 7 (a)]; contracted East Coast fever, and died on the 35th day.

(i) *Injections on the 22nd July, 1910, with 20 c.c. gland pulp (fine grain) of Heifer 913.*

(I).—Cow 1032, aged; purchased in the Transvaal; history unknown.

NOTE.—This animal had been previously used on the 4th May, 1910 (*vide* Annual Report, 1909-10, p. 47), for an intralymphal injection of gland juice of cow 596, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Irregular reaction, but in no way typical for East Coast fever.
- (b) Microscopical examination of blood: Lymphocytosis and eosinophilia were noted on the 16th and 19th days respectively. Puncture of the lymphatic glands on the 13th, 18th, and 19th days gave negative results.

NOTE.—This animal was used subsequently on the 22nd August, 1910 [*vide* Experiment No. 5 (*d*)], for an intrajugular injection of spleen and gland pulp of heifer 908, and also on the 15th November, 1910 [*vide* Experiment No. 7 (*f*)], for an intrajugular injection of spleen pulp of heifer 1107, without contracting East Coast fever. Finally tested with brown adult ticks on the 14th December, 1910 [*vide* Experiment No. 7 (*l*)], and died of East Coast fever on the 26th day.

(j) *Injections on the 22nd July, 1910, with 5 c.c. gland pulp (coarse grain) of Heifer 913.*

(J).—*Cow 1023, aged; purchased in the Transvaal; history unknown.*

NOTE.—This animal had been used previously on the 4th May, 1910 (*vide* Annual Report, 1909–10, p. 49), for an intralymphal injection of gland juice of cow 596, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Irregular reactions followed, but not typical for East Coast fever.
- (b) Microscopical examination of blood: Eosinophilia and leucocytosis were noted on the 8th day. Puncture of the lymphatic glands on the 17th day gave negative results.

NOTE.—This animal was used subsequently on the 22nd August, 1910 [*vide* Experiment No. 5 (*a*)], for an intrajugular injection of spleen and gland pulp of heifer 908, and also on the 3rd January, 1911 [*vide* Experiment No. 8 (*p*)], for an intrajugular injection of spleen pulp of ox 179, without contracting the disease. Finally tested on the 30th January, 1911, with brown nymphae [*vide* Experiment No. 8 (*p*)], and died of East Coast fever on the 27th day.

(C).—INTRAJUGULAR INJECTIONS.

(k) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (fine grain) of Heifer 913.*

(K).—*Bull 1052, aged; purchased in the Transvaal; history unknown.*

NOTE.—This animal had been used previously on the 25th May, 1910 (*vide* Annual Report, 1909–10, page 49), for an intrajugular injection of gland juice of heifer 928, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An immediate reaction followed, with a maximum temperature of 104° F. on the 2nd and 3rd days, and regaining normal on the 12th day. From the next day another reaction set in, reaching its maximum of 104 F. on the 19th and 20th days, and returning to normal eight days later.
- (b) Microscopical examination of blood: Leucocytosis and eosinophilia were noted on the 15th day. Puncture of the lymphatic glands on the 13th, 17th, and 22nd days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (*a*); these ticks were not infective.]

Remarks.—Thirty-two ticks were fast the following day. No reaction followed. Microscopical examination of the blood and glands on the 17th day gave negative results.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Eight ticks were fast the following day. No temperature reaction followed; microscopical examination of the blood on the 13th day showed the presence of small piroplasms. The animal died on the 14th day of poverty.

- (I) *Injections on the 22nd July, 1910, with 20 c.c. spleen pulp (coarse grain) of Heifer 913.*

- (I).—*Heifer 1015*, about two years old; purchased in the Cape Province, and arrived at the Laboratory in March, 1910.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight reaction started immediately, returning to normal about the 9th day; a second reaction commenced from the 13th day, with a maximum temperature of 104° F. on the 19th day, and regaining normal on the 21st day.
- (b) Microscopical examination of blood: Small piroplasms in rare numbers were noted from the 17th to 20th days. Puncture of the lymphatic glands on the 17th, 20th, and 22nd days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Thirty-nine ticks were fast the following day; no reaction followed; small piroplasms were seen on the 17th day, but examination of gland juice obtained by puncture on the same day proved negative.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Seven ticks were fast the following day. A slight reaction followed, indicated by an oscillating temperature, reaching 102° F. on two occasions.

- (3) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Three ticks were fast the following day, one having died; no temperature reaction followed.

- (4) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two adults off heifer 1111 (Reference No. 426). [*Note.*—See Experiment No. 25 (d) and 25 (e); both batches of ticks were infective.]

Remarks.—Three ticks were fast the following day. Temperature reaction from the 6th to the 13th day, with a maximum of 104·6° F. on the 7th day.

- (5) Exposed on the farm Burnside on the 7th January, 1911, and died on the 12th day of debility and tick irritation. Microscopical examination of the spleen and glands gave negative results.

(m) *Injections on the 22nd July, 1910, with 20 c.c. gland pulp (fine grain) of Heifer 913.*

(M).—*Heifer 1017, about two and a half years old; purchased in the Cape Province, and arrived at the Laboratory in March, 1910.*

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Slight oscillations were noted between the 10th and 18th days, but no indications of a definite reaction.
- (b) Microscopical examination of blood: Puncture of the lymphatic glands on the 12th and 18th days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Forty-two ticks were fast the following day. No reaction followed. With the exception of the lesions of anisocytosis on the 17th day, all microscopical examination gave negative results; examination of gland juice obtained by puncture on the 17th day proved negative.

- (2) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); ticks of this batch failed to transmit the disease in several instances, but proved to be pathogenic for at least two animals.]

Remarks.—Temperature reaction from the 13th day: *Theileria parva* appeared in the blood for the first time on the 12th day; plasma bodies (agamogonous forms) were noted in the glands on the 18th day. The animal died of East Coast fever on the 20th day.

(n) *Injections on the 22nd July, 1910, with 5 c.c. gland pulp (coarse grain) of Heifer 913.*

(N).—*Heifer 1016, about two and a half years old; purchased in the Cape Province, and arrived at the Laboratory in March, 1910.*

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight reaction ensued, lasting for about five days after injection.
- (b) Microscopical examination of blood: Eosinophilia was noted on the 16th day. Puncture of the lymphatic glands on the 13th and 20th days gave negative results.

Immunity Tests.—

- (1) Infested on the 5th September, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experimental No. 25 (a); these ticks were not infective.]

Remarks.—Twenty-six ticks were fast the following day; a slight reaction followed. All microscopical examinations gave negative results, and examination of gland juice obtained by puncture on the 17th day also proved negative.

- (2) Infested on the 20th October, 1910, with brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Seven ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—All four ticks were fast the following day. No temperature reaction followed.

- (4) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Six ticks were fast the following day. No reaction followed.

- (5) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two adults off heifer 1111 (Reference No. 426). [*Note.*—See Experiment No. 25 (d) and 25 (i); both batches of ticks were infective.]

Remarks.—Two ticks were fast the following day. A reaction ensued from the 16th day, reaching 104·6° F. on the following evening; a remission to normal was noted on the 20th day, immediately followed by a second rise, the temperature reaching 104·5° F. on the 22nd day, and returning to normal seven days later. Small piroplasms were noted on the 19th and 21st days, but examination of gland juice on the 21st day proved negative.

- (6) Exposed on the farm Burnside on the 7th January, 1911, and died on the 20th day of East Coast fever, plasma bodies being frequently found in the smears from the spleen.

SUMMARY OF EXPERIMENT NO. 2, With Material from Heifer 913.

NUMBER OF DAYS WHICH ELAPSED BETWEEN INFESTATION OF ANIMAL WITH TICKS AND DEATH: 24

NUMBER OF DAYS WHICH ELAPSED BETWEEN THE DATE OF THE FIRST RISE OF TEMPERATURE AND DEATH: 11.

DETAILS OF INJECTIONS AND RESULTS.

DETAILS OF TESTS ON IMMUNITY.

Ref. No.	Animal injected.	No. of times the animal was injected previously or subsequently.	References to these injections.	Method of injection.	Quantity injected.	Material injected.		Result.	No. of times tested with ticks.	Result.		Result of Exposure at Burnside.	REMARKS.
						Pulp of	Grain.			No. of tick infestation.			
A.	Bull 1051	1	A. R. 1909-10	Intrathoracal	20 c.c.	Spleen	Fine	I.R. † O.C.	—	—	—	Cause of death, purule pneumonia, 32nd da	
B.	Ox 1046	1	" "	"	15 " grs.	"	Coarse Chopped	R.R.	4	4	R.P. †		
C.	Cow 1030	1	" "	"	"	"	"	R.R.	2	2	R.R. † Poverty on the 15th day		
D.	Bull 1049	1	" "	"	20 c.c.	Gland	Fine	I.R.	5	1, 2, & 3	I.R.	Still alive (236 days)	
E.	Ox 1042	1	" "	"	5 c.c.	"	Coarse	I.R.	5	2	I.R.		
F.	Ox 1043	1	" "	"	15 grs.	"	Coarse Chopped	I.R.	2	2	R.P. †	" "	
G.	Ox 1038	3	A. R. 1909-10; Expts. 5 E & 7 C	Intralymphal	20 c.c.	Spleen	Fine	I.R.	—	—	—	For final history see Experiment 7 C (R.P. † Burnside).	
H.	Ox 1048	3	A. R. 1909-10; Expts. 5 G & 7 A	"	"	"	Coarse	I.R.	—	—	—		For final history see Experiment 7 A (R.P. † ticks).
I.	Cow 1032	3	A. R. 1909-10; Expts. 5 D & 7 F	"	"	Gland	Fine	I.R.	—	—	—	For final history see Experiment 7 F (R.P. † ticks).	
J.	Cow 1023	3	A. R. 1909-10; Expts. 5 A & 8 P	"	5 c.c.	"	Coarse	I.R.	—	—	—	For final history see Experiment 8 P (R.P. † ticks).	
K.	Bull 1052	1	A. R. 1909-10	Intrajugular	20 c.c.	Spleen	Fine	R.R.	2	2	† Poverty on 14th day	—	
L.	Heifer 1015	—	—	"	"	"	Coarse	R.R.	4	2 & 4	I.R.	† Debility 12th day	
M.	Heifer 1017	—	—	"	"	Gland	Fine	I.R.	2	2	R.P. †	—	
N.	Heifer 1016	—	—	"	5 c.c.	"	Coarse	R.R.	5	1	I.R.	R.P. †	
										5	R.R.		

EXPLANATION OF SYMBOLS.

R.P. †.—Indicates that the animal had a reaction, accompanied with the presence of plasma bodies, and died of East Coast fever.
R.R.—Indicates that the animal had a reaction and recovered, but that plasma bodies were not detected.

I.R.—Indicates that the animal had an irregular reaction and recovered.
N.R.—Indicates that the animal did not react to the injection.
† O.C.—Indicates that the animal died later of other causes, and that East Coast fever could not be considered to be in any way responsible for death.

RESULTS.

Of fourteen animals injected with material taken from heifer 913 (which was killed twenty-four days after tick infestation, or eleven days after the first rise of temperature), none contracted the disease. (One animal died of purulent pneumonia on the 32nd day). Of the remaining thirteen animals, four showed reactions indicative of East Coast fever, but plasma bodies could not be detected. When exposed to tick infestation or natural infection, one died of East Coast fever and the remaining three died of poverty.

The other nine animals showed irregular reactions or no reactions, and when tested later, seven died of East Coast fever, and two survived. These two animals were injected intrathoracally with gland pulp of heifer 913, but as they had been used previously it is difficult to say which injection conveyed immunity.

The intralymphal, intrajugular, and intrathoracal injections again failed to transmit the disease.

 EXPERIMENT No. 3.

TO NOTE THE EFFECT OF THE INJECTION OF MATERIAL OF OX 1026.

NOTE.—Ox 1026, aged ; purchased in the Transvaal ; history unknown.

Treatment.—Infested on the 27th June, 1910, with twenty brown nymphae off cattle 923, 917, and 700 (Reference Nos. 268, 335, and 309).

Remarks.—The temperature commenced to rise on the 13th day, reaching the maximum of 106° F. on the 15th day ; a descent now took place until the 20th day, when the minimum record of 101.2° F. was registered. The following day a second rise ensued, with the maximum of 106° F. on the 25th day, and remaining above 105° F. for the next three days, when the animal was killed (25th July, 1910).

The lymphatic glands were examined on the 15th day, when free agamonts were found in rare numbers ; on the 18th, 22nd, and 24th days there were still only a few noted, but on the date of slaughter they were very numerous. *Theileria parva* were noted in the blood for the first time on the 17th day, increasing rapidly in numbers during the following three days, but becoming less frequent until death. Plasma bodies were noted in the blood on the 28th day.

NOTE.—This animal was killed before the conclusion of the second reaction, probably a day or two before death would have occurred under ordinary conditions.

(A).—INTRAJUGULAR INJECTIONS OF SPLEEN AND GLAND PULP (COARSE GRAIN).

(a) *Injections on the 25th July, 1910, with 5 c.c. spleen and gland pulp (coarse grain) of Ox 1026.*

(A).—Cow 1074, about two years old ; purchased in the Transvaal ; history unknown.

Treatment.—Injected as before.

Remarks.—A reaction started immediately after injection reaching 105° F. on the 7th day. The animal died of gangrenous pneumonia.

(b) *Injections on the 25th July, 1910, with 10 c.c. spleen and gland pulp (coarse grain) of Ox 1026.*

(B).—Cow 1061, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction followed from the 4th day, reaching its maximum of 104° F. on the 9th day. The animal died as a sequel of cirrhosis of the liver on the 13th day.

(b) Microscopical examination of blood: Leucocytosis and lymphocytosis were noted on the 5th day.

(c) *Injections on the 25th July, 1910, with 15 c.c. spleen and gland pulp of Ox 1026 (coarse grain).*

(C).—Cow 1065, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction started almost immediately after injection, reaching 105° F. on the 7th day; a second reaction ensued from the 13th to 27th days, with a maximum record of 105° F. on the 18th and 19th days.

(b) Microscopical examination of blood: Eosinophilia was noted on the 12th day; lymphocytosis was seen on the 19th day. Puncture of the lymphatic glands on the 14th and 19th days gave negative results.

Immunity Tests.—

(1) Infested on the 7th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—About sixty-five ticks were fast the following day; no reaction followed; all microscopical examination gave negative results, and examination of gland and spleen on the 26th day also proved negative.

(2) Infested on the 15th September, 1910, with brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b). It is doubtful if these ticks were infective.]

Remarks.—Twenty ticks were fast the following day; some irregular temperatures followed and small piroplasms were detected in the blood on the 11th day, but examination of gland juice obtained by puncture on the same day also proved negative.

(3) Infested on the 20th October, 1910, with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—No temperature reaction followed.

(4) Infested on the 16th November, 1910, with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c) above.]

Remarks.—One tick was fast the following day; no reaction followed.

- (5) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (a); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Six ticks were fast the following day; no temperature reaction followed.

- (6) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411) and two adults off heifer 1111 (Reference No. 426). [*Note.*—See Experiment No. 25 (a) above and 25 (i); both batches of ticks were infective.]

Remarks.—Three adults were fast the following day; no temperature reaction followed.

- (7) Exposed on the farm Burnside on the 7th January, 1911, and died of poverty on the 16th March (68th day); microscopical examination of the blood and glands after death gave negative results.

- (d) *Injections on the 25th July, 1910, with 20 c.c. spleen and gland pulp (coarse grain) of Ox 1026.*

- (D).—Ox 1075, about two years old; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—Temperature: An immediate reaction followed, culminating in the death of the ox on the 8th day of gangrenous pneumonia.

- (e) *Injections on the 25th July, 1910, with 30 c.c. spleen and gland pulp (coarse grain) of Ox 1026.*

- (E).—Cow 1073, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—An irregular temperature reaction followed and the animal died on the 10th day of gangrenous pneumonia.

(B).—INTRAJUGULAR INJECTIONS OF SPLEEN AND GLAND PULP (FINE GRAIN).

- (f) *Injections on the 25th July, 1910, with 5 c.c. spleen and gland pulp (fine grain) of Ox 1023.*

- (F).—Ox 1076, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An irregular reaction followed, continuing until the 25th day.

- (b) Microscopical examination of blood: lymphocytosis was noted on the 11th day. Two plasma bodies were seen on the 19th day. Puncture of the lymphatic glands on the 4th, 12th, 15th, and 19th days gave negative results.

Immunity Tests.—

- (1) Infested on the 17th August, 1910, with brown nymphae off heifer 1013 (Reference No. 319). [*Note.*—See Experiment No. 25 (a); these ticks were not infective.]

Remarks.—Forty ticks were fast the following day; no reaction followed; all microscopical examination gave negative results, and examination of gland juice obtained by puncture also proved negative.

- (2) Infested on the 15th September, 1910, with twenty brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Twenty ticks were fast the following day; no temperature reaction followed, and examination of gland juice obtained by puncture on the 14th day proved negative.

- (3) Infested on the 30th September, 1910, with brown nymphae off heifers 913 and 914 (Reference Nos. 355 and 356). [*Note.*—See Experiment No. 25 (h); these ticks were not infective.]

Remarks.—Some irregular temperature reactions followed from the 8th to 20th days. Examination of gland juice on the 11th day proved negative.

- (4) Infested on the 20th October, 1910, with six brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Five ticks were fast the following day. No reaction followed.

- (5) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). (*Note.*—See above.)

Remarks.—Three ticks were fast the following day. Slight indications of a reaction were noted from the 15th to 21st days with 103° F. as an average evening record.

- (6) Infested on the 8th December, 1910, with brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Four ticks were fast the following day. No temperature reactions followed.

- (7) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411) and two adults off heifer 1111 (Reference No. 426). (*Note.*—Both these batches of ticks were infective.)

Remarks.—Some irregular records were noted from the 7th to 16th days.

- (8) Exposed at the farm Burnside on the 7th January, 1911, and was still alive at the date of writing (31st August, 1911).

(g) *Injections on the 25th July, 1910, with 15 c.c. spleen and gland pulp (fine grain) of Ox 1026.*

(G).—Ox 1099, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—Temperature: An immediate reaction followed, with evening records of 105° F.; the animal died on the 9th day of gangrenous pneumonia.

(h) *Injections on the 25th July, 1910, with 10 c.c. spleen and gland pulp (fine grain) of Ox 1026.*

(H).—Cow 1079, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) *Temperature:* An irregular reaction started almost immediately after injection; the maximum record of 105° F. was reached on the 14th, 15th, and 18th days; the animal died on the 19th day of gangrenous pneumonia.

(b) *Microscopical examination of blood:* Leucocytosis was noted on the 4th and 9th days. Puncture of the lymphatic glands on the 15th and 19th days gave negative results.

(i) *Injections on the 25th July, 1910, with 20 c.c. spleen and gland pulp of Ox 1026.*

(I).—Ox 1098, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) *Temperature:* A reaction ensued immediately, with a maximum record of 105° F. on the 4th, 7th, and 8th days, culminating in the death of the animal on the 11th day of gangrenous pneumonia.

(b) *Microscopical examination of blood:* Anisocytosis was noted on the 4th day. Puncture of the lymphatic glands on the 5th day gave negative results.

(j) *Injections on the 25th July, 1910, with 30 c.c. spleen and gland pulp (fine grain) of Ox 1026.*

(J).—Cow 1095, aged; purchased locally; history unknown.

Treatment.—Injected as above.

Remarks.—A temperature reaction started almost immediately and the animal died on the 9th day of gangrenous pneumonia.

SUMMARY OF EXPERIMENT No. 3,

With Material from Ox 1026.

NUMBER OF DAYS WHICH ELAPSED BETWEEN INFESTATION OF ANIMAL WITH TICKS AND DEATH: 28.

NUMBER OF DAYS WHICH ELAPSED BETWEEN THE DATE OF THE FIRST RISE OF TEMPERATURE AND DEATH: 15.

DETAILS OF INJECTIONS AND RESULTS.							DETAILS OF TESTS ON IMMUNITY.				REMARKS.
Ref. No.	Animal injected.	Method of injection.	Quantity injected.	Material injected.		Result.	No. of times tested with ticks.	Result.		Result of exposure at Burnside.	
				Pulp of	Grain.			No. of tick infestation.			
A.	Ox 1074	Intrajugular	5 c.c.	Spleen and gland	Coarse	† G.P. 7th day.	—	—	—	—	Cause of death attributed to poverty.
B.	Cow 1061	"	10 c.c.	" "	"	† C.L. 13th day.	—	—	—	—	
C.	Cow 1065	"	15 c.c.	" "	"	R.R.	6	2	I.R.	† O.C. 68th day.	
D.	Ox 1075	"	20 c.c.	" "	"	† G.P. 8th day.	—	—	—	—	
E.	Cow 1073	"	30 c.c.	" "	"	† G.P. 10th day.	—	—	—	—	
F.	Ox 1076	"	5 c.c.	" "	Fine	R.P.R.	7	3, 5, & 7	I.R.	Still alive (236 days).	
G.	Ox 1099	"	15 c.c.	" "	"	† G.P. 9th day.	—	—	—	—	
H.	Cow 1079	"	10 c.c.	" "	"	† G.P. 19th day.	—	—	—	—	
I.	Ox 1098	"	20 c.c.	" "	"	† G.P. 11th day.	—	—	—	—	
J.	Cow 1095	"	30 c.c.	" "	"	† G.P. 9th day.	—	—	—	—	

EXPLANATION OF SYMBOLS.

R.P.R.—Indicates that the animal had a reaction, accompanied with the presence of plasma bodies, and recovered.

R.R.—Indicates that the animal had a reaction and recovered, but that plasma bodies were not detected.

I.R.—Indicates that the animal had an irregular reaction and recovered.

† O.C.—Indicates that the animal died later of other causes, and that East Coast fever could not be considered to be in any way responsible for death.

† G.P.—Indicates that the animal died of gangrenous pneumonia.

† C.L.—Indicates that the animal died as a sequel of cirrhosis of the liver.

RESULTS.

Of ten animals injected intrajugularly with material taken from ox 1026 (which was killed twenty-eight days after tick infestation or fifteen days after the first rise of temperature), seven died of gangrenous pneumonia and one as a sequel of cirrhosis of the liver. Of the remaining two, one had a reaction indicative of East Coast fever, but no plasma bodies could be detected in the glands, and the other had a typical East Coast fever reaction accompanied with the presence of plasma bodies. When exposed, the former survived natural infection for sixty-eight days, and then died of poverty, and the latter was still alive at the date of writing (236 days).

Both these survivors had been injected with spleen and gland pulp, the former with 15 c.c. coarse grain and the latter with 5 c.c. fine grain.

EXPERIMENT No. 4.

TO NOTE THE EFFECT OF THE INJECTION OF MATERIAL FROM HEIFER 561.

NOTE.—Heifer 561 was infested on the 26th July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

Remarks.—On the 13th day the temperature rose, reaching the maximum of 106.4° F. on the 17th day, from which date a gradual descent occurred, until the 22nd day, on which date the morning record was 102° F., when the heifer was killed (17th August, 1910).

The examination of the glands on the 13th and 14th days gave negative results, but on the 20th and 21st days both gamogonous and agamogonous forms of *Theileria parva* were present in fairly large numbers. Agamogonous forms were also present in the spleen at *post-mortem* examination.

Numbers of *Theileria parva* were found in the blood on the 15th and 16th days.

NOTE.—The animal was killed at the end of the first half of the reaction.

(a) INTRATHORACAL INJECTIONS on the 17th August, 1910, with 20 c.c. spleen and gland pulp (medium grain) of Heifer 561.

(A).—Cow 1070, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: An irregular reaction followed for the first six days; a second irregular reaction was noted between the 16th and 24th days, but the evening records never exceeded 102° F.

(b) Microscopical examination of blood: Negative. Puncture of the lymphatic glands on the 16th and 26th days, and of the spleen on the latter date, gave negative results.

Immunity Tests.—

(1) Infested on the 20th October, 1910, with brown nymphae off heifer 908 (Reference No. 364). [Note.—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Four ticks were fast the following day. A reaction followed with some irregular records, later going over into a definite curve, and returning to normal on the 25th day. The maximum temperature noted was 103.6° F.

- (2) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Four ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Four ticks were fast the following day. No temperature reaction followed.

- (4) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two adults off heifer 1111 (Reference No. 426). [*Note.*—See Experiment Nos. 25 (d) and 25 (h); both batches of ticks were infective.]

- (5) This heifer left Pretoria for Burnside on the 7th January, 1911, but died of debility in the truck en route.

(B).—Cow 1072, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Some irregular records were noted for the first seven days.
- (b) Microscopical examination of blood: Eosinophilia and leucocytosis were noted on the 6th and 16th days. Puncture of the lymphatic glands on the 16th and 26th days, and of the spleen on the latter date, gave negative results.

Immunity Tests.—

- (1) Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Five ticks were fast the following day. Some irregular readings were noted for the first few days. Later a definite curve was traced, and the temperature returned to normal on the 27th day. The maximum evening record was 104° F. Small piroplasms in very rare numbers were detected in the blood on the 14th day.

- (2) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—One tick was fast the following day. No temperature reaction followed.

- (3) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1152 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—No temperature reaction followed.

(4) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two adults off heifer 1111 (Reference No. 426). [*Note*.—Both these batches of ticks proved infective in Experiments Nos. 25 (*d*) and 25 (*i*).]

Remarks.—No reaction followed.

(5) Exposed on the farm Burnside on the 7th January, 1911, and died on the 2nd April, 1911 (85th day), of poverty. Microscopical examination of the blood, glands, and spleen proved negative.

(C).—Ox 1097, aged; purchased locally; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: An irregular reaction followed, but in no way typical for East Coast fever.

(b) Microscopical examination of blood: Gave negative results on the 17th and 26th days. Puncture of the lymphatic glands on the same day also proved negative.

NOTE.—This animal was used subsequently on the 3rd January, 1911 [*vide* Experiment No. 8 (*h*)] for a subcutaneous injection of spleen pulp of ox 179, and died eighteen days later of anaemia.

(D).—Ox 1100, aged; purchased locally; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: No reaction.

(b) Microscopical examination of blood: Eosinophilia and leucocytosis appeared on the 26th day. Examination of lymphatic gland on the 17th and 26th days gave negative results.

NOTE.—This animal was used subsequently on the 15th November, 1910 [*vide* Experiment No. 7 (*e*)], for an intrajugular injection of 20 c.c. spleen pulp of heifer 1107, and was killed on the 19th December, 1910 (34th day), on account of poverty.

(E).—Ox 1101, aged; purchased locally; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: No reaction.

(b) Examination of the blood and lymphatic glands on the 17th and 26th days gave negative results.

NOTE.—This animal was used subsequently on the 15th November, 1910 [*vide* Experiment No. 7 (*i*)], for an intrajugular injection of spleen pulp of heifer 1107, with negative results. Later (14th December, 1910) infested with ticks without contracting the disease: finally was exposed at Burnside on the 7th January, 1911 [*vide* Experiment No. 7 (*j*)], and was still alive on the 31st August, 1911.

(F).—Cow 1104, aged; purchased locally; history unknown.

Treatment.—Injected as above.

Remarks.—

(a) Temperature: A reaction followed almost immediately, reaching 104° F. on several occasions, and regaining normal limits on 14th day.

- (b) Examination of the blood and lymphatic gland on the 17th and 26th days gave negative results. The animal died on the 25th October of poverty (69th day).

(f) INTRAJUGULAR INJECTIONS on the 17th August, 1910, with 20 c.c. spleen and gland pulp (medium grain) of Heifer 561.

(G).—Ox 1050, aged; purchased in the Transvaal; history unknown.

NOTE.—This animal had been used previously on the 17th May, 1910 (*vide* Annual Report, 1909–10, p. 48), for an intralymphal injection of gland juice of heifer 1018, without contracting East Coast fever.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: Slight reaction lasted a few days after inoculation; another reaction ensued from the 21st to 32nd days, with a maximum temperature of 103° F.
- (b) Microscopical examination of blood: Small piroplasms were noted on the 16th and 19th days, but puncture of the lymphatic glands on the same days gave negative results.

Immunity Test.—Infested on the 20th October, 1910, with ten brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); these ticks failed to transmit the disease in several instances.]

Remarks.—Seven ticks were fast the following day; a slight irregular reaction followed, developing into a definite reaction and lasting from the 14th to 22nd days; a second rise was noted directly afterwards, reaching 106° F. on two occasions. The animal died of East Coast fever on the 32nd day. *Theileria parva* appeared in the blood for the first time on the 15th, and on the same date plasma bodies (agamogonous forms) were detected in the lymphatic glands.

(H).—Cow 1062, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight irregular reaction followed, lasting until 15th day.
- (b) Microscopical examination of blood: With the exception of the lesions of anisocytosis, blood and gland smears gave negative results. The cow was killed on the 6th October, 1910, on account of poverty (50th day).

(I).—Cow 1063, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: A slight irregular reaction from about the 11th to 24th days, with a maximum evening temperature of 102° F.
- (b) Microscopical examination of blood: Negative. Puncture of the lymphatic glands on the 13th and 19th days also gave negative results.

Immunity Tests.—

- (1) Infested on the 20th October, 1910, with brown nymphae off heifer 908 (Reference No. 364). [*Note.*—See Experiment No. 25 (b); it is doubtful if these ticks were infective.]

Remarks.—Eight ticks were fast the following day. An irregular reaction followed from the 13th to 23rd days, with maximum evening records of 103° F.

- (2) Infested on the 16th November, 1910, with four brown adults, origin Natal (Reference No. 349). [*Note.*—See Experiment No. 25 (c); heifer 1084 contracted East Coast fever from the infestation of four ticks of the same batch.]

Remarks.—Three ticks were fast the following day. No temperature reaction followed.

- (3) Infested on the 8th December, 1910, with six brown adults off heifer 1053 (Reference No. 411). [*Note.*—See Experiment No. 25 (d); heifer 1157 contracted East Coast fever from the infestation of six ticks of the same batch.]

Remarks.—Four adults were fast the following day. No temperature reaction followed.

- (4) Infested on the 14th December, 1910, with two brown adults off heifer 1053 (Reference No. 411), and two adults off heifer 1111 (Reference No. 426). [*Note.*—See Experiment No. 25 (d) and 25 (i); both batches of ticks were infective.]

Remarks.—One adult was fast the following day. A slight temperature reaction from the 8th to 18th days, with a maximum of 102° F. on one occasion.

- (5) Exposed on the farm Burnside on the 7th January, 1911, and was still alive on the 31st August, 1911.

(J).—Cow 1067, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—

- (a) Temperature: An irregular reaction followed, with a rise on the 3rd day to 104° F. A slight curve was distinguishable from the 11th to 25th day.
- (b) Microscopical examination of blood and glands on the 13th and 19th days gave negative results. The animal died on the 16th October of poverty (60th day).

(K).—Cow 1069, aged; purchased in the Transvaal; history unknown.

Treatment.—Injected as above.

Remarks.—A temperature reaction set in from the 11th day, reaching 103° F. two days later; irregular records were noted for the next three weeks, but the evening temperature never exceeded 102° F. The cow died on the 3rd October of poverty (47th day).

SUMMARY OF EXPERIMENT NO. 4, With Material from Heifer 561.

NUMBER OF DAYS WHICH ELAPSED BETWEEN INFESTATION OF ANIMAL WITH TICKS AND DEATH: 22.

NUMBER OF DAYS WHICH ELAPSED BETWEEN THE DATE OF THE FIRST RISE OF TEMPERATURE AND DEATH: 9.

(No bacteria were found in the smears taken immediately after death.)

DETAILS OF INJECTIONS AND RESULTS.									DETAILS OF TESTS ON IMMUNITY.				REMARKS.
Ref. No.	Animal injected.	No. of times the animal was injected previously or subsequently.	References to these injections.	Method of injection.	Quantity injected.	Material injected.		Result.	No. of times tested with ticks.	Result.		Result of exposure at Burnside.	
						Pulp of	Grain.			No. of tick infestation.			
A.	Cow 1070	—	—	Intrathoracal	20 c.c.	Spleen and gland	Medium	I.R.	4	1	R.R.	—	Died of debility in truck en route to Burnside. Death attributed to poverty.
B.	Cow 1072	—	—	"	"	" "	"	I.R.	4	1	R.R.	† O.C. 85th day.	
C.	Ox 1097	1	Expt. 8 H	"	"	" "	"	I.R.	—	—	—	—	For final history see Experiment 8 H († anaemia 18th day).
D.	Ox 1100	1	Expt. 7 E	"	"	" "	"	N.R.	—	—	—	—	
E.	Ox 1101	1	Expt. 7 I	"	"	" "	"	N.R.	—	—	—	—	For final history see Experiment 7 I (still alive at Burnside).
F.	Cow 1104	—	—	"	"	" "	"	I.R. † O.C. 85th day	—	—	—	—	
G.	Ox 1050	1	A. R. 1909-10	Intrajugular	"	" "	"	R.R.	1	1	R.P. †	—	Killed on account of poverty.
H.	Cow 1082	—	—	"	"	" "	"	I.R. † O.C. 50th day	—	—	—	—	
I.	Cow 1083	—	—	"	"	" "	"	I.R.	4	4	I.R.	Still alive (236 days)	Death attributed to poverty.
J.	Cow 1087	—	—	"	"	" "	"	R.R. † O.C. 60th day	—	—	—	—	
K.	Cow 1069	—	—	"	"	" "	"	I.R. † O.C. 47th day	—	—	—	—	Death attributed to poverty.

EXPLANATION OF SYMBOLS.

R.P. †.—Indicates that the animal had a reaction, accompanied with the presence of plasma bodies, and died of East Coast fever.
R.R.—Indicates that the animal had a reaction and recovered, but that plasma bodies were not detected.

I.R.—Indicates that the animal had an irregular reaction and recovered.
N.R.—Indicates that the animal did not react to the injection.
† O.C.—Indicates that the animal died later of other causes, and that East Coast fever could not be considered to be in any way responsible for death.