

# Some Observations concerning the Transmission of East Coast Fever by Ticks.

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It has been proved that *Theileria parva*, the cause of East Coast fever, can be transmitted by at least five different species of ticks of the genus *Rhipicephalus* (viz. *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Rhipicephalus simus*, *Rhipicephalus nitens*, and *Rhipicephalus capensis*). Of these *Rhipicephalus appendiculatus* is the commonest in South Africa and in the regions in which East Coast fever is known generally (Uganda, British and German East Africa). They were almost exclusively used in my experiments on East Coast fever.

The following observations, which were made in connection with experiments undertaken for other purposes (mainly to get material for the artificial transmission of the disease and to test the resulting immunity), form an interesting addition to and confirmation of facts which have already been established concerning the transmission of East Coast fever by means of ticks.

## EXPERIMENT No. 1.

A).—TO DEMONSTRATE THE FACT THAT BROWN TICK IMAGINES, WHICH AS LARVA HAD BECOME INFECTED WITH EAST COAST FEVER AND HAD TRANSMITTED THE DISEASE IN THEIR NYMPHAL STAGE, ARE NO LONGER INFECTIVE FOR SUSCEPTIBLE CATTLE.

(a) *Rhipicephalus appendiculatus imago* (Reference No. 268).

NOTE.—The adult females originated from Natal on the 12th January, 1910; the eggs were laid on the 18th January, 1910, and hatched into larvae in the Laboratory on the 18th February, 1910.

*Injecting the Ticks.*—The larvae were fed on heifer 923 from the 20th to 23rd March, 1910, that is from the 20th to 23rd day of the disease and at a time when the parasites of East Coast fever were very frequent in the blood.

*Cleaning the Ticks.*—The larvae moulted in due time, and the nymphae were placed on cow 596 on the 7th April, 1910. Cow 596 developed the disease after an incubation time of fifteen days, and was killed for experimental purposes on the 12th day of the East Coast fever illness.

*Remarks.*—The nymphae collected as engorged larvae off heifer 923, whilst undergoing an East Coast fever reaction, proved to be infective for cow 596.

NOTE.—The nymphae which communicated the disease to cow 596 engorged and were collected off this cow from the 13th to 15th April, 1910. They moulted into adults on the 20th May, 1910, and were used for the infestation of cattle Nos. 1022 and 1060 [vide Experiment No. 1 (C), Nos. 21 and 22].

(b) *Rhipicephalus appendiculatus imagoes* (Reference No. 309).

NOTE.—The engorged females originated from Natal on the 9th December, 1909. The eggs were laid on the 24th December, 1909, and the larvae hatched out on the 30th January, 1910.

*Infesting the Ticks.*—The larvae were fed on calf 700 from the 27th February, 1910, and dropped between the 2nd and 7th March; that is from the 7th to 8th days of the disease and at a time when *Theileria parva* were frequently met with in the blood.

*Cleaning the Ticks.*—The engorged larvae moulted on the 17th March, 1910, and the nymphae were placed on calf 917 on the 30th March, 1910. This calf developed East Coast fever after the usual period and died. The examination of the blood proved the presence of *Theileria parva* and the glands contained agamonts and gamonts in large numbers.

*Remarks.*—The ticks, which as larvae were fed on calf 700 during the time of the East Coast fever reaction, proved virulent for calf 917 in their nymphal stage.

NOTE.—The nymphae which communicated the disease to calf 917 engorged and were collected from the 3rd to 7th April, 1910, namely, a week after infestation. They moulted into adults on the 4th May, 1910, and were placed on heifer 1088 on the 15th December, 1910, and on calf 1145 on the 6th January, 1911, in order to test their infectivity [*vide* later, Experiment No. 1 (C), Nos. 23 and 24].

(c) *Rhipicephalus appendiculatus* imagines (Reference No. 335).

*Infesting the Ticks.*—The larvae originated from females collected in Natal on the 19th December, 1909; the eggs were laid on the 24th December, 1909, and the larvae hatched out on the 30th January, 1910. On the 27th February, 1910, the larvae were placed on calf 917 [calf 917 contracted East Coast fever from the ticks of calf 700, *vide* sub-head (b)]. The larvae were collected off calf 917 between the 11th and 14th April, 1910, at a time when *Theileria parva* were frequently met with in the blood and the plasma bodies were present in the organs.

*Cleaning the Ticks.*—The engorged larvae moulted in due time and, together with nymphae off cattle 700 (Reference No. 309) and 923 (Reference No. 268), were used for the infestation of the following eight cattle: Nos. 561, 908, 919, 1011, 914, 1012, 1026, and 1040.

(d) *Rhipicephalus appendiculatus* (Reference Nos. 309, 268, and 335).

(1) *Heifer* 561.—Was infested on the 26th July, 1910, with brown nymphae as above, and developed East Coast fever after an incubation time of twelve days. This heifer was killed for experimental purposes ten days later, when microscopical examination proved the presence of *Theileria parva* in the blood and the plasma bodies in the glands.

*Remarks.*—The ticks, which as larvae had fed on cattle 700, 917, and 923, proved virulent in their nymphal stage for heifer 561.

NOTE.—The engorged brown nymphae were all collected by the 2nd August, 1910. They moulted into adults on the 19th September, 1910, and were placed on heifer 1088 on the 15th December, 1910, and on calf 1145 on the 6th January, 1911 [*vide* Experiment No. 1 (C), Nos. 23 and 24].

(2) *Heifer* 908.—Was infested on the 26th July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335), as above. This heifer developed East Coast fever after an incubation time of thirteen days, and was killed for experimental purposes on the 22nd August, 1910, after an illness of fourteen days. The blood contained *Theileria parva*, and in the glands and spleen plasma granules were frequently found.

NOTE.—The engorged brown nymphae were collected by the 2nd August, 1910, and had moulted into adults on the 19th September, 1910. The adults were placed on heifer 1088 on the 15th December, 1910, and on calf 1145 on the 6th January, 1911, in order to test their infectivity [*vide* later, Experiment No. 1 (C), Nos. 23 and 24].

(3) *Calf* 919.—Was infested on the 27th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. An East Coast fever reaction set in after an incubation time of fourteen days, and the calf died on the 28th July, 1910, the 16th day of illness. The blood contained *Theileria parva* in large numbers, and plasma bodies were frequently met with in the glands.

NOTE.—The engorged brown nymphae were all collected by the 12th July, 1910, and moulted into adults in due time, when they were placed on heifer 1088 and calf 1145 in order to test their infectivity [*vide* later, Experiment No. 1 (C), Nos. 23 and 24].

(4) *Heifer* 914.—Was infested on the 28th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. It developed East Coast fever after an incubation time of thirteen days and recovered. The blood contained *Theileria parva*, and plasma bodies were found in the glands.

NOTE.—The engorged brown nymphae were all collected by the 12th July, 1910, and moulted into adults by the 19th September, 1910. They were placed on heifer 1088 and calf 1145 on the 15th December, 1910, and on the 6th January, 1911, respectively, in order to test their infectivity [*vide* later, Experiment No. 1 (C), Nos. 23 and 24].

(5) *Heifer* 1012.—Was infested on the 27th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. It developed East Coast fever after an incubation time of twelve days and died on the 26th July, 1910. The blood was found to contain *Theileria parva*, and plasma bodies were noted in the glands.

NOTE.—The engorged nymphae were collected in due time, and were finally placed on heifer 1088 and on calf 1145 in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 13 and 14].

(6) *Heifer* 1011.—Was infested on the 27th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. After an incubation time of thirteen days, an East Coast fever reaction set in, and the heifer was killed for experimental purposes on the 8th day of the disease (18th July, 1910). The blood contained *Theileria parva* in rare numbers, but plasma bodies were found to be very frequent in the glands.

NOTE.—The engorged nymphae were collected in due time, and had moulted by the 19th September, 1910. They were placed on heifer 1088 on the 15th December, 1910, and on calf 1145 on the 6th January, 1911, in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 23 and 24].

(7) *Ox* 1026.—Was infested on the 27th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. East Coast fever developed after an incubation time of twelve days, and the ox was killed on the 16th day of illness for experimental purposes (25th July, 1910). The blood contained *Theileria parva* and the glands showed the presence of plasma bodies in large numbers.

NOTE.—The engorged nymphae were collected in due time and moulted into adults by the 19th September, 1910. They were used for the infestation of heifer 1088 and calf 1145 on the 15th December, 1910, and 6th January, 1911, respectively, in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 23 and 24].

(8) *Ox* 1040.—Was infested on the 27th June, 1910, with brown nymphae off cattle 700, 917, and 923 (Reference Nos. 309, 335, and 268), as above. Ox 1040 developed East Coast fever after an incubation time of thirteen days, and died on the 15th day of illness (25th July, 1910). The blood showed a strong infection of *Theileria parva*, but plasma bodies were found in rare numbers only in the glands.

NOTE.—The nymphae engorged in due time, and moulted into adults by the 19th September, 1910. They were used for the infestation of heifer 1088 and calf 1145 [*vide* Experiment No. 1 (C), Nos. 23 and 24].

(B).—TO DEMONSTRATE THE FACT THAT BROWN TICK IMAGINES, WHICH AS LARVAE HAD BECOME INFECTED WITH EAST COAST FEVER, AFTER HAVING PASSED THEIR NYMPHAL STAGE ON CATTLE RENDERED IMMUNE TO EAST COAST FEVER BY INOCULATION, NO LONGER TRANSMIT THE DISEASE TO SUSCEPTIBLE CATTLE.

(e) *Rhipicephalus appendiculatus* (Reference Nos. 309, 268, and 335).

[See previous sub-head (d).]

NOTE.—The animals referred to later (Nos. 829, 883, 836, 895, 1047, 1033, 871, 621, 679, and 615) had all been rendered immune to East Coast fever by inoculation, details of which were given in the Annual Report of the Government Veterinary Bacteriologist for 1909–10, under the title of “The Artificial Transmission of East Coast Fever” (see pp. 1–55).

(9) *Ox* 829.—Was rendered immune to East Coast fever by inoculation (see above).

*Infested* on the 24th March, 1910, with brown nymphae off calf 700 (Reference No. 309).

NOTE.—The engorged nymphae were collected on the 30th March, 1910; they moulted into adults on the 21st April, 1910, and were placed on heifers 1057 and 1058 on the 5th August, 1910, in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(10) *Heifer* 883.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 24th March, 1910, with brown nymphae off calf 700 (Reference No. 309).

NOTE.—The engorged nymphae were collected on the 30th March, 1910; they moulted into adults on the 21st April, and in their adult stage were used for the infestation of heifers 1057 and 1058 [*vide* Experiment No. 1 (C), Nos. 25 and 26] on the 5th August, 1910, in order to test their infectivity.

(11) *Heifer* 836.—Rendered immune by inoculation (*vide* above).

*Infested* on the 24th March, 1910, with brown nymphae off calf 700 (Reference No. 309).

NOTE.—The engorged nymphae dropped on the 30th March, 1910, and moulted into adults on the 21st April, 1910. The adults were placed on heifers 1057 and 1058 on the 5th August, 1910, in order to test their infectivity *vide* Experiment No. 1 (C), Nos. 25 and 26].

(12) *Heifer* 895.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 24th March, 1910, with brown nymphae off calf 700 (Reference No. 309).

NOTE.—The engorged nymphae dropped on the 30th March, 1910, and moulted into adults on the 21st April, 1910. They were placed on heifers 1057 and 1058 on the 5th August, 1910, in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(13) *Ox* 1047.—Rendered immune by inoculation (*vide* above).

*Infested* on the 26th July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected on the 2nd August, 1910, and moulted into adults on the 19th September, 1910. The adults were used for the infestation of heifer 1088 and calf 1145 [*vide* Experiment No. 1 (C), Nos. 25 and 26] in order to test their infectivity.

(14) *Cow* 1033.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 26th July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected on the 2nd August, 1910, and moulted into adults on the 19th September, 1910. Later, they were placed on cattle 1088 and 1145 [*vide* Experiment No. 1 (C), Nos. 25 and 26] in order to test their infectivity.

(15) *Heifer* 871.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 28th June, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected and moulted into adults in due time; later, they were used for the infestation of cattle 1088 and 1145 in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(16) *Ox* 621.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 2nd July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected within the usual time and moulted into adults on the 19th September, 1910. The adults were later placed on cattle 1088 and 1145 in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(17) *Heifer* 895.—Was rendered immune by inoculation.

*Infested* on the 2nd July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected from the 4th to 14th July, 1910, and moulted into adults on the 19th September, 1910. They were later placed on cattle 1088 and 1145 in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(18) *Heifer* 836.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 2nd July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected from the 4th to 14th July, 1910, and moulted into adults on the 19th September, 1910. They were later placed on cattle 1088 and 1145 in order to test their infectivity [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(19) *Cow* 679.—Was rendered immune by inoculation (*vide* above).

*Infested* on the 2nd July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected from the 4th to 14th July, 1910, and moulted into adults on the 19th September, 1910. In order to test their infectivity they were placed on cattle 1088 and 1145 [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(20) *Bull* 615.—Rendered immune by inoculation (*vide* above).

*Infested* on the 2nd July, 1910, with brown nymphae off cattle 700, 923, and 917 (Reference Nos. 309, 268, and 335).

NOTE.—The engorged nymphae were collected from the 4th to 14th July, 1910, and moulted into adults on the 19th September, 1910. Later, in order to test their infectivity they were placed on cattle 1088 and 1145 [*vide* Experiment No. 1 (C), Nos. 25 and 26].

(C).—TO SHOW THAT THE TICKS, WHICH AS NYMPHAE HAD COMMUNICATED THE DISEASE TO SUSCEPTIBLE CATTLE NOS. 596, 561, 908, 919, 914, 1012, 1026, AND 1040, OR WHICH HAD PASSED THEIR NYMPHAL STAGE ON ANIMALS ARTIFICIALLY IMMUNIZED (NOS. 829, 883, 836, 895, 1047, 1033, 871, 621, 895, 836, 679, AND 615), DO NOT TRANSMIT THE DISEASE IN THEIR ADULT STAGE TO SUSCEPTIBLE CATTLE.

(f) *Rhipicephalus appendiculatus* (Reference No. 268).

NOTE.—The following cattle, 1022, 1060, 1088, 1045, 1057, and 1058, were all born and bred in areas free from East Coast fever, and accordingly were susceptible to the disease.

(21) *Heifer* 1022.—Was infested on the 23rd June, 1910, with brown adults off heifer 923 (Reference No. 268). This infestation was repeated on the 28th June, 15th July, 20th July, 28th July, 3rd August, and 20th August; altogether 100 ticks were placed on this heifer, but not all of them became attached.

*Remarks.*—This animal remained quite healthy.

(22) *Heifer* 1060.—Was infested on the 29th August, 1910, with brown adults of heifer 923 (Reference No. 268), and was reinfested on the 14th September, 1910; eight ticks of the first infestation were counted to be fast and eighteen of the second lot.

*Remarks.*—This heifer remained healthy. Later, she was used for inoculation purposes, and was finally exposed to East Coast fever infection on the farm Burnside, Natal, and died on the 21st day of East Coast fever [*vide* article on "Progress Report on the Possibility of Vaccinating Cattle against East Coast Fever", Experiment No. 6 (d)].

(23) *Heifer* 1088.—Was infested on the 15th December, 1910, with a large number of brown adults, which as nymphae had given the disease to cattle 561, 908, 919, 914, 917, 1011, 1012, 1026, 1040, or which as nymphae had fed on immune cattle 1047, 1033, 871, 621, 895, 836, 679, 615, 829, and 883; the ticks were fast on the 16th December, 1910.

*Remarks.*—Heifer 1088 did not contract East Coast fever from this infestation, but was used later for inoculation purposes [*vide* article cited above, Experiment No. 9 (j)]; finally was exposed at Burnside, and died of East Coast fever complicated with redwater.

(24) *Calf* 1145.—Was infested on the 6th January, 1911, with large numbers of brown adults off cattle 561, 908, 909, 914, 917, 1011, 1012, 1026, 1040, 1047, 1033, 871, 621, 895, 836, 679, 615, 883, and 829.

*Remarks.*—The calf did not contract East Coast fever from this infestation, but died from enteritis on the 14th February, 1911.

(25) *Heifer* 1057.—Was infested on the 5th August, 1910, with large numbers of adults which had passed their nymphal stage on cattle 829, 883, 836, and 895 (Reference No. 309); she was reinfested on the 19th and 29th August with ticks of the same batch. A good many ticks were found fast the following day. The engorged females dropped on the 13th and 23rd August and 5th September.

*Remarks.*—Heifer 1057 did not contract East Coast fever from this infestation. It was used later for inoculation experiments [*vide* article quoted above, Experiment No. 6 (b)].

(26) *Heifer* 1058.—Was infested on the 5th August, 1910, with large numbers of brown adults off cattle 829, 883, 836, and 895 (Reference No. 309). The ticks were found to be fast the following day, and large numbers of engorged females were collected from the 23rd August.

*Remarks.*—Heifer 1058 did not contract East Coast fever from this infestation. It was used later for inoculation experiments (*vide* article cited above, Experiment No. 6 (b)), finally exposed in Burnside, and died of East Coast fever. Plasma bodies were found in the blood, spleen, and glands on *post-mortem* examination.

*Results.*—None of the six animals which were infested with “cleaned” ticks developed East Coast fever from the infestation.

#### CONCLUSIONS.

The adult ticks which as larvae had fed on sick animals, and which as nymphae had communicated the disease to nine susceptible cattle, or which had been feeding on twelve artificially immunized cattle, did not communicate East Coast fever when placed in large numbers on six susceptible cattle.

These observations were made on a fair number of animals and with a great many ticks, so that all coincidences can be excluded.

#### EXPERIMENT No. 2.

TO SHOW THAT TICKS WHICH HAVE BEEN FEEDING ON CATTLE SUFFERING FROM EAST COAST FEVER DO NOT ALWAYS TRANSMIT THE DISEASE IN THEIR SUBSEQUENT STAGE.

#### PARTICULARS OF TICKS USED.

##### I.—*Rhipicephalus appendiculatus* (Reference No. 319).

The larvae originated from mothers collected in Natal on the 18th March, 1910; the eggs were laid in the Laboratory on the 25th March, and the larvae hatched out on the 26th April, 1910. The larvae were placed on heifer 1013 on the 7th day of the fever reaction (30th May, 1910), and were collected during the three days previous to death, which occurred from East Coast fever on the 7th June, 1910. During the time the ticks were feeding on heifer 1013 the parasites of East Coast fever were frequently met with, and all stages of development were found in the lymphatic glands.

The larvae moulted into nymphae on the 5th August, 1910.

II.—*Rhipicephalus appendiculatus* (Reference No. 364).

The larvae originated from mothers collected in Natal on the 18th March, 1910; the eggs were laid at the Laboratory on the 25th March, and the larvae hatched out on the 26th April, 1910. The larvae were placed on heifer 908 on the 12th August, 1910, viz., the 5th day after the rise of temperature, and were collected five days before death, which occurred on the 22nd August, 1910, during which period the blood showed *Theileria parva* in large numbers, and in the spleen and glands plasma bodies were very frequently met with.

The larvae moulted into nymphae on the 10th September, 1910.

III.—*Rhipicephalus appendiculatus* (Reference No. 355).

The larvae originated from mothers collected in Natal on the 18th March, 1910, and the eggs were laid in the Laboratory on the 25th March; the larvae hatched out on the 26th April, 1910. The larvae were placed on heifer 913 on the 14th July, 1910, at a time when the parasites of East Coast fever were frequently met with, and the engorged larvae were collected from the 19th to 21st July, 1910. Heifer 913 was killed on the 22nd July, 1910. The examination of the blood since the 14th July had shown the presence of the East Coast fever parasites in but rare numbers; free plasma bodies (agamogonous forms) had been noted in the glands.

The larvae moulted into nymphae on the 1st September, 1910.

IV.—*Rhipicephalus appendiculatus* (Reference No. 356).

The larvae originated from mothers collected in Natal on the 12th March, 1910; the eggs were laid in the Laboratory on the 25th March, and the larvae emerged on the 18th April, 1910. The larvae were placed on heifer 914 on the 14th July, 1910, and were collected from the 17th to 20th July. On the 18th July, *Theileria parva* were noted on the blood of heifer 914, and plasma bodies were seen in the glands. The heifer recovered.

The engorged larvae moulted into nymphae on the 15th August, 1910.

V.—*Rhipicephalus appendiculatus* (Reference No. 349).

The engorged nymphae were collected off sick cattle in Natal on the 29th June, 1910, and moulted in the Laboratory on the 11th September, 1910.

VI.—*Rhipicephalus appendiculatus* (Reference No. 411).

The larvae originated from mothers collected in Natal on the 12th March, 1910; the eggs were laid at the Laboratory on the 14th March, 1910, and the larvae hatched on the 18th April, 1910. The larvae were placed on heifer 914 (suffering from East Coast fever) on the 14th July, 1910. The engorged larvae were collected from the 17th to 20th July, and moulted into nymphae on the 15th August, 1910. They were placed on heifer 1053 on the 18th October, 1910, at a time when this heifer was suffering from East Coast fever and when *Theileria parva* were noted in the blood and plasma bodies were seen in the glands. The engorged nymphae dropped from the 22nd to 27th October, 1910, and moulted into adults on the 21st November, 1910.

NOTE.—These ticks had been feeding in both their larval and nymphal stages on animals suffering from East Coast fever.

VII.—*Rhipicephalus appendiculatus* (Reference No. 426).

The larvae originated from mothers collected in Natal on the 18th February, 1910, and hatched by the 30th March, 1910. The larvae were reared on "clean" heifer 868 and dropped from the 12th to 16th July, 1910. They



moulted on the 23rd August, 1910, and the nymphae were placed on heifer 1111 on the 26th October, at a time when this heifer was suffering from East Coast fever, *Theileria parva* being found in the blood and plasma bodies in the glands. The engorged nymphae dropped from the 31st October to 5th November, 1910, and moulted into adults on the 26th November, 1910.

#### VIII.—*Rhipicephalus appendiculatus* (Reference No. 373).

The adult females originated from cattle 906, 1088, 1009, and 1021, animals which were kept at the Laboratory. The eggs hatched on the 18th November, 1910, and the larvae were placed on heifer 909 on the 2nd January, 1911, at a time when this heifer was undergoing an attack of East Coast fever and when *Theileria parva* and plasma bodies were noted in the blood and glands respectively.

The engorged larvae were collected from the 5th to 7th January, 1911, and moulted about the 20th January, 1911, into nymphae.

#### TESTING OF THE TICKS.

(a) *Brown Nymphae* (Reference Nos. 363, 364, 355, 356, and 349).

(1) *Heifer 627*.—Had previously been used for inoculation experiments in connection with the transmission of East Coast fever, but with negative results.

*Infested* with brown nymphae (Reference No. 363) on the 17th August, 1910; about twenty were found attached the following day, and the engorged nymphae were collected on the 23rd August. No reaction ensued.

*Reinfested* on the 15th September, 1910, with twenty brown nymphae (Reference No. 364). All twenty ticks were found attached on the following day. No reaction followed.

*Reinfested* on the 30th September, 1910, with brown nymphae (Reference Nos. 355 and 356). On the following day fourteen ticks were found attached. No reaction ensued.

*Reinfested* on the 20th October with six brown adults, originating from Natal (Reference No. 349). Three adults were found fast on the 21st October. The heifer contracted East Coast fever, and died on the 27th November, 1910.

(2) *Heifer 911*.—Had been previously used for inoculation purposes in connection with the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 17th August with a number of brown nymphae (Reference No. 319). About seventy ticks were found fast the following day, and the engorged nymphae were collected on the 23rd August. No reaction followed.

*Reinfested* on the 15th September with brown nymphae (Reference No. 364). Twenty ticks were found attached the following day. No reaction followed.

*Reinfested* on the 30th September, 1910, with brown nymphae (Reference Nos. 355 and 356). On the 1st October twenty ticks were found fast. No reaction ensued.

*Reinfested* on the 20th October, 1910, by the infestation of six brown adults; origin Natal (Reference No. 349). The heifer contracted East Coast fever, and died on the 6th November, 1910.

(3) *Heifer 1014*.—Had been previously used in connection with the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 17th August, 1910, with brown nymphae (Reference No. 319). Twenty-four ticks were found fast the following day, and the engorged nymphae dropped on the 23rd August, 1910. No reaction resulted.

*Reinfested* on the 15th September, 1910, with twenty brown nymphae (Reference No. 364). Twenty ticks were fast on the 16th September, 1910. No reaction ensued.

*Reinfested* on the 30th September, 1910, with brown nymphae (Reference Nos. 355 and 356). Ten ticks were found fast the following day. No reaction followed.

*Reinfested* on the 20th October, 1910, by the infestation of six brown adults; origin Natal (Reference No. 349). Heifer 1014 contracted East Coast fever, and died on the 21st November, 1910.

(4) *Cow* 1068.—Had been previously used in connection with the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 17th August, 1910, with brown nymphae (Reference No. 363). On the following day about seventy were found fast. The engorged nymphae were collected on the 23rd August, 1910. No reaction ensued.

*Reinfested* on the 15th September, 1910, with brown nymphae (Reference No. 364). Twenty ticks were found attached the following day. No reaction resulted.

*Reinfested* on the 30th September, 1910, with brown nymphae (Reference Nos. 355 and 356). Fourteen ticks were fast the next day. No reaction.

*Reinfested* on the 20th October, 1910, with six brown adults; origin Natal (Reference No. 349). Five adults were fast the next day. This cow contracted East Coast fever and died on the 21st November, 1910.

(b) *Brown Nymphae* (Reference Nos. 363 and 373).

(5) *Ox* 1037.—Had been previously used for the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 5th September, 1910, with brown nymphae (Reference No. 319). Twenty-seven ticks were found to be attached the following day. No reaction ensued.

*Reinfested* on the 30th January, 1911, with twenty brown nymphae off heifer 909 (Reference No. 373). Twenty ticks were found fast the following day. Ox 1037 contracted East Coast fever, and died on the 22nd February, 1911.

(c) *Brown Nymphae* (Reference Nos. 363, 364, and 349).

(6) *Ox* 1046.—Had been previously used for the artificial transmission of East Coast fever, with negative results.

*Infested* on the 5th September, 1910, with brown nymphae (Reference No. 319). Twenty-eight ticks were found attached the following day. No reaction developed.

*Reinfested* on the 20th October, 1910, with ten brown nymphae (Reference No. 364). On the following day eight ticks were found fast. No reaction followed.

*Reinfested* on the 16th November, 1910, with four brown adults; origin Natal (Reference No. 349). Three adults were fast the following day. No reaction ensued.

*Reinfested* on the 8th December, 1910, with six brown adults off East Coast fever animal 1053 (Reference No. 411), and reinfested six days later with two brown adults off 1053 (Reference No. 411) and two brown adults of heifer 1111 (Reference No. 426). Four ticks of the first batch and one tick of the second batch were found fast on the day after infestation. Ox 1046 contracted East Coast fever, and died on the 8th January, 1911.

(d) *Brown Nymphae* (Reference Nos. 363 and 364).

(7) *Ox* 1043.—Had been used previously for the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 5th September, 1910, with brown nymphae (Reference No. 319). Thirty-three ticks were found attached the following day. No reaction resulted.

*Reinfested* on the 20th October, 1910, with ten brown nymphae (Reference No. 364). Six nymphs were fast the following day. *Ox* 1043 contracted East Coast fever, and died on the 18th November, 1910.

(8) *Heifer* 1017.—Had been previously used in connection with the artificial transmission of East Coast fever, but with negative results.

*Infested* on the 5th September, 1910, with brown nymphae (Reference No. 363). Forty-two ticks were found attached the next day. No reaction ensued.

*Reinfested* on the 20th October, 1910, with ten brown nymphae (Reference No. 364). All ten ticks were fast the following day. A fever reaction set in and heifer 1017 died of East Coast fever on the 9th November, 1910.

(e) *Rhipicephalus appendiculatus* (Reference Nos. 364, 349, 411, and 426).

(9) *Heifer* 1090.—Had not been used previously in any experiment, and was purchased in an area free from East Coast fever.

*Infested* on the 20th October, 1910, with ten brown nymphae (Reference No. 364). On the following day six nymphs were found fast, but no reaction developed.

*Reinfested* on the 16th November, 1910, with four brown adults (Reference No. 349); origin Natal. Two adults were fast the following day, but no reaction ensued.

*Reinfested* on the 8th December with six brown adults off heifer 1053 (Reference No. 411). Five ticks were attached the following day.

*Reinfested* on the 14th December with two brown adults off heifer 1053 (Reference No. 411) and two adults off 1111 (Reference No. 426). Two adults were fast the following day. A reaction set in, and the animal died of East Coast fever on the 27th December, 1910.

(f) *Rhipicephalus appendiculatus* (Reference Nos. 363, 364, and 349).

(10) *Heifer* 1082.—An animal which had been born in an area free from East Coast fever and had not been used previously.

*Infested* on the 30th August, 1910, with brown nymphae (Reference No. 319). Twenty-three ticks were found attached on the 6th September, 1910, but no reaction followed.

*Reinfested* on the 15th September, 1910, with twenty brown nymphae (Reference No. 364). No reaction followed.

*Reinfested* on the 20th October, 1910, with six brown adults; origin Natal (Reference No. 349). On the following day five adult ticks were found fast. A reaction set in and the animal died of East Coast fever on the 25th November, 1910.

(g) *Rhipicephalus appendiculatus nymphae* (Reference No. 364).

(11) *Ox* 1050.—Had been previously used in connection with the artificial transmission of East Coast fever, without results.

*Infested* on the 20th October, 1910, with ten brown nymphae (Reference No. 364). Seven ticks were found attached the following day. A reaction set in, and the ox died on the 21st November, 1910, of East Coast fever.

## RESULTS.

*Rhipicephalus appendiculatus nymphae* (Reference No. 319).

These ticks failed to transmit the disease in every instance, and the nine animals which were infested were proved subsequently to be susceptible to East Coast fever. The larvae hatched out on the 26th April, 1910, and became infested at the end of May and beginning of June; that is, about five to six weeks after they had hatched. They moulted into nymphae on the 5th August, viz., about two months after they had become replete on heifer 1013. The shortest moulting period in this stage is sixteen days in the summer. They were placed on the susceptible animals as follows:—

Heifer	627	infested on the 17th August, 1910.....	20	ticks were fast.
„	911	infested on the 17th August, 1910.....	70	„ „
„	1014	infested on the 17th August, 1910.....	24	„ „
„	1068	infested on the 17th August, 1910.....	70	„ „
„	1082	infested on the 30th August, 1910.....	20	„ „
„	1037	infested on the 5th September, 1910....	27	„ „
„	1046	infested on the 5th September, 1910....	28	„ „
„	1017	infested on the 5th September, 1910....	42	„ „
„	1043	infested on the 5th September, 1910....	33	„ „

That is to say, twelve days, twenty-five days, and one month after they had moulted.

*Rhipicephalus appendiculatus nymphae* (Reference No. 364).

These ticks failed to transmit the disease in seven instances out of ten, the three positive cases being cattle 1043, 1017, and 1050. These ticks hatched on the 26th April, 1910 (the same as batch No. 363, belonging to the same original lot) and were placed on heifer 908 on the 12th August, namely, about 107 days after they had hatched. They moulted into nymphae on the 10th September, 1910.

They were placed on the following animals:—

Heifer	627	on the 15th September, 1910, and....	20	became attached.
„	911	on the 15th September, 1910, and....	20	„
„	1068	on the 15th September, 1910, and....	20	„
„	1050	(died) on the 15th September, 1910, and..	7	„
„	1082	on the 15th September, 1910, and....	20	„
„	1014	on the 15th September, 1910, and....	20	„
„	1046	on the 20th October, 1910, and.....	8	„
„	1043	(died) on the 20th October, 1910, and... 6	6	„
„	1090	on the 20th October, 1910, and.....	6	„
„	1017	(died) on the 20th October, 1910, and... 10	10	„

Of the animals infested on the 15th September, one died, and of the animals infested on the 20th October, two died. It is remarkable that in this instance the minimum number of ticks (six and ten) transmitted the disease.

*Rhipicephalus appendiculatus nymphae* (Reference Nos. 355 and 356).

These ticks failed to transmit the disease in four instances when used together for the purposes of infestation.

The larvae of batch 355 hatched on the 26th April, 1910, and when they were seventy-nine days old were infested with East Coast fever. They moulted into nymphae on the 1st September, 1910.

Larval ticks number 356 hatched on the 18th April, 1910, and when ninety-five days old were infested with East Coast fever. They moulted into nymphae on the 15th August, 1910.

They were placed on the following cattle:—

627 on the 30th September, 1910, and .....	14	became attached.
911 on the 30th September, 1910, and .....	20	„
1014 on the 30th September, 1910, and .....	10	„
1068 on the 30th September, 1910, and .....	14	„

*Rhipicephalus appendiculatus adults* (Reference No. 349).

These ticks transmitted the disease to five animals out of seven. The engorged nymphae were collected off sick cattle in Natal on the 29th June, 1910, and moulted into adults at the Laboratory on the 11th September, 1910, or seventy-four days after collection. They were used for the infestation of

Cattle 627 on the 20th October, 1910.....	3	became attached.
„ 911 on the 20th October, 1910.....	6	„
„ 1014 on the 20th October, 1910.....	6	„
„ 1068 on the 20th October, 1910.....	5	„
„ 1082 on the 20th October, 1910.....	5	„
„ 1046 on the 20th October, 1910.....	3	„
„ 1090 on the 16th November, 1910.....	2	„

*Rhipicephalus appendiculatus nymphae* (Reference No. 373).

These ticks transmitted the disease to one animal (1037). As larvae they were infected on heifer 909 on the 7th January, 1910. They moulted into nymphae on the 20th January, 1911, and were used on the 30th January, 1911, when ten days old. They were placed on heifer 1037 on the 30th January, 1911, and twenty became attached.

*Rhipicephalus appendiculatus adults* (Reference Nos. 426 and 411).

Batch number 426 transmitted the disease to two animals, 1040 and 1090. They were infected as nymphae on the 26th October, 1910, and moulted into adults on the 26th November, 1910.

Batch reference 411 were infected as nymphae on the 18th October, 1910. They moulted into adults on the 21st November, 1910.

The adults of batches 426 and 411 were eighteen days old when used. Ticks (Reference No. 411) were placed on heifer 1046 on the 8th December, 1910, of which four became attached, and on the 14th December (Reference Nos. 411 and 426) were infested, of which one became attached. Reference No. 411 undoubtedly transmitted the disease. Reference No. 411 were also placed on heifer 1090 on the 8th December, 1910, of which five became fast, and on the 14th December, 1910, 411 and 426 were infested, of which two became fast. The last batch transmitted the disease.

### EXPERIMENT No. 3.

TO NOTE WHETHER BROWN ADULTS WHICH WERE FEEDING AS LARVAE ON CATTLE SUFFERING FROM EAST COAST FEVER, AND WHICH PROVED TO BE VIRULENT AS NYMPHAE, WILL TRANSMIT THE DISEASE TO ADULTS AFTER HAVING PASSED THEIR NYMPHAL STAGE ON A RABBIT.

#### PARTICULARS OF THE TICKS.

##### I.—*Rhipicephalus appendiculatus* (Reference No. 342).

The larvae originated from mothers collected in Natal on the 12th January, 1910: the eggs were laid on the 18th January and the larvae hatched on the 18th February, 1910, and were placed on 923 on the 20th March, 1910

(Reference No. 268), at a time when the parasites of East Coast fever were frequently noted in the blood. The engorged larvae were collected on the 23rd March, 1910, and hatched out in due time. One lot was placed on cow 596 on the 7th April, 1910, and transmitted the disease, *vide* Experiment No. 1 (a). A second lot was placed on a rabbit, and the engorged nymphae were collected from the 13th to 29th April, 1910.

## II.—*Rhipicephalus appendiculatus* (Reference No. 309).

The larvae originated from mothers collected in Natal; the eggs were laid on the 24th December, and the larvae hatched on the 30th January, 1910; they were placed on calf 700 on the 27th February, 1910, *vide* Experiment No. 1 (b). The engorged larvae were collected during the period when the parasites of East Coast fever were frequently met with in the blood. These engorged larvae hatched on the 15th March, 1910, and the nymphae were placed on calf 917 [*vide* Experiment No. 1 (b)] and transmitted the disease. Another lot was placed on rabbits, and the engorged nymphae were collected from the 3rd to 7th June, 1910. They moulted on the 4th May, 1910.

### TESTING THE TICKS.

(1) *Heifer* 1059.—Was infested on the 29th August, 1910, with adult brown ticks (Reference No. 342) of the first rabbit. A reinfestation was made on the 14th September with ticks off the second rabbit (Reference No. 309). On the 19th September, 1910, twenty-six ticks were found attached. No reaction followed; the heifer eventually died of East Coast fever at Burnside.

(2) *Heifer* 1019.—Was infested on the 23rd June with twenty brown adults off the first rabbit (Reference No. 342). On the 28th June it was reinfested with twenty brown adults off the second rabbit (Reference No. 309). On the following day eight adults were found attached. On the 15th and 20th July, and on the 3rd and 20th August, this heifer was reinfested with brown adults off the first rabbit (Reference No. 342). On the 28th July, nine ticks were found fast. On the 4th August no ticks could be seen. No reaction followed, but the animal eventually died at Burnside of East Coast fever.

### CONCLUSION.

The infestation of two susceptible heifers with adult brown ticks which in their larval stage had been feeding on cattle suffering from East Coast fever (and as nymphae proved infective for cattle) did not convey East Coast fever as adults after they had passed their nymphal stage on a rabbit.

### EXPERIMENT No. 4.

TO NOTE WHETHER BROWN ADULTS WHICH FED AS NYMPHAE ON CATTLE IMMUNE TO EAST COAST FEVER WILL TRANSMIT THE DISEASE TO SUSCEPTIBLE CATTLE.

*Particulars of Ticks.*—Heifer 914 had been infested with brown nymphae (Reference Nos. 268, 335, and 309) on the 28th June, 1910, and contracted East Coast fever, from which it recovered. Plasma bodies were found in the glands and *Theileria parva* in the blood. The heifer showed a typical East Coast fever reaction, and by the end of May had completely recovered.

*History of Ticks used to infest the immune Heifer.*—Brown nymphae (Reference No. 298) off heifer 868, susceptible to East Coast fever, were placed on heifer 914 on the 2nd September, 1910. These nymphae engorged and dropped on the 6th and 7th September, 1910.

*Testing of the Ticks.*—They moulted into adults on the 19th October, 1910, and on the 15th December these adult ticks were placed on heifer 1021. The following day the ticks were found fast. No reaction followed.

Heifer calf 1130 was infested on the 6th January with ticks of the same batch as above (brown adults, Reference No. 298) and the following day they were found attached. No reaction followed.

#### CONCLUSIONS.

The infestation of two susceptible cattle with adult brown ticks which had passed their nymphal stage on a heifer immune to East Coast fever did not convey the disease. This observation supports some conclusions made as far back as 1903.

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#### SUMMARY OF CONCLUSIONS.

In the foregoing experiments it has been proved

(1) That brown tick imagines which as larvae had become infected with East Coast fever and which had transmitted the disease in their nymphal stage were no longer infective for susceptible cattle. Four batches of ticks proved their infectivity in the nymphal stage on eight animals, but in their adult stage failed to transmit the disease to two susceptible animals.

(2) Ticks belonging to the same batches which were feeding on two animals rendered immune to East Coast fever by inoculation in the nymphal stage did not transmit the disease in their adult stage to six animals, thus proving that the brown tick which has become infected in one stage cleans itself in the following stage by feeding on an immune or susceptible animal.

(3) Ticks which became infected with East Coast fever in their larval stage, and which passed their nymphal stage on a rabbit did not prove to be infective in their adult stage for susceptible cattle. This conclusion bears out that given above (2), showing that a tick loses its infectivity the first time it feeds on an animal susceptible or immune to East Coast fever.

(4) Clean or infective ticks feeding on an animal which has recovered from an attack of East Coast fever do not transmit the disease in their next stage. This conclusion is in support of experiments undertaken eight years ago (*vide* Annual Report of the Government Veterinary Bacteriologist, 1904-05).

(5) It has been demonstrated that certain batches of ticks collected at the same time and which fed under similar conditions do not transmit the disease in their next stage, even when infested in great numbers and on numerous animals. Other batches of ticks reared in exactly the same way and under similar conditions only infected a few animals, whilst again other ticks proved infective in almost every instance even when a minimum number were used.

It is difficult to give an explanation of this fact, but it is quite likely that outside conditions have some influence. The ticks which did not transmit the disease were bred during the coldest time of the year. This may perhaps explain the fact that during the winter months the infection in the field is not so active as during the summer months, remembering at the same time that during the winter months tick life is generally weaker than in the summer.