The Transmission of Tickborne Diseases by the Intrajugular injection of the Emulsified intermediary Host itself.

By Sir ARNOLD THEILER, K.C.M.G., D.Sc., Dr. Med. Vet., etc., Director of Veterinary Education and Research, and P. J. DU TOIT, Dr. Med. Vet., etc., Deputy-Director of Veterinary Education and Research, Onderstepoort.

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I.—EXPERIMENTS WITH HEARTWATER.

Recently Cowdry has shown that in ruminants suffering from the South African disease commonly known as heartwater, certain bodies are present that in all respects correspond to the description of Rickettsias, as found in man and animal suffering from Rocky Mountain fever, and which are considered to be the causal agency of the latter disease. Cowdry called the organism *Rickettsia ruminantium*. He also found identical organisms in the body of the transmitting host, *Amblyomma hebraeum*, after it had become infected by sucking blood on sick animals. This similarity with Rocky Mountain fever gave the impetus to further investigations analogous to those carried out by Spencer and Parker (R. R. Spencer and R. R. Parker. Rocky Mountain Spotted Fever. Public Health Reports, Washington. Reprint 976) to transmit heartwater by means of the injection of emulsified ticks; to which experiments Cowdry had drawn our attention.

There are two intermediate hosts of Rocky Mountain fever, viz., *Dermacentor andersoni* (Stiles) and *Haemaphysalis leporis-palustris* (Packard). Spencer and Parker worked exclusively with *Dermacentor andersoni*. This is a three-host tick, and in this respect resembles the *Ambloymma hebraeum*.

Of the several findings of these authors, the following are at present of interest to us:—

Dermacentor andersoni can receive the infection in the adult stage and transmit it through the eggs to the next generation, viz., to the larvae and nymphae. Any of the immature stages can also receive the infection and transmit it to the succeeding one, viz., larvae to nymphae and adults, and nymphae to adults. In this respect the transmission of heartwater by *Ambloymma hebraeum* is somewhat different. This tick receives the infection in the immature stages and transmits it to the succeeding ones, as is the case in Rocky Mountain fever. However, it has never been observed that the adult female that receives the infection transmits it to the progeny through the egg.

Of further importance are the following notes:—

The progeny of a female infected in the adult stage, viz., the egg, the larva, and the nympha, the latter soon after moulting, when

emulsified and injected will transmit the Rocky Mountain fever infection. Adult ticks infected in the adult stage often fail to transmit the fever, although the injection of the viscera soon after feeding is usually successful. The contents of adults infected in any of the immature stages, and which have passed through the winter or have been kept on ice, are only infective when the ticks have first been incubated or fed, although the adults do readily infect by feeding. The injection of the unfed, not incubated tick, however, may produce a certain amount of immunity. It was also found that a 24-hour incubation at 37° C. of unfed nymphae and adults infected as larvae give a higher percentage of positive infection than the injection of ticks not incubated. The similarity of the two diseases in regard to the aetiological agency being present in both Rickettsias, led us to undertake a few preliminary experiments to note whether in heartwater also artificial transmission of the disease is possible by means of emulsified ticks.

A.—Experiment 2305*a*, 29.10.25. *Nymphae, batch* 697, *reared as larvae on calf* 1024, *from* 26.3.25 *to* 1.4.25, *suffering from heartwater.*

1. Calf 1015 served as control to test the virulency of the nymphae and to feed the nymphae for the experiment.
Infested 29.10.25 nymphae 173 days old.

Result.—After an incubation period of 16 days the fever commenced. It lasted for 6 days, and the calf recovered. Sub-inoculations of blood from this calf taken during the fever reaction were made into two susceptible sheep on the 21.11.25. Sheep 13841 received intrajugularly 10 c.c. blood. Fever commenced after 10 days, and lasted 8 days, and the sheep recovered. Sheep 13893, also injected 10 c.c. intrajugularly. Fever commenced after 9 days, lasted 6 days, and the sheep recovered. The diagnosis heartwater was made in both cases.

2. Sheep 13840, 29.10.25. Injected intrajugularly emulsified infected, unfed nymphae, 173 days old.

Result.—After an incubative period of 14 days the fever commenced. It lasted 9 days, and the animal recovered. Sub-inoculations were made with the blood of this sheep taken during the fever into two susceptible sheep. Sheep 13950, on the 18.11.25, was injected intrajugularly with 10 c.c. blood taken on the same day. After 9 days, fever commenced and lasted six days. The sheep died of heartwater on 4.12.25. Sheep 13994 was injected as above. Fever commenced after 10 days and lasted for 10 days. The sheep recovered.

3. Sheep 13874, 6.11.25. Injected intrajugularly with emulsified infected nymphae that had been feeding on calf 1015 from 29.10.25 to 5 and 6.11.25. The nymphae were fully engorged, 181 days old.

Result.—After 8 days the fever commenced and lasted 12 days. The sheep recovered after severe illness. Sub-inoculations were made into two susceptible sheep. On the 18.11.25 sheep 13954 was injected intrajugularly with 10 c.c. blood. Fever commenced 9 days later and lasted 12 days. It recovered. Sheep 13992 showed fever after 8 days, it lasted 11 days. The sheep recovered. Both reactions were typical of heartwater

Résumé of Results of Experiment 2305*a*.—Nymphae of *Amblyomma hebraeum* infected with heartwater, unfed and fed, emulsified and injected intrajugularly produced heartwater in susceptible sheep. The disease was in no instance fatal; mild in the sheep injected with unfed and severe in the sheep with fed ticks. The diagnosis heartwater was in all cases supported by sub-inoculation as well as in the control calf that had served to feed the ticks.

B.—Experiment 2305*b*. 4.1.26. *Nymphae,* Amblyomma hebraeum, *batch* 688, *reared from* 21.3.25 *until* 27.3.25 *as larvae on heifer* 1024 *suffering from heartwater. Moulted to nymphae on* 9.5.25.

1. Cow 629 served as control to test the virulency of the infected ticks and to feed the nymphae collected after engorging and dropping. She was infested on 4.1.26. Nymphae 227 days old.

Result.—This cow did not contract the disease. From the previous and succeeding history of this cow it must be concluded that she was immune against heartwater, having been running on heartwater veld, where she is still running at present.

2. Sheep 11971 on the 4.1.26 was injected intrajugularly with emulsified unfed nymphae 227 days old.

Result.—The sheep was kept under observation for 4 weeks and remained healthy. On 5.2.26 the sheep was tested for immunity against heartwater by the intrajugular injection of 5 c.c. virulent blood. Fever commenced after 8 days. The sheep died of heartwater 4 days later (17.2.26).

3. Sheep 12080 on the 12.1.26 was injected with emulsified nymphae fed from 4.1.26 until 12.1.26 on cow 629. Nymphae 235 days old.

Result.—Fever commenced after six days and lasted 10 days. The sheep recovered. Sub-inoculations were made into 2 sheep with blood taken during the reaction. On the 23.1.26 sheep 14294 was injected intrajugularly 10 c.c. After 4 days the fever commenced and lasted 10 days. The sheep recovered. Sheep 14341 received intrajugularly 10 c.c. The fever commenced after 5 days and lasted 7 days. The sheep recovered. On account of the somewhat unusual short incubation period, further sub-inoculations were made from each sheep into two others. In all four cases fever reactions occurred, ending with death in three and in recovery from heartwater in one case.

Résumé of Results in Experiment 2305*b*.—The nymphae that were used in this experiment were undoubtedly infected, although the control cow did not contract the disease. She was in all probability an immune animal. The interesting feature is that unfed nymphae did not transmit heartwater to a susceptible sheep, but fed nymphae did, and produced a disease after a rather short incubation period. Subsequent sub-inoculation showed that the virus transmitted was of a rather severe virulency.

C.—Experiment 2512. *Nymphae, batch* 796 *reared* 2.3.26 *until* 7.3.26 *as larvae on calf* 1776 *suffering from heartwater, moulted on* 1.4.26.

Result.—Calf 1199 served as control for the virulency of the infected ticks and to feed the nymphae. It was infested on the 26.4.26. Nymphae 25 days old. After an unusual long incubative

period of 23 days, the fever commenced. It lasted for 5 days. There was some doubt about the nature of this reaction, and sub-inoculations were made into two sheep on the 25.5.26. Sheep 13770 was injected intrajugularly with 5 c.c. blood. This sheep developed a somewhat irregular temperature curve during the incubative period, and died 13 days after injection of heartwater (7.6.26). Sheep 13773 was injected intrajugularly with 5 c.c. blood. After 7 days, fever commenced, lasting 14 days, and the sheep recovered. The diagnosis heartwater was made. Sub-inoculations from this sheep were made into two others, 14259 and 14283, of this experiment, which had not reacted to the injection of unfed ticks. Both contracted heartwater.

2. Sheep 14259 on the 26.4.26 was injected intrajugularly with unfed emulsified ticks. Nymphae 25 days old.

Result.—An atypical reaction commenced after 18 days and the diagnosis heartwater was excluded. On 4.6.26 sheep 14259 was tested for its immunity with blood of sheep 13773. It contracted heartwater after an incubation period of 8 days. The reaction lasted 10 days and the sheep died of heartwater on 22.6.26. The failure of transmission was thus not due to any immunity in sheep 14259.

3. Sheep 14283 on the 1.5.26 was injected intrajugularly with emulsified nymphae incubated at 37° C. for 110 hours. Nymphae 30 days old.

Result.—No reaction was noted in this sheep. On the 4.6.26 this sheep was tested for its immunity with blood of sheep 13773. It contracted heartwater after an incubation period of 7 days. The reaction lasted 2 days, and the sheep recovered. The failure of transmitting the disease was thus not due to the immunity of sheep 14283.

4. Sheep 14305 on the 1.5.26 was injected intrajugularly with emulsified nymphae fed on calf 1199 for 110 hours. Nymphae 30 days old.

Result.—There was a somewhat irregular course of temperature, three different reactions following each other. The second one commenced on the 8th day and lasted 8 days. It was diagnosed as heartwater. After the third reaction the sheep recovered. In order to support the diagnosis heartwater a sub-inoculation was made on 13.5.26 into sheep 14346. After an incubation period of 10 days the sheep showed fever lasting 8 days, culminating in death from heartwater (31.5.26).

5. Sheep 14316 on the 1.5.26 was injected intrajugularly with emulsified nymphae fed on calf 1199 and collected after they had been fully engorged. Nymphae 30 days old.

Result.—After an incubation period of 6 days, during which there were slight temperature disturbances, a fever reaction commenced lasting 11 days, and the sheep recovered. Sub-inoculations into susceptible sheep were made, viz., on 14.5.26 into sheep 13946, which received 5 c.c. blood intrajugularly. This sheep after an incubation period of 8 days showed a reaction lasting 12 days, and recovered. A further sub-inoculation into sheep 5068 was made on 27.5.26. After an incubation period of 5 days it showed a fever lasting 12 days, and recovered. Both these sheep showed other clinical symptoms, and the diagnosis heartwater was made in both instances.

Résumé of Results in Experiment 2512.—The infected nymphae transmitted heartwater to the control calf by feeding. Intrajugular injection of emulsified unfed nymphae or unfed incubated nymphae did not transmit heartwater. Emulsion of fed nymphae injected intrajugularly transmitted the disease. Sheep that did not show a reaction did not acquire immunity against heartwater.

D.—Experiment 2620. 29.6.26. *To transmit Heartwater by means of intrajugular injection of infected fed nymphae. Nymphae, batch* 796, *reared* 2.3.26 *until* 7.3.26 *as larvae on calf* 1776 *suffering from heartwater. Moulted on* 1.4.26.

1. Calf 1925 served as control for the virulency of the infected ticks and to feed the nymphae. It was infested on the 29.6.26. The nymphae were 117 days old.

Result.—After an unusually long incubative period of 18 days the fever commenced. It lasted 10 days and took a regular course, at the end of which the calf died. It had shown typical nervous symptoms towards the end of the illness. The ticks were collected on the 7.7.26 and injected intrajugularly into sheep 13844, 13845, 13940, and 13857, and again on the 10.7.26 injected into sheep 13872. During the reaction, calf 1925 was bled, and the blood injected on 22.7.26 into sheep 7829 and sheep 9293. Both contracted heartwater, from which one died and one recovered.

2. Sheep 13844. 7.7.26. Injected intrajugularly 5 c.c. of an emulsion of almost engorged nymphae for 8 days fed on calf 1925. The nymphae were ground up in physiological water and then decanted. Each c.c. liquid contained one tick. The nymphae were 125 days old.

Result.—After an incubative period of 5 days the fever commenced and lasted about 14 days. The sheep was severely ill, but recovered. During the reaction blood was withdrawn and injected into sheep 6093 and 6819 on 19.7.26. Both contracted heartwater, one sheep (6093) died and sheep 6819 recovered.

3. Sheep 13845. On 7.7.26 this sheep received intrajugularly 1 c.c. of an emulsion of almost engorged nymphae, 125 days old, fed on calf 1925, ground up finely with sand and then decanted. The emulsion contained 1 nympha per c.c. of liquid.

Result.—After an incubative period of 9 days the fever commenced and lasted 8 days. The sheep showed severe illness, but recovered.

4. Sheep 13857. On 7.7.26 this sheep received intrajugularly 0·1 c.c. of an emulsion of almost engorged nymphae, fed on calf 1925, ground up in sand and decanted. The emulsion contained one tick per c.c. liquid. Nymphae 125 days old.

Result.—After an incubative period of 9 days the fever commenced, and lasted 8 days. The sheep was severely ill, but recovered.

5. Sheep 13940. On 7.7.26 this sheep received intrajugularly 0·01 c.c. of an emulsion of almost engorged nymphae, fed on calf 1925, ground up with sand and decanted. The emulsion contained one tick per c.c. liquid. Nymphae 125 days old.

Result.—The sheep showed no reaction. To test its immunity it was injected on 31.7.26 with 5 c.c. virulent blood of sheep 6093, suffering at the time from heartwater (transmitted by the injection sub. 2). Sheep 13940 contracted heartwater, and died of this disease.

6. 10.7.26. Sheep 13872 received an intrajugular injection of 5 c.c. emulsion of nymphae fed on calf 1925, ground up, almost fully engorged, with sand, and then filtered through filter-paper. The liquid contained 1 nympha per c.c. Age 128 days.

Result.—The incubative period lasted 9 days and the disease 11 days. The sheep was severely ill, but recovered.

Résumé of Results of Experiment 2620.—The infected nymphae were when placed on calf 1925 117 days old. They transmitted heartwater to this calf, of which it died. The ticks that had been feeding for 8 days and 10 days respectively transmitted the disease after intrajugular injection in the quantities of 5 c.c., 1 c.c., 0·1 c.c. tick when ground up with sand and simply decanted, and in the quantity of 5 ticks after passing a paper filter. The quantity of 0·01 c.c. decanted tick emulsion was not virulent, the respective sheep subsequently contracting heartwater after an intrajugular injection of virulent blood.

E.—29.6.26. *Experiment* 2621. *To transmit heartwater by means of emulsified infected* (a) *unfed nymphae and* (b) *unfed and incubated nymphae.*

The emulsion was made in saline solution, and was injected intrajugularly, both filtered and not filtered. The liquid contained 1 nympha per c.c. The nymphae were of batch 796 that in experiment 2620 proved infective for calf 1925. The ticks were 117 days old.

(a) *Experiments with unfed nymphae—*

Nos. of Sheep.	*Quantity Injected.*	*Result.*
6474	5 c.c., not filtered	Negative.
6516	1 c.c. „	„
6811	0·1 c.c. „	„
7418	0·01 c.c. „	„
10600	5 c.c., filtered	„

(*b*) *Experiments with unfed incubated nymphae,* 118 *days old—*

Nos. of Sheep.	*Quantity Injected.*	*Result.*
12194	5 c.c., not filtered	Negative.
13759	1 c.c. „	„
13781	0·1 c.c. „	„
13827	0·01 c.c. „	„
13837	5 c.c., filtered	„

Résumé of Results.—The unfed nymphae although infective as the results in experiment 2620 with calf 1925 show, did not transmit the disease when emulsified and intrajugularly injected . It is remarkable that an emulsion of ticks of the same batch when fed in experiment 2620 produced the disease but not an emulsion of unfed ticks. This result is contrary to that obtained in experiment 2305, in which both fed and unfed nymphae produced the disease when emulsified and injected intrajugularly.

F.—29.7.26. *Experiment* 2614. *To transmit heartwater by means of emulsified infected nymphae* (a) *not fed,* (b) *not fed and incubated, and* (c) *fed. The emulsion was made by grinding the ticks in a saline-glycerine solution* aa *and in the dilution of* 1 *nympha per* 2 *c.c. liquid. The emulsion was injected intrajugularly, both filtered and not filtered.*

The nymphae belonged to batch 796 that were fed in experiment 2620 (v. supra) on calf 1925 and proved to be virulent. The nymphae were 147 days old.

(a) *Experiments with not fed nymphae—*

Nos. of Sheep.	*Quantity Injected.*	*Result.*
10851	4 c.c., not filtered	Negative.
10799	2 c.c. ,,	,,
13917	0·2 c.c. ,,	,,
13923	0·02 c.c. ,,	,,
13851	4 c.c., filtered	,,

(b) *Experiments with not fed nymphae incubated for* 24 *hours—*

Nos. of Sheep.	*Quantity Injected.*	*Result.*
13968	4 c.c., not filtered	Negative.
13793	2 c.c. ,,	,,
10407	0·2 c.c. ,,	,,
11113	0·02 c.c. ,,	,,
13788	4 c.c., filtered	,,

(c) *Experiments with fed nymphae collected almost engorged,* 7.7.26 (vide *experiment* 2620) *from calf* 1925 *and on* 10.7.26—

Nos. of Sheep.	*Quantity Injected.*	*Result.*
13986	2 c.c., not filtered	Negative.
14347	0·2 c.c. ,,	,,
13871	0·02 c.c. ,,	,,
13882*	4 c.c., filtered	,,

Résumé of Results.—All results were negative; also the experiments with the fed nymphae, which in experiment 2620 gave positive results injected in identical quantities intrajugularly. The reason for this failure must be looked for in the difference of the medium used for the emulsion. In experiment 2620 this was normal saline solution; in this experiment saline and glycerine in equal quantities, viz., a 50 per cent. of glycerine, which apparently kills the virus.

* Collected 10.7.26.

TABULATED SUMMARY OF RESULTS WITH A. HEBRAEUM NYMPHAE.

Experiment.	No. of Batch.	Age of Ticks.	No. of Animal.	No. of Ticks Used.	Fed on.	Control.	72 h.–96 h.	96 h.–120 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2305	697	Days. 173	1015	00		R						2305 = bovine.
,,	,,	173	13840	?						R		
,,	,,	181	13874	?	1015				R			Ticks injected 8 days after attachment fully engorged.
2305	688	227	629	00								629 = immune cow.
,,	,,	227	11971	?						O	Died	
,,	,,	235	12080	?	629				R			Ticks injected 8 days after attachment fully engorged.
2512	696	25	1199	00		R						
,,	,,	25	14259	?						O	Died	
,,	,,	30	14283	?						O	R	Incubated 110 hours before the injection.
,,	,,	30	14305	?	1199			R				,, ,, ,, ,,
,,	,,	30	14316	?	1199				R			Ticks injected fully engorged.
2620	796	117	1925	00		Died						
,,	,,	125	13844	1	1925				R			The nymphae were almost engorged.
,,	,,	125	13845	1	1925				R			Emulsified with sand and decanted.
,,	,,	125	13857	0·1	1925				R			1 c.c. of liquid contained 1 tick.
,,	,,	125	13940	0·01	1925				O		Died	,, ,, ,, ,,
,,	,,	128	13872	5	1925				R			Almost fully engorged, emulsified with sand and filtered.

Explanation of Table: 00 = many ticks; ? = several ticks; R = Reaction; O = No reaction.

TABULATED SUMMARY OF RESULTS WITH A. HEBRAEUM NYMPHAE.—(*continued*).

Experiment.	No. of Batch.	Age of Ticks.	No. of Animal.	No. of Ticks Used.	Fed on.	Control.	72 h.–96 h.	96 h.–120 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2621	796	Days. 117	6474	5						O		Emulsified and not filtered.
,,	,,	117	6516	1						O		
,,	,,	117	6811	0·1						O		
,,	,,	117	7418	0·01						O		
,,	,,	117	10600	5						O		Emulsified and filtered.
2621	796	118	12194	5						O		Incubated ticks for one day.
,,	,,	118	13759	1						O		,, ,, ,,
,,	,,	118	13781	0·1						O		,, ,, ,,
,,	,,	118	13827	0·01						O		,, ,, ,,
,,	,,	118	13837	5						O		Incubated ticks for one day, filtered.
2664	796	147	10851	4 c.c.						O		Saline-glycerine emulsion, not filtered.
,,	,,	147	10799	2 c.c.						O		(2 c.c. = 1 tick.)
,,	,,	147	13917	0·2 c.c.						O		,, ,,
,,	,,	147	13923	0·02 c.c.						O		,, ,,
,,	,,	147	13851	4 c.c.						O		Saline-glycerine emulsion, filtered.
,,	,,	147	13968	4 c.c.						O		Incubated—24 hours.
,,	,,	147	13793	2 c.c.						O		
,,	,,	147	10407	0·2						O		
,,	,,	147	11113	0·02						O		
,,	,,	147	13788	4						O		Filtered.
,,	,,	147	13186	2	1925			O				Almost fully engorged; not filtered.
,,	,,	147	14347	0·2	1925			O				
,,	,,	147	13871	0·02	1925			O				
,,	,,	147	13832	4	1925			O				Filtered—Saline glycerine.

Explanation of Table: 00 = many ticks; ? = several ticks; R = Reaction; O = No reaction.

Conclusion.—The artificial transmission by intrajugular injection of emulsified infected *Amblyomma hebraeum* nymphae succeeded only in one instance with unfed nymphae. It succeeded in eight out of nine instances with fed nymphae. The minimal successful quantity was 0·1 tick per c.c. liquid. The emulsion was infective when made by grinding the ticks in saline solution with and without the medium of sand, and both filtered and not filtered. The emulsion was not infective when a 50 per cent. glycerine-saline solution was used. The virus is apparently killed by the glycerine. In the instances where infection did not succeed, no immunity was established. The feeding of the nymphae seems to increase the virulency of the virus they contain.

II.—EXPERIMENTS WITH EAST COAST FEVER.

The successful transmission experiments with heartwater immediately suggested the undertaking of similar experiments with East Coast fever. There is some similarity in the mode of transmission in these two diseases. In both instances a three-host tick is responsible. They differ in their aetiological agency, East Coast fever being caused by *Theileria parva,* belonging to the *Piroplasmidae*; the infection is received by the immature stages, i.e. by nymphae that have been feeding as larvae and adults that have been feeding as nymphae on infected cattle. Infection cannot be transmitted from adult to larvae through the egg or from larvae to adult if the nympha has been feeding on a non-infected host. It is a remarkable fact that infected nymphae or adults only transmit the disease in the interval from 72 to 120 hours after attachment to the host, to which fact full consideration was given in the transmission experiments with emulsified ticks.

(1) *Experiments with adults of* Rhipicephalus appendiculatus.

A.—*Experiment* 2407. *Ticks of batch* 771, *infected as nymphae on ox* 1170 (*suffering from East Coast fever*) *from* 2.1.26 *to* 6.1.26, *moulted to adults on* 28.1.26. *Bull* 972 *and ox* 1666 *had to act as controls for the virulency of the ticks.*

1. Bull 972 infested on the 9.2.26 with adult ticks. Adults 12 days old.

Result.—After six days incubation the fever commenced. It lasted sixteen days, and the calf succumbed to East Coast fever on 4.3.26. Agamonts and gamonts were seen during life in the lymph glands and *Theileria parva* were present in the blood.

2. Ox 1666 infested on the 9.2.26 as above. This calf served to feed the nymphae injected into bull 994 and bull 978. Adults 12 days old.

Result.—After six days incubation the fever commenced. It lasted 17 days, and the calf succumbed to East Coast fever on 5.3.26. Agamonts and gamonts were seen during life in the lymph glands and *Theileria parva* were noted in the blood.

3. Bull 996 was injected on the 9.2.26. Unfed adults were emulsified and injected intrajugularly. Adults 12 days old.

Result.—The animal was kept under observation for 4 weeks. It remained healthy. Bull 996 was subsequently tested in experiment 2527 on 11.5.26 with six infected males of batches 750 and 760. The bull succumbed to East Coast fever.

4. Bull 1012. Injected intrajugularly as above with emulsified unfed adults of the same batch. Adults 12 days old.

Result.—Bull 1012 was kept under observation for four weeks. It remained healthy. Subsequently bull 1012 was injected (experiment 2484) on the 10.4.26 intrajugularly with an emulsion of infected nymphae of batch 759 fed for 72 hours on an East Coast fever immune heifer 725. Bull 1012 showed a double reaction, the first beginning after 6 days and lasting 4 days, the second four days later and lasting 7 days. Agamonts and gamonts were not found in the lymph glands, but small piroplasms were found in the blood during the second reaction. It was tested on the 17.6.26 with infected ticks, batch 783, that in ox 1668, acting as a control in experiment 2599, produced East Coast fever. These ticks did not convey the disease to calf 1012, thus proving that the previous reaction in calf 1012 was one of East Coast fever.

5. Bull 978 on the 13.2.26 was injected intrajugularly with six emulsified adults fed on ox 1666 for 90 hours, and removed before complete engorgement. Adults 16 days old.

Result.—After 5 days incubation the fever commenced, and lasted 19 days, when the calf succumbed to East Coast fever (10.3.26). Agamonts and gamonts were found during life in the lymph glands and *Theileria parva* were present in the blood.

6. Bull 994, on the 13.2.26, was injected into jugular vein with six emulsified adults fed for 90 hours on ox 1666. Adults 16 days old.

Result.—After one day's incubation the fever commenced and lasted 16 days. The bull succumbed on 3.3.26 to East Coast fever. Agamonts and gamonts were seen during life in the lymph glands and *Theileria parva* were present in the blood.

Résumé of Results.—The adults transmitted East Coast fever by feeding, and so proved their virulency. Unfed adults did not transmit the disease when intrajugularly injected, nor did this injection give immunity. The injection of adults fed for 72 hours gave rise to a fever that was most likely abortive East Coast fever, as subsequent tests to immunity with infected ticks proved. Adults fed for 90 hours, emulsified and injected, transmitted East Coast fever in both instances.

B.—Experiment 2528. 11.5.26. *Adults of batches* 750 *and* 760. *Batch* 750 *was feeding as nymphae from* 18.6.25 *to* 22.6.25 *on heifer* 745, *suffering from East Coast fever. Moulted on* 27.8.25. *Batch* 760 *was feeding as nymphae from* 1.10.25 *to* 5.10.25 *on ox* 1670 *suffering from East Coast fever. Moulted on* 4.11.25. *Bull* 1049, *ox* 1600, *and bull* 996 *acted as controls for the virulency of the ticks. Bull* 1049 *also served to feed the tick for the intrajugular injection.*

1. Bull 1049, 11.5.26, infested with 30 males and 30 females. Adults 258 and 189 days old respectively.

Result.—After an incubation period of 14 days the fever commenced. It lasted 18 days, and the calf succumbed to East Coast fever on 13.6.26. Agamonts and gamonts were seen in the lymph glands during life and *Theileria parva* were present in the blood.

2. Ox 1600, 11.5.26, infested with six females. Adults 258 and 189 days old respectively.

Result.—After an incubation period of 15 days the fever commenced. It lasted 17 days, and the ox succumbed to East Coast fever on 13.6.26. *Theileria parva* were found in the blood and agamonts and gamonts were seen during life in the lymph glands.

3. Bull 996, on the 11.5.26, was infected with six males. Adults 258 and 189 days old respectively.

Result.—After an incubation period of 14 days the fever commenced. It lasted 15 days, and the bull succumbed to East Coast fever on the 9.10.26. *Theileria parva* were seen in the blood and agamonts and gamonts were present in the lymph glands during life.

4. Heifer 1194, on the 11.5.26, was injected intrajugularly with an emulsion of 6 males and 6 females, not fed. Adults 258 days and 189 days old respectively.

Result.—Heifer 1194 was kept under observation for one month and remained healthy. Heifer 1194 subsequently used in experiment 2601 and 2749 and 2806 with negative results.

Immunity Test—Heifer 1194. Infested *Rhip. appendiculatus* adults, batch 848, infected with East Coast fever. The fever menced after an incubation period of 14 days, and the heifer died of East Coast fever on the 19.11.26. *Theileria parva* were found in the blood.

5. Heifer 1197, on the 14.5.26, was injected intrajugularly with an emulsion of 6 males and 6 females, feeding for 72 hours on calf 1049. Adults 261 and 192 days old respectively.

Result.—Heifer 1197 was kept under observation for one month and remained healthy. Subsequently used in experiments 2601 and 2749 with negative results.

Immunity Test.—Heifer 1197, on 26.10.26, infested *Rhip. appendiculatus*, adults batch 784. After an incubation period of 11 days the fever commenced, and the heifer died of East Coast fever on 18.11.26.

6. Heifer 1128, on the 15.5.26, was injected intrajugularly with an emulsion of 6 males and 6 females feeding for 90 hours on bull 1049. Adults 262 and 193 days old respectively.

Result.—Heifer 1128 was kept under observation for 4 weeks and remained healthy. Subsequently used in experiment 2601 with negative results.

Immunity Test.—9.9.26. Heifer 1128 was infested *Rhip. appendiculatus*, adults batch 784. After an incubation of 9 days the fever commenced, and the heifer died on 9.10.26 of East Coast fever.

7. Bull 1015, on the 20.5.26, was injected intrajugularly with an emulsion of 6 males and 6 females feeding for 120 hours on bull 1049. Adults 267 and 198 days old respectively.

Result.—Bull 1015 was kept under observation for 4 weeks and remained healthy. Subsequently used in experiments 2750 and 2807 with negative results.

Immunity Test.—Bull 1015, on 20.11.26 infested *Rhip. appendiculatus* nymphae, batch 781. After an incubation of 9 days the fever commenced, and the bull died, 22.12.26, of East Coast fever.

8. Bull 853, on the 25.5.26, was injected intrajugularly with an emulsion of 6 engorged males and 6 females removed on the 10th day after infestation from bull 1049. Adults 272 and 203 days old respectively.

Result.—Bull 853 was kept under observation for 4 weeks and remained healthy. Subsequently injected in experiments 2606 and 2750 with negative results.

Immunity Test.—Bull 853, on 27.10.26, infested *Rhip. appendiculatus* nymphae, batch 781. After an incubation of 9 days the fever commenced, and the bull died of East Coast fever on 26.11.26.

Résumé of Results.—The adult ticks used in this experiment transmitted East Coast fever when fed on three different calves. The natural infection was thus successful, not so the artificial by the intrajugular injection of the emulsified ticks. The calves utilized for the injection were not immune, as subsequent tests with the feeding of infected ticks proved definitely. In view of the positive result of experiment 2407, we are at present not able to explain the failure.

Experiment 2601 (a). 17.6.26.

1. *Adults of batches* 760 *and* 775. *Batch* 760 *had been feeding as nymphae on ox* 1670 *suffering from East Coast fever from* 1.10.25 *to* 5.10.25 *and the nymphae moulted on* 4.11.25. *Batch* 775 *had been feeding as nymphae on ox* 1171 on 5.1.26 *to* 9.1.26 *and moulted on* 28.1.26.

1. Bull 1133 served as a control to test the infectivity of the adults of batches 760 and 775. (Batch 760 had previously proved to be infective in experiment 2528 on cattle 1049, 1600 and 996.) The attached ticks were to be removed between the 96th and 108th hours and to be injected intrajugularly into the susceptible heifer 1197. Bull 1133 infested, 17.6.26, adults batch 760 and 775. Adults of batch 760 were 236 days old and adults of batch 775 were 140 days old. After an incubation period of 11 days the fever commenced. It lasted 19 days, and the calf died of East Coast fever on 18.7.26.

2. Heifer 1197. 21.6.26 injected 6 adults fed for 96 hours on bull 1133 emulsified with sand in 5 c.c. saline solution, and subsequently allowed to settle and be decanted. The ticks were 240 and 144 days old respectively when injected.

Result.—Calf 1197 did not contract East Coast fever.

Immunity Test.—Heifer 1197 was infested, 26.10.26, adults *Rhip. appendiculatus*, batch 784 infected East Coast fever. It contracted East Coast fever and succumbed 18.11.26.

Experiment 2601 (b). 21.6.26.

Adults of batches 760 and 775, for particulars *vide supra*, experiment 2601 (*a*). In that experiment batches 760 and 775 proved to be virulent for bull 1133. In this experiment adults of the same batch were fed on heifer 893 (immune to East Coast fever; recovered in 1925). Heifer 893 had been infested on 17.6.26 with the adults under consideration (236 and 140 days old), and were removed on the 21.6.26, viz., 96 hours later. Heifer 893 being immune did not contract the disease.

21.6.26. Heifer 1193 injected intrajugularly emulsion of 6 adults fed on immune heifer 893. The emulsion was made by grinding the ticks with sand, then added saline solution, and the liquid allowed to settle. The supernatant liquid was injected. The adults were 240 and 144 days old when injected.

Result.—Heifer 1193 did not contract East Coast fever.

Immunity Test.—9.9.26. Heifer 1193 was infested with nymphae of *Rhip. appendiculatus* of batch 774. It contracted East Coast fever after an incubation period of 10 days; the disease lasted 19 days, and the animal died, 8.10.26, of East Coast fever.

Résumé of Results: Experiments 2601 (*a*) *and* (*b*).—The ticks used in this experiment proved to be virulent when fed on a susceptible bovine. Ticks that had been fed for 96 hours on a susceptible or an immune bovine were then emulsified and injected into the jugular vein without transmitting the disease. This result is in conformity with that of experiment 2528, where injected ticks also failed to transmit the disease after intrajugular injection. The minimal age of the ticks in experiments 2528 and 2601 was 144 days. In the successful experiment 2407 it was 16 days.

Experiment 2606.

1. Bull 853. 21.6.26. Injected intrajugularly with 1 East Coast fever infected adult fed for 96 hours on heifer 893 of batches 760 and 775 after it had been emulsified with sand and the liquid passed through filter-paper. The ticks were 240 and 144 days old.

Result.—Bull 853 did not contract East Coast fever.

Immunity Test.—27.10.26. Bull 853 was infested East Coast fever infected nymphae *Rhip. appendiculatus*, batch 781. It contracted and succumbed to East Coast fever on 27.11.26.

2. *Heifer* 1135. 21.6.26 injected intrajugularly 1 East Coast fever infected adult fed for 96 hours on heifer 893 of batches 760 and 775 after it had been emulsified with sand and the liquid simply decanted.

Result.—Heifer 1135 did not contract East Coast fever.

Immunity Test.—Heifer 1135 was tested on 26.10.26 with infected adults of *Rhip. appendiculatus*, batch 784. It contracted East Coast fever and died on 24.11.26.

Experiment 2749. Rhip. appendiculatus *adults batch* 784. *They had been infected as nymphae on ox* 1173 *on* 14.1.26 *to* 19.1.26, *suffering from East Coast fever. They moulted on* 6.2.26. *The adults were tested for virulency on heifer* 1128 (*in experiment* 2739). *Some of the adults were to be removed from heifer* 1128 *after feeding for* 100 *hours and to be used for subcutaneous and intrajugular injections.*

1. Heifer 1128. 9.9.26. Infested adults *Rhip. appendiculatus*, batch 784. The adults were 203 days old.

Result.—After an incubation period of 9 days the fever commenced. It lasted 21 days. The heifer succumbed to East Coast fever on 9.10.26, and the diagnosis of East Coast fever was confirmed on post-mortem. *Theileria parva* were present during the reaction.

2. Heifer 1135. 13.9.26. Injected intrajugularly 1 c.c. emulsion of adults removed on same date (i.e. fed for 100 hours) from heifer 1128. The ticks were emulsified with sand and the emulsion diluted so that 1 c.c. contained one tick. The adults were 207 days old.

Result.—Heifer 1135 was under observation for one month. She remained healthy.

Immunity Test.—26.10.26. Heifer 1135 was infested *Rhip. appendiculatus* adults, batch 784. After an incubation period of 9 days the fever commenced, and the heifer died on 24.11.26.

3. Heifer 1197. 13.9.26. Injected intrajugularly 5 c.c. emulsion of adults removed on same date (i.e. fed for 100 hours) from heifer 1128. The adults were 207 days old. The ticks were emulsified as described sub. 2.

Result.—Heifer 1197 was kept under observation for one month. It remained healthy.

Immunity Test.—26.10.26. Heifer 1197 was infested adults *Rhip. appendiculatus*, batch 784, infected with East Coast fever. After an incubation period of 10 days, fever commenced, and the heifer succumbed to East Coast fever on 18.11.26.

4. Ox 1109. 13.9.26. Injected subcutaneously 1 c.c. emulsion of adults removed on same date (i.e. fed for 100 hours) from heifer 1128. The adults were 207 days old. The ticks were emulsified as described sub. 2.

Result.—Ox 1109 was kept under observation for one month. It remained healthy. Subsequently injected in experiment 2806 with negative results.

Immunity Test.—20.11.26. Tested adults *Rhip. appendiculatus*, batch 848. After an incubation period of 9 days the fever commenced, and the ox recovered from the disease after an illness of 18 days.

5. Heifer 1194. 13.9.26. Injected subcutaneously 5 c.c. emulsion of adults removed on same date (i.e. fed for 100 hours) from heifer 1128. The adults were 207 days old. The ticks were emulsified as described sub. 2.

Result.—Heifer 1194 was kept under observation for one month. It remained healthy. 11.10.26. Heifer 1194 was reinjected (experiment 2806) with negative results.

Immunity Test.—20.11.26. Heifer 1194 infested adults *Rhip. appendiculatus*, batch 848. This heifer succumbed to East Coast fever.

Résumé of Results.—The ticks utilized in this experiment were infective, and conveyed East Coast fever to a susceptible bovine after feeding on it. The emulsion of ticks that were fed for 96-100 hours and removed did not transmit the disease when injected either intrajugularly or subcutaneously. This result is in accordance with that of experiments 2528 and 2601. The minimal age of the ticks used was 207 days.

Experiment 2806. 31.10.26.

Adults of batches 784. For details *vide supra* experiment 2749.

1. Heifer 1197, 26.10.26, infested batch 784. Served as a control for the virulency of batch 784 and to feed the ticks, some of which were removed after 100-hours feeding. The nymphae were 253 days old.

Result.—The fever commenced after an incubation period of 10 days. It lasted 14 days, and the heifer died on 18.11.26 of East Coast fever. Agamonts and gamonts of *Theileria parva* were seen during the course of the fever.

2. 26.10.26. Infested heifer 1135. The heifer served as a control for the virulency of batch 784 and to feed the adults, some of which were to be removed after 100 hours. The adults were 253 days old.

Result.—The fever commenced after an incubation period of 9 days; it lasted 20 days, and the animal died, 24.11.26, of East Coast fever. Agamonts and gamonts were seen during the course of the illness.

3. 31.10.26. Heifer 1194 was injected intrajugularly emulsion of six adults infected East Coast fever of batch 784 fed for 100 hours on heifers 1135 and 1197 (three ticks from each heifer). The ticks were 258 days old. They were emulsified without using sand, and the emulsion was filtered before the injection.

Result.—The heifer was kept under observation for one month, and remained healthy.

Immunity Test.—20.11.26. Heifer 1194 was infested adults *Rhip. appendiculatus,* batch 848, infected East Coast fever. After an incubation period of 14 days the fever commenced, and the heifer died of East Coast fever on 19.12.26. During the course of the disease *Theileria parva* were seen in the blood.

4. 31.10.26. Ox 1109 was injected with an emulsion of six adults collected as sub. 3. The ticks were 258 days old. They were emulsified with sand, and the emulsion was filtered before the injection.

Result.—The ox was kept under observation for 4 weeks, and remained healthy.

Immunity Test.—20.11.26. Ox 1109 was infested with adults *Rhip. appendiculatus,* batch 848, infested with East Coast fever. After an incubation period of 9 days the fever commenced, and after an illness of 18 days the ox recovered from East Coast fever. It was discharged on 23.12.26. *Theileria parva* were seen during the course of the disease.

Résumé of Results of Experiment 2806.—The adults used in this experiment conveyed the disease to susceptible cattle when fed. An emulsion of adults fed for 100 hours did not convey the disease when injected intrajugularly into susceptible cattle. The minimal age of the ticks utilized was 258 days. The negative results are in conformity with those obtained in experiments 2528, 2601, 2606, and 2749.

TABULATED SUMMARY OF RESULTS WITH ADULTS OF "RH. APPENDICULATUS."

Experiment.	No. of Batch.	Age of Ticks.	No. of Animal.	No. of Ticks Used.	Fed on.	Control.	72 h.	96 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2407	771	Days. 12	972	50		Died						
,,	,,	12	1666	50		Died						
,,	,,	12	996	6						O	Died	
,,	,,	12	1012	6						O	R	Recovered in experiment 2484.
,,	,,	16	978	6	1666		Died					Emulsion in saline.
,,	,,	16	994	6	1666		Died					,, ,,
2528	750 & 760	258 & 189	1049	30 ♂ 30 ♀		Died						
,,	,,	258 & 189	1600	6 ♀		Died						
,,	,,	258 & 189	996	6 ♂		Died						
,,	,,	258 & 189	1194	6 ♀ 6 ♂						O	Died	
,,	,,	261 & 192	1197	6 ♀ 6 ♂			O				Died	
,,	,,	262 & 193	1128	6 ♀ 6 ♂	1049			O			Died	Emulsified in saline.
,,	,,	267 & 198	1015	6 ♀ 6 ♂	1049				O		Died	,, ,,
,,	,,	272 & 203	853	6 ♀ 6 ♂	1049				O		Died	Fed for 10 days.

Explanation of Table: R = Reaction; O = No Reaction.

TABULATED SUMMARY OF RESULTS WITH ADULTS OF "RH. APPENDICULATUS."—(*continued*).

Experiment.	No. of Batch.	No. of Ticks.	Age of Animal.	No. of Ticks Used.	Fed on.	Control:	72 h.	96 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2601	760 & 775	Days. 236 & 140	1133	?		Died						
,,	,,	236 & 140	1197	6 ♀	1133			O			Died	Emulsified with sand.
,,	,,	240 & 144	1193	6 ♂	893			O			Died	Emulsified with sand and decanted.
2606	760 & 775	240 & 144	853	1 ♀	893			O			Died	Emulsified with sand and filtered.
,,	,,	240 & 144	1135	1 ♀	893			O			Died	Emulsified with sand and decanted.
2749	784	203	1128	00		Died						
,,	,,	207	1135	1 ♀	1128			O			Died	Emulsified with sand and decanted.
,,	,,	207	1197	5	1128			O			Died	,, ,, ,, ,,
,,	,,	207	1109	1 ♀	1128			O			R	,, ,, ,, ,,
,,	,,	207	1194	5	1128			O			Died	Subcutaneous injection.
2806	784	253	1197	00		Died						
,,	,,	253	1135	00		Died						
,,	,,	258	1194	6	1135 & 1197			O			Died	Emulsified and filtered.
,,	,,	258	1109	6	1135 & 1197			O			R	Emulsified with sand and filtered.

Explanation of Table: 00 = Many ticks; ? = Several ticks; R = Reaction; O = No reaction.

Summary of Results Obtained with Adults in Experiments 2407, 2528, 2601, 2606, 2749, 2806.

All the adults used in these experiments proved to be infective when fed on susceptible cattle. The emulsion of fed ticks, however, proved to be infective when injected intrajugularly only in experiment 2407, in which six adults fed for 72 hours conveyed the disease in the manner indicated. In experiment 2528 six adults, also fed for 72 hours, failed to transmit the disease in the same way, and also ticks fed for longer periods failed to do so. In all subsequent experiments the ticks fed and emulsified failed to transmit the disease. There is accordingly only one successful experiment, viz., 2407. In this experiment the ticks were only 16 days old; in all other experiments they were not younger than 144 days. It would appear, therefore, that the age of the adult tick is the determining factor whether an emulsion is infective or not, viz., young ticks are infective, ticks 140 days old or older are no longer infective when injected intrajugularly.

(2) *Experiments with Nymphae of* Rhipicephalus appendiculatus.

A.—Experiment 2430. *Nymphae of batch* 759, *infected as larvae on ox* 1670 *from* 1.10.25 *to* 5.10.25 *suffering from East Coast fever, moulted on* 30.10.25. *Calves* 1611 *and* 1646 *had to act as controls for the virulency of the nymphae. Some of the fed nymphae were to be used for intrajugular injection.*

1. Ox 1611 on the 26.2.26 was infested with nymphae 119 days old.

Result.—After an incubation period of 9 days the fever commenced. It lasted 16 days. The ox succumbed to East Coast fever on the 24.3.26. Agamonts and Gamonts were seen in the glands during life and *Theileria parva* were found in the blood.

2. Bull 1646 on the 26.2.26 was infested with nymphae 119 days old.

Result.—After an incubation period of 5 days the fever commenced. It lasted 23 days, and the bull succumbed to East Coast fever 27.3.26. Agamonts and gamonts were seen during life in the glands and *Theileria parva* were noted in the blood.

3. Ox 1600 on the 26.2.26 was injected with an emulsion of six unfed nymphae into the jugular vein. Nymphae 119 days old.

Result.—Ox 1600 was kept under observation for one month. It remained healthy. Subsequently ox 1600 was used in experiment 2484, 7.4.26, batch 759, and again injected intrajugularly with unfed infected nymphae, with negative results.

Ox 1600 was tested for immunity on 11.5.26 by feeding six females of batches 750 and 760. It succumbed to East Coast fever.

Result.—The injection of emulsified, unfed, infected nymphae did not transmit East Coast fever nor gave immunity, since this calf succumbed to East Coast fever conveyed by the bite of ticks in the subsequent experiment 2528.

4. Ox 1627 on the 2.3.26 was injected with an emulsion of six nymphae fed for 90 hours on control bull 1646. Nymphae 123 days old.

Result.—There were two successive fever reactions. The first commenced after 14 days, and lasted for 6 days, with a maximum

temperature of 106° F. The second reaction lasted from 27 to 31 days with a maximum temperature of 104·8° F. The examination of glands gave negative results. Notwithstanding these negative results, it is likely that they were abortive East Coast fever reactions. Subsequently, on the 17.6.26, in experiment 2599, ox 1627 was tested for its immunity with ticks of batch 783. These ticks caused the disease in calf 1668, which acted as a control, but not in ox 1627, thus proving that the reaction in this calf had been one of East Coast fever.

5. Bull 1616, on the 4.3.26, was injected with an emulsion of six nymphae fed on ox 1611 and collected fully engorged after dropping. Nymphae 125 days old.

Result.—Bull 1616 was kept under observation for one month, and remained healthy. Subsequently bull 1616 was injected (experiment 2484) on the 11.4.26 intrajugularly with 12 crushed nymphae, batch 759, fed for 96 hours on heifer 725. It succumbed to East Coast fever.

Résumé of Results.—The infected nymphae feeding on the controls transmitted the disease in the natural way. The unfed nymphae did not transmit the disease when injected intrajugularly, neither did the nymphae that were collected after dropping. The nymphae that were feeding for 90 hours gave rise to a fever that must be considered to be East Coast fever, since a subsequent test with infected ticks proved the calf to be immune. The injection of infected ticks did not produce immunity against East Coast fever in the two cases that failed to react.

B.—Experiment 2484. 7.4.26. *The nymphae utilized in this experiment were of batch* 759, *which proved infective for two controls in experiment* 2430. *Accordingly, there were no controls used in this experiment to test the virulency, and the feeding of the nymphae was done on an East Coast fever immune heifer* 725.

1. Ox 1600 on the 7.4.26 was injected intrajugularly with an emulsion of 12 unfed nymphae 160 days old.

Result.—This ox was kept under observation for 5 weeks, and remained healthy.

Immunity Test.—Ox 1600 was infested on the 11.5.26 with six females of batches 750 and 760 (experiment 2528). The animal succumbed to East Coast fever.

2. Bull 996 on the 9.4.26 was injected intrajugularly with an emulsion of 12 nymphae kept in the incubator at 37° C. for 24 hours. Nymphae 162 days old.

Result.—The bull was kept under observation for 5 weeks and remained healthy.

Immunity Test.—Bull 996 was tested on the 11.5.26 by infestation of six males of batches 750 and 760. The animal succumbed to East Coast fever.

3. Bull 1012 on the 11.4.26 was injected intrajugularly with an emulsion of 12 partly engorged nymphae feeding for 72 hours on an East Coast fever immune animal 725. Nymphae 164 days old.

Result.—This bull showed a double reaction. The first began after 6 days and lasted 4 days, the second began 4 days later and lasted 7 days. Agamonts and gamonts were not found, but small piroplasms were seen in the blood during the second reaction. This calf was tested subsequently with ticks of batch 783, the batch that

gave ox 1668, acting as a control, East Coast fever; but these ticks did not give the disease to bull 1012, thus proving its immunity, due to the intrajugular injection of the emulsified ticks.

4. Bull 1616 on the 11.4.26 was injected intrajugularly with an emulsion of 12 nymphae fed for 96 hours on an East Coast fever immune heifer 725. Nymphae 164 days old.

Result.—After an incubation perioid of 8 days the fever commenced and lasted 17 days. The bull succumbed to East Coast fever on 7.5.26. Agamonts and gamonts were seen during life in the lymph glands and *Theileria parva* were present in the blood.

5. Heifer 1121 on the 12.4.26 was injected intrajugularly with an emulsion of 12 nymphae fed on East Coast fever immune heifer 725, collected after engorging and dropping. The ticks had been feeding for 120 hours. Nymphae 165 days old.

Result.—After an incubative period of 8 days the fever commenced and lasted 19 days, and the heifer died of East Coast fever on 10.5.26. Agamonts and gamonts were seen during life in the lymph glands and *Theileria parva* were noted in the blood.

6. Heifer 1135 on the 26.4.26 was injected intrajugularly with an emulsion of 12 nymphae fed on East Coast fever immune heifer 725, collected after engorging and dropping, and kept for 14 days. Nymphae 179 days old.

Result.—The heifer was kept under observation for one month and remained healthy. Subsequently injected in experiments 2606 and 2749 with negative results.

Immunity Test.—26.10.26. Heifer 1135 infested *Rhip. appendiculatus*, adults batch 784. The fever commenced after 10 days, and the heifer succumbed to East Coast fever on 18.11.26.

Résumé of Results.—Emulsion of unfed nymphae and of unfed incubated nymphae did not transmit East Coast fever by intrajugular injection, nor did the injection give immunity. Nymphae fed for 72 hours injected intrajugularly gave rise to a fever, which was abortive East Coast fever. The injection of emulsified nymphae fed for 96 hours and of nymphae collected after dropping, feeding for 120 hours, produced East Coast fever. Fully engorged nymphae that had dropped after 120 hours' attachment and had been kept for 14 days did not transmit the disease.

C.—Experiment 2750. *Nymphae of batch* 774 *infested as larvae on ox* 1171 *from* 4.1.26 *to* 8.1.26, *suffering from East Coast fever. The larvae moulted to nymphae on* 27.1.26. *Heifer* 1193 *had to act as host to feed the nymphae and to serve as a control for the virulency of the nymphae.*

1. *Heifer* 1193 (in experiment 2740). 9.10.26. Infested *Rhip. appendiculatus* nymphae, batch 774. The nymphae were 230 days old.

Result.—After an incubation period of 10 days the fever commenced. It lasted 19 days, and the animal succumbed to East Coast fever on the 8.10.26.

2. Bull 1015. 13.9.26. Injected subcutaneously 1 c.c. emulsion of *Rhip. appendiculatus* nymphae, batch 774, fed for 100 hours on heifer 1193. The nymphae were 234 days old. The emulsion was made by grinding the ticks with sand, and diluted so as to contain one tick per c.c. The solution was filtered before injection.

Result.—The bull was kept for one month under observation and remained healthy. It was subsequently reinjected in experiment 2807 with negative results.

Immunity Test.—20.11.26. Bull 1015 infested nymphae, batch 781. After an incubation period of 9 days the bull succumbed to East Coast fever on 22.12.26.

3. Bull 1045. 13.9.26. Injected subcutaneously 5 c.c. emulsion of *Rhip. appendiculatus* nymphae, batch 774, fed for 100 hours on heifer 1193. The nymphae were 234 days old. The emulsion was made as under 2.

Result.—The bull remained under observation for one month and remained healthy. It was subsequently reinjected in experiment 2807 with negative results.

Immunity Test. 20.11.26. Bull 1045 was infested with nymphae *Rhip. appendiculatus*, batch 781. After an incubation period of 9 days the fever commenced, and the bull died of East Coast fever on 18.12.26.

4. Bull 853. 13.9.26. Injected intrajugularly 1 c.c. emulsion of *Rhip. appendiculatus* nymphae, batch 774, fed for 100 hours on heifer 1193. The nymphae were 234 days old. The emulsion was made as under 2.

Result.—The calf remained under observation for one month, and remained healthy.

Immunity Test.—27.10.26. Bull 853 was infested with nymphae *Rhip. appendiculatus*, batch 781, infected with Each Coast fever. The fever commenced 9 days later, and the bull died on 27.11.26 of East Coast fever. *Theileria parva* were seen in the blood during the reaction.

5. Bull 1102. 13.9.26. Injected intrajugularly 5 c.c. emulsion of *Rhip. appendiculatus* nymphae, batch 774, fed for 100 hours on heifer 1193. The nymphae were 234 days old. The emulsion was made as under 2.

Result.—The bull remained under observation for one month, and remained healthy.

Immunity Test.—27.10.26. This bull was infested with East Coast fever infected nymphae *Rhip. appendiculatus*, batch 781. It contracted East Coast fever after an incubation period of 9 days, and succumbed on 21.11.26. *Theileria parva* were seen in the blood during the reaction.

Résumé of Results of Experiment 2750.—The nymphae which were utilized were infective when tested by feeding on a susceptible bovine. The engorged nymphae fed for ± 96 hours, emulsified and injected intrajugularly did not convey the disease. This result is contrary to that obtained in experiment 2484, and it was thought that the method of preparing the emulsion by means of grinding the ticks up with sand and filtering the liquid was responsible.

D.—Experiment 2807. 1.11.26. *Nymphae* Rhip. appendiculatus *of batch* 781. *The larvae of batch* 781 *had been feeding on ox* 1173 *from* 8.1.26 *to* 12.1.26 *during the period the ox was suffering from East Coast fever. The larvae moulted into nymphae on* 28.1.26.

1. 27.10.26. Bull 853 was infested with nymphae of batch 781 infected East Coast fever. The ticks were 273 days old. Some of the nymphae were to be removed after 100-120 hours' feeding, to be emulsified and injected intrajugularly into susceptible cattle.

Result.—Bull 853 contracted East Coast fever after an incubation period of 9 days, and succumbed to East Coast fever on 27.11.26. *Theileria parva* were present in the blood in the course of the disease.

2. 27.10.26. Bull 1102 was infested as sub. 1. The nymphae were 273 days old.

Result.—The fever commenced after an incubation period of 9 days, and the animal died on 21.11.26 of East Coast fever. *Theileria parva* were seen in the blood during the course of the disease.

3. Bull 1045. 1.11.26. Injected intrajugularly emulsion of six nymphae *Rhip appendiculatus*, batch 781, fed for 100-120 hours on bull 853 and bull 1102, three ticks from each animal. The nymphae were 277 days old. The nymphae were emulsified in saline (without sand), and the emulsion was filtered before the injection.

Result.—Bull 1045 remained under observation for one month, and remained healthy.

Immunity Test.—20.11.26. Bull 1045 was infested with nymphae *Rhip. appendiculatus*, batch 781, infected with East Coast fever. After an incubation of 9 days the fever commenced, and the bull died 18.12.26. *Theileria parva* were present during the course of the disease.

4. Bull 1015. 1.11.26. Injected as sub. 3. The nymphae were 277 days old. The nymphae were emulsified with sand in 5 c.c. saline, three ticks from each animal. The emulsion was filtered before the injection.

Result.—Bull 1015 remained under observation for one month, and remained healthy.

Immunity Test. —20.11.26. Bull 1015 was infested with nymphae *Rhip. appendiculatus*, batch 781, infected with East Coast fever. After an incubation period of 9 days the fever commenced, and the bull died 22.12.26 of East Coast fever. During the reaction *Theileria parva* were found in the blood.

Résumé of Results in Experiment 2807.—The nymphae were infective and transmitted the disease to the susceptible animal on which they were fed. The emulsion of nymphae that had been feeding for 96-120 hours and more did not produce the disease after intrajugular injection. It was thought that the filtration of the emulsion might be responsible for the negative results.

E.—Experiment 2859. 7.12.26. *Nymphae of* Rhip. appendiculatus *of batch* 781. *For details of history of batch* 781, vide *Experiment* 2807. *The nymphae of batch* 781 *had proved virulent for bull* 853 *and bull* 1102 *in Experiment* 2807.

1. 7.12.26. Ox 1742. Injected intrajugularly 5 c.c. emulsion of 10 nymphae infected with East Coast fever fed for 96-120 hours on East Coast fever immune cattle 825, 893, 894, 1012, 1627. The nymphae were 313 days old. The nymphae were emulsified with sand in saline solution and *not filtered.*

Result.—After an incubation period of 15 days the fever commenced, and the ox recovered from an attack of East Coast fever. The diagnosis was confirmed by the demonstration of *Theileria parva* in the blood and gamonts and agamonts in the lymph glands during the reaction.

2. 7.12.26. Heifer 1195. Injected 5 c.c. emulsion of 10 nymphae fed for 96 to 120 hours on East Coast fever immune cattle

825, 893, 894, and 1627. The nymphae were 313 days old. The nymphae were emulsified with sand in saline solution, but *not filtered.*

Result.—The heifer remained under observation for one month, and remained healthy. Tested subsequently with ticks for immunity, it contracted East Coast fever.

Résumé of Results in Experiment 2859.—The nymphae utilized were infective and transmitted the disease to susceptible cattle in experiment 2807. The emulsion was made with ten infected ticks by grinding them in sand and filtering the liquid. In one case the disease was transmitted, and in the second case it gave no result. It is worthy of note that ticks of the same batch treated in exactly an identical manner gave different results.

F.—Experiment 2864.—10.12.26. *Nymphae* Rhip. appendiculatus, *batch* 782. *The larvae of batch* 782 *had been feeding on ox* 1173 *from* 11.1.26 *to* 15.1.26 *during the period the animal was suffering from East Coast fever. The larvae moulted into nymphae on* 28.1.26.

1. 10.12.26. Cow 162 was infested with nymphae *Rhip. appendiculatus,* batch 782. Some of the nymphae were to be removed after 72 and 96 hours' feeding, to be emulsified and injected intrajugularly into susceptible cattle. The nymphae were 316 days old.

Result.—Cow 162 contracted East Coast fever and succumbed. The incubation period lasted 10 days, the fever 17 days, and the cow succumbed 16-17.1.27. *Theileria parva* were numerous in the blood as well as gamonts and agamonts in the spleen.

2. 14.12.26. Ox 1723 was injected intrajugularly 5 c.c. emulsion of 25 nymphae *Rhip. appendiculatus* infected with East Coast fever fed as sub. 1. The nymphae were 320 days old. The emulsion was made by grinding the nymphae in saline solution *without* the use of sand.

Result.—After an incubation period of 9 days fever commenced, and the ox succumbed to East Coast fever on 9.1.27.

3. 14.12.26. Heifer 1577 was injected intrajugularly with emulsion of 25 nymphae *Rhip. appendiculatus* infected East Coast fever fed as sub. 1. The nymphae were 320 days old. The emulsion was made by grinding the nymphae in saline solution *with sand.*

Result.—After an incubation period of 10 days the fever commenced, and the heifer succumbed to East Coast fever on 9.1.27.

Résumé of Results in Experiment 2864.— The nymphae were infective, and conveyed the disease after feeding to a susceptible bovine. The emulsion was made with 25 c.c. engorged nymphae feeding for 72-96 hours by grinding with sand. It was not filtered before the injection. The injection produced the disease in both animals injected.

G.—Experiment 2895. 3.1.27. *The nymphae belong to batch* 782. *Details of feeding of this batch,* vide supra *Experiment* 2864.

1. Heifer 1195. 3.1.27 infested nymphae, batch 782. The nymphae were 340 days old. Some of the nymphae were to be removed after feeding for 72 hours to be emulsified and injected intrajugularly.

Result.—Heifer 1195 contracted East Coast fever after an incubation period of 11 days, and the animal died of East Coast fever. *Theileria* parva were present in the blood and glands.

2. Ox 1114. 6.1.27 injected intrajugularly emulsion of 20 c.c. nymphae fed for 72 hours. The emulsion was made by grinding the nymphae in sand, and the supernatant liquid was not filtered before the injection. The ticks were 343 days old.

Result.—Ox 1114 remained under observation until the incubation period had elapsed, and did not contract East Coast fever. Its immunity was not tested.

3. Heifer 1196. 6.1.27 injected intrajugularly with emulsion of 20 engorged nymphae fed for 72 hours. The emulsion was made as above, but was filtered before the intrajugular injection. The nymphae were 343 days old.

Result.—Heifer 1196 remained under observation until the incubation period had elapsed, and did not contract East Coast fever. Its immunity was not tested.

4. Heifer 1199. 6.1.27 injected intrajugularly with emulsion of 20 engorged nymphae fed for 72 hours. The emulsion was made as sub. 2 and filtered before the intrajugular injection. The nymphae were 343 days old.

Result.—Heifer 1199 remained under observation until the incubation period had elapsed, and did not contract East Coast fever. It was not tested for immunity.

5. Heifer 1656. 6.1.27. Injected intrajugularly with emulsion of 20 engorged nymphae fed for 72 hours. The emulsion was made as sub. 2, but not filtered before the intrajugular injection. The nymphae were 343 days old.

Result.—Heifer 1656 remained under observation until the incubation period had elapsed, and did not contract East Coast fever. It was not tested for immunity.

Résumé of Results in Experiment 2895.—The nymphae were infective and transmitted the disease to the susceptible animal on which they were fed. The cattle that were injected were undoubtedly susceptible, notwithstanding the fact that their susceptibility has not yet been tested.

The injection of the emulsion of nymphae fed for 72 hours did not produce the disease. The method of preparation of the emulsion can not be made responsible.

In this experiment the nymphae had been fed for exactly 72 hours when they were removed.

Summary of Results Obtained with Nymphae in Experiments 2430, 2484, 2750, 2807, 2859, 2864, *and* 2895.—All the nymphae used in these experiments proved to be infective when fed on susceptible cattle. The emulsion of nymphae fed for 72-96 hours injected intrajugularly proved to be infective for five animals out of nine injected. The failures were all in the same batch that had been fed exactly for 72 hours before being removed. The nymphae that were fed for 96 hours and more proved to be infective only in two out of nine cases. Unfed and unfed incubated nymphae were not infective. Also in this experiment one batch failed to transmit the disease. The age of the nymphae has apparently nothing to do with their infectivity, since the batch that failed was 230 days old and the one that succeeded was 320 days old. The animals that did not react to the injection did not prove to be immune when tested with infected ticks.

TABULATED SUMMARY OF RESULTS WITH NYMPHAE OF "RH. APPENDICULATUS."

Experiment.	No. of Batch.	Age of Ticks.	No. of Animal.	No. of Ticks Used.	Fed on.	Control.	72 h.–96 h.	96 h.–120 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2430	759	Days. 119	1611	00		Died						
,,	,,	119	1646	00		Died						
,,	,,	119	1600	6						O	Died	
,,	,,	123	1627	6	1646		R				O	
,,	,,	125	1616	6	1611				O		Died	In experiment 2484.
2484	759	160	1600	12						O	Died	
,,	,,	162	996	12						O	Died	Nymphae kept in incubation for 24 hours.
,,	,,	164	1012	12	725		R					725—E.C.F. immune.
,,	,,	164	1616	12	725		Died					
,,	,,	165	1121	12	725			Died				
,,	,,	179	1135	12	725				O		Died	Nymphae kept for 14 days before injection.
2750	774	230	1193	00		Died						
,,	,,	234	1015	1	1193			O			Died	Emulsified with sand and filtered subcutaneous injection.
,,	,,	234	1045	5	1193			O			Died	Emulsified with sand and filtered subcutaneous injection.
,,	,,	234	853	1	1193			O			Died	(Subcutaneous injection). Emulsified with sand and filtered.

Explanation of Table: 00 = Many ticks; R. = Reaction; O = No reaction.

TABULATED SUMMARY OF RESULTS WITH NYMPHAE OF "RH. APPENDICULATUS.—(*continued*)."

Experiment.	No. of Batch.	Age of Ticks.	No. of Animal.	No. of Ticks Used.	Fed on.	Control.	72 h.–96 h.	96 h.–120 h.	120 h. and Over.	Unfed.	Immunity Test.	Remarks.
2750	774	234	1802	5	1193			O			Died	Emulsified with sand and filtered.
2807	781	273	853	00		Died						
,,	,,	273	1102	00		Died						
,,	,,	277	1045	6	853 & 1102			O			Died	Emulsified in saline and filtered.
,,	,,	277	1015	6	853 & 1102			O			Died	Sand filtered.
2859	781	313	1742	10	T.C.			R				T.C.—825, 893, 894, 1012, 1627. Emulsified with sand and not filtered.
,,	,,	313	1195	10	T.C.			O			Died	T.C.—825, 893, 894, 1012, 1627. Emulsified with sand and not filtered.
2864	782	316	162	00		Died						
,,	,,	320	1723	25	162		Died					Emulsified without sand and not filtered.
,,	,,	320	1577	25	162		Died					Emulsified with sand, not filtered.
2895	782	340	1195	00		Died						
,,	,,	343	1114	20	1195		O				Not tested	Emulsified with sand, not filtered.
,,	,,	343	1196	20	1195		O				Not tested	Emulsified with sand and filtered.
,,	,,	343	1199	20	1195		O				Not tested	,, ,, ,,
,,	,,	343	1656	20	1195		O				Not tested	Emulsified with sand, not filtered.

Explanation of Table: 00 = Many ticks; R. = Reaction; O = No reaction.

Summary of Conclusions.

Rickettsia ruminantium can be transmitted by intrajugular injection of emulsified infected nymphae of *Amblyomma hebraeum*, and corresponds in this respect to Rocky Mountain fever. The experiments with fed nymphae were in eight out of nine cases positive, and the one failure was observed in the case that received the minimal quantity of emulsion. The experiments with unfed nymphae succeeded once only.

Similarly, *Theileria parva* of East Coast fever can be transmitted by intrajugular injection of both emulsified infected fed adults and nymphae of *Rhipicephalus appendiculatus*. The transmission succeeded only with adults that had recently moulted and had been feeding for 72-100 hours. The transmission with nymphae did not succeed with every batch of ticks, and not with all ticks within one and the same batch. No reason can be given for these failures. It is possible that the method of preparing the emulsion may be partly responsible. It is also suggested that in one instance the period of feeding (exactly 72 hours) was too short.