

## The Transmission of Tick-Bite Fever by the Dog Tick *Rhipicephalus sanguineus*, Labr.

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It has been pointed out previously (Mason and Alexander, 1939) that very little experimental work has been carried out to identify the arthropod vectors of tick-bite fever. The statement by Brumpt (1927) that *Amblyomma hebraeum*, *Rhipicephalus simus*, and *Boophilus decoloratus* are transmitters appears to have been based on circumstantial evidence, and to our knowledge is not supported by direct experimental proof. From time to time different species of ticks have been implicated as a result of the establishment of a strain of rickettsia in guinea-pigs following the intraperitoneal injection of emulsified ticks, e.g., Pijper and Dau (1934) with *Rhipicephalus appendiculatus* larvae, Gear and Douthwaite (1938) with an adult *Haemaphysalis leachi*, and Finlayson, Grobler and Smithers (1940) with a mixture of larval, nymphal, and adult *Hyalomma aegyptium*. The fact that the emulsified viscera of ticks contain viable rickettsias affords some evidence that the tick is a vector but it certainly does not furnish proof that the species plays a rôle in the natural transmission of the disease.

In 1939, Alexander, Mason, and Neitz reported the isolation of a strain of rickettsia by feeding *Hyalomma aegyptium* var. *impressum* nymphae on a guinea-pig; these ticks had been collected in a partially engorged state from a hare. Neitz and du Toit (1938) reported that the resulting adult ticks also produced the disease but that the larvae and nymphae of the next generation were not infective. Gear and de Meillon (1939) established a strain by feeding on a guinea-pig adult *Haemaphysalis leachi* removed from a dog which belonged to a patient found to be suffering from an acute attack of tick-bite fever. As far as we are aware these are the only positive results obtained in tick-feeding experiments.

It is not out of place to mention that Roberts (1935), in the course of his investigations into tropical typhus in Kenya, reported the production of a febrile reaction and a scrotal enlargement in a guinea-pig following the intraperitoneal injection of an emulsion of engorged females and eggs of *Rhipicephalus sanguineus*. Negative results were obtained with *R. pulchellus*. In 1939 Roberts established a strain of rickettsia in a guinea-pig by injecting an extract from

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the local lesion excised from a patient suffering from a complaint diagnosed as Kenya Fever. Presumably the specific *tâche noire* was produced at the site of a bite by a tick. However, the relation of Kenya Fever to South African tick-bite fever, and to other diseases caused by rickettsias has not been worked out.

### TECHNIQUE.

The rickettsia used in these experiments was the Robertson strain obtained from Dr. J. H. S. Gear of the South African Institute for Medical Research, Johannesburg. Originally it was isolated from the blood of a human patient who became ill after being associated with the deticking of dogs. The morphology, characteristics, cultivation on the chorio-allantoic membrane of the developing chick embryo, and immunological relation to other strains of rickettsias have been described in a number of publications (Gear and Bevan 1936, Gear and Douthwaite 1939, Alexander and Mason 1939, Mason and Alexander 1939).

In our hands this strain was maintained with some difficulty in guinea-pigs by brain to peritoneum passage; frequently inoculated guinea-pigs showed indefinite reactions and on many occasions individuals failed to react at all. Consequently the guinea-pigs on which the ticks were fed to pick up infection received intraperitoneal injections of egg-membrane cultures shown by microscopic examination to contain large numbers of rickettsias. By this method we were certain of producing a marked reaction; thus the ticks, if they attached, would be engorging at a time when the circulating blood was known to be infective.

The general scheme of the experiments was to place "clean" ticks on an infected guinea-pig so that they would engorge during the reaction. Thus larvae were placed in position about 36 hours after injection, and the slower feeding nymphae and adults at the time of injection. When the ticks detached after engorgement they were placed in test tubes stoppered with cotton wool plugs and stored in glass jars in a constant temperature-humidity room (79° F. and 79 per cent. moisture) to hasten moulting. After moulting ticks were fed on each of 2 guinea-pigs. One guinea-pig was retained for an immunity test which was carried out after an interval of one month; the other was killed at a stage when the temperature record or general habitus indicated that it was passing through a reaction. A saline emulsion of the whole brain was then injected into three guinea-pigs by the intraperitoneal route. Subinoculations were carried out for three passages and positive transmission was claimed only on demonstration of rickettsias in tunica smears, together with resistance to an immunity test of some of the surviving guinea-pigs. Male guinea-pigs only were used so that the incidence of scrotal reactions could be noted; the temperature of all guinea-pigs was recorded twice daily.

Two different batches of ticks, identified as *Rhipicephalus sanguineus*, were used:—

1. Batch 1534.—Engorged adults were collected from dog 2513 on 12.5.39. Oviposition commenced on 17.5.39 but no record was kept of the date of hatching. A number of the

larvae were fed on two guinea-pigs on 1.2.40, and failed to initiate any infection, thus indicating that the collected ticks were "clean". The nymphae which developed from the engorged larvae collected on 5.2.40 were used for experiments on transmission from nymph to adult and subsequently from adult through the egg to larvae of the succeeding generation.

2. Batch 1539.—Engorged nymphae were collected from a dog in the kennels. On 6.7.39 the adults were fed on dog 2531. Engorgement was completed by 15.7.39 and the eggs laid commenced to hatch on 25.8.39. A number of the larvae were fed on guinea-pigs and failed to transmit any infection. The remainder were used in experiments on transmission from larva to nymph.

In the feeding experiments the ticks were placed on the backs of guinea-pigs from which the hair had been clipped short by means of an electric clipper. The ticks were controlled by placing them beneath a piece of calico stuck round the periphery to the guinea-pig by means of an adhesive paste prepared from the following formula:—gelatine 20 g., zinc oxide 15 g., glycerine 25 c.c., water 40 c.c. This paste is very adhesive and none of the ticks even larvae escaped. Moreover the pliability of the calico allowed adequate space for increase in size of the engorging ticks. The attached ticks could be examined from time to time by slitting open the calico and subsequently closing the slit with a fresh patch of calico and paste. At the conclusion of an experiment the strip of adhering calico may be removed by bathing with water.

The guinea-pigs were housed singly in enamel bins covered with stout wire gauze under conditions which excluded the possibility of accidental tick infestation.

#### EXPERIMENTAL PROCEDURE.

##### A. *Transmission from Larva to Nymph.*

- (a) 26.9.39. Guinea-pig 4.—Received intraperitoneally egg-membrane culture, generation 2.  
27.9.39. Interim 30 hours; *R. sanguineus* larva, batch 1539A, placed on back.

The guinea-pig showed a marked febrile reaction up to 105.6° F. accompanied by scrotal enlargement commencing after an incubation period of 48 hours and lasting 7 days.

- 3.10.39. 50 engorged larvae were collected, all attached ticks having dropped.

These ticks commenced their moult to nymphae on 31.10.39.

- (b) 1.10.39. Guinea-pig 9.—Inoculated intraperitoneally with egg-membrane culture, generation 3.  
3.10.39. Interim 48 hours; *R. sanguineus* larvae, batch 1539A, placed on back.

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At the time the ticks were placed on the guinea-pigs, the febrile reaction accompanied by scrotal reaction had commenced.

6.10.39. 30 engorged larvae collected.

7.10.39. 20 engorged larvae collected.

These ticks commenced their moult to nymphae on 31.10.39.

(c) 13.11.39. Guinea-pig 4A.—Infested on back with 50 nymphae, batch 1539A.

16.11.39. A number of ticks found dead but a large number attached.

18.11.39. 11 engorged nymphae collected.

19.11.39. 20 engorged nymphae collected; all ticks detached.

20.11.39. A mild febrile reaction with a maximum temperature of 104° F. commenced.

23.11.39. Guinea-pig killed and brain emulsion injected intraperitoneally into three guinea-pigs (=generation 2). The site of engorgement of the ticks was markedly inflamed, the skin thickened and somewhat oedematous.

*Generation 2.*

26.11.39. One guinea-pig died; peritonitis.

29.11.39. Both surviving guinea-pigs commenced febrile reaction; maximum temperature 105° F.; no scrotal reaction.

1.12.39. Both guinea-pigs killed. On post-mortem examination spleen enlarged, inguinal lymph glands swollen. No rickettsias seen in tunica and peritoneum smears. Brain emulsion injected into each of four guinea-pigs (=generation 3).

*Generation 3.*

11.12.39. One guinea-pig showed a unilateral scrotal reaction; killed by ether anaesthesia. On post-mortem examination the only macroscopic lesion was a marked hyperaemia of one testicle. Examination of tunica smears stained by Giemsa, and Macchiavello's (1937) basic fuchsin-methylene blue stain showed the presence of definite intracytoplasmic rickettsias although they were exceedingly rare.

Each of the remaining three guinea-pigs showed a mild diphasic temperature reaction commencing on the 5th day and lasting 6-8 days; highest temperature 105° F. These guinea-pigs were kept and were given an immunity test on 19.12.39.

(d) 13.11.39. Guinea-pig 9B was infected with approximately 40 nymphae, batch 1539B.

16.11.39. A number of ticks had attached but some were dead.

19.11.39. 30 engorged nymphae had detached and were collected.

The guinea-pig showed a very mild temperature reaction commencing on the 8th day and lasting for 8 days. The highest recorded temperature was 104.2° F. The portion of the skin to which the ticks attached became markedly haemorrhagic and thickened. Later, erosions were formed and the area healed slowly. The guinea-pig was retained for immunity test together with the three guinea-pigs from experiment (c) above.

#### *Immunity Test.*

The four guinea-pigs received an intraperitoneal injection of egg-membrane culture material on 19.12.39. None reacted while two control guinea-pigs reacted severely and showed scrotal reactions.

#### *Result.*

*R. sanguineus* larvae picked up infection when fed on reacting guinea-pigs and passed on the infection when subsequently fed as nymphae on susceptible guinea-pigs.

#### *B. Transmission from Nymph to Adult.*

- (a) 26.9.39. Guinea-pig 5 infected with tick-bite fever by intraperitoneal injection of egg-membrane culture. On the same day a number of *R. sanguineus* nymphae, batch 1534Aa, were placed on the back. The guinea-pig showed a good febrile reaction with scrotal enlargement. The ticks fed well, 30 engorged nymphae being collected on the 7th and 8th days. The ticks commenced their moult to adults on 31.10.39.
- (b) 1.10.39. Guinea-pig 10 received an injection of egg-membrane culture. When infested with *R. sanguineus* nymphae, batch 1534Ab, 48 hours later it was showing a scrotal reaction. On 9.10.39 twelve engorged nymphae were collected; moulting to adults was in progress on 31.10.39.
- (c) 13.11.39. Guinea-pig 5 infested with adults from (a) above. A number of ticks failed to attach but eight engorged females were collected on the 7th day and one on the 8th day. A marked febrile reaction commenced on the 4th day, i.e., at least three days before the ticks had completed engorging. The highest recorded temperature was 105.2° F. and the reaction persisted for eight days. There was no scrotal reaction. This guinea-pig was retained for immunity test.
- (d) 13.11.39. Guinea-pig 10b infested with adult ticks from (b) above. The ticks attached and fed well, three engorged females being collected on the 7th day and seven on the 8th day. A febrile reaction without scrotal involvement commenced on the 5th day and persisted

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for five days when the guinea-pig was killed for sub-inoculation (generation 2). At this stage the skin on the back was inflamed, thickened and oedematous. The only other macroscopic lesion was slight enlargement of the spleen.

### *Generation 2.*

23.11.39. Three guinea-pigs received the brain emulsion of guinea-pig 10b.

G.P. 1. Ran a consistently high temperature in the region of 104° F. from the commencement of the experiment. On 7th day a slight unilateral scrotal reaction which persisted for two days was noticed. The guinea-pig was retained for immunity test.

G.P. 2. Also ran a consistently high temperature fluctuation about 104° F. On the 6th day a decided unilateral scrotal reaction was noticed. The guinea-pig was killed. The spleen and inguinal lymphatic glands were enlarged. One testicle appeared normal; the other was hyperaemic and showed one haemorrhage about 3 mm. in diameter. Smears from this testicle showed fairly numerous intracellular rickettsias of the *Dermocentroxenus* type.

G.P. 3. Showed no thermal or scrotal reaction, and was saved for immunity test.

### *Immunity Test.*

11.12.39. The three guinea-pigs and two controls received an intraperitoneal injection of egg-membrane culture.

Guinea-pig 3 of generation 2 above and the two controls showed the characteristic febrile and scrotal reaction. Guinea-pig 5a of experiment (c) and guinea-pig 1 of generation 2 above showed no reaction.

### *Result.*

*R. sanguineus* nymphae picked up the rickettsial infection and transmitted it when feeding as adults.

### *C. Transmission from Adult through the Egg to Larva of the Next Generation.*

1.2.40. Two guinea-pigs were each infested with approximately 100 larvae which were part of the progeny of the engorged females collected from guinea-pig 5a in the previous series of experiments. These females commenced oviposition on 26.12.39 and hatching commenced on 3.1.40.

One guinea-pig developed acute diarrhoea and succumbed apparently to enteritis of unknown origin four days after the commencement of the experiment.

On the second guinea-pig the ticks fed well and approximately 100 engorged larvae were collected on the 4th day. On the 7th day a mild febrile reaction commenced. The temperature never exceeded 104° F. and there was no scrotal reaction. The skin on the back, however became inflamed and thickened and several ugly erosions developed. On the 13th day the guinea-pig was killed for subinoculation (generation 2). The only post-mortem lesion was slight swelling of the spleen.

#### *Generation 2.*

14.2.40. Three guinea-pigs received brain emulsion. In all three a temperature reaction started on the 5th day. The reactions were of the diphasic type and reached a maximum of 105.4° F. The testicles were not involved. On the 9th day two guinea-pigs were destroyed and the pooled brain emulsion subinoculated into three guinea-pigs (generation 3). On post-mortem examination the spleen and inguinal lymphatic glands were decidedly enlarged. No rickettsias were seen in smears from the peritoneum or tunica vaginalis.

The surviving guinea-pig was subsequently given an immunity test and proved solidly resistant.

#### *Generation 3.*

23.2.40. Three guinea-pigs received brain emulsion intraperitoneally from generation 2 above.

- G.P. 1. Showed a slight but definite scrotal reaction accompanied by a rise in temperature to 104.2° F. on the 5th day. It was killed and rare intracytoplasmic rickettsias were demonstrated in smears from the tunica of the hyperaemic testicles.
- G.P. 2. Showed a fairly severe temperature reaction which began on the 5th day, reached a height of 104.6° F. on the 7th day, and persisted for five days. Subsequently it was shown on immunity test to be solidly immune.
- G.P. 3. Showed a marked thermal reaction which commenced on the 5th day, but no scrotal swelling. It was killed on the 9th day for the purpose of maintaining a strain of rickettsia by passage in guinea-pigs for other experimental work. Rickettsias could not be found in tunica smears.

#### *Result.*

The rickettsial infection passed from adult *R. sanguineus* through the egg to the larvae of the next generation, i.e., there was an hereditary transmission similar to that recorded for Rocky Mountain spotted fever by species of *Dermacentor*. In the experiment described it is not clear whether infection persisted from the nymphal stage through the adult to the resulting larvae or whether the ticks picked up infection afresh as adults since they were feeding at a stage when the blood of the guinea-pigs would be infective.

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### SUMMARY.

1. The technique adopted for the feeding of ticks on guinea-pigs in investigations into the tick transmission of South African tick-bite fever is described.

2. Since the reactions produced in guinea-pigs by the intraperitoneal injection of guinea-pig passage brain material are irregular, the host guinea-pigs were infected with egg membrane cultures rich in rickettsias.

3. The criteria for positive transmission were the microscopic demonstration of rickettsias in smears from the tunica vaginalis, and the production of a well defined febrile reaction with scrotal enlargement on passage, or the development of a solid immunity in the guinea-pig.

4. It was shown that in the case of *R. sanguineus* infection was picked up by larvae and transmitted as nymphae, picked up by nymphae and transmitted as adults, and passed through the eggs to larvae of the next generation.

### CONCLUSIONS.

The rickettsia, for which the name *Dermacentroaenus rickettsi* var. *piperi* has been suggested (Mason and Alexander 1939), was transmitted by the dog tick *R. sanguineus*. Larvae which were fed on two reacting guinea-pigs transmitted the infection when fed on susceptible guinea-pigs as nymphae. Nymphae picked up infection which was transmitted to guinea-pigs as adults. These adults had not completed engorgement by the time the host guinea-pigs had commenced their reaction; the larvae which hatched from eggs laid by these adults in turn were shown to be infective.

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