Rocky Mountain spotted fever

Failure of Triatomid bugs to transmit the virus experimentally

by

Cornelius B. Philip and Emmanuel Dias

Entomologist, Rocky Mountain Laboratory of the National Institute of Health, United States Public Health Service, Hamilton, Montana

Chief of Laboratories, Oswaldo Cruz Institute, Rio de Janeiro, Brazil

Although the fundamental role of ticks in the transmission of Rocky Mountain spotted fever among small mammals and to man is well established, the possibility of occasional transmission by other parasites having suitable host relationships deserves further investigation. Such relationships are shown in varying degrees by certain triatomid bugs, particularly in Brazil where domestic house-frequenting species are widely distributed, and in the southern United States where rodent nests and often houses are infested. Given the opportunity, most species will bite a wide variety of vertebrate hosts including man.

Some of these bugs occur in areas where the very closely related and possibly identical infections, Rocky Mountain and Brazilian spotted fever are respectively endemic. The only experiments with these bugs are reported by Dias and Martins (1937) who found that the virus of Brazilian spotted fever (a Minas Geraes strain) did not persist in the gut of Panstrongylus megistus for 48 hours and longer.

The following experiments with Rocky Mountain spotted fever (western Montana strains) were conducted in part at the Rocky Mountain Laboratory in Montana, and in part at the Oswaldo Cruz Institute in Brazil. These tests of transmission and of survival of the virus in triatomids have been made in accordance with the species and number of bugs available.

The species of bugs used were: Eutriatoma uhleri (Neiva) collected in a desert rat's nest near Las Cruces, New Mexico, Triatoma protracta (Uhler) from a wood rat's nest near Seven Oaks, California; and T. infestans (Klug), P. megistus (Burm.) and R. prolincus Stal. from laboratory stock of one of us (E. D.). Only one living specimen of each of the first 2 species was available.

Virus transfer was attempted both by feeding and by injection of bug tissues at stated intervals after an initial blood-meal on infected
guinea pigs during fever. The designation «negative» as used below, means that the animal under test was without clinical evidence of disease during an observation period of at least 14 days, and developed a typical infection following a subsequent injection of controlled virus.

EXPERIMENTAL OBSERVATIONS

_E. uhleri._—On July 26, 1937, a nymph was fully engorged on an infected guinea pig in the third day of fever, making 7 punctures on the scrotum and belly. It refused subsequently offered feedings and molted during the next 33 days. Test feedings on normal guinea pigs were permitted 33, 47, 75 and 141 days after the original feed, with negative results.

_T. protracta._—May 12, 1937, a large nymph was partially fed on an infected guinea pig with typical scrotal lesions and in the third day of fever, and allowed to complete its feeding on a normal guinea pig. Interruptions were effected during the above feedings to make 2 bitings on each animal. Results were negative.

Thirty-two days later the same insect was allowed to feed to repletion on another infected guinea pig, and test feedings (without intervening molt) on test animals followed at 15 and 37 days. These were also negative.

_T. infestans._—On Oct. 26, 1937, bugs of this species were fed on an infected guinea pig in its fourth day of fever. Eight days later, 15 bugs of this lot accepted blood-meals; the results were again negative.

Tests of survival of the virus in this species were made with another part of the same lot at 24, 48, 72, 96, 120, and 192-hour intervals after the initial, «infective» feeding. Two normal guinea pigs were each injected with the gut contents of a separate bug at the above intervals except the first when only one animal was used. The tests made at 24 and 48 hours were positive. The incubation periods were somewhat lengthened being 6 days for the 24-hour test, and 5 and 9 days respectively for the 48-hour tests. All 3 animals died showing typical lesions. The 72-hour and succeeding tests were all negative, the last having been made with 2 of the 15 insects used in the above feeding test.

_P. megistus._—On Apr. 23, 1937, an infected guinea pig with scrotal lesions and on the third day of fever, was bitten by a stock of these bugs. No feeding tests were attempt in view of previous negative experience with other species. At intervals of 24, 48, 72 and 144 hours, 2 guinea pigs were each injected intraperitoneally with the viscera of separate bugs. Both animals at 24 and one at 48 hours developed characteristic infections; one of the first pair recovered and was immune to subsequently injected blood-virus; the second 48-hour guinea pig died of pneumonia. The subsequent tests were either negative or rendered valueless by intercurrent infection (see table 1).

May 5, 1937, a fresh group of _megistus_ was given an infective feeding as before, and at intervals of 24, 48 and 72 hours, and at 12 days fresh
guinea pigs were injected with the gut contents of one bug each. The first 3 animals died of typical spotted fever with incubation periods of 4 to 6 days. The 12-day test was invalidated by intercurrent infection.

The experiment was again repeated January 4, 1938, at 48, 72, and 96-hour periods after an infective blood-meal by a fresh group of these insects. The first 2 tests involved single bugs and test animals, while in the 96-hour test, 2 guinea pigs were inoculated with the pooled visceral contents of 4 nymphs. The 72-hour and one of the 96-hour tests were negative. The other tests were valueless.

*R. prolixus.*—Since only one insect (*T. protracta*) was used in the previous undelayed mechanical transmission experiment, 2 more tests were performed in February, 1938, using 15 and 7 adults, respectively, of this species. The insects of each group were allowed to make undelayed alternate feedings first on an infected guinea pig, then on a normal one. The feedings were continued to satiation, the same infected and normal hosts being used, for each group. Of the first group, 7 bugs made one, 5 made 2, and 3 made 3 such alternate bitings. Of the second, 6 insects bit each animal once and one fed alternately 3 times on each. Both tests were again negative.

The visceral contents of 2 insects of the first group, when injected into a normal guinea pig approximately 3 hours after feeding on both donor and test animals, produced typical spotted fever, thus proving the bugs had ingested virus though unable to transmit it by interrupted feeding. Four additional bugs, not used in the first test, became engorged on the first donor each day thereafter, the gut contents of one of these was injected into a normal guinea pig. The 24, 48 and 72-hour tests were positive, the 96-hour test, negative. The 48 hour bug, however, was allowed to feed on a normal animal before being used for injection, and droplets of fresh feces which were deposited incident thereto, were suspended in a small amount of saline and injected intraperitoneally into an additional test guinea pig. Both tests were negative.

Protocols of all of the above experiments are given in Table I.

SUMMARY AND CONCLUSIONS

1. — The following species of blood-sucking triatomids failed to transmit the virus of Rocky Mountain spotted fever to susceptible guinea pigs by feeding at the following respective time intervals after the infective feeding: *Eutriatoma ahleri*, 33, 47, 75, and 141 days (one bug); *Triatoma protracta*, 15 and 37 days (one bug); *T. infestans*, 8 days (15 bugs); and *Rhodnius prolixus*, 2 days (1 bug). The last was shown to contain virus.

2. — Mechanical transmission tests by undelayed, interrupted feedings of 2 species, *T. protracta* and *R. prolixus*, were also negative. One insect of the former species accepted 2 infective and 2 normal (test) feedings, while 22 bugs of the latter species accepted alternate blood-meals one to 3 times each on infected and normal guinea pigs.
3. — Fecal droplets collected from one *R. prolixus* 2 days after an infected feeding failed to infect when injected into a susceptible guinea pig, although virus was shown to be present in the bug by subsequent injection of the viscera into another test animal.

4. — The period of survival of the virus in the bugs was determined by injection of gut contents at various short intervals after infected feedings.

*T. infestans*: Positive once at 24 hours and twice at 48 hours; negative twice at 72, 96, 120 and 192 hours each. *Panstrongylus megistus*: Positive 3 times at 24 hours, twice at 48 hours, and once at 72 hours; negative once each at 72 and 96 hours; tests doubtful or valueless once at 48 hours, and twice each at 72, 96 and 144 hours. *R. prolixus*: Positive once each at 24, 48 and 72 hours, and negative at 96 hours.

5. — From these data, involving species of 4 genera of the Triatomidae, it appears unlikely that triatomids can either transmit Rocky Mountain spotted fever by their bites, or retain virulent virus within their bodies for longer than 2 to 4 days.

REFERENCE

**Dias, E. & Martins, A. V.**