

EXPERIMENTAL TRANSMISSION OF Q FEVER BY AMBLYOMMA CAJENNENSE

ENID C. DE RODANICHE, Ph.D.¹

Since Davis and Cox (1) first isolated the causative agent of Q fever from *Dermacentor andersoni*, several species of ticks have been incriminated in the epidemiology of this disease in various parts of the world. Investigations implicating the following species were reviewed by Kohls in 1948 (2): in Australia, *Haemaphysalis humerosa* and *H. bispinosa*, *Ixodes holocyclus*, *Rhipicephalus sanguineus* and *Boophilus microplus*; and in the United States, *Dermacentor occidentalis*, *Amblyomma americanum*, *Haemaphysalis leporis-palustris*, *Ixodes dentatus*, *Ornithodoros moubata* and *O. hermsi*.

Recently Q fever has been demonstrated to occur sporadically in Panama (3, 4). However, there are no data available concerning the possible vectors in this area. We decided, therefore, to attempt experimental transmission by *Amblyomma cajennense*, a tick ideally suited for this purpose in Panama, due to its abundance and wide host range (5). The JD strain of *Rickettsia burneti*, isolated locally (6), was employed as the infective agent and the guinea-pig as the experimental animal.

The identity of the tick-transmitted infection with Q fever was established by testing recovered animals for immunity, transmission of the disease to other guinea-pigs and fertile eggs by inoculation with acute phase blood, and pathological changes in sacrificed animals.

An engorged female *Amblyomma cajennense* was obtained from a dairy cow in the town of Juan Diaz, R.P. on June 10, 1948. It began to deposit eggs on June 16, terminating 3 days later, when it was triturated with saline solution and inoculated intraperitoneally into duplicate normal guinea-pigs, neither of which showed symptoms or subsequent immunity to Q fever.

On July 12, 23 days after the termination of oviposition, the first larval ticks were observed to have hatched. Two groups of 25 each were ground with physiological salt solution for inoculation of paired normal guinea-pigs without production of symptoms or immunity to Q fever.

On August 13, about 100 of the remaining larvae were used to infest a guinea-pig which had been inoculated immediately before with *R. burneti* (JD strain) in yolk sac suspension. The animal showed high fever on August 16 and 17 and died on August 23. Active engorged larvae dropped off the pig on August 20. Typical rickettsiae were observed in stained smears of the gut and its contents in one of these larvae, and inoculation of paired normal guinea-pigs with a saline suspension of 6 triturated larvae readily reproduced the infection in both, whereas 2 immune pigs similarly inoculated remained unaffected.

Nymphal feeding experiments. On September 2 and 3, the larva-nymph moult was completed. A suspension of 2 macerated nymphs in salt solution proved

¹ Gorgas Memorial Laboratory, Panama, R.P.

fully infectious to two normal guinea-pigs and innocuous to two immunes. Abundant rickettsiae were observed in the epithelial lining cells of the gut and the feces.

Groups of 4 to 25 nymphs were used at various times to infest four normal guinea-pigs. However, only a few, never more than five, were observed to attach to one pig, selecting exclusively the hairless parts of the body. The sluggish engorged nymphs dropped from the host in 5 to 7 days. All four guinea-pigs developed fever of 3 to 7 days duration within 5 to 10 days of infestation. Blood drawn from guinea-pig No. 2 on the second day of fever yielded typical growth in the yolk sacs of chick embryos, and reproduced the symptoms of Q fever in two normal pigs without affecting two immunes. Guinea-pig No. 3 was sacrificed on the first day of defervescence with observation of gross pathological changes

TABLE 1

Results of nymphal-feeding of infected Amblyomma cajennense on normal guinea-pigs

GUINEA-PIG NO.	1	2	3	4
Date of infestation	Sept. 13, '48	Sept. 28, '48	Sept. 28, '48	Dec. 2, '48
No. nymphs engorging	4	3	3	2
First day of fever	Sept. 23	Oct. 5	Oct. 3	Dec. 7
Subsequent immunity	Immune	Immune	Sacrificed	Immune

TABLE 2

Results of adult-feeding of infected Amblyomma cajennense on normal guinea-pigs

GUINEA-PIG NO.	5	6	7	8	9
No. adults feeding	1 F	1F & 1M	1F & 1M	1F & 1M	1F & 1M
Date of attachment	Oct. 21, '48	F—Nov. 5 M—Nov. 6	F—Nov. 16 M—Nov. 30	F—Nov. 18 M—Nov. 11	M & F—Jan. 6
First day of fever	Nov. 4	Nov. 10	Nov. 24	Nov. 24	Jan. 12, '49
Subsequent immunity	Immune	Immune	Immune	Immune	Sacrificed

F indicates female, M male.

characteristic of this strain. The surviving pigs all showed complete immunity when tested 12 to 30 days after the onset of symptoms. Data concerning the nymphal feedings are presented in Table 1.

Adult feeding experiments. Of the 12 engorged nymphs obtained in this experiment, 11 moulted as adults in 18 to 20 days yielding 5 males and six females. Considerable difficulty was experienced in inducing these adults to feed. They often remained for long periods in the feeding-boxes without making any apparent effort to attach to the host. Mating was observed to take place in one instance while male and female were attached in the same spot on the animal. Five normal guinea-pigs were infested at various times with 2 to 3 adults each. Febrile reactions of 2 to 5 days duration were observed in all in from 8 to 14 days after attachment of one or more of the parasites. Blood drawn on the second

day of fever from guinea-pig No. 7 was infective to paired normal pigs and to fertile eggs, but did not produce symptoms in 2 immune pigs. Guinea-pig No. 9 showed typical gross pathological changes when sacrificed on the first day of defervescence. The surviving animals were immune to the homologous strain of *R. burneti* when tested 15 to 20 days after the onset of fever. Data concerning the adult feedings appear in Table 2.

Only one completely engorged female was recovered in this series. This female engorged in 12 days and dropped off the host on November 30. It began to oviposit on December 6, terminating on December 13. One of the partially engorged females was macerated for the preparation of smears of the gut and contents, and to inoculate two normal guinea-pigs, with positive results. The others were either killed accidentally or failing of fertilization, engorged only partially and later died.

The first larvae were observed to hatch on December 29, and hatching continued slowly until January 9. Only a relatively small proportion of the eggs yielded larvae. Three normal guinea-pigs were infested with groups of 15, 20 and 20 respectively of these larvae on January 13, 20 and 22, 1949. Small numbers of engorged larvae, 2, 5 and 6 respectively were recovered in 5 to 7 days after application. None of the host animals developed fever or any other symptom and none showed subsequent immunity when tested with the homologous strain of *R. burneti* 19 to 24 days after infestation. Intraperitoneal inoculation of a suspension of 12 triturated larvae in salt solution failed to produce infection or immunity in two normal guinea-pigs, and smears of the gut of three were completely negative.

Thus, no evidence was obtained of transovarian passage of the rickettsia, as successfully demonstrated for *Dermacentor andersoni* by Parker and Davis (7) and for *Ornithodoros moubata* by Davis (8).

CONCLUSIONS

Amblyomma cajennense experimentally infected with *R. burneti* during the larval stage readily transmit Q fever to normal guinea-pigs by feeding during the nymphal and adult stages. Transovarian passage of the infection was not obtained.

REFERENCES

1. DAVIS, G. E. AND COX, H. R.: A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni*, reactions in animals and filtration experiments. Pub. Health Rep., 53: 2259, 1938.
2. KOHLS, G. M.: Vectors of Rickettsial Disease. Symposium on the Rickettsial Diseases of Man. A. A. A. S., Washington, D. C., 1948.
3. CHENEY, G. AND GREIB, W. A.: The identification of Q fever in Panama. Am. J. Hyg., 44: 158, 1946.
4. RODANICHE, E. DE AND RODANICHE, A.: Studies on Q fever in Panama. Am. J. Hyg., 49: 67, 1949.

5. FAIRCHILD, G. B.: An annotated list of the bloodsucking insects, ticks and mites known from Panama. *Am. J. Trop. Med.*, **23**: 569, 1943.
6. RODANICHE, E. DE AND RODANICHE, A.: Q fever. Report of a case and study of the etiologic agent. *Arch. Hosp. Sto. Tomas*, **2**: 327, 1947.
7. PARKER, R. R. AND DAVIS, G. E.: A filter-passing infectious agent isolated from ticks. II. Transmission by *Dermacentor andersoni*. *Pub. Health Rep.* **53**: 2267, 1938.
8. DAVIS, G. E.: American Q fever. Experimental transmission by the Argasid tick *Ornithodoros moubata* and *O. hermsi*. *Pub. Health Rep.* **58**: 984, 1943.