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NATURAL INFECTION OF THE TICK, *AMBLYOMMA CAJENNENSE*,
WITH *RICKETTSIA RICKETTSII* IN PANAMA

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Gorgas Memorial Laboratory, Panama, R. P.

The occurrence of Rocky Mountain spotted fever on the Isthmus of Panama was established for the first time in 1950 when a highly virulent strain of *Rickettsia rickettsii* was isolated by the author from the blood of a 26 year old Panamanian farmer who died February 8 of that year, shortly after admission to the hospital. The patient evidently had contracted his infection near the town of Ollas Arriba about seven miles north of Capira in the Province of Panama. Since then similarly virulent strains of *R. rickettsii* have been isolated from two additional cases, hospitalized in May 1950 and February 1951, both male, aged 13 and 26 years respectively, who were employed in agricultural work on farms located in the same area as the previous case. The 13-year old boy died. The 26-year old man survived after a severe and protracted illness. His blood showed complement-fixing antibodies against Rocky Mountain spotted fever antigen in high titer during convalescence.

These findings indicated that the vicinity of Ollas Arriba was an endemic focus of Rocky Mountain spotted fever. However, nothing was known of the possible vectors. In order to determine the identity of the vector, we began to collect ticks in the town of Ollas Arriba and neighboring sections, including the towns of Caimito, Caimitillo and Valdesa. The collection was begun in February 1952 near the beginning of the dry season and continued through July of the same year. The cooperation of Drs. G. B. Fairchild and P. Galindo of the staff of the Gorgas Memorial Laboratory was received in planning and conducting the field work. The area was divided into four zones which were swept periodically for ticks by dragging a white flannel cloth over the ground. The ticks which accumulated on the cloth were killed by freezing, brushed off, classified and used for guinea pig inoculations. The drag technique yielded large numbers of larvae and nymphs, but relatively few adults. Later we began to pick ticks from horses which were brought to the Ollas Arriba station from the neighboring areas with a high yield of adults.

No attempt was made to isolate *Rickettsia* by feeding the ticks on experimental animals due to the fear of escape. Instead groups of these arthropods were ground with physiological salt solution. The resultant suspension was centrifuged lightly and the supernatant solution used to inoculate paired guinea pigs of which one received one cc. of the inoculum by the subcutaneous and the other by the intraperitoneal route. Temperatures were taken once daily for a period of one month, the animals being observed for any abnormality at the same time.

All animals were bled for serological study both prior to inoculation and 30 to 35 days afterwards. The complement-fixation reaction with Rocky Mountain spotted fever antigen (soluble type) purchased from Lederle Laboratories was uniformly employed, the technique used being that of overnight ice-box incuba-

tion as recommended by Cox. It was hoped by this means to detect any inapparent infections such as those described originally by Spencer and Parker (1923) who found that the inoculation of guinea pigs with suspensions of macerated fasting infected ticks may produce an asymptomatic but immunizing form of the disease. Tests with Q and murine typhus fever antigens also were performed, as controls, and in hope of identifying a natural vector of Q fever in this area.

Only three different species of ticks were recovered, *Amblyomma cajennense*, *Amblyomma oblongoguttatum* and *Otocentor nitens*. A total of 2436 adult *A. cajennense*, 194 *O. nitens* and 24 *A. oblongoguttatum* were obtained, also about 8000 nymphal *Amblyomma* and an innumerable quantity of larvae. We are indebted to Dr. G. B. Fairchild for all tick identifications. Adult ticks were classified as to species and nymphs as to genera. No attempt was made to classify larval ticks.

TABLE 1

Data concerning guinea pigs giving positive results after inoculation with suspensions of macerated ticks

NO. OF GUINEA-PIG	INOCULUM	DATE OF INOCULATION	ISOLATION OF <i>R. rickettsii</i>	TITER OF COMPLEMENT-FIXING ANTIBODIES AGAINST RMSF ANTIGENS IN SERUM
709 & 710	<i>A. cajennense</i> , 11 adults from horse	March 19	Yes	—
694 & 695	<i>A. cajennense</i> , 25 adults, drag	March 3	No	1-32 & 1-16
728	1000 non-identified drag larvae	March 22	No	1-16
829	<i>A. cajennense</i> , 58 adults, drag	June 10	No	1-8
903	<i>A. cajennense</i> , 23 adults from horse	July 12	No	1-32

RESULTS

A strain of *R. rickettsii* highly virulent for the guinea pig according to the criteria established by Topping (1941) was isolated from a group of 11 adult *A. cajennense* picked on March 19, 1952, from a horse which had been brought from an orange grove located near the town of Caimito to the Ollas Arriba station. Both guinea pigs inoculated with a suspension of these ticks developed fever 5 and 6 days respectively after inoculation. The strain was readily established in passage guinea pigs by serial subinoculations, producing high fever of 3 to 8 days duration after an incubation period of 3 to 4 days, a marked testicular reaction and high mortality (90 per cent). Postmortem examination revealed characteristic gross pathology including enlargement of the spleen, inflammation of the inguinal and axillary lymph nodes and inflammation of the testicles and scrotum accompanied frequently by adhesions between the visceral and parietal lamina of the tunica vaginalis, hemorrhages into the tunica and scrotum and necrosis of the scrotum. Scrapings of the tunica vaginalis revealed small numbers of typical rickettsiae. Cross-immunity studies between this tick strain and a strain of *R. rickettsii* previously isolated by the author from a fatal case of the

disease showed complete reciprocal cross-immunity. Furthermore, 8 guinea-pigs vaccinated with a commercial vaccine all survived a challenge inoculation with the tick strain whereas 8 controls all died.

No symptoms suggestive of Rocky Mountain spotted fever were obtained in any of the other guinea pigs inoculated with tick suspensions. However, 5 animals showed a change in serology from negative prior to inoculation to positive in titers of 1-8 to 1-32 thirty to thirty-five days after inoculation. These positive animals included guinea pigs no. 694 and 695, both injected on March 3 with the same suspension of 25 adult drag *A. cajennense*; no. 728 inoculated on March 22 with a pool of approximately 1000 non-identified drag larvae, no. 829 inoculated on June 10 with a suspension of 58 adult drag *A. cajennense* and no. 903 inoculated on July 12 with a suspension of 23 adult *A. cajennense* picked from a horse. Results of this study are presented in Table 1. The complement-fixation reactions with Q and murine typhus fever antigens gave completely negative results.

DISCUSSION

The isolation of a strain of *Rickettsia rickettsii* from one lot of adult *Amblyomma cajennense*, as well as the demonstration of complement-fixing antibodies against Rocky Mountain spotted fever antigen in the serums of guinea pigs inoculated with other lots of this species, provides evidence that *A. cajennense* is a vector in Panama. This species has been found naturally infected also in Colombia by Patiño-Camargo (1941), in Mexico by Bustamante and Varela (1946) and on various occasions in Brazil according to data summarized by Dias and Martins (1939). A related species, *Amblyomma americanum*, was found spontaneously infected in the United States by Parker, Kohls and Steinhaus (1943). Although *Otocentor nitens* has been shown by Patiño-Camargo (1941) to transmit the infection under experimental conditions, no evidence of natural infection was found in the limited number examined here. Furthermore, the habits of this species render it improbable that it would play an important role as a vector. We have as yet had no opportunity to examine specimens of *Rhipicephalus sanguineus*, a species which was found naturally infected with Rocky Mountain spotted fever in Mexico by Ortiz Mariotte and co-workers (1944). However, as Fairchild (1943) reports that this species is very rarely found on any animal except the dog in Panama, it is of doubtful importance as a possible vector here.

Amblyomma cajennense has all the qualifications of the perfect vector in Panama. It is not only one of the most abundant ixodid species in this area but also exhibits a wide host range and attacks man readily in all stages. Under the circumstances it is difficult to understand why clinical Rocky Mountain spotted fever is not encountered with greater frequency. Evidently further investigation of the many epidemiological factors involved is required.

CONCLUSIONS

A strain of *R. rickettsii* highly virulent for the guinea pig was isolated from a lot of 11 adult *Amblyomma cajennense* removed from a horse in an endemic area

of Rocky Mountain spotted fever in Panama. Guinea pigs inoculated with three other lots of this species remained asymptomatic but developed complement-fixing antibodies against Rocky Mountain spotted fever antigens in their serums.

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