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Natural History of Tick-borne Spotted Fever in the USA

Susceptibility of Small Mammals to Virulent *Rickettsia rickettsii*

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In the ecology of spotted fever rickettsiae, one of the as yet unsolved problems concerns the significance of small animals in the distribution of Rickettsia rickettsii *in nature. In the Bitter Root Valley of western Montana, a great variety of rodents, rabbits and hares are known to serve as the preferred hosts for the immature stages of the vector tick,* Dermacentor andersoni.

The authors analyse the susceptibility of various species of small mammals to virulent R. rickettsii *and evaluate their efficiency as sources of infection for larval ticks. The results demonstrate that meadow-mice, Columbian ground-squirrels, golden-mantled ground-squirrels, chipmunks and snowshoe hares (the latter to a lesser extent), when bitten by infected ticks, respond with rickettsiaemias of sufficient length and degree to infect normal larval* D. andersoni. *High infection rates were obtained in ticks that fed during periods of high rickettsial concentrations in the blood.*

During his studies of Rocky Mountain spotted fever in the Bitter Root Valley of western Montana from 1906 to 1908, Howard T. Ricketts (1909) demonstrated the significance of the Rocky Mountain wood tick, *Dermacentor andersoni*, as the principal vector and advanced the hypothesis that the etiological agent, unknown to him, is maintained in nature in a continuous cycle between infected ticks and one or several of their host animals. In limited laboratory experiments, he established the susceptibility to Rocky Mountain spotted fever of several small animals, including the ground-squirrel, rock-squirrel, chipmunk and woodchuck, and showed that ticks feeding on these did acquire the infectious agent and could transmit it to guinea-pigs. These findings led him to the assumption that new lines of infected ticks were started each season by simultaneous feeding of infected and non-infected ticks on susceptible host animals, primarily rodents, rabbits and hares. Ricketts' plans for more detailed studies, including detection of infected and immune animals in nature, could not be carried out because of his death in 1910.

Although Ricketts' observations concerning the ecology of *Rickettsia rickettsii* have been re-emphasized by many investigators, several questions already raised by him remained unsolved or have not been answered until recently. Isolation of *R. rickettsii* from naturally infected animals in North America, for instance, was not made until 1954, when Gould & Miesse recovered a mild strain from the tissues of a meadow-mouse (*Microtus pennsylvanicus*) in Virginia. One additional strain from a naturally infected cottontail rabbit (*Sylvilagus floridanus*) and several potential strains from wild mice (*Peromyscus leucopus, Pitymys pinetorum*) trapped in the same State were recovered in 1961 by Shirai et al.

More recently, members of the Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases conducted a survey in the Bitter Root Valley of western Montana, in which various species of small animals that serve as hosts for immature *D. andersoni* were periodically trapped and investigated for complement-fixing antibodies to spotted fever antigen or for rickettsiae in their blood and tissues (Burgdorfer et al., 1962). Previous exposure to *R. rickettsii* was detected in large percentages of ground squirrels, chipmunks and snowshoe hares; bushy-tailed woodrats (*Neotoma c. cinerea*) were consistently negative, although they originated from areas where spotted fever is highly endemic. Seven

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strains of spotted fever rickettsiae were recovered: one from the blood of a snowshoe hare (*Lepus americanus*), one from the spleen of a golden-mantled ground-squirrel (*Citellus lateralis tescorum*) and five from spleen tissues of chipmunks (*Eutamias amoenus*).

The question remained unanswered whether all these animals or only certain species represent efficient sources for infection of ticks. The present report deals with a quantitative analysis of susceptibility to virulent *R. rickettsii* for various species of small animals and evaluates their role as possible sources for infecting larval *D. andersoni*.

MATERIAL AND METHODS

The animals studied included golden-mantled ground-squirrels (*C. lateralis tescorum*), Columbian ground-squirrels (C. *columbianus columbianus*), chipmunks (*E. amoenus*), snowshoe hares (*L. americanus*), bushy-tailed woodrats (*N. c. cinerea*) and meadow-mice (*Microtus* spp.). Specimens obtained from areas free of spotted fever were exposed to nymphal *D. andersoni* infected with the " Sawtooth " strain of *R. rickettsii*, five infected ticks usually being used (exceptions are noted). This strain of *R. rickettsii* was detected in the progeny of a female tick collected off vegetation in the Sawtooth Canyon on the west side of the Bitter Root Valley in 1961 and has since been maintained in ticks by transovarial passage. Male guinea-pigs fed on by infected ticks develop fever (39.8°C—41.5°C) for 5-12 days and severe reactions typical of virulent spotted fever.

Beginning on the third day after attachment of ticks, and on alternate days thereafter, rodents were bled either by heart puncture or by the orbital bleeding technique of Riley (1960); snowshoe hares were bled daily. Blood was titrated in fivefold or 10-fold dilutions in male guinea-pigs by intraperitoneal injection of 0.5 ml.

To evaluate host animals as sources for infection of ticks, normal larval *D. andersoni* were allowed to feed during initial, peak and final stages of rickettsiaemias produced by infected nymphal ticks. When the larvae had moulted to nymphs, they were dissected and examined for rickettsiae and degree of infection by the fluorescent-antibody technique (Burgdorfer, 1961).

RESULTS

Susceptibility of small animals to virulent R. rickettsii

Columbian ground-squirrels and chipmunks developed rickettsiaemias that appeared on the third or fourth day and lasted from six to seven days (Fig. 1). In one instance, rickettsiae were detected in a chipmunk for as long as nine days. The largest concentrations of rickettsiae in both species of rodent were found on day 6 or 7, when blood dilutions of 10^{-3} still produced infections in guinea-pigs. For comparison, rickettsiaemias were observed in guinea-pigs that were fed on by the same number of infected ticks as were Columbian ground-squirrels and chipmunks. The concentration of rickettsiae in the blood of guinea-pigs was much higher, with a prolonged period of at least six days in which 100 or more infectious doses per 0.5 ml of blood were present.

FIG. 1

RICKETTSIAEMIA IN COLUMBIAN GROUND-SQUIRREL, CHIPMUNK AND GUINEA-PIG FOLLOWING FEEDING OF *DERMACENTOR ANDERSONI* INFECTED WITH VIRULENT *RICKETTSIA RICKETTSII*

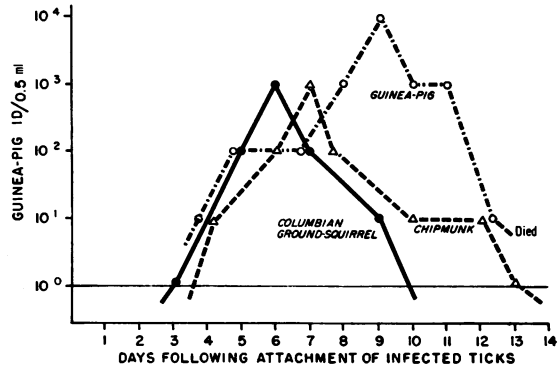
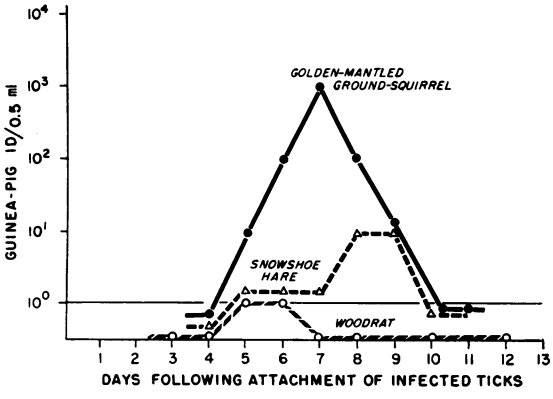


Fig. 2 presents data obtained with golden-mantled ground-squirrels, snowshoe hares and woodrats. The golden-mantled ground-squirrels experienced rickettsiaemias with maximum titres of at least 10^8 for a relatively short period of time. In snowshoe hares rickettsiaemias lasted as long as five days but the concentrations of rickettsiae were much lower and rarely exceeded 10 infectious guinea-pig doses. One specimen, hare No. 7561, bitten by 30 infected *D. andersoni*, responded with a rickettsiaemia that lasted from the sixth through the 14th day and reached its highest titre (10^2) on day 7. Least susceptible to virulent *R. rickettsii* were bushy-tailed woodrats, in which rickettsiae could be demonstrated only following attachment of hundreds of infected ticks.

Fig. 3 summarizes observations made on three meadow-mice which circulated rickettsiae for as long as six to eight days in concentrations that, in some

FIG. 2

RICKETTSIAEMIA IN GOLDEN-MANTLED GROUND-SQUIRREL, SNOWSHOE HARE AND WOODRAT FOLLOWING FEEDING OF *DERMACENTOR ANDERSONI* INFECTED WITH VIRULENT *RICKETTSIA RICKETTSII*



specimens, reached at least 1000 infectious doses per 0.5 ml of blood.

The role of small animals as sources for infecting larval D. andersoni

Experiments were conducted with Columbian and golden-mantled ground-squirrels, meadow-mice and snowshoe hares. The results indicate that normal *D. andersoni* larvae that fed on these hosts during peak rickettsiaemias invariably showed high infection rates while those that fed during the initial or final stages ingested rickettsiae insufficient in numbers to establish permanent infection of tick tissues (Fig. 4 and 5). Of 42 larvae placed on Columbian ground-

FIG. 3

RICKETTSIAEMIA IN THREE MEADOW-MICE FOLLOWING FEEDING OF *DERMACENTOR ANDERSONI* INFECTED WITH VIRULENT *RICKETTSIA RICKETTSII*

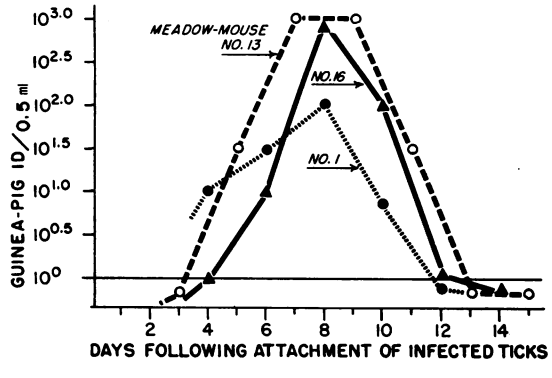
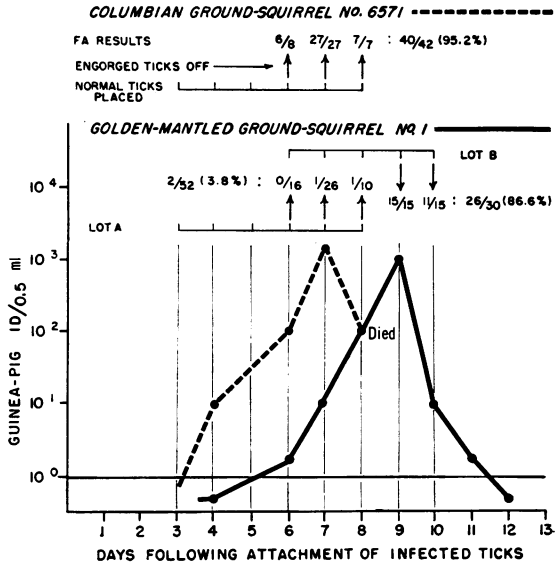


FIG. 4

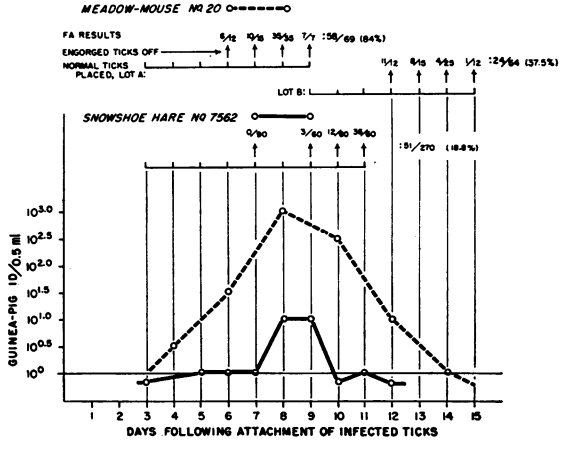
INFECTION RATES IN TICKS FOLLOWING FEEDING ON RICKETTSIAEMIC COLUMBIAN GROUND-SQUIRREL AND GOLDEN-MANTLED GROUND-SQUIRREL [a]



[a] FA results: the numerator indicates the number of ticks found positive for *R. rickettsii* by fluorescent antibody technique, and the denominator the number of ticks examined.

FIG. 5

INFECTION RATES IN TICKS FOLLOWING FEEDING ON RICKETTSIAEMIC MEADOW-MOUSE AND SNOWSHOE HARE [a]



[a] FA results: the numerator indicates the number of ticks found positive for *R. rickettsii* by fluorescent antibody technique, and the denominator the number of ticks examined.

squirrel No. 6571, 40 (95.2%) developed a generalized infection. Only two of 52 (3.8%) became positive after feeding during the initial stages of rickettsiaemia in golden-mantled ground-squirrel No. 1. However, permanent infection resulted in 26 of 30 (86.6%) ticks that ingested blood from the same animal during the peak of rickettsiaemia.

Similar observations were made with meadow-mouse No. 20. Of 12 larvae that fed during the period in wich the rickettsial concentration did not exceed $10^{1.5}$ infectious doses, only six became carriers of *R. rickettsii*. Infection rates increased to 100% of ticks that ingested blood, with titres ranging from $10^{2.0}$ to $10^{3.0}$. A second lot of normal ticks was placed on the same animal at the time when the rickettsial concentration was expected to decrease. Again, high infection rates were found in ticks that had completed engorgement by the time the titre had decreased to $10^{1.0}$ guinea-pig infectious doses, but of those ticks that fed during the final stages of rickettsiaemia only a few became infected.

In the blood of snowshoe hare No. 7562, rickettsiae circulated in low quantities not exceeding 10 infectious doses. None of the ticks became infected that fed during the period in which only undiluted blood proved infectious; of those that engorged when rickettsial concentration was higher, up to 60% became positive.

DISCUSSION

Previous laboratory experience has shown that infection rates of immature *D. andersoni* that fed on infected guinea-pigs varied considerably, depending on whether the ticks completed engorgement during the initial or final stages of the disease. This indicated that the ability of ticks to become infected with *R. rickettsii* may depend on a certain concentration of rickettsiae ingested.

This hypothesis was confirmed in the present study, in which it was found that the minimal dosage requirement to infect 50% of *D. andersoni* larvae ranges between 10 and 100 guinea-pig infectious doses per 0.5 ml of blood. Although this figure has to be regarded as relative, since the prolonged and irregular feeding pattern of wood-ticks does not permit an exact determination of blood and rickettsiae ingested, it provides a basis for establishing and comparing the efficiency of various host animals as sources for infecting ticks. Accordingly, meadow-mice, Columbian ground-squirrels, chipmunks and golden-mantled ground-squirrels must be considered highly efficient sources of infection, at least for those ticks that feed during the periods when large quantities of rickettsiae are present in the blood. In meadow-mice, which appeared to be the most susceptible, rickettsiae circulated in concentrations of 10^2 to 10^3 guinea-pig infectious doses for as long as four days. Similar titres, although for one or two days only, were detected in chipmunks and Columbian and golden-mantled ground-squirrels.

Meadow-mice have long been suspected to play a significant role in the natural history of spotted fever. Jellison (1934) demonstrated tick-to-tick transmission *via* this host and reported that fatalities and scrotal involvement were frequent in mice injected with blood from infected guinea-pigs. In the present study, in which rickettsial infection was introduced by tick-bite, a few specimens died but their deaths could not be attributed with certainty to rickettsial infection. Scrotal involvement was rarely seen; when it occurred it consisted merely of swelling and reddish discoloration.

Serological surveys (Parker et al., 1951; Burgdorfer et al., 1962) have shown that large percentages of snowshoe hares from the Bitter Root Valley possess specific complement-fixing antibodies to *R. rickettsii* and suggested that this animal—which serves as host for both the Rocky Mountain wood tick, *D. andersoni*, and the rabbit tick, *Haemaphysalis leporispalustris*—may be a significant source for the infection of ticks in nature. The present study demonstrates that hares do respond to infectious tick-bites with rickettsiaemias that in general are much milder than those observed in ground-squirrels, chipmunks or meadow-mice. However, for at least one or two days, infectious titres may reach the level necessary to infect 50% or more of larval *D. andersoni*. Titres as high as 10^2 were detected in hare No. 7561, but this specimen was fed on by a relatively large number of infected ticks.

Finally, the bushy-tailed woodrat, a common host of immature *D. andersoni*, was the only species of animal that did not circulate rickettsiae in the blood following feedings of infected ticks. This observation, as well as the fact that previous serological surveys never detected an individual with complement-fixing antibodies to *R. rickettsii* suggest that this rodent is not susceptible to spotted fever and is of no significance for infecting ticks in nature.

RÉSUMÉ

Depuis que Ricketts démontra le rôle de *Dermacentor andersoni* dans la transmission de la fièvre pourprée des Montagnes Rocheuses, de nombreuses inconnues subsistent concernant l'écologie de *Rickettsia rickettsii*. Dans la vallée de Bitter Root, Montana occidental, une grande variété de rongeurs sont connus comme hôtes habituels des formes immatures de *D. andersoni*; les auteurs ont analysé la réceptivité de diverses espèces de ces petits animaux à *R. rickettsii* virulente et évalué leur rôle comme source d'infection possible des larves de tiques.

Ils ont exposé des spécimens de ces animaux provenant de régions indemnes de fièvre pourprée à des nymphes de *D. andersoni* infectées avec la souche Sawtooth de *R. rickettsii*. Le cobaye mâle, exposé dans les mêmes conditions, présente une fièvre à 39,8°C-41,5°C pendant 5-12 jours avec de fortes réactions caractéristiques de la fièvre pourprée. Le sang prélevé sur les animaux en expérimentation a été titré sur le cobaye, par injection intrapéritonéale. Des larves normales de *D. andersoni* ont été nourries au début, à l'acmé et à la fin de la rickettsémie provoquée par les nymphes infectées. Les rickettsies ont été recherchées par dissection après la mue nymphale de ces larves et le degré de l'infection a été évalué par la technique de l'immuno-fluorescence.

Les taux d'infection des *D. andersoni* nourris sur animaux infectés ont varié considérablement suivant que la tique se gorgeait au stade de forte rickettsémie ou aux stades initial et final de la maladie, ce qui semble indiquer que l'infection de la tique dépend probablement de la concentration des rickettsies ingérées.

Après exposition à des tiques infectées, *Citellus columbianus columbianus*, *C. lateralis tescorum*, *Eutamias amoenus* et *Microtus montanus* ont présenté des rickettsémies apparaissant dès le 3^{e} jour et durant jusqu'à 5-9 jours. Les plus fortes concentrations de rickettsies dans leur sang ont été d'au moins 10^{3} doses infectantes pour le cobaye par 0,5 ml. Les rickettsies ont été trouvées dans le sang de *Lepus americanus* pendant neuf jours mais à des titres plus faibles, dépassant rarement 10^{1} doses infectantes. *Neotoma c. cinerea* ne s'est pas montré sensible à *R. rickettsii*.

Toutes les espèces animales étudiées, sauf *Neotoma c. cinerea*, peuvent être considérées comme des sources actives d'infection pour les larves de *D. andersoni*.

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