INFECTION AND IMMUNITY, Sept. 1970, p. 256-259
Vol. 2, No. 3

Susceptibility of Various Species of Rodents to the Relapsing Fever Spirochete, *Borrelia hermsii*

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Received for publication 25 May 1970

In a study to determine susceptiblity to *Borrelia hermsii* of various rodents commonly found in or near places where human cases of relapsing fever occurred, chipmunks (*Eutamias amoenus*), pine squirrels (*Tamiasciurus hudsonicus richardsoni*), flying squirrels (*Glaucomys sabrinus*), Columbian ground squirrels (*Spermophilus columbianus columbianus*), golden-mantled ground squirrels (*S. lateralis tescorum*), wood rats (*Neotoma cinerea cinerea*), white-footed deer mice (*Peromyscus maniculatus*), and meadow voles (*Microtus pennsylvanicus*) were exposed to spirochetes either by bites of infected *Ornithodoros hermsi* or by injection of infectious tick suspensions. Daily microscopic examination of blood samples revealed that pine squirrels, chipmunks, and meadow voles responded with spirochetemias of various degrees and length. Pine squirrels were most susceptible and experienced long-lasting and severe spirochetemias accompanied by typical signs of illness. Flying squirrels, Columbian ground squirrels, golden-mantled ground squirrels, wood rats, and white-footed deer mice did not develop demonstrable spirochetal infection.

Recently, 11 of 42 Boy Scouts and scoutmasters contracted tick-borne relapsing fever while sleeping in poorly constructed cabins on Browne Mountain near Spokane, Wash. (5). Investigations of the cabins revealed infestation with *Ornithodoros hermsi*, two of which were infected with spirochetes. Numerous rodent nests were also found, particularly in a basement pit, in the attics, and behind mop boards. The wooded area around the cabins abounded with a large variety of rodents, especially ground squirrels (*Spermophilus* sp.), pine squirrels (*Tamiasciurus hudsonicus richardsoni*), and chipmunks (*Eutamias* sp.).

Pine squirrels and chipmunks are considered the natural reservoirs of *Borrelia hermsii*, the etiological agent of this type of tick-borne relapsing fever. This belief is based on isolation of spirochetes from these animals during early investigations of relapsing fever in California (1, 4) and on recovery of infected *O. hermsi* from chipmunk nests in both California and Colorado (3). To the best of our knowledge, there has been only one experimental study of the susceptibility of chipmunks to *B. hermsii* (1). In that study, two of three specimens of *Eutamias amoenus* developed mild and transient spirochetemias after inoculation with mouse blood containing *B. hermsii* isolated from a naturally infected chipmunk.

The outbreak on Browne Mountain rekindled interest in relapsing fever and induced us to determine susceptibility of various species of rodents including chipmunks and pine squirrels. The results are presented in this paper.

MATERIALS AND METHODS

Species of animals included chipmunks (*E. amoenus*), pine squirrels (*T. hudsonicus richardsoni*), flying squirrels (*Glaucomys sabrinus*), Columbian ground squirrels (*S. columbianus columbianus*), golden-mantled ground squirrels (*S. lateralis tescorum*), wood rats (*Neotoma cinerea cinerea*), white-footed deer mice (*Peromyscus maniculatus*), and meadow voles (*Microtus pennsylvanicus*). All these animals were trapped near Hamilton, Mont., where relapsing fever has never occurred. Three to five specimens of each rodent species were infected with *B. hermsii* by inoculating them intramuscularly with suspensions of infected *O. hermsi* or by allowing two to five experimentally infected ticks to feed on them. The strain of spirochetes originated from one of the two infected ticks collected in the cabins on Browne Mountain.

For 30 days after inoculation or tick-feeding, drops of blood were taken daily from tail, ear, or toes of each animal and prepared as thick drops and thin smears. These were then stained by Giemsa's method and examined under oil immersion (1,000× magnification). Degree of infection was determined by counting the spirochetes detected during 5 min of examination.

RESULTS

Spirochetes were not detected in flying squirrels, ground squirrels, wood rats, or deer mice. On the other hand, pine squirrels, chipmunks, and meadow voles had spirochetemias of various

TABLE 1. *Spirochetemias in pine squirrels (Tamiasciurus hudsonicus richardsoni), chipmunks (Eutamias amoenus), and meadow voles (Microtus pennsylvanicus) after injection of tick suspension or feeding of ticks infected with Borrelia hermsii*[a]

Days after injection or feeding	Pine squirrels			Chipmunks			Meadow voles		
	No. 1 (injection)	No. 2 (feeding)	No. 3 (injection)	No. 1 (injection)	No. 2 (injection)	No. 3 (feeding)	No. 1 (injection)	No. 2 (injection)	No. 3 (injection)
1	−	−	−	−	−	−	−	−	−
2	−	−	−	−	(+)	−	−	(+)	+
3	(+)	−	(+)	++	+++	(+)	++	+++	+++
4	+	+	+	++++	++++	+	+	+	+
5	+++	++++	+++	(+)	(+)	+++	++	++	+
6	+++	++++	++++	(+)	(+)	++++	+	(+)	+
7	+++	++++	+++	+	++	+++	(+)	+	(+)
8	+++	+++	++++	(+)	+	++	−	(+)	−
9	++++	++++	++++	+	++	(+)	+	−	(+)
10	++++	++++	++++	++	++	(+)	++	−	(+)
11	+	++++	++++	+++	(+)	(+)	−	−	−
12	+	(+)	+	−	−	(+)	(+)	−	−
13	+++	+++	++++	−	−	(+)	++	(+)	(+)
14	++++	++++	++++	−	+	(+)	−	(+)	(+)
15	++++	++++	++++	−	+	+	−	−	−
16	++++	−	+	(+)	+++	(+)	−	−	−
17	−	Dead	++	(+)	+	(+)	−	(+)	−
18	(+)		+++	(+)	−	−	−	−	−
19	+		++++	(+)	−	−	−	−	−
20	+++		++++	+	−	(+)	−	−	−
21	+		++++	++	(+)	(+)	−	−	−
22	+		++++	++	+	(+)	−	−	−
23	++		++++	(+)	(+)	−	−	−	−
24	+++		++	−	−	(+)	−	−	−
25	+++		++++	−	−	(+)	−	−	−
26	++++		++++	−	−	−	−	−	−
27	+		++++	(+)	(+)	−	−	−	−
28	+++		++++	−	−	−	−	−	−
29	(+)		++++	−	−	−	−	−	−
30	Dead		(+)	−	−	−	−	−	−

[a] Symbols indicate no. of spirochetes counted per 5 min: −, negative; (+), 1 to 5; +, 6 to 25; ++, 26 to 100; +++, 101 to 300; ++++, >300.

length and degrees (Table 1). Pine squirrels were most susceptible; after an incubation period of 2 to 3 days, spirochetes, often in large quantities, circulated in the blood stream. Pine squirrel 1, after receiving infectious tick material, had a continuous spirochetemia from the 3rd through the 16th day, was negative on day 17 but was again positive from day 18 to day 30 when it died. The number of spirochetes in the blood of this animal varied considerably. They were low on days 3 and 4, but increased sharply thereafter, and reached a peak on day 10. A sharp decrease occurred on days 11 and 12 followed by a steady increase to a second peak on day 16. From the 18th day to time of death, three additional cycles occurred with peaks of spirochetemia on days 20, 26, and 28.

A similar, but even more severe, spirochetemia was noted in pine squirrel 2 which was infected through feeding of infectious *O. hermsi*. Spirochetes were first seen in thick-drop preparations on day 4. Within 24 hr, the number of spirochetes increased to such an extent that from 15 to 25 organisms could regularly be counted per microscopic field (Fig. 1). Degree of infection remained high until day 12, at which time most spirochetes disappeared from the blood stream. They reappeared in increasing numbers on day 13 and reached a peak on day 15 when approximately 30 organisms were present in each microscopic field. Blood smears became negative shortly before the animal died on the 17th day.

After receiving infectious tick suspension, pine squirrel 3 developed a spirochetemia which was almost identical to that in the other two specimens during the first 15 days. The blood was continuously positive from the 3rd through the 30th day, and possibly even longer. After the first period of severe spirochetemia, there were three

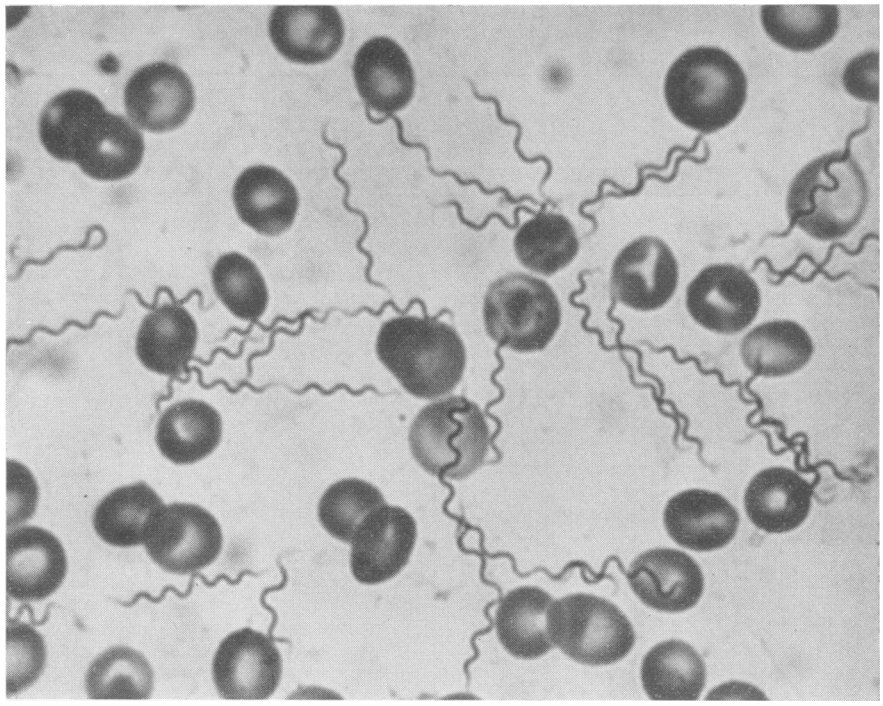

FIG. 1. *Borrelia hermsii in blood of pine squirrel no. 2. Giemsa stain, ×2,700.*

relapses, each after 1 or 2 days, at which point the number of circulating spirochetes was low. Peak infection occurred on days 26 and 27, when more than 50 spirochetes were counted per microscopic field.

Each pine squirrel exhibited typical signs of illness as soon as large numbers of spirochetes circulated in the blood. Throughout the disease, the animals were hunched and frequently experienced shivering spells. Shortly before they died, pine squirrels 1 and 2 became very weak and drowsy and were severely anemic. Pine squirrel 3 survived the period of examination but was found dead 7 days after the experiment was terminated.

Chipmunks and meadow voles experienced much milder spirochetemias and did not show signs of illness. In chipmunks, maximum numbers of spirochetes were noted at beginning of spirochetemias, i.e., in chipmunks 1 and 2 on day 4 after injection of infectious tick material, and in chipmunk 3 on day 6 after tick bite. Chipmunks 1 and 2 had mild to moderate infections until day 12, when their blood was free of spirochetes. A mild spirochetemia reoccurred in chipmunk 1 on day 16 and lasted for an additional 7 days before it disappeared. In chipmunk 2, the blood became positive again on day 14 and was heavily infected on day 16, but was free of spirochetes on day 18. A 3rd and a 4th rather mild relapse lasting for 3 and 1 days, respectively, was noted in this animal on days 21 and 27. Chipmunk 3, on the other hand, had a continuous low-grade spirochetemia from days 3 through 17. Spirochetes could not be detected for the following 2 days, but they reappeared on day 20, and persisted for 3 additional days before the blood again became negative. A final relapse occurred on day 24 and lasted for only 2 days.

Of the three species of rodents which experienced spirochetemias, meadow voles were least susceptible to *B. hermsii*, although large numbers of organisms circulated in their blood for at least 1 day (day 3). Each animal experienced three periods of spirochetemia which lasted 1 to 7 days. The longest period occurred early in infection.

DISCUSSION

The results demonstrate the susceptibility of pine squirrels, chipmunks, and meadow voles to *B. hermsii* and suggest that these species may represent sources for infecting ticks in nature. Pine squirrels, in particular, experienced long-lasting and severe spirochetemias. Little is known of the role this animal plays in the ecology of *B. hermsii*, and, to the best of our knowledge, it has never been established that pine squirrels are hosts of *O. hermsi*. However, since

spirochetes were isolated from Tamarack squirrels in California (1, 4), and pine squirrels are closely associated with chipmunks, they could be involved in the natural history of *B. hermsii*, possibly even as the most important source for infecting ticks. It is questionable whether pine squirrels succumb to spirochetal infection under natural conditions, as did the three experimentally infected specimens in our studies. This rodent is extremely excitable and very difficult to handle in captivity. Possibly, abnormal stress in the laboratory was a factor responsible for the fatal course of the disease.

Chipmunks, as far as is known, are the primary host of *O. hermsi*. During studies of relapsing fever in Colorado (2), 213 ticks were collected from a chipmunk nest in a decaying Douglas fir stump, and 51 ticks were collected from nesting material in another stump in the same locality. More recently, during investigation of relapsing fever in Ferry County, Washington (Jones, Russell, and Burgdorfer, *unpublished data*), 37 *O. hermsi* were found in decaying fir tree snags at a logging site. Four strains of *B. hermsii* were isolated from these ticks.

Chipmunks are the most important host by which ticks are brought into cabins and summer homes, at which sites these attractive rodents build their nests in poorly constructed walls, attics, and storage places. Although less susceptible to *B. hermsii* than pine squirrels, chipmunks nevertheless experience prolonged spirochetemia of sufficient degree to infect normal ticks. In experiments with Swiss mice (Burgdorfer, *unpublished data*), a spirochetemia of not more than five spirochetes per thick-drop preparation was sufficient to infect *O. hermsi*.

Also worth mentioning is that flying squirrels, which also nest in hollow, rotten stumps and downed logs, were not susceptible to *B. hermsii*. There are no records that would indicate infestation of this animal by *O. hermsi*.

Meadow voles were included in this study because a large colony was found near the two cabins on Browne Mountain. These rodents are generally restricted to grassy or marshy habitats although they are known to invade shacks and wooden dwellings. They, too, could serve as sources for infection of ticks because of their susceptibility to *B. hermsii*.

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