The New Zealand White Rabbit: An Experimental Host for Infecting Ticks with Lyme Disease Spirochetes

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Efficiency of the New Zealand white rabbit as a host for infecting larval Ixodes dammini, I. pacificus, and I. ricinus with Lyme disease spirochetes was evaluated. Rabbits inoculated with infected midgut suspensions of I. dammini from Shelter Island, New York, or fed upon by infected ticks from the same area, responded with spirochetemias of sufficient concentrations to infect as many as 30 percent of the ticks. When infected ticks were used as indicators, it appeared that spirochetemias persisting for up to ten days occurred as early as the tenth day after inoculation or feeding of ticks.

INTRODUCTION

The New Zealand white rabbit (Oryctolagus cuniculus) has been shown [1; Burgdorfer W: unpublished information] to be a suitable host for maintaining colonies of Ixodes dammini, I. pacificus, and I. ricinus, the three species of ixodid ticks found to be carriers of Lyme disease spirochetes [2]. In the course of our studies, hundreds of field-collected male and female ticks of those three species were fed on rabbits not only to determine the susceptibility of this animal to the spirochete but also to establish tick colonies for experimental investigations.

As reported previously [3,4], the feeding of I. dammini from Shelter Island, New York, and of I. ricinus from the Seewald forest in Switzerland, appeared to have no immediate adverse effects on the rabbits; all attempts to detect spirochetes microscopically in thick drops and thin smears of peripheral blood taken daily for 14 days after placement of ticks, failed. However, from as early as four to 12 weeks after the ticks had engorged and dropped from the hosts, skin lesions appeared on the back and sides of each rabbit, but rarely at the site of tick feeding. At first, the lesions were small annular papules, that gradually enlarged to annular and irregularly shaped erythematous areas up to 5 cm in diameter and surrounded by a narrow, dark-red border. In most affected rabbits, lesions persisted for at least eight to 12 weeks before they gradually faded and disappeared.

Because only rabbits with cutaneous lesions developed high titers of antibodies to the Lyme disease spirochete, I thought the lesions might be a clinical manifestation of a spirochete-related disease. This observation led us to determine whether normal I. dammini, I. pacificus, and I. ricinus would become infected when fed on such rabbits. The results of this study are the subject of this paper.

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MATERIAL AND METHODS

The New Zealand white rabbits were from the Junelove Rabbitry in Missoula, Montana. Upon arrival at the Rocky Mountain Laboratories, each rabbit was tested by indirect immunofluorescence for antibodies to the Lyme disease spirochete and was found to be negative.

Two rabbits were inoculated intravenously and intraperitoneally with 0.5 ml of a suspension prepared from midgut tissues of five infected I. dammini females (Shelter Island, New York) triturated in 3.0 ml of 1/15 M phosphate-buffered saline, pH 7.0. Spirochetal infections in these ticks were demonstrated by darkfield microscopy of dissected midgut tissues.

In one additional experiment, ten infected I. dammini females, contained in a metal capsule, were allowed to feed on the shaven abdomen of a rabbit. The ticks originated from a Shelter Island focus (Hilo Drive), where 83 percent of those examined were found to have spirochetes. After each tick became engorged, it was dissected and examined by darkfield microscopy; all ten had spirochetes in their midgut diverticula.

At varying intervals after the rabbits had been inoculated or exposed to infected ticks, several hundred larval I. dammini, I. pacificus, or I. ricinus, derived from normal ticks, were placed freely on each rabbit for feeding. To prevent escape of ticks unwilling to feed and to collect engorged ticks, each rabbit was put in a wire cage over a tray of water. Engorged larvae were collected daily and stored in cotton-stoppered vials in desiccator jars (90 to 100 percent relative humidity) until they had molted to nymphs. When ready for examination, about six to eight weeks after molting, they were evaluated for spirochetal infections by direct fluorescent antibody staining of tissue smears. Conjugates were prepared according to Peacock et al. [5] from sera of rabbits that had been immunized with the Shelter Island isolate of the spirochete.

RESULTS

Exposure of the rabbits to Lyme disease spirochetes either by inoculation of infected tick tissues or by feeding of infected ticks resulted in spirochetemias of suffi-
R No 3501: Suspension of 5 +++ I. dammini (Shelter Island)

|       | 3/10  | 5/20  | 3/10  | 0/5  | 8/40  | 2/40  | 10/40 | 1/20 |
|-------|-------|-------|-------|------|-------|-------|-------|------|
|       | (30)  | (25)  | (15)  |      | (20)  | (5)   | (25)  | (5)  |

1 2 3 4 5 10 11 12 13 14 15 16 17 18 19 20 days

* : Placement of normal I. ricinus larvae
** : Engorged I. ricinus larvae off
***: Number of infected ticks over number of ticks examined
(% in parenthesis)

FIG. 2. New Zealand white rabbit used to infect larval Ixodes ricinus with Lyme disease spirochetes (Shelter Island, New York).

Sufficient concentrations to infect variable percentages of larval I. dammini, I. pacificus, and I. ricinus.

Figure 1 summarizes the results for larval I. dammini that were placed on rabbit No. 3550 on days 1, 3, 5, 11, 13, and 14 after it had been inoculated with infected midgut suspensions. None of 150 ticks that had fed during the first nine days after inoculation became infected. On the other hand, spirochetal infections were detected in 12 of 80 (15 percent) ticks that had become engorged by the twelfth through fifteenth day after inoculation of the rabbit. Ticks that had dropped later were negative. Because larval I. dammini requires three to five days' feeding before they become replete, concentrations of spirochetes sufficient to infect ticks most likely had been circulating in the rabbit's peripheral blood on about the tenth through the fourteenth day after inoculation.

Rabbit No. 3501 also had been infected by inoculation of spirochete-containing midgut suspensions. It had a far longer and possibly heavier spirochetemia. As indicated in Fig. 2, 32 of 195 (16.4 percent) I. ricinus larvae that had completed engorgement and had dropped from the thirteenth through the twentieth day after inoculation were infected when examined as nymphs, suggesting a spirochetemia of at least eight days.

R No 3441: Feeding by infected I. dammini females (Shelter Island)

|        | 0/40 | 0/40 | 0/30 | 0/40 | 0/20 | 0/20 |
|--------|------|------|------|------|------|------|
|        |      |      |      |      |      |      |
|        | 7/40 | 2/40 | 6/40 |
|        | (17.5)| (5)  | (15) |

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 days

* : Placement of normal I. pacificus larvae
** : Engorged I. pacificus larvae off
***: Number of infected ticks over number of ticks examined
(% in parenthesis)

FIG. 3. New Zealand white rabbit used to infect larval Ixodes pacificus with Lyme disease spirochetes (Shelter Island, New York).
Figure 3 summarizes the results for *I. pacificus* larvae off rabbit No. 3441 that had been exposed to the feeding of ten *I. dammini* females from the Hilo Drive focus on Shelter Island, New York. None of 12 larvae that had been placed on the rabbit for feeding on days 1, 3, and 5 became infected. The spirochetemia occurred later, as indicated by the presence of spirochetes in 15 of 120 (12.5 percent) ticks that had become completely engorged by the fifteenth through the seventeenth day.

**DISCUSSION**

These limited, preliminary observations confirm that the New Zealand white rabbit is susceptible to the Lyme disease spirochete and suggest that this laboratory animal is a suitable host for the experimental infection of the hitherto recognized tick vectors, *I. dammini*, *I. pacificus*, and *I. ricinus*. Even so, only low percentages of ticks became infected, whereas in certain natural foci, such as Shelter Island, New York, up to 100 percent of ticks were found to harbor spirochetes [Burgdorfer W, Benach JL: unpublished information]. This disparity suggests the existence in nature of animal hosts that have spirochetemias of greater concentrations and possibly also of longer duration than those recorded in laboratory rabbits. This assumption is strongly supported by previously reported evidence suggesting that generalized infection of ticks leading to transovarian transmission is rare; when it occurs, it does not result in filial ticks with generalized spirochetal infections, only restricted ones.

Undoubtedly persistence and development of spirochetes in their tick vectors depends on the number of spirochetes ingested. Ticks feeding during peak spirochetemias may readily become infected, whereas those ingesting few spirochetes may not. A "minimal dosage requirement" similar to that for other arthropod-borne disease agents may exist.

The present study was limited to infecting rabbits with Lyme disease spirochetes from Shelter Island, New York. As yet, we do not know whether spirochetes in the same species of ticks from other geographic regions behave similarly in this laboratory animal. We also do not know whether spirochetes in *I. pacificus* from the west coast or in *I. ricinus* from Switzerland produce spirochetemias of similar durations and magnitudes in rabbits. Indeed, it would not be surprising to find remarkable differences in the biological behavior (pathogenicity, virulence) of these microorganisms in view of the differences in parasite/vector/host relationship(s).

So far, Lyme disease spirochetes have been recovered from deer, raccoons, and deer mice [6,7], but as yet there is no information on the duration and concentration of spirochetemias that occur in these tick hosts. Thus, for now at least, the New Zealand white rabbit appears to be a suitable animal for continued investigations on the parasite/vector relationships of these spirochetes.

**REFERENCES**

1. Krinsky WL: Development of the tick *Ixodes dammini* (Acarina: Ixodidae) in the laboratory. J Med Entomol 16:354-355, 1979
2. Burgdorfer W: Discovery of the Lyme disease spirochete and its relation to tick vectors. Yale J Biol Med 57:515-520, 1984
3. Burgdorfer W, Barbour AG, Hayes SF, et al: Lyme disease—a tick-borne spirochetosis? Science 216:1317-1319, 1982
4. Burgdorfer W, Barbour AG, Hayes SF, et al: Erythema chronicum migrans—a tickborne spirochetosis. Acta Tropica 40:79-83, 1983
5. Peacock M, Burgdorfer W, Ormsbee RA: Rapid fluorescent-antibody conjugation procedure. Infect Immun 3:355-357, 1971
6. Bosler EM, Coleman JL, Benach JL, et al: Natural distribution of the *Ixodes dammini* spirochete. Science 220:321-322, 1983