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Peromyscus leucopus and *Microtus pennsylvanicus* Simultaneously Infected with *Borrelia burgdorferi* and *Babesia microti*

JOHN F. ANDERSON,[1]* RUSSELL C. JOHNSON,[2] LOUIS A. MAGNARELLI,[1] FRED W. HYDE,[2] AND JAMES E. MYERS[3]

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504[1]; Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455[2]; and Department of Environmental Management, Division of Fish and Wildlife, West Kingston, Rhode Island 02892[3]

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***Borrelia burgdorferi*, the etiologic agent of Lyme disease, and *Babesia microti*, the causative agent of human babesiosis, were isolated from 71 and 57%, respectively, of 14 specimens of *Peromyscus leucopus* and *Microtus pennsylvanicus* collected from Prudence and Patience Islands, R.I. Both pathogens were isolated from five individual rodents. The presence of these two infectious organisms in the same mammal suggests that individual larval *Ixodes dammini* may ingest both pathogens and subsequently transmit them in the nymphal stage.**

Borrelia burgdorferi (15) and *Babesia microti*, etiologic agents of Lyme disease (7) and human babesiosis (22), respectively, are transmitted in the northeastern United States by the tick *Ixodes dammini* (7, 20, 21). Larval ticks acquire these pathogens while feeding on infected hosts, and, subsequently, nymphs and adults may transmit them to other animals, including humans (3, 6, 20). Although rates of infection by or exposure to these spirochetal and protozoan pathogens may be relatively high (≥50%) in rodents (1, 2, 9, 12, 17), it is unknown whether an individual mammal can be simultaneously infected with both microbes and thus serve as a reservoir for the two agents. We report the isolation and identification of *Borrelia burgdorferi* and *Babesia microti* from individual specimens of *Peromyscus leucopus* (white-footed mouse) and *Microtus pennsylvanicus* (meadow vole) captured on two islands in Narragansett Bay, R.I.

MATERIALS AND METHODS

Thirty Sherman box traps baited with peanut butter, apple, and sunflower seeds were placed in forests and marshes on Prudence and Patience Islands in Narragansett Bay on 20 November 1984 and retrieved the following day. Captured rodents were individually placed in cages until they were sacrificed in the laboratory on 26 and 27 November 1984.

Blood was drawn from the heart of each rodent, and 0.5 ml was injected intraperitoneally into a Syrian hamster in an attempt to isolate *Babesia microti* (9). At weekly intervals for 6 weeks postinoculation, a drop of blood obtained from the tail of each inoculated hamster was smeared onto a glass slide, fixed in methanol for 30 s, and overlaid with Giemsa stain. Erythrocytes were examined for *Babesia microti* at ×650 magnification. Hamsters were considered negative if no parasites were observed in blood smears 6 weeks after inoculation.

Attempts to isolate *Borrelia burgdorferi* were made from blood, kidney, and spleen tissues of each rodent as described previously (1, 14). Briefly, 1 or 2 drops of whole blood were drawn from the heart of each animal and inoculated into 8 ml of Barbour-Stoenner-Kelly (BSK) medium containing 0.1% agarose (Seakem LE, FMC Corp., Rockland, Maine) (4, 14). Spleen and kidneys were aseptically excised and triturated in 2 ml of medium without agarose, and 0.1 ml was inoculated into 8 ml of BSK medium with agarose. Inoculated tubes of media were kept at 31°C and examined for spirochetes by dark-field microscopy 3 to 4 weeks after inoculation. The remaining triturated tissues were placed in 7-ml polystyrene screw-capped tubes and shipped by overnight courier to the University of Minnesota, where duplicate 1:10 dilutions of the tissues were cultured at 30°C in BSK medium containing agarose.

Swiss mouse antisera prepared against the Ct 2591 strain of *Borrelia burgdorferi* (3) and murine monolconal antibody H5332 reactive with the 31,000-molecular-weight surface protein of *Borrelia burgdorferi* B-31 (5) were used in indirect fluorescent antibody tests to identify spirochetes cultured from rodent tissues. The DNA filter method was used to genetically characterize one of the spirochete isolates from *M. pennsylvanicus* (13).

RESULTS AND DISCUSSION

Four meadow voles were captured on each of the two islands; 17 white-footed mice were also obtained on Prudence Island. *Babesia microti* and *Borrelia burgdorferi* were recovered from members of both species of rodents. One *M. pennsylvanicus* (no. 98) captured on Patience Island was simultaneously infected with both pathogens (Table 1); two others (no. 97 and 99) were infected with spirochetes only. Of the 10 rodents tested from Prudence Island (the remaining 11 captured rodents were released), both pathogens were isolated from 1 specimen of *M. pennsylvanicus* (no. 103) and from 3 of *P. leucopus* (no. 2695, 2696, and 2697). Two other specimens of *M. pennsylvanicus* (no. 101 and 102) and one of *P. leucopus* (no. 2694) were infected with *Babesia microti*, and three specimens of *P. leucopus* (no. 2698, 2699, and 2700) were infected with borreliae.

Spirochetes isolated from both rodent species reacted with monoclonal antibody H5332 and produced titers of ≥1:512 with Swiss mouse antisera to *Borrelia burgdorferi* Ct 2591. Also, spirochetes isolated from one specimen of *M. pennsylvanicus* exhibited a 68% DNA homology with

* Corresponding author.

TABLE 1. Isolation of *Borrelia burgdorferi* and *Babesia microti* from *M. pennsylvanicus* and *P. leucopus*

Source, rodent, specimen no.	Isolation of[a]:				
	Borrelia burgdorferi in[b]:				*Babesia microti*
	Blood	Spleen	Left kidney	Right kidney	
Patience Island					
M. pennsylvanicus					
97	−	+	+	+	−
98	−	+	+	+	+
99	−	+	+	+	−
100	−	−	−	−	−
Prudence Island					
M. pennsylvanicus					
101	−	−	−	−	+
102	−	−	−	−	+
103	−	+	−	−	+
P. leucopus					
2694	−	−	−	−	+
2695	−	+	+	+	+
2696	−	+	+	+	+
2697	+	+	+	+	+
2698	−	−	+	−	−
2699	−	+	+	+	−
2700	−	+	−	−	−

[a] Rodent specimens were collected on 21 November 1984 from Prudence and Patience Islands, R.I.

[b] Symbols: −, pathogen not isolated; +, pathogen isolated. Results of isolations of *Borrelia burgdorferi* made in Connecticut and Minnesota laboratories are combined.

Borrelia burgdorferi (ATCC 35210). The results of these tests establish the identity of these spirochetes as *Borrelia burgdorferi* (5, 13). Intraerythrocytic protozoa isolated in Syrian hamsters were morphologically consistent with the description of *Babesia microti* (11).

The frequent isolation of spirochetes from spleen and kidney tissues and the relatively high prevalence of infected *P. leucopus* on Prudence Island parallel our earlier findings in Connecticut (1). Although spirochetes were previously detected in *M. pennsylvanicus* (6), our serologic and genetic characterizations of four isolates and one isolate, respectively, demonstrate that this rodent, along with *P. leucopus*, is an important reservoir for *Borrelia burgdorferi* (1, 3, 6, 16). Our recovery of *Babesia microti* confirms earlier reports (2, 9, 12, 20a) that relatively large numbers of rodents may also be infected by this parasite.

Although *Babesia microti* and *Borrelia burgdorferi* may be universal in the same rodent populations, Lyme disease is more prevalent than babesiosis in humans (8, 19). The relatively low numbers of human cases of the latter have been attributed to unawareness of the disease and misdiagnosis (18). Our finding both pathogens in five individual rodents suggests that *I. dammini* larvae feeding on infected hosts may ingest these parasites and may transmit both agents to the nymphal stage. Since humans may also be exposed to multiple tick bites (e.g., in July 1985, a woman from Guilford, Conn., sent us 4 larval specimens and 1 nymphal specimen of *I. dammini* that she had removed from her body and reported that she had 15 other similar ticks attached) and since concurrent infections occur (10; L. C. Marcus, A. C. Steere, A. E. Anderson, and E. B. Mahoney, Abstr. Joint Meet. R. Am. Soc. Trop. Med., p. 195, 1984), consideration should be given to more thorough analyses of serum samples obtained from patients suspected of having either disease.

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LITERATURE CITED

1. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde.** 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). J. Clin. Microbiol. **22**:36–38.
2. **Anderson, J. F., and L. A. Magnarelli.** 1983. Spirochetes in *Ixodes dammini* and *Babesia microti* on Prudence Island, Rhode Island. J. Infect. Dis. **148**:1124.
3. **Anderson, J. F., L. A. Magnarelli, W. Burgdorfer, and A. G. Barbour.** 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. Am. J. Trop. Med. Hyg. **32**:818–824.
4. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
5. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41**:795–804.
6. **Bosler, E. M., J. L. Coleman, J. L. Benach, D. A. Massey, J. P. Hanrahan, W. Burgdorfer, and A. G. Barbour.** 1983. Natural distribution of the *Ixodes dammini* spirochete. Science **220**: 321–322.
7. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick borne spirochetosis? Science **216**:1317–1319.
8. **Dammin, G. J., A. Spielman, J. L. Benach, and J. Piesman.** 1981. The rising incidence of clinical *Babesia microti* infection. Hum. Pathol. **12**:398–400.
9. **Etkind, P., J. Piesman, T. K. Ruebush, A. Spielman, and D. D. Juranek.** 1980. Methods for detecting *Babesia microti* infection in wild rodents. J. Parasitol. **66**:107–110.
10. **Grunwaldt, E., A. G. Barbour, and J. L. Benach.** 1983. Simultaneous occurrence of babesiosis and Lyme disease. N. Engl. J. Med. **308**:1166.
11. **Healy, G. R., and T. K. Ruebush.** 1980. Morphology of *Babesia microti* in human blood smears. Am. J. Clin. Pathol. **73**:107–109.
12. **Healy, G. R., A. Spielman, and N. Gleason.** 1976. Human babesiosis: reservoir of infection on Nantucket Island. Science **192**:479–480.
13. **Hyde, F. W., and R. C. Johnson.** 1984. Genetic relationship of Lyme disease spirochetes to *Borrelia*, *Treponema*, and *Leptospira* spp. J. Clin. Microbiol. **20**:151–154.
14. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20**:1099–1101.
15. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34**:496–497.
16. **Levine, J. F., M. L. Wilson, and A. Spielman.** 1985. Mice as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg. **34**:355–360.
17. **Magnarelli, L. A., J. F. Anderson, W. Burgdorfer, and W. A. Chappell.** 1984. Parasitism of *Ixodes dammini* (Acari: Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, U.S.A. J. Med. Entomol. **21**:52–57.
18. **Ruebush, T. K., P. B. Cassaday, H. J. Marsh, S. A. Lisker, D. B. Voorhees, E. B. Mahoney, and G. R. Healy.** 1977. Human babesiosis on Nantucket Island. Ann. Intern. Med. **86**:6–9.
19. **Schmid, G. P.** 1985. The global distribution of Lyme disease.

Rev. Infect. Dis. **7**:41–50.

20. **Spielman, A.** 1976. Human babesiosis on Nantucket Island: transmission by nymphal *Ixodes* ticks. Am. J. Trop. Med. Hyg. **25**:784–787.

20a. **Spielman, A., P. Etkind, J. Piesman, T. K. Ruebush II, D. D. Juranek, and M. S. Jacobs.** 1981. Reservoir hosts of human babesiosis on Nantucket Island. Am. J. Trop. Med. Hyg. **30**:560–565.

21. **Steere, A. C., and S. E. Malawista.** 1979. Cases of Lyme disease in the United States: locations correlated with distribution of *Ixodes dammini*. Ann. Intern. Med. **91**:730–733.

22. **Western, K. A., G. D. Benson, N. N. Gleason, G. R. Healy, and M. G. Schultz.** 1970. Babesiosis in a Massachusetts resident. N. Engl. J. Med. **283**:854–856.