INFECTION AND IMMUNITY, Apr. 2004, p. 2442–2444 Vol. 72, No. 4
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.4.2442–2444.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

Adaptation of Diverse Lyme Disease Spirochetes in a Natural Rodent Reservoir Host

Dania Richter,[1]* Birte Klug,[1] Andrew Spielman,[2] and Franz-Rainer Matuschka[1]

Abteilung Parasitologie, Institut für Pathologie, Charité, Medizinische Fakultät der Humboldt-Universität zu Berlin, 12249 Berlin, Germany,[1] and Laboratory of Public Health Entomology, Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts 02115[2]

Received 14 October 2003/Returned for modification 18 November 2003/Accepted 13 January 2004

We compared the relative reservoir competence of European wood mice for two genospecies of Lyme disease spirochetes by analyzing susceptibility, intrinsic incubation period, and degree and duration of infectivity. *Borrelia afzelii*, specializing in particular reservoir hosts, is better adapted to those hosts than is the more generalist genospecies *B. burgdorferi* sensu stricto.

Diverse Lyme disease spirochetes infect European vector ticks. The local distribution of genospecies varies. In seven sites in Germany and France that we have examined, *Borrelia afzelii* consistently infects ticks more frequently than does *B. burgdorferi* sensu stricto (14, 17, 18). For each tick infected by *B. burgdorferi* sensu stricto, more than six ticks are infected by *B. afzelii*.

The composition of reservoir hosts in a site appears to affect the local genospecies distribution. *B. afzelii* is said to perpetuate mainly in rodents, whereas *B. garinii* is said to perpetuate in birds (7). No such host association, however, has been suggested for *B. burgdorferi* sensu stricto. Indeed, both murine and avian hosts are reservoir competent for *B. burgdorferi* sensu stricto in the northeastern United States (1, 19), where it is the sole genospecies that is pathogenic for humans. White-footed mice, *Peromyscus leucopus*, and American robins, *Turdus migratorius*, readily acquire *B. burgdorferi* sensu stricto infection from infected nymphal ticks, maintain the infection, and infect most ticks feeding on them. If both murine and avian hosts were similarly reservoir competent for *B. burgdorferi* sensu stricto in Central Europe, it would seem paradoxical that this variant is less prevalent in questing ticks than is *B. afzelii*, which parasitizes rodents but not birds. The differential reservoir competence of rodents for *B. burgdorferi* sensu stricto and *B. afzelii* remains unknown.

It may be that European rodents are more competent reservoir hosts for *B. afzelii* than for *B. burgdorferi* sensu stricto. Accordingly, we analyzed the susceptibility, the intrinsic incubation period, and the degree and duration of infectivity of these spirochetes in rodents. In particular, we compared the competence of a natural European reservoir rodent, the wood mouse *Apodemus sylvaticus*, to that of an experimental host, the Mongolian jird *Meriones unguiculatus*.

(Portions of this research were conducted in partial fulfillment of the requirements for a doctoral degree [to B.K.] from the Freie Universität Berlin, Berlin, Germany.)

Each of the *B. afzelii* and *B. burgdorferi* sensu stricto isolates originated from an individual naturally infected nymphal tick collected from vegetation in suburban Berlin, Germany. The genospecies of each was identified by amplification and sequence analysis of their 16S rRNA genes (18).

To infect rodents and to determine their infectivity for ticks, we permitted nymphal ticks infected by *B. afzelii* or by *B. burgdorferi* sensu stricto to feed on each host. Hosts were exposed to ticks as described previously (12). Noninfected larvae were permitted to attach simultaneously to each animal and again every 2 or 3 days throughout the first 3 weeks. This xenodiagnostic procedure was repeated every 6 weeks for 7 months thereafter. Spirochetal infection in xenodiagnostic ticks was diagnosed after molting by examining their gut contents by dark-field microscopy.

First, we determined whether *B. afzelii* infects rodents more readily than does *B. burgdorferi* sensu stricto. Twelve nymphal ticks infected by one or another spirochete genospecies were permitted to attach to wood mice, and 12 *B. burgdorferi* sensu stricto-infected ticks or 8 *B. afzelii*-infected ticks were allowed to attach to jirds. About half as many engorged ticks were recovered from the wood mice as from jirds (Table 1). At least one infected tick engorged on each of the rodents, as verified by dark-field microscopy. All hosts exposed to *B. afzelii*-infected ticks became infectious to at least one xenodiagnostic tick. Somewhat fewer of the rodents that had been exposed to tick-borne *B. burgdorferi* sensu stricto infected xenodiagnostic ticks.

We then compared the intrinsic incubation periods of *B. afzelii* and *B. burgdorferi* sensu stricto in rodents, as measured from the time of initial exposure of a rodent to infected nymphs until the time that xenodiagnostic larvae first acquired infection from these rodents. About half of the larvae acquired *B. afzelii* from infected jirds as early as 2 days after infected nymphs had been permitted to attach (Fig. 1). Wood mice, in contrast, infected appreciable numbers of xenodiagnostic larvae with this spirochete 6 days after initial exposure to infected ticks. *B. burgdorferi* sensu stricto-infected rodents infected numerous xenodiagnostic larvae about 12 days after initial infection, and wood mice became infectious more slowly than did jirds. We conclude that the incubation period of *B. afzelii* in rodents is shorter than that of *B. burgdorferi* sensu stricto ($P =$

---

* Corresponding author. Mailing address: Abteilung Parasitologie, Institut für Pathologie, Charité, Medizinische Fakultät der Humboldt-Universität zu Berlin, Malteserstraβe 74-100, 12249 Berlin, Germany. Phone: 49-30-83-87-03-72. Fax: 49-30-77-62-085. E-mail: drichter@charite.de.

TABLE 1. Susceptibility of wood mice, *A. sylvaticus*, and jirds, *M. unguiculatus*, to infection by tick-borne *B. afzelii* and *B. burgdorferi* sensu stricto

Host		Data for infecting nymphs				No. of hosts infecting ≥1 xenodiagnostic tick
Species	No.	Containing genospecies	% Infected	No.	% Recovered	
A. sylvaticus	6	*B. afzelii*	100	74	31.1	6
	7	*B. burgdorferi*	70	84	20.2	5
M. unguiculatus	6	*B. afzelii*	100	48	60.4	6
	6	*B. burgdorferi*	70	72	38.9	5

0.0024, Mann-Whitney test) and that jirds become infectious to ticks more rapidly than do wood mice ($P = 0.036$, Mann-Whitney test).

We determined how infectious rodents may become after exposure to spirochete-infected ticks. Virtually all larvae became infected when feeding on *B. afzelii*-infected rodents (Fig. 1). Jirds reached this degree of infectivity during the first week after exposure to infected nymphs, whereas wood mice lagged about 2 weeks behind. *B. burgdorferi* sensu stricto-infected rodents never became so infectious to larval ticks, and infected jirds were more infectious than wood mice during the acute first 3 weeks of infection. *B. afzelii*-infected wood mice appear to be more infectious to larval ticks than are *B. burgdorferi* sensu stricto-infected rodents.

We observed the infectivity of rodents chronically infected by *B. afzelii* or *B. burgdorferi* sensu stricto. Infectivity generally has waned by about 15 weeks (Fig. 2). Although infectivity of *B. burgdorferi* sensu stricto in jirds waned to nil, that of the other combinations of spirochetes and hosts appeared to plateau. Particular rodents retain infectivity of *B. afzelii* and of *B. burgdorferi* sensu stricto long enough to perpetuate the infection between transmission seasons.

Finally, we compared the cumulative potential of *B. afzelii*- or *B. burgdorferi* sensu stricto-infected rodents to infect the ticks that might parasitize them throughout an 8-month transmission season. *B. afzelii*-infected rodents infected at least twice as many of the larval ticks as did *B. burgdorferi* sensu stricto-infected rodents, regardless of the rodent species ($P < 0.0001$, Fisher's exact test) (Table 2). At least in the case of *B. afzelii*, more ticks became infected after feeding on infected jirds than on wood mice ($P < 0.0001$, Fisher's exact test). *B. afzelii*-infected rodents would infect more ticks in the course of a transmission season than would *B. burgdorferi* sensu stricto-infected rodents.

TABLE 2. Efficiency of the natural reservoir host, wood mouse (*A. sylvaticus*) and the laboratory model, Mongolian jird (*M. unguiculatus*) to infect larval *I. ricinus* ticks with *B. afzelii* or *B. burgdorferi* sensu stricto over a period of 8 months

Host		Genospecies	Data for xenodiagnostic ticks	
Species	No.		No. examined	% Infected
A. sylvaticus	6	*B. afzelii*	604	48.0
	5	*B. burgdorferi*	339	21.2
M. unguiculatus	6	*B. afzelii*	493	68.8
	4	*B. burgdorferi*	415	27.5

Infected wood mice remain infectious to ticks long enough to span the transmission season. Considering that such reservoir rodents encounter an infected nymphal tick only infrequently (13), such persistent infectivity would be required for efficient perpetuation of the pathogen. Indeed, naturally infected wood mice remain infectious to ticks in the laboratory for several years, which is longer than such mice normally live (2). Natural rodent hosts, therefore, may contribute infected ticks throughout the entire season of tick activity. Such an extended period of infectivity may also facilitate overwinter survival of the pathogen within its reservoir host.

Our demonstration that *B. afzelii* is better adapted to mice than is *B. burgdorferi* sensu stricto corresponds to field observations. The majority of spirochete-infected, field-derived mice harbor *B. afzelii* infections (4, 6). Although *B. burgdorferi* sensu stricto is much less prevalent in Central Europe than is *B. afzelii*, these spirochetes have been isolated from a broad array of hosts, including various rodents and birds as well as their

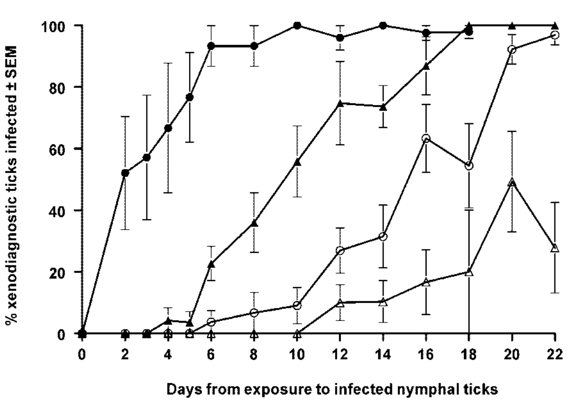

FIG. 1. Infectivity to larval vector ticks of wood mice (*A. sylvaticus* [triangles]) and jirds (*M. unguiculatus* [circles]) throughout the first 3 weeks following exposure to nymphal *I. ricinus* ticks infected with *B. afzelii* (solid symbols) or *B. burgdorferi* sensu stricto (open symbols). SEM, standard error of the mean.

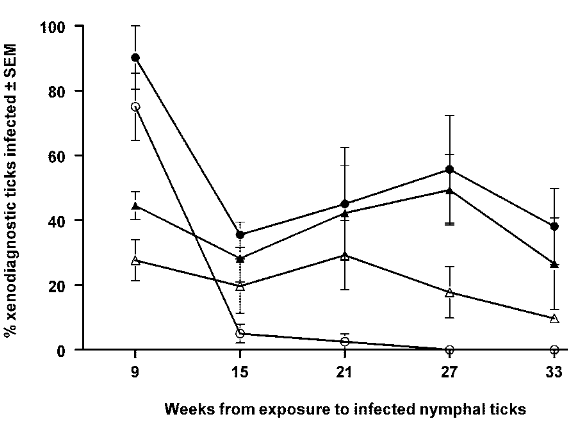

FIG. 2. Duration of infectivity to larval vector ticks of wood mice (*A. sylvaticus* [triangles]) and jirds (*M. unguiculatus* [circles]) exposed to nymphal *I. ricinus* ticks infected with *B. afzelii* (solid symbols) or *B. burgdorferi* sensu stricto (open symbols). SEM, standard error of the mean.

attached ticks (4, 15, 17). Other genospecies, such as *B. garinii* and *B. valaisiana*, seem better adapted to birds. Indeed, isolates taken from European birds most frequently prove to be one of these spirochetes (3, 5, 8, 15). Numerous ticks acquire spirochetes from pheasants experimentally infected by *B. garinii*, but only few ticks do so from *B. burgdorferi* sensu stricto-infected pheasants (9). Whereas *B. afzelii* appears to be well adapted to rodents and *B. garinii* seems to be adapted to birds, *B. burgdorferi* sensu stricto is less likely to perpetuate in either of these hosts. Our observations constitute the first experimental demonstration of relative competence of particular genospecies in rodents. We conclude that the more specialized genospecies, *B. afzelii*, appears better adapted to rodents than is the more generalized genospecies, *B. burgdorferi* sensu stricto.

The *B. burgdorferi* sensu stricto genospecies appears to behave differently in North America than in Europe. The American *B. burgdorferi* sensu stricto generally infects about one-third of nymphal vector ticks. It perpetuates in birds and mice (10, 11, 16). In the rodent population, prevalence of infection becomes virtually universal. In Europe, *B. burgdorferi* sensu stricto infects only about 3% of questing nymphal ticks (18, 20, 21) and is far less prevalent in rodents (4, 17) and birds (15). Although they are considered the same species, their host adaptations appear to differ between continents. American *B. burgdorferi* sensu stricto passes readily in avian as well as rodent experimental hosts (1, 19). Although the European *B. burgdorferi* sensu stricto form is more closely related to its American equivalent than to *B. afzelii* or *B. garinii*, it is transmitted less efficiently. The force of transmission of *B. burgdorferi* sensu stricto in Europe is weaker than it is in North America.

The prevalence of *B. afzelii* in Central Europe exceeds that of *B. burgdorferi* sensu stricto but is similar to that of *B. garinii* (18). *B. valaisiana* and *B. burgdorferi* sensu stricto infect far fewer ticks, about 1 in 11 and 1 in 14 infected ticks, respectively. These relationships most likely reflect differences in the competence of their mutually shared reservoir hosts. Indeed, we found that one of the important European reservoir hosts, the wood mouse, is far more competent for *B. afzelii* than for *B. burgdorferi* sensu stricto. Because prevalence of infection reflects the force of transmission, which in turn reflects adaptation, *B. afzelii* would be better adapted than *B. burgdorferi* sensu stricto in much of Europe. The more specialized genospecies, *B. afzelii*, appears better adapted to rodents than is the more generalized genospecies, *B. burgdorferi* sensu stricto.

This study was supported by grant Ma 942/10-1 from the Deutsche Forschungsgemeinschaft.

Editor: D. L. Burns

REFERENCES

1. **Donahue, J. G., J. Piesman, and A. Spielman.** 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg. **36:**92–96.
2. **Gern, L., M. Siegenthaler, C. M. Hu, S. Leuba-Garcia, P. F. Humair, and J. Moret.** 1994. *Borrelia burgdorferi* in rodents (*Apodemus flavicollis* and *A. sylvaticus*): duration and enhancement of infectivity for *Ixodes ricinus* ticks. Eur. J. Epidemiol. **10:**75–80.
3. **Hanicova, K., V. Taragelova, J. Koci, S. M. Schäfer, R. Hails, A. J. Ullmann, J. Piesman, M. Labuda, and K. Kurtenbach.** 2003. Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. Appl. Environ. Microbiol. **69:**2825–2830.
4. **Hu, C. M., P.-F. Humair, R. Wallich, and L. Gern.** 1997. *Apodemus* sp. rodents, reservoir hosts for *Borrelia afzelii* in an endemic area in Switzerland. Zentbl. Bakteriol. **285:**558–564.
5. **Humair, P.-F., D. Postic, R. Wallich, and L. Gern.** 1998. An avian reservoir (*Turdus merula*) of the Lyme borreliosis spirochetes. Zentbl. Bakteriol. **287:** 521–538.
6. **Humair, P. F., O. Rais, and L. Gern.** 1999. Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. Parasitology **118:**33–42.
7. **Kurtenbach, K., S. De Michelis, S. Etti, S. M. Schäfer, H.-S. Sewell, V. Brade, and P. Kraiczy.** 2002. Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. Trends Microbiol. **10:**74–79.
8. **Kurtenbach, K., M. Peacy, S. G. T. Rijpkema, A. N. Hoodless, P. A. Nuttall, and S. E. Randolph.** 1998. Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. Appl. Environ. Microbiol. **64:**1169–1174.
9. **Kurtenbach, K., S. M. Schäfer, H.-S. Sewell, M. Peacey, A. Hoodless, P. A. Nuttall, and S. E. Randolph.** 2002. Differential survival of Lyme borreliosis spirochetes in ticks that feed on birds. Infect. Immun. **70:**5893–5895.
10. **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.
11. **Mather, T. N., M. L. Wilson, S. I. Moore, J. M. Ribeiro, and A. Spielman.** 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). Am. J. Epidemiol. **130:**143–150.
12. **Matuschka, F.-R., S. Endepols, D. Richter, and A. Spielman.** 1997. Competence of urban rats as reservoir hosts for Lyme disease spirochetes. J. Med. Entomol. **34:**489–493.
13. **Matuschka, F.-R., P. Fischer, M. Heiler, D. Richter, and A. Spielman.** 1992. Capacity of European animals as reservoir hosts for the Lyme disease spirochete. J. Infect. Dis. **165:**479–483.
14. **Ohlenbusch, A., F.-R. Matuschka, D. Richter, H.-J. Christen, R. Thomssen, A. Spielman, and H. Eiffert.** 1996. Etiology of the acrodermatitis chronica atrophicans lesion in Lyme disease. J. Infect. Dis. **174:**421–423.
15. **Olsén, B., T. G. Jaenson, and S. Bergström.** 1995. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. Appl. Environ. Microbiol. **61:**3082–3087.
16. **Rand, P. W., E. H. Lacombe, R. P. Smith, and J. Ficker.** 1998. Participation of birds (*Aves*) in the emergence of Lyme disease in southern Maine. J. Med. Entomol. **35:**270–276.
17. **Richter, D., S. Endepols, A. Ohlenbusch, H. Eiffert, A. Spielman, and F.-R. Matuschka.** 1999. Genospecies diversity of Lyme disease spirochetes in rodent reservoirs. Emerg. Infect. Dis. **5:**291–296.
18. **Richter, D., D. B. Schlee, and F.-R. Matuschka.** 2003. Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg. Infect. Dis. **9:**697–701.
19. **Richter, D., A. Spielman, N. Komar, and F.-R. Matuschka.** 2000. Competence of American robins as reservoir hosts for Lyme disease spirochetes. Emerg. Infect. Dis. **6:**133–138.
20. **Rijpkema, S. G. T., D. Golubic, M. Molkenboer, N. Verbeek-de Kruif, and J. F. P. Schellekens.** 1996. Identification of four genomic groups of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic region of northern Croatia. Exp. Appl. Acarol. **20:**23–30.
21. **Rijpkema, S. G. T., M. J. C. H. Molkenboer, L. M. Schouls, F. Jongejan, and J. F. P. Schellekens.** 1995. Simultaneous detection and genotyping of three genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S rRNA genes. J. Clin. Microbiol. **33:**3091–3095.