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Habitat-Specific Diversity of *Borrelia burgdorferi* Sensu Lato in Europe, Exemplified by Data from Latvia

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The distribution of *Borrelia burgdorferi* sensu lato genospecies in questing *Ixodes ricinus* ticks from ecologically distinct habitats in Latvia was analyzed. A significant variation in the frequency of the genospecies across sites was observed, pointing to the importance of the host community in the ecology of Lyme borreliosis.

Borrelia afzelii, *Borrelia garinii,* and *Borrelia valaisiana* are the most prevalent genospecies of *Borrelia burgdorferi* sensu lato in questing *Ixodes ricinus* ticks from Europe (4, 5, 7). Little is known about the scale of the geographical variation in the distribution of the *B. burgdorferi* sensu lato genospecies and their strains (10, 11). The genospecies of *B. burgdorferi* sensu lato, and some of their variants, are known to be host associated, which is determined by host complement (9, 12, 14–16, 21). Differences in the composition of the host community could, therefore, lead to a pronounced local variation in the diversity of *B. burgdorferi* sensu lato. However, migration of larger mammals or birds that carry immature ticks could have adverse effects, homogenizing the frequency distribution of spirochetal strains across sites. The aim of this study was to analyze the spatial variation in the distribution of the *B. burgdorferi* sensu lato genospecies in questing ticks in a region of East Europe.

Three forest sites around Riga, Latvia, were selected. Site 1 (Jaunciems; longitude, 24° 09′; latitude, 57° 03′) is peridomestic and close to a suburb of Riga. Site 2 (Kemeri; longitude, 23° 29′; latitude, 56° 56′) is also peridomestic and represents a mixed forest habitat along marshes. Site 3 (Babite; longitude, 23° 48′; latitude, 56° 50′) is truly sylvatic. Questing *I. ricinus* nymphs and adults were collected in May 1999 and 2000 by blanket dragging (13). The 5S-23S intergenic spacer of *B. burgdorferi* sensu lato was amplified from ticks by PCR, and the genospecies were determined using DNA probes specific for the whole group of Lyme borreliosis spirochetes as well as for *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana,* and *Borrelia lusitaniae* (14, 20). As *B. garinii* comprises different ecotypes (12), the *ospA* gene of randomly selected *B. garinii* was also amplified, and DNA sequences were determined.

Of the 505 questing *I. ricinus* ticks analyzed, 141 (28%) were infected with *B. burgdorferi* sensu lato, with no significant difference between the years (Table 1). When comparing site and developmental stage of the tick, significant differences in the infection prevalence between site and stage, but not between the years, were observed with a significant site-to-stage interaction ($\chi^2 = 14.5$; $df = 2$; $P = 0.0007$). *B. burgdorferi* sensu lato was more prevalent in nymphs than in adult ticks in site 1 but more prevalent in adult ticks than in nymphs in sites 2 and 3. The four genospecies *B. afzelii*, *B. garinii*, *B. valaisiana,* and *B. burgdorferi* sensu stricto were identified. Some amplicons that reacted with the *B. burgdorferi* sensu lato-specific probe did not react with the genospecies-specific probes and were considered to be untypeable. For *B. afzelii*, no significant difference in prevalence of infection between nymphs and adults was observed in any site, but the overall infection prevalence of this genospecies was significantly lower in site 3 than in sites 1 and 2 ($\chi^2 = 20.8$; $df = 2$; $P = 0.00003$). For *B. garinii*, both the site and the developmental stage of the tick were significantly different ($\chi^2 = 11.5$, $df = 1$, $P = 0.0007$; $\chi^2 = 14.93$, $df = 2$, $P = 0.0006$; scale parameter = 2.31). A similar pattern has been observed for *B. valaisiana* ($\chi^2 = 8.73$; $df = 1$; $P = 0.003$; for site: $\chi^2 = 14.6$; $df = 2$; $P = 0.0007$). No significant trend was found for *B. burgdorferi* sensu stricto, as this genospecies was too rare. The overall increase in prevalence of infection of *B. burgdorferi* sensu lato from nymphs to adults as seen in sites 2 and 3 is, therefore, due to the increase of *B. garinii* and *B. valaisiana*. The *ospA* genes of 12 randomly selected *B. garinii* samples were sequenced, and 9 alleles were found. Eight alleles were found to represent OspA serotypes other than OspA serotype 4 (3, 6). One sample derived from a questing nymph collected in site 1 represented OspA serotype 4 (Table 2).

The study shows a high degree of local variation in the distribution of *B. burgdorferi* sensu lato genospecies. The distances between the sites studied (>40 km) suggest that the populations of small mammals are effectively separated from each other (1, 2). However, since larger mammals and many bird species have greater activity ranges, it is likely that tick migration occurs between the sites (17, 18). Therefore, we had expected to find a more homogeneous distribution pattern of *B. burgdorferi* sensu lato genospecies across the sites. The substantial heterogeneity in the genospecies distribution suggests

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TABLE 1. *B. burgdorferi* sensu lato genospecies in questing *I. ricinus* ticks collected in the years 1999 and 2000[a]

Year or site	Stage	No. tested	No. (%) of ticks infected with:								No. (%) of UT
			*B. b.*s.l.	*B. afz.*	*B. ga.*	*B. va.*	*B. b.*s.s	*B. b.*s.s./*B. afz.*	*B. b.*s.s/*B. va.*	*B. ga./B. va.*	
1999											
1	Nymphs	16	3 (19)	2 (67)	1 (33)	0	0	0	0	0	0
	Adults	50	9 (18)	6 (67)	2 (22)	1 (11)	0	0	0	0	0
2	Nymphs	19	1 (5)	1 (100)	0	0	0	0	0	0	0
	Adults	50	24 (48)	9 (37)	7 (29)	5 (21)	1	1	1	0	0
3	Nymphs	20	3 (15)	1 (33)	0	1 (33)	0	0	0	0	1 (33)
	Adults	50	24 (48)	0	17 (71)	5 (21)	0	0	0	2 (8)	0
2000											
1	Nymphs	50	14 (28)	13 (93)	0	0	0	0	0	0	1 (7)
	Adults	50	8 (16)	7 (87)	1 (13)	0	0	0	0	0	0
2	Nymphs	50	8 (16)	6 (75)	0	0	0	0	0	0	2 (25)
	Adults	50	15 (30)	3 (20)	4 (27)	4 (27)	0	1 (7)	0	0	3 (20)
3	Nymphs	50	12 (24)	2 (17)	3 (25)	1 (8)	3 (25)	1 (8)	0	1 (8)	1 (8)
	Adults	50	20 (40)	1 (5)	9 (45)	2 (10)	4 (20)	1 (5)	0	2 (10)	1 (5)

[a] *B. b.*s.l., *Borrelia burgdorferi* sensu lato; *B. afz.*, *B. afzelii*; *B. ga.*, *B. garinii*; *B. va.*, *B. valaisiana*; *B. b.*s.s., *B. burgdorferi* sensu stricto. columns 9 to 11 present mixed infections. UT, untypeable amplicons using the DNA probes described in references 14 and 20.

that strong ecological factors operate which maintain a habitat-specific diversity of the bacteria. Since *B. burgdorferi* sensu lato is host associated, it is predicted that the structure of the host community differs substantially between the sites and that rodents are dominant in site 1 and ground-feeding birds are dominant in sites 2 and 3. Preliminary, qualitative observations support this as they indicate that ground-feeding or ground-nesting birds are highly abundant in sites 2 and 3 (A. Bormane, personal observations). The most frequently observed species include the chaffinch (*Fringilla coelebs*), robin (*Erithacus rubecula*), wood warbler (*Phylloscopus sibilatrix*) and thrush species (*Turdus* spp.). Furthermore, most of the sequenced *B. garinii* samples represent OspA serotypes other than serotype 4, as deduced from their sequences. In vivo and in vitro evidence indicates that such strains are bird associated (3, 9, 12, 14, 15, 16, 21). Furthermore, larvae of *I. ricinus* are more likely to feed on small mammals, but nymphs are more likely to feed on certain birds and larger mammals, such as rabbits, hares, or deer (18, 19). Thus, it is likely that the relative proportion of each life stage of *I. ricinus* feeding on each host species and the different efficiencies with which the strains of *Borrelia* are transmitted are the main factors shaping the local genospecies diversity in the developmental stages of questing ticks.

OspA serotype 4 strains of *B. garinii* are hyperinvasive, and isolates have so far mainly been obtained from human patients. Therefore, it seems to be rare in Europe (6, 8). The detection of one OspA serotype 4 sample in field-collected ticks in this study may indicate that such strains become more prevalent towards Asia. Unlike the other OspA serotypes of *B. garinii*, OspA serotype 4 strains are associated with rodents (6, 8), suggesting that their ecology is similar to that of *B. afzelii* (5). However, the reason for the low prevalence of OspA serotype 4 strains in Europe remains obscure.

The present study shows that the migration rates of *I. ricinus* are not sufficient to homogenize the frequency distribution of *B. burgdorferi* sensu lato genotypes between ecologically distinct sites. We conclude that the structure of the host community determines the population structure of Lyme borreliosis spirochetes in Europe.

TABLE 2. *OspA* alleles of 12 *B. garinii* samples amplified from questing ticks and their relationships with reference strains

Allele	No. of samples	% Similarity to reference strain (strain)[a]	OspA serotype[b]	Sensitivity or resistance to complement[c]	Associated host[d]
1	3	100 (VSBP)	6	Sens. to human	ND
2	1	100 (VSDA)	6	Sens. to human	ND
3	1	100 (VSBM)	5	Sens. to human	ND
4	2	100 (ZQ1ox)[e]	3	Sens. to rodent, human	Bird
5	1	99 (T25)	7	ND[f]	ND
6	1	97 (ZQ1ox)[e]	3	Sens. to rodent, human	Bird
7	1	97 (ZQ1ox)[e]	3	Sens. to rodent, human	Bird
8	1	94 (T25)	7	ND	ND
9	1	100 (A91s)	4	Res. to rodent, human	Rodent

[a] Described in GenBank and in references 3, 15, 16, and 21.
[b] The OspA serotype is given for the reference strains.
[c] Sens., sensitivity; Res., resistance.
[d] From references 6, 14, and 15.
[e] Resequenced clone derived from ZQ1 (16) with an *ospA* allele identical to that of the Rio 2 strain (3, 15).
[f] ND, not determined.

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REFERENCES

1. **Bergstedt, B.** 1966. Home ranges and movements of the rodent species *Clethrionomys glareolus* (Schreber), *Apodemus flavicollis* (Melchior) and *Apodemus sylvaticus* (L.) in southern Sweden. Oikos **17:**150–157.
2. **Cerny, V.** 1975. The role of mammals in natural foci of tick-borne encephalitis in Central Europe. Folia Parasitol. **22:**271–273.
3. **Escudero, R., M. Barral, A. Pérez, M. M. Vitutia, A. G. García-Pérez, S. Jiménez, R. E. Sellek, and P. Andra.** 2000. Molecular and pathogenic characterisation of *Borrelia burgdorferi* sensu lato isolates from Spain. J. Clin. Microbiol. **38:**4026–4033.
4. **Gern, L., C. M. Hu, E. Kocianova, V. Vyrostekova, and J. Rehacek.** 1999. Genetic diversity of *Borrelia burgdorferi* sensu lato isolates obtained from *Ixodes ricinus* ticks collected in Slovakia. Eur. J. Epidemiol. **15:**665–669.
5. **Hanincová, K., S. M. Schäfer, S. Etti, H.-S. Sewell, V. Taragelová, D. Ziak, M. Labuda, and K. Kurtenbach.** 2003. Association of *Borrelia afzelii* with rodents in Europe. Parasitology **126:**11–20.
6. **Hu, C. M., B. Wilske, V. Fingerle, Y. Lobet, and L. Gern.** 2001. Transmission of *Borrelia garinii* OspA serotype 4 to BALB/c mice by *Ixodes ricinus* ticks collected in the field. J. Clin. Microbiol. **39:**1169–1171.
7. **Hubalek, Z., and J. Halouzka.** 1997. Distribution of *Borrelia burgdorferi* sensu lato genomic groups in Europe, a review. Eur. J. Epidemiol. **13:**951–957.
8. **Huegli, D., C. M. Hu, P.-F. Humair, B. Wilske, and L. Gern.** 2002. *Apodemus* species mice are reservoir hosts of *Borrelia garinii* OspA serotype 4 in Switzerland. J. Clin. Microbiol. **40:**4735–4737.
9. **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.
10. **Kirstein, F., S. Rijpkema, M. Molkenboer, and J. S. Gray.** 1997. The distribution and prevalence of *B. burgdorferi* genomospecies in *Ixodes ricinus* ticks in Ireland. Eur. J. Epidemiol. **13:**67–72.
11. **Kirstein, F., S. Rijpkema, M. Molkenboer, and J. S. Gray.** 1997. Local variations in the distribution and prevalence of *Borrelia burgdorferi* sensu lato genomospecies in *Ixodes ricinus* ticks. Appl. Environ. Microbiol. **63:**1102–1106.
12. **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.
13. **Kurtenbach, K., H. Kampen, A. Dizij, S. Arndt, H. M. Seitz, U. E. Schaible, and M. M. Simon.** 1995. Infestation of rodents with larval *Ixodes ricinus* L. (Acari:Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* sensu lato in German woodlands. J. Med. Entomol. **32:**807–817.
14. **Kurtenbach, K., M. Peacey, 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.
15. **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.
16. **Kurtenbach, K., H.-S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall.** 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. **66:**1248–1251.
17. **Olsen, B., T. G. T. Jaenson, and S. Bergstrom.** 1995. Prevalence of *Borrelia burgdorferi* sensu lato infected ticks on migrating birds. Appl. Environ. Microbiol. **61:**3082–3087.
18. **Randolph, S. E.** 1998. Ticks are not insects: consequences of contrasting vector biology for transmission potential. Parasitol. Today **5:**186–192.
19. **Randolph, S. E., and N. G. A. Craine.** 1995. A general framework for comparative quantitative studies on the transmission of tick-borne diseases using Lyme borreliosis in Europe as an example. J. Med. Entomol. **32:**765–777.
20. **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 3 genomic groups of *Borrelia burgdorferi* sensu lato in Dutch *Ixodes ricinus* ticks by characterization of the amplified intergenic spacer region between 5S and 23S ribosomal RNA genes. J. Clin. Microbiol. **33:**3091–3095.
21. **Van Dam, A. P., A. Oei, R. Jaspars, C. Fijen, B. Wilske, L. Spanjaard, and J. Dankert.** 1997. Complement-mediated serum activity among spirochetes that cause Lyme disease. Infect. Immun. **65:**1228–1236.