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Local Variations in the Distribution and Prevalence of *Borrelia burgdorferi* Sensu Lato Genomospecies in *Ixodes ricinus* Ticks

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Unfed nymphal and adult *Ixodes ricinus* ticks were collected from five locations within the 10,000-ha Killarney National Park, Ireland. The distribution and prevalence of the genomospecies of *Borrelia burgdorferi* sensu lato in the ticks were investigated by PCR amplification of the intergenic spacer region between the 5S and 23S rRNA genes and by reverse line blotting with genomospecies-specific oligonucleotide probes. The prevalence of ticks infected with *B. burgdorferi* sensu lato was significantly variable between the five locations, ranging from 11.5 to 28.9%. Four genomospecies were identified as *B. burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, and VS116. Additionally, untypeable *B. burgdorferi* sensu lato genomospecies were identified in two nymphs. VS116 was the most prevalent of the genomospecies and was identified in 50% of the infected ticks. Prevalences of *B. garinii* and *B. burgdorferi* sensu stricto were similar (17 and 18%, respectively); however, significant differences were observed in the prevalence of these genomospecies in mixed infections (58.8 and 23.5%, respectively). Notably, the prevalence of *B. afzelii* was low, comprising 9.6 and 7.4%, respectively, of single and mixed infections. Significant variability was observed in the distribution and prevalence of *B. burgdorferi* sensu lato genomospecies between locations in the park, and the diversity and prevalence of *B. burgdorferi* sensu lato genomospecies was typically associated with woodland. The distributions of *B. burgdorferi* sensu lato genomospecies were similar in wooded areas and in areas bordering woodland, although the prevalence of *B. burgdorferi* sensu lato infection was typically reduced. Spatial distributions vegetation composition, and host cenosis of the habitats were identified as factors which may affect the distribution and prevalence of *B. burgdorferi* sensu lato genomospecies within the park.

Lyme borreliosis (LB) is a zoonosis, the etiological agent being a spirochete species complex, *Borrelia burgdorferi* sensu lato, which is transmitted by *Ixodes ricinus* ticks (5). The clinical symptoms of LB in humans vary from an expanding skin rash (erythema migrans) at the site of the tick bite to severe arthritic, neurological, dermatological, and cardiac manifestations. European *B. burgdorferi* sensu lato isolates have been divided into five genomospecies: *B. burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii* (3), VS116, and PotiB2 (20). There is strong evidence that the division in the genomospecies has clinical relevance for European LB. Studies in Belgium, France, Germany, and The Netherlands have presented both direct and indirect evidence of an association of *B. garinii* with neurological symptoms, *B. burgdorferi* sensu stricto with arthritis, and *B. afzelii* with acrodermatitis chronica atrophicans (1, 2, 7, 23, 24). Because of this apparent association between the genomospecies of *B. burgdorferi* sensu lato and the clinical symptoms of LB, knowledge of the factors affecting the distribution of the genomospecies in ticks may provide important information for the identification and classification of habitats according to the risk of acquiring LB.

Significant variability has been observed in the geographic distribution and prevalence of genomospecies *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and VS116 in the Irish *I. ricinus* tick population (15). The variability may be representative of geographic location or due to geographical variability in tick densities, vertebrate host cenosis (8, 16), or vegetational type (18) or it may be an artefact of sampling.

In this study, the distribution and prevalence of *B. burgdorferi* sensu lato genomospecies in *I. ricinus* ticks in five sites in Killarney National Park were compared to investigate the possible influence of geographic location, habitat vegetation, and habitat host cenosis.

MATERIALS AND METHODS

Study area and tick collection. In late May and early June 1995, ticks were collected from five sites in three locations (Fig. 1) in the Killarney National Park, County Kerry, Ireland (52°N, 9°30′W) between 4:00 and 6:00 p.m. by blanket dragging (ticks per hour) as previously described (9). Ticks were collected from all parts of each site. This time of the year was selected because it represents a major risk period in that most of the ticks of the spring feeding cohort are still active and visitors to the area are becoming numerous. Two sites (locations 1 and 3) were within Muckross Estate in a parkland environment of 4,000 ha about 5 km to the south of Killarney town, and the third site (location 2), Knockreer Estate, consisting of 1,000 ha, was just to the west of the town and within the town boundaries. The large vertebrates primarily responsible for the maintenance of the tick populations are red deer, *Cervus elaphus*, and/or sika deer, *Cervus nippon*. Medium-sized vertebrates include rabbits, *Oryctolagus cuniculus*, hedgehogs, *Erinaceus europaeus*, and squirrels, *Sciurus vulgaris*. The latter two species are significant tick hosts but neither seemed to be particularly numerous in the habitats studied. The most abundant tick hosts are the small mammals, mostly consisting of the wood mouse, *Apodemus sylvaticus*, which is about three times more numerous than the bank vole, *Clethrionomys glareolus*. The pygmy shrew (*Sorex minutus*), a poor tick host, is also present. Ground-feeding birds also feed many ticks and at these sites consist mainly of blackbirds, *Turdus merula*, and robins, *Erithacus rubecula*. The three locations were divided into five different sampling sites.

Location 1. Location 1 consisted of a 180,000-m^2 wood, predominantly coniferous in the first third of the western end but becoming dominated by deciduous trees, especially beech, oak, and ash, in the remaining two thirds. Little undergrowth is present at the western end, and the ground consists of much-fissured limestone with a thick covering of moss. In the deciduous portion at the eastern end the undergrowth is very varied and includes bramble and bracken fern.

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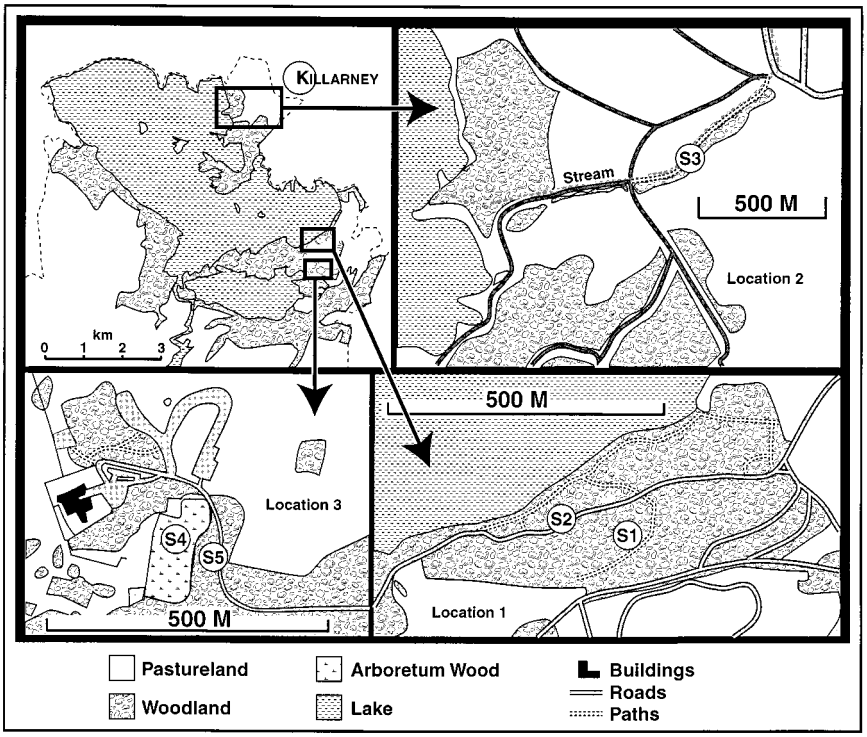

FIG. 1. The positions of the sampling sites 1 to 5 (S1 to S5) within the three locations in Killarney National Park.

Extensive growths of moss are evident on exposed rocks and on fallen trees. There are many small paths in this part of the wood. The northern side of the wood is bordered by a road for wheeled traffic, while the southern side is bordered by a field of permanent pasture used by cattle. Rabbits (*O. cuniculus*) are numerous on this southern edge. Other large vertebrates in the location are Japanese sika deer (*C. nippon*), usually only 2 to 4 animals, and a similar number of wild goats. Surveys carried out from 1994 to 1996 in May and June resulted in rodent trapping rates of between 52 and 89%. Sampling site 1 was the interior of the wood and extended the whole length, and sampling site 2 was the roadside.

Location 2. Location 2 consisted of a towpath alongside the southern edge of a small stream about 6-m wide which ran for about 2 km westward to the lake from the town. After about 1 km the towpath becomes a road for wheeled traffic (mainly horse-drawn carts), and the sampling area was extended for another 500 m until the road turns south. The area sampled (sampling site 3) was the southern edge of the roadside. This edge consists of short variable vegetation and is bordered by a thick hedge. Red deer have access to this area and are most in evidence at the western end, farthest from the town. Rodent trapping rates (percent successful traps) vary from 40 to 80%.

Location 3. Location 3 was a section of the arboretum adjacent to Muckross House and consisted of an area of about 15,000-m^2 containing many and varied exotic garden shrubs and trees. The area is much intersected with broad footpaths and is enclosed by a 2-m-high deer fence. The ground cover is dominated by ivy, and there is a thick leaf litter in most areas. The area outside the deer fence is mixed deciduous and coniferous woodland. The undergrowth is highly variable, but a thick litter of dead vegetation and debris from fallen and rotting wood is present throughout. Moss growth is much in evidence. Many red deer make use of this area in early spring. Rodent trapping rates vary from 35 to 70%. Sampling site 4 was inside the arboretum, and sampling site 5 was outside the deer fence.

***B. burgdorferi* sensu lato PCR.** Prior to PCR, ticks were air dried on filter paper, homogenized in 100 μl of 0.7 M ammonium hydroxide by using a sterile tip, and denatured for 15 min at 100°C. The ammonia was evaporated by heating the samples at 100°C for a further 15 min (12). An aliquot of 5 μl of tick lysate was added per reaction. The *B. burgdorferi* sensu lato PCR was performed with 5S-23S ribosomal primers, Promega *Taq* polymerase, and reagents under the conditions described previously (22). Strains of *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and VS116 (obtained from S. J. Rijpkema [22]) were amplified in tandem as positive controls. Cultured uninfected *I. ricinus* ticks were included in each tick batch as negative controls. The biotinylated 225-bp products were visualized on ethidium bromide-stained 2% agarose gels. Tick samples which were negative by PCR were spiked with 100 fg of *B. burgdorferi* B31 DNA, and the samples were reamplified to ensure that no inhibiting substances were present in the tick homogenates.

Reverse line blotting (RLB). Following PCR amplification, positive samples were reverse line blotted against five 5S-23S rDNA probes: one *B. burgdorferi* sensu lato probe which reacts with all genomospecies of the complex and four probes respectively specific for the genomospecies *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and VS116 as described previously (22). Briefly, with the Miniblotter 45 system (Immunetics, Cambridge, Mass.) 10 μl of the denatured biotinylated PCR products was incubated at 90° to the position of the covalently bound genomospecies probes (100 pmol) on a Biodyne C membrane (Pall Europe Ltd., Portsmouth, United Kingdom). PCR products of the four genomospecies were used as positive controls to verify specificities of the hybridized products. Hybridized products were detected by conjugation with streptavidin-peroxidase (Dakopatts, Glostrup, Denmark) followed by chemiluminescent detection with the Amersham ECL detection system and visualized by exposure of the blot to X-ray film.

RESULTS

A total of 512 unfed *I. ricinus* ticks were collected from five different sampling sites in Killarney National Park. Of these, 411 were nymphs, 53 were adult female ticks, and 48 were adult male ticks. The overall prevalence of *B. burgdorferi* sensu lato-infected ticks was 18.4%. There was no significant difference between the prevalence of *B. burgdorferi* sensu lato in the nymphal population and that in the adult *I. ricinus* population. However, statistical differences were observed in the prevalence of *B. burgdorferi* sensu lato among the five locations (Table 1).

Four *B. burgdorferi* sensu lato genomospecies were specifically identified by RLB: *B. burgdorferi* sensu stricto, *B. garinii*, VS116, and *B. afzelii*. Additionally, untypeable *B. burgdorferi* sensu lato genomospecies were identified in two nymphs (Fig. 2). VS116 was the most prevalent genomospecies, and single and mixed infections of VS116 accounted for 50% of the total number of *B. burgdorferi* sensu lato infections identified in the ticks in the park. Similar prevalences of *B. burgdorferi* sensu stricto (18.01%) and *B. garinii* (17.02%) were identified; however, their respective contributions to mixed infections varied

TABLE 1. Distribution and prevalence of *B. burgdorferi* sensu lato species in nymphal and adult *I. ricinus* ticks in Killarney National Park

Location	Stage and sex	No. of ticks collected	No. of ticks positive by PCR	Distribution of *B. burgdorferi* sensu lato genomospecies identified by RLB (% prevalence)								
				B. burgdorferi sensu stricto	*B. afzelii*	*B. garinii*	VS116	*B. afzelii* and VS116	*B. garinii* and VS116	*B. burgdorferi* sensu stricto and *B. afzelii*	*B. burgdorferi* sensu stricto, *B. afzelii*, and VS116	Untypeable
Wood (site 1)	Nymph	62	15	4	1	4	3	1	2			
	Adult female	4	1	1								
	Adult male	3	1								1	
	Total	69	17	5 (29.4)	1 (5.9)	4 (23.5)	3 (17.7)	1 (5.9)	2 (11.8)		1 (5.9)	
Roadside (site 2)	Nymph	100	14	3		6	4		1			
	Adult female	18	4				3		1			
	Adult male	21	4			1	2	1				
	Total	139	22	3 (13.6)		7 (31.8)	9 (40.9)	1 (4.6)	2 (9.1)			
Tow path (site 3)	Nymph	79	11			1	8					2
	Adult female	12	1				1					
	Adult male	12	0									
	Total	103	12			1 (8.33)	9 (75)					2 (16.67)
Arboretum (site 4)	Nymph	100	28	7	4	2	9		3	3		
	Adult female	10	4	1	2		1					
	Adult male	4	1		1							
	Total	114	33	8 (24.2)	7 (21.2)	2 (6.1)	10 (30.3)		3 (9.1)	3 (9.1)		
Outside of arboretum (site 5)	Nymph	70	8	1		2	2	1	2			
	Adult female	9	1		1							
	Adult male	8	1						1			
	Total	87	10	1 (10.0)	1 (10.0)	2 (20.0)	2 (20.0)	1 (10.0)	3 (30.0)			
Killarney National Park		512	94	17 (18.1)	9 (9.6)	16 (17.02)	33 (35.1)	3 (3.2)	10 (10.6)	3 (3.19)	1 (1.1)	2 (2.1)

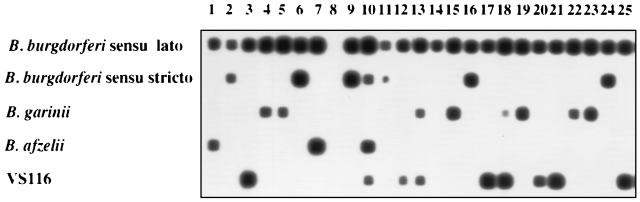

FIG. 2. RLB identification of *B. burgdorferi* sensu lato genomospecies in *I. ricinus* ticks in Killarney National Park. Lanes 1 to 4 contain positive control PCR products for the following *Borrelia* isolates: *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and VS116, respectively. Lanes 5 to 25, genotyping of *B. burgdorferi* sensu lato. PCR products amplified from *I. ricinus* ticks. *B. burgdorferi* sensu stricto was identified in lanes 6, 9, 11, 16, and 24. *B. garinii* was identified in lanes 5, 15, 19, 22, and 23. *B. afzelii* was identified in lane 7. VS116 was identified in lanes 12, 17, 20, 21, and 25. Mixed infections of *B. garinii* and VS116 were identified in lanes 13 and 18. A triple infection of *B. burgdorferi* sensu stricto, *B. afzelii*, and VS116 was identified in lane 10. Lane 8 contains a negative *B. burgdorferi* sensu lato PCR sample. Lane 14 contains *B. burgdorferi* sensu lato PCR products which were untypeable by RLB.

considerably (23.5 and 58.8%). The prevalence of *B. afzelii* in single and mixed infections was 9.6 and 4.3%, respectively (Table 1).

B. burgdorferi sensu lato genomospecies were not uniformly distributed. Four genomospecies were identified at both locations 1 and 3. Two genomospecies, VS116 and *B. garinii*, and untypeable genomospecies of *B. burgdorferi* sensu lato were identified in location 2 (Table 1). Variation was also observed in the prevalence of genomospecies within locations.

In location 1, VS116 and *B. garinii* were more prevalent on the roadside (site 2) than in the wood (site 1), whereas *B. burgdorferi* sensu stricto was more prevalent in the wood. VS116 was represented in all coinfections at this location. Single and mixed infections of *B. afzelii* were rare, and a single infection was identified in only one nymph in the wood at this location. Coinfections with genomospecies *B. afzelii* and VS116 were limited to one male tick on the roadside and one nymph in the wood. One adult male tick on the roadside supported a triple infection of genomospecies *B. burgdorferi* sensu stricto, group VS116, and *B. afzelii*. Coinfections of VS116 and *B. garinii* were identified in two ticks in both sites (Table 1).

In location 2 (the tow path), only two genomospecies were identified, *B. garinii* and VS116. VS116 was the most prevalent and accounted for 75% of the infection in this location. No mixed infections of *B. burgdorferi* sensu lato genomospecies were identified; however, untypeable *B. burgdorferi* sensu lato genomospecies were detected in two nymphs (Table 1).

In location 3, *B. burgdorferi* sensu lato genomospecies *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and VS116 were identified (Table 1). Prevalences of *B. burgdorferi* sensu stricto, *B. afzelii*, and group VS116 were higher inside the arboretum than outside. The prevalence of *B. garinii* was lower inside the arboretum (6.1%) than outside (20%); however, only 2 ticks were infected in both of these locations. Coinfections of *B. burgdorferi* sensu lato genomospecies were observed in both locations, representing 18.2% of the infection in the arboretum and 40% of the infection outside. Coinfections of group VS116 and *B. garinii* were identified in 9.1% of the infected ticks in the arboretum and in 30% of the infected ticks outside the arboretum. Notably, coinfections of genomospecies *B. burgdorferi* sensu stricto and *B. afzelii* were observed inside the arboretum (9.1%) but not outside. A single instance of a nymph coinfected with genomospecies *B. afzelii* and VS116 was identified outside the arboretum.

DISCUSSION

Significant variability was observed in the prevalence of infection among the five sites examined, with the prevalence of infection ranging from 11.5 to 28.9%. Similar variations in the prevalence of *B. burgdorferi* sensu lato infection have been observed geographically (10, 15), seasonally (13), and annually among sampling sites (18, 21).

The overall prevalence and distribution of *B. burgdorferi* sensu lato genomospecies differed considerably even in adjacent habitats. The predominance of VS116 may indicate the susceptibility of a broad range of host species for this genomospecies, as mixed infections of *B. garinii* and VS116 have recently been isolated from birds (14) and from the woodmouse, *A. sylvaticus* (15a). Although the prevalences of *B. burgdorferi* sensu stricto and *B. garinii* infections were similar, *B. garinii* was predominant in mixed infections. The latter observation may be attributed to constant association of *B. garinii* with VS116, whereas *B. burgdorferi* sensu stricto was more frequently identified in mixed infections with *B. afzelii*. The reduced prevalence of *B. afzelii* in Killarney and elsewhere in Ireland (15) compared to its prevalence Europe may be due to the restricted vertebrate host cenosis of islands. The fact that *B. garinii* never occurred together with *B. afzelii* or *B. burgdorferi* sensu stricto but only with VS116 may be partially explained by the suggestion that *B. garinii* and VS116 are mostly associated with birds, whereas *B. afzelii* and *B. burgdorferi* sensu stricto are mostly associated with rodents. An increasing amount of data is becoming available to support this view.

Although five different *B. burgdorferi* sensu lato genomospecies were identified in the park, not all were represented in any one location sampled. Four genomospecies were identified in single and mixed infections in ticks collected from four of the five sampling sites in Killarney National Park. However, only two typeable genomospecies, *B. garinii* and VS116, and untypeable *B. burgdorferi* sensu lato genomospecies were identified in the fifth location. These results indicate that the spatial distribution of the *B. burgdorferi* sensu lato genomospecies can vary significantly within a geographic location. Higher diversities and mixed infections of *B. burgdorferi* sensu lato genomospecies were associated with areas of woodland.

The distributions of *B. burgdorferi* sensu lato genomospecies in ticks in adjacent habitats were similar, whereas the prevalence of *B. burgdorferi* sensu lato genomospecies varied considerably. Higher prevalences were identified in nymphal and adult *I. ricinus* ticks collected in habitats which had complex vegetational compositions, i.e., the wood (24.6%) and the arboretum (28.9%). Lower prevalences of *B. burgdorferi* sensu lato genomospecies were identified in ticks in adjacent habitats with reduced vegetational complexity despite the availability of suitable tick habitat. The lowest prevalences of *B. burgdorferi* sensu lato genomospecies were identified along the tow path and also appear to be associated with a reduction in the vegetational complexity of the habitat. Habitats with diverse floral compositions typically support a wide variety of fauna, and the host cenosis of the habitat may affect the prevalence of *B. burgdorferi* sensu lato genomospecies.

The reservoir competence of birds as hosts for *B. burgdorferi* sensu lato genomospecies was recently demonstrated (14, 19). Both the arboretum and the woods support large numbers of ground-feeding birds such as blackbirds (*T. merula*) and robins (*E. rubecula*), and their residence time in these areas may augment the infection level. Furthermore, there is evidence that the exclusion of deer increases the prevalence of *B. burgdorferi* sensu lato-infected ticks (8, 17). In this study, the prevalence of *B. burgdorferi* sensu lato infections in the nymphal

and adult *I. ricinus* populations was significantly higher in the arboretum than in the adjacent habitat outside the arboretum fence, which is utilized by red deer (*C. elaphus*). Reduced prevalences of *B. burgdorferi* sensu lato genomospecies were also identified in the tow path habitat, which is extensively grazed by deer. These results indicate that the host cenosis and vegetational composition may affect the distribution and prevalence of the *B. burgdorferi* sensu lato genomospecies even in adjacent habitats.

The greatest risk of infection in this geographic location was associated with nymphal and adult *I. ricinus* ticks in both artificially planted and natural mixed deciduous woodland vegetation. Similar habitat associations have been made by other authors (18). The risk of infection was reduced in areas bordering dense woodland due to the lower prevalence of *B. burgdorferi* sensu lato infection in the tick population. However, the ticks in the bordering vegetation were infected with a diverse range of *B. burgdorferi* sensu lato genomospecies and also had a high incidence of multiple infections. Notably, woodland habitats such as those described in this study are typically utilized by both residents and tourists for recreational purposes in all parts of Ireland. Although at present the clinical implications of multiple infections of *B. burgdorferi* sensu lato genomospecies in the pathology of LB is undefined, mixed infections have been identified in clinical cases of LB (6, 23). Further studies on the variability of *B. burgdorferi* sensu lato genomospecies infections in ticks may provide insights into the possible roles that specific genotypes play in infectivity and disease manifestation. The variable symptoms associated with LB may even be due to polymorphisms within *B. burgdorferi* sensu lato genomospecies (4, 11).

In conclusion, these results suggest that both the prevalence of infection in ticks and genomospecies heterogeneity increase with increasing heterogeneity of the habitat, and this is probably due largely to the occurrence of a wider range of hosts in heterogeneous habitats. Even within a geographically localized area the distribution and prevalence of the endemic *B. burgdorferi* sensu lato genomospecies seems to be variable, and to obtain reliable estimates of both parameters extensive sampling of several habitats is required. Furthermore, the magnitude of variation in these parameters should be taken into account in the future identification or classification of risk habitats and in the design of epidemiological models for LB.

REFERENCES

1. **Anthonissen, F. M., M. Dekesel, P. P. Hoet, and G. H. Bigaignon.** 1994. Evidence for the involvement of different genospecies of *Borrelia* in the clinical outcome of Lyme disease in Belgium. Res. Microbiol. **145:**327–331.
2. **Assous, M. V., D. Postic, G. Paul, P. Névot, and G. Baranton.** 1993. Western blot analysis of sera from Lyme borreliosis patients according to the genomic species of the *Borrelia* strains used as antigens. Eur. J. Clin. Microbiol. Infect. Dis. **12:**261–268.
3. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J. C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
4. **Bunkis, J., B. Olsén, V. Fingerle, J. Bonnedahl, B. Wilske, and S. Bergström.** 1996. Molecular polymorphism of the Lyme disease agent *Borrelia garinii* in Northern Europe is influenced by a novel enzootic *Borrelia* focus in the North Atlantic. J. Clin. Microbiol. **34:**364–368.
5. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davies.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:** 1317–1319.
6. **Demaerschalck, I., A. Ben Massoud, M. De Kesel, B. Hoyois, Y. Lobet, P. Hoet, G. Bigaignon, A. Bollen, and E. Godfroid.** 1995. Simultaneous presence of different *Borrelia burgdorferi* sensu lato genospecies in biological fluids of Lyme disease patients. J. Clin. Microbiol. **33:**602–608.
7. **Dressler, F., R. Ackermann, and A. C. Steere.** 1994. Antibody responses to three genomic subgroups of *Borrelia burgdorferi* in European Lyme Borreliosis. J. Infect. Dis. **169:**313–318.
8. **Gray, J. S., O. Kahl, C. Janetzki, and J. Stein.** 1992. Studies of the ecology of Lyme disease in a deer forest in County Galway, Ireland. J. Med. Entomol. **29:**915–920.
9. **Gray, J. S., O. Kahl, J. Janetzki, J. Stein, and E. C. Guy.** 1995. The spatial distribution of *Borrelia burgdorferi* infected *Ixodes ricinus* in the Connemara region of County Galway, Ireland. Exp. Appl. Acarol. **19:**163–172.
10. **Gustafson, R.** 1994. Epidemiological studies on Lyme borreliosis and tick-borne encephalitis. Scand. J. Infect. Dis. **92**(Suppl.)**:**1–63.
11. **Guttman, D. S., P. W. Wang, I. N. Wang, E. M. Bosler, B. J. Luft, and D. E. Dykhuizen.** 1996. Multiple infections of *Ixodes scapularis* ticks by *Borrelia burgdorferi* as revealed by single-stranded conformation polymorphism analysis. J. Clin. Microbiol. **34:**652–656.
12. **Guy, E. C., and G. Stanek.** 1991. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. J. Clin. Pathol. **44:**610–611.
13. **Hubálek, Z., J. Halouzka, Z. Juřicová, and Š. Svobodová.** 1994. Seasonal distribution of Borreliae in Ixodes ricinus ticks. Zentralbl. Bakteriol. **280:** 423–431.
14. **Humair, P. F., et al.** 1996. Reservoir hosts for the Lyme disease spirochetes: what about blackbirds. Fifth Biennial Meeting of the European Tick Study Group, April 1996. NERC Institute of Virology & Environmental Microbiology, Oxford, United Kingdom.
15. **Kirstein, F., S. Rijpkema, M. Molkenboer, and J. S. Gray.** The distribution and prevalence of *B. burgdorferi* genomospecies in *Ixodes ricinus* ticks in Ireland. Eur. J. Epidemiol., in press.

15a. **Kirstein, F.** Unpublished data.

16. **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* s.l. in German woodlands. J. Med. Entomol. **32:**807–817.
17. **Lacombe, E., P. W. Rand, and R. P. Smith, Jr.** 1993. Disparity of *Borrelia burgdorferi* infection rates of adult *Ixodes dammini* on deer and vegetation. J. Infect. Dis. **167:**1236–1238.
18. **Mejlon, H. A., and T. G. T. Jaenson.** 1993. Seasonal prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* in different vegetation types in Sweden. Scand. J. Infect. Dis. **25:**449–456.
19. **Olsen, B., T. G. T. Jaenson, and S. Bergström.** 1995. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. Appl. Envir. Microbiol. **61:**3082–3087.
20. **Postic, D., M. Assous, P. A. D. Grimont, and G. Baranton.** 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of *rrf* (5S) *-rrl* (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. **44:**743–752.
21. **Rijpkema, S., J. Nieuwenhuijs, F. F. J. Franssen, and F. Jonejan.** 1994. Infection rates of Borrelia burgdorferi in different instars of Ixodes ricinus ticks from the Dutch North Sea Island of Ameland. Exp. Appl. Acarol. **18:** 531–542.
22. **Rijpkema, S., M. Molkenboer, L. Schouls, F. Jongejan, and J. 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.
23. **Rijpkema, S. G. T., D. J. Tazelaar, M. J. C. H. Molkenboer, G. T. Noordhoek, G. Plantinga, L. M. Schouls, and J. Schellekens.** 1995. Unpublished data.
24. **Van Dam, A. P., H. Kuiper, K. Vos, A. Widjojokusumo, B. M. De Jongh, L. Spanjaard, A. C. P. Ramselaar, M. D. Kramer, and J. Dankert.** 1993. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. Clin. Infect. Dis. **17:**708–717.