

## Differential Transmission of the Genospecies of *Borrelia burgdorferi* Sensu Lato by Game Birds and Small Rodents in England

KLAUS KURTENBACH,<sup>1,2\*</sup> MICK PEACEY,<sup>1,2</sup> SJOERD G. T. RIJPKEMA,<sup>3†</sup> ANDREW N. HOODLESS,<sup>1,4</sup>  
PATRICIA A. NUTTALL,<sup>2</sup> AND SARAH E. RANDOLPH<sup>1</sup>

Department of Zoology, University of Oxford, OX1 3PS, Oxford,<sup>1</sup> NERC Institute of Virology and Environmental Microbiology, OX1 3SR, Oxford,<sup>2</sup> and The Game Conservancy Trust, Fordingbridge, SP6 1EF, Hampshire,<sup>4</sup> United Kingdom, and Research Laboratory for Infectious Diseases, National Institute of Public Health and the Environment, 3720BA, Bilthoven, The Netherlands<sup>3</sup>

Received 24 September 1997/Accepted 1 January 1998

**The genetic diversity of *Borrelia burgdorferi* sensu lato was assessed in a focus of Lyme borreliosis in southern Britain dominated by game birds. Ticks, rodents, and pheasants were analyzed for spirochete infections by PCR targeting the 23S-5S rRNA genes, followed by genotyping by the reverse line blot method. In questing *Ixodes ricinus* ticks, three genospecies of *B. burgdorferi* sensu lato were detected, with the highest prevalences found for *Borrelia garinii* and *Borrelia valaisiana*. *B. burgdorferi* sensu stricto was rare (<1%) in all tick stages. *Borrelia afzelii* was not detected in any of the samples. More than 50% of engorged nymphs collected from pheasants were infected with borreliae, mainly *B. garinii* and/or *B. valaisiana*. Although 19% of the rodents harbored *B. burgdorferi* sensu stricto and/or *B. garinii* in internal organs, only *B. burgdorferi* sensu stricto was transmitted to xenodiagnostic tick larvae (it was transmitted to 1% of the larvae). The data indicate that different genospecies of *B. burgdorferi* sensu lato can be maintained in nature by distinct transmission cycles involving the same vector tick species but different vertebrate host species. Wildlife management may have an influence on the relative risk of different clinical forms of Lyme borreliosis.**

Lyme borreliosis is a tick-borne disease of humans in temperate climates of the northern hemisphere, whose causative agent, a spirochete belonging to the genus *Borrelia*, was described and named *Borrelia burgdorferi* in 1984 (13). On the basis of DNA-DNA relatedness and other molecular criteria, *B. burgdorferi* sensu lato is now considered to comprise at least nine genospecies and genomic groups (1, 30, 33). Phylogenetic analyses of various genes have suggested that the population structure of *B. burgdorferi* sensu lato is clonal (6). Here we ask whether the diverse spirochete strains have differential transmission patterns.

In Eurasia, six genospecies of *B. burgdorferi* sensu lato have been recorded; *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* are causative agents of Lyme disease in humans (38), while the pathogenic potentials of *Borrelia japonica*, *Borrelia valaisiana* (formerly genomic group VS116 [39]), and *Borrelia lusitaniae* (formerly genomic group PotiB2 [18]) have not yet been demonstrated. Culturing *Borrelia* is commonly considered the “gold standard” for detection of *B. burgdorferi* sensu lato. Approaches based on the PCR (33), however, appear to be more accurate in assessing the diversity and distribution of borreliae in nature, as culturing may favor particular genotypes (27). Furthermore, strains of *B. burgdorferi* sensu lato prevalent in the United Kingdom (known to be variants of *B. garinii*, *B. afzelii*, *B. burgdorferi* sensu stricto, and *B. valaisiana*) have been found to be unusually difficult to isolate and culture from ticks and hosts by standard techniques (20). There

is increasing evidence that the kinds of borreliae in ticks and hosts vary considerably (9, 14, 28, 29, 32, 33). In The Netherlands, for example, *B. afzelii* appears to be the most frequent genospecies in ticks, whereas in Ireland *B. garinii* and *B. valaisiana* seem to dominate (14, 32, 33). Surprisingly, *B. burgdorferi* sensu stricto, the most common genospecies in northeastern North America, appears to be comparatively rare in Europe and virtually absent in central and east Asia (7, 24, 26). The reasons for this variation remain unknown but may be related to the structure of the vertebrate host census; it has been postulated that genospecies are associated with particular groups of vertebrate hosts, such as birds or rodents (24). This suggestion appears to conflict with the observation that different genospecies of *B. burgdorferi* sensu lato may coexist in individual vertebrate hosts (7, 26). Such concurrent infections, however, do not imply that the transmissibilities of the genospecies or strains between hosts and ticks are equal; any differential transmission of the genospecies in the various natural tick-host systems would influence the prevalence of the genospecies and the degree of ecological diversity observed. While the transmission behavior of *B. burgdorferi* sensu stricto has been studied in detail with both laboratory and natural rodent hosts (5, 16, 22), the relative transmissibilities of other genospecies of *B. burgdorferi* sensu lato in rodents and other hosts have not been investigated previously.

Small mammals, particularly mice, have always been considered the principal hosts of *B. burgdorferi* sensu lato (10, 16, 17, 22, 25), but a role for avian hosts as reservoirs of *B. burgdorferi* sensu lato is gradually gaining credence (11, 12, 15, 31, 36) despite early claims to the contrary (21, 23). The potential role of birds in the transmission dynamics of *B. burgdorferi* sensu lato is substantial. In England, approximately 20 million farm-reared pheasants (*Phasianus colchicus*) are released into the woodlands each year to supplement natural populations for recreational shooting. As a result, pheasants constitute the vast majority of the land-based avifauna, especially in woodlands of

\* Corresponding author. Mailing address: The Wellcome Trust Center for the Epidemiology of Infectious Diseases, Department of Zoology, University of Oxford, OX1 3PS, Oxford, United Kingdom. Phone: 0044-1865-281630. Fax: 0044-1865-281696. E-mail: kku@mail.nerc.oxford.ac.uk.

† Present address: National Institute of Biological Standards and Controls, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, United Kingdom.

TABLE 1. Levels of infection for four genospecies of *B. burgdorferi* sensu lato in *I. ricinus* collected in 1995 and 1996 and in xenodiagnostic ticks

| Tick developmental stage           | Collection season              | No. examined     | % Infected with:                 |                                     |                   |                   |                      |  |                                   |
|------------------------------------|--------------------------------|------------------|----------------------------------|-------------------------------------|-------------------|-------------------|----------------------|--|-----------------------------------|
|                                    |                                |                  | <i>B. burgdorferi</i> sensu lato | <i>B. burgdorferi</i> sensu stricto | <i>B. garinii</i> | <i>B. afzelii</i> | <i>B. valaisiana</i> | <i>B. garinii</i> + <i>B. valaisiana</i> | Untypeable borreliae <sup>f</sup> |
| Questing larvae                    | Summer '95                     | 100              | 1.0                              | 0                                   | 1.0               | 0                 | 0                    | 0  | 0                                 |
| Questing nymphs <sup>a</sup>       | Spring, summer, and autumn '95 | 380              | 2.1                              | NA <sup>g</sup>                     | NA                | NA                | NA                   | NA                                       | NA                                |
|                                    | Spring '95                     | 100              | 3.0                              | 0                                   | 3.0               | 0                 | 0                    | 0  | 0                                 |
|                                    | Spring '96                     | 100              | 4.0                              | 1.0                                 | 2.0               | 0                 | 1.0                  | 0  | 0                                 |
| Questing adults                    | Autumn '96                     | 100              | 16.0                             | 1.0                                 | 10.0 <sup>d</sup> | 0                 | 6.0 <sup>d</sup>     | 3.0 <sup>e</sup>                         | 2.0                               |
| Larvae fed on rodents <sup>b</sup> | Spring '96                     | 771              | 1.3                              | 1.3                                 | 0                 | 0                 | 0                    | 0  | 0                                 |
| Nymphs fed on pheasants            | Spring '96                     | 122 <sup>c</sup> | 56.6                             | 0                                   | 37.7 <sup>d</sup> | 0                 | 27.0 <sup>d</sup>    | 10.6 <sup>e</sup>                        | 2.5                               |

<sup>a</sup> The mean level of infection of *B. burgdorferi* sensu lato in all questing nymphs was 2.6% (15 of 580 nymphs).

<sup>b</sup> Xenodiagnostic larvae were introduced to 47 wild rodents trapped alive.

<sup>c</sup> Number of pheasant-derived engorged nymphs tested.

<sup>d</sup> Ticks had single and mixed infections.

<sup>e</sup> Ticks had only mixed infections.

<sup>f</sup> The borreliae did not hybridize with species-specific DNA probes.

<sup>g</sup> NA, not applicable.

southern England (34), and are present alongside high densities of mammals, such as woodmice, voles, squirrels, and deer. All of these hosts feed considerable numbers of *Ixodes ricinus*, the European vector of *B. burgdorferi* sensu lato (4, 15, 31).

In this paper, we present field data that reveal differential transmission of *B. burgdorferi* sensu lato genospecies through pheasant and rodent populations to different developmental stages of *I. ricinus* ticks.

#### MATERIALS AND METHODS

**Study site.** Animals were caught in a Dorset woodland 10 miles west of Fordingbridge (1°56'W, 50°53'N) within a focus of Lyme borreliosis in southern England. This site contains mainly oak (*Quercus* spp.), ash (*Fraxinus excelsior*), and patchy conifer plantations (mainly *Pinus sylvestris*), and the undergrowth is dominated by bluebells (*Hyacinthoides non-scripta*) and dog's mercury (*Mercurialis perennis*) in the spring and bracken fern (*Pteridium aquilinum*) from late summer until winter.

**Rodents.** In May 1996, 47 rodents belonging to the species *Apodemus sylvaticus* (woodmouse) and *Clethrionomys glareolus* (bank vole) were trapped alive with Longworth traps (Penlon Ltd., Abingdon, United Kingdom) and taken to a laboratory. The spirochetal infectivity of the rodents for ticks was assayed by larval xenodiagnosis (16) commencing 1 week after trapping; 30 uninfected *I. ricinus* larvae (colony maintained at the NERC Institute of Virology and Environmental Microbiology, Oxford, United Kingdom) were introduced to each animal. After repletion, the engorged ticks were kept for 14 days above a saturated solution of MgSO<sub>4</sub> at 18°C and then frozen at -20°C. The rodents were finally autopsied under aseptic conditions in a building in which no *Borrelia* DNA was handled. Biopsies from internal organs (heart, urinary bladder, kidney, brain) and earlobes were taken and stored at -20°C until PCR analysis.

**Birds.** Thirty adult male pheasants were shot in April 1996. All of the *I. ricinus* ticks infesting these birds were recorded and allowed to drop off naturally. No fully engorged larvae or adult ticks were recovered, but 150 fed nymphs from the 30 birds (five ticks per bird) were collected and kept alive for 2 weeks before they were preserved in 70% ethanol at -20°C. Of these, the 122 most fully engorged ticks were selected and analyzed for the presence of spirochetal DNA.

**Questing ticks.** *I. ricinus* ticks were collected fortnightly by blanket dragging from the same site as their vertebrate hosts. Some of these ticks (larvae collected in the summer of 1995, nymphs collected in the spring, summer, and autumn of 1995 and spring of 1996, and adults collected in the autumn of 1996) were analyzed to determine whether they were infected with particular genospecies of *B. burgdorferi* sensu lato by PCR and the reverse line blot method.

**PCR and reverse line blotting.** Genomic DNA was extracted from ticks and animal biopsies by alkaline hydrolysis (8). A PCR with a nested set of primers (primers 23SN1 [5'-ACCATAGACTCTTATTACTTTGAC], 23SC1 [5'-TAAGCTGACTAATACTAATTACC], 23SN2 [5'-ACCATAGACTCTTATTACTTTGAC], and 5SC2 [5'-biotin-GAGAGTAGGTTATTGCCAGGG]) was performed by targeting the tandemly duplicated *rrf* (5S)-*rrl* (23S) rRNA gene clusters (19, 30, 33). All of the steps were separated temporally and spatially (different laboratories) and were performed under strictly aseptic conditions. As *B. japonica* is not present in Europe, a culture of this genospecies was used as a

positive control in order to avoid DNA contamination. Negative controls at a ratio of approximately 2:3 were incorporated into the PCR procedures at the DNA extraction level and at the first- and second-round amplification levels and into the electrophoresis of PCR products. All amplicons were electrophoresed with 2% agarose gels, stained with ethidium bromide, and visualized by UV transillumination. All samples that produced bands at approximately 380 and/or 225 to 270 bp were subjected to DNA-DNA hybridization by the reverse line blot method, a modification of the reverse dot blot method performed with a line blotter (Miniblotter 45; Immunetics, Cambridge, Mass.). Briefly, biotin-labelled amplicons were hybridized with DNA probes which were covalently bound to an activated membrane by the 5' aminolink (a) group. The probes were specific for *B. burgdorferi* sensu lato (5'-a-CTTTGACCATATTTTTATCTTCCA), *B. burgdorferi* sensu stricto (5'-a-AACACCAATATTTAAAAACATAA), *B. afzelii* (5'-a-AACATTTAAAAAATAAATCAAGG), *B. garinii* (5'-a-AACATGAACATCTAAAAACATAAAA), and *B. valaisiana* (5'-a-CATTAATAAAAAATATAAAAATAAATTTAAGG). PCR products of DNA templates derived from cloned cultures of *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. japonica* were included as positive controls for the reverse line blot. After incubation with streptavidin-peroxidase conjugate (Boehringer Mannheim GmbH, Mannheim, Germany), hybrids were visualized with an enhanced chemiluminescence system (type ECL; Amersham Life Sciences, Amersham, United Kingdom).

Inhibition of specific DNA amplification by an excess of host-derived and/or tick-derived tissue was tested with flat and engorged ticks and rodent tissues spiked with variable numbers of cultured *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. japonica*. Consistent with previous studies (2, 35, 36), high concentrations of tissue in a lysate (>10 mg/ml), particularly tissue from blood-fed ticks, proved to be inhibitory for the PCR (data not shown), so all DNA lysates were diluted appropriately to adjust the sensitivity of the PCR to the amount of DNA equivalent to two spirochetes in a given reaction mixture.

#### RESULTS

A total of 780 questing *I. ricinus* ticks (100 larvae, 100 adults, and 580 nymphs) were analyzed for *B. burgdorferi* sensu lato infection by PCR. The levels of infection with *B. burgdorferi* sensu lato in larvae and nymphs were 1 and 2.6%, respectively (not significantly different), but the level of infection in questing adult ticks was significantly higher (16%) ( $\chi^2 = 14.70$ ,  $P < 0.001$ ) (Table 1). All of the larvae and adults and 200 of the nymphs were subjected to genotyping, which revealed three genospecies, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. valaisiana* (Table 1). The most abundant genospecies was *B. garinii* (found in all developmental stages of *I. ricinus*), followed by *B. valaisiana*, while *B. burgdorferi* sensu stricto was rare ( $\leq 1\%$ ) and there was no detectable difference in the levels of *B. burgdorferi* sensu stricto infection in nymphs and adult ticks. The levels of *B. garinii* and *B. valaisiana* infection in questing larvae and nymphs were low ( $\leq 3.0\%$ ), but the level of infection

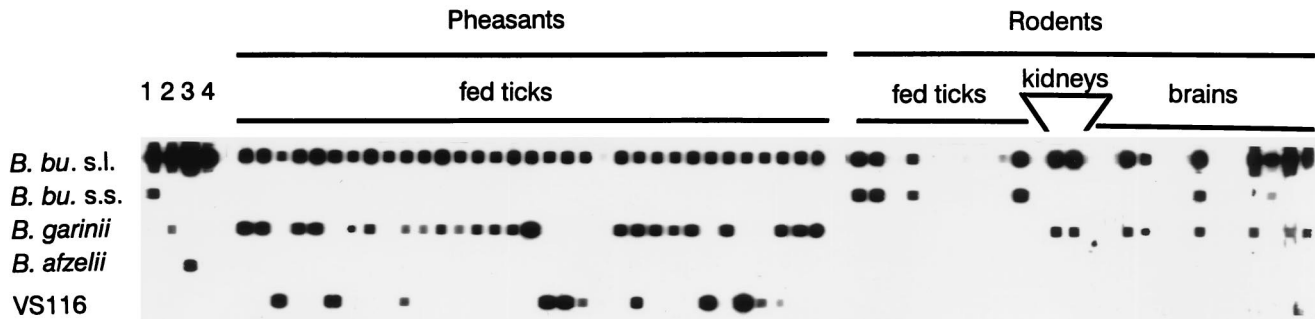


FIG. 1. Reverse line blot to identify the genospecies of *B. burgdorferi* sensu lato prevalent in ticks and host tissues. Lanes 1 to 4 contained positive controls that were PCR products of cloned isolates (lane 1, *B. burgdorferi* sensu stricto; lane 2, *B. garinii*; lane 3, *B. afzelii*; lane 4, *B. japonica*). The results for hybrids of representative PCR products from pheasant- and rodent-derived ticks and from tissues are also shown. Untypeable samples reacted with the *B. burgdorferi* sensu lato-specific DNA probe, but not with any of the genospecies-specific probes. Genomic group VS116 has recently been named *B. valaisiana* (39) and was not included as a positive control in the blot shown. Abbreviations: *B. bu. s.l.*, *B. burgdorferi* sensu lato; *B. bu. s.s.*, *B. burgdorferi* sensu stricto.

in questing adults was significantly higher (13%) ( $\chi^2 = 25.85$ ,  $P < 0.001$ ). Three of the infected adult ticks were infected with both *B. garinii* and *B. valaisiana*, and two *B. burgdorferi* sensu lato infections in adult ticks could not be identified to genospecies. *B. afzelii* was not detected in any tick.

Spirochetal DNA was detected in 5 of 26 *C. glareolus* individuals (19.2%) and in 4 of 21 *A. sylvaticus* individuals (19.0%). Two genospecies were detected, *B. burgdorferi* sensu stricto and *B. garinii*. All nine infected rodents were positive for *B. garinii* (seven brain infections and two kidney infections) (Fig. 1), while two animals had mixed infections with *B. garinii* and *B. burgdorferi* sensu stricto in their brains (Table 2). None of the skin, urinary bladder, and heart samples proved to be infected.

Spirochete-free *I. ricinus* larvae were introduced to the 47 rodents (30 larvae per animal). A total of 771 engorged larvae were analyzed by PCR. Ten of these were positive for *B. burgdorferi* sensu lato, giving an overall level of infection in xenodiagnostic ticks of 1.3% (Table 1). All positive samples were identified as containing *B. burgdorferi* sensu stricto (Fig. 1). The infected xenodiagnostic larvae came from four individual rodents, only one of which had tested positive for tissue infections with *B. burgdorferi* sensu lato (Table 2).

Of the 122 fed *I. ricinus* nymphs from pheasants that were analyzed, 69 (56.6%) were infected with *B. burgdorferi* sensu lato. The organisms in all but three of these samples could be identified to species; 33 ticks were infected with *B. garinii*, 20 ticks were infected with *B. valaisiana*, and 13 ticks were found to have mixed infections with both of these genospecies (Table 1). Of the 30 birds, 27 yielded at least one *Borrelia*-infected tick; 14 birds yielded at least one tick infected with *B. garinii* and another tick infected with *B. valaisiana* or ticks infected concurrently with both genospecies, while 13 birds yielded ticks infected with only one of the two genospecies.

None of the negative controls incorporated into the PCR procedures performed throughout the present study gave a positive signal.

In summary, the transmission of spirochetes to ticks from rodents and the transmission of spirochetes to ticks from pheasants differed both qualitatively and quantitatively. Not only were different genospecies of *B. burgdorferi* sensu lato transmitted, but the level of infection by any genospecies was significantly lower in xenodiagnostic larvae that fed on rodents (1.3%) than in nymphs that had fed on pheasants (56.6%) ( $\chi^2 = 398.6$ ,  $df = 1$ ,  $P \ll 0.001$ ).

## DISCUSSION

This study showed that three different genospecies of the *B. burgdorferi* sensu lato species complex, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. valaisiana* (formerly genomic group VS116), are circulating in an endemic focus of Lyme borreliosis in southern England. *B. afzelii*, one of the most abundant genospecies in continental Europe, was not detected. Although 19% of the rodents harbored *B. garinii*, only *B. burgdorferi* sensu stricto was transmitted by the rodents to ticks. The infectivity of the rodent population was surprisingly low; only 1.3% of the xenodiagnostic ticks were infected. In contrast, more than 50% of the nymphal ticks derived from pheasants were infected with *B. garinii* and *B. valaisiana*. This pattern and the matching genotypic composition of *B. burgdorferi* sensu lato in the questing ticks indicate that *B. garinii* and *B. valaisiana* are preferentially transmitted to ticks by pheasants, while *B. burgdorferi* sensu stricto appears to be maintained at a low level by a rodent-tick cycle.

The detection of spirochetes in the present study was based on successful amplification of spirochetal DNA, which cannot distinguish between viable and nonviable borreliae. However, as all of the ticks were allowed to engorge and digest their bloodmeal for 14 days postrepletion, it is unlikely that the PCR detected only naked DNA from spirochetes in the ticks' midguts, particularly because the target of this PCR is located on the chromosome rather than on a plasmid (19, 30). Similarly, the presence of naked chromosomal DNA in host tissues was unlikely as the animals were autopsied more than 2 weeks after trapping, their last possible contact with infected ticks.

The observed level of infection with *B. burgdorferi* sensu lato in questing larval *I. ricinus*, 1%, is within the range previously described for endemic foci of Lyme borreliosis in Europe (17, 32), while the level of infection in questing nymphs, 2.6%, appears to be rather low compared with the levels of infection in endemic foci in North America (37) and continental Europe (10, 17, 31). The overall level of infection in adults was fivefold higher than the level of infection in nymphs, indicating that hosts of *I. ricinus* nymphs were particularly infective. Within the questing tick population, *B. garinii* was the most frequent genospecies and was detected at all developmental stages, followed by *B. valaisiana*. The levels of infection for both of these genospecies were markedly higher in adults than in questing nymphs. In contrast, the level of infection with *B. burgdorferi* sensu stricto, the most abundant genospecies in northern North America, did not exceed 1% even in adult ticks. This



TABLE 2. Results of PCR and genotyping of tissues and xenodiagnostic tick larvae from wild-trapped *A. sylvaticus* and *C. glareolus*

| Animal species       | Animal no. | Results of PCR and genotyping <sup>a</sup> |         |        |                      |                      | Xenodiagnostic ticks |
|----------------------|------------|--|---------|--------|----------------------|----------------------|----------------------|
|                      |            | Heart                                      | Bladder | Kidney | Brain                | Skin                 |                      |
| <i>A. sylvaticus</i> | 1          | -  | NT      | -      | -                    | -                    | -                    |
|                      | 2          | -  | -       | -      | -                    | -                    | -                    |
|                      | 3          | -  | -       | -      | -                    | -                    | -                    |
|                      | 4          | -  | NT      | -      | -                    | -                    | -                    |
|                      | 5          | -  | NT      | -      | -                    | -                    | -                    |
|                      | 6          | -  | -       | -      | -                    | -                    | -                    |
|                      | 7          | -  | NT      | -      | -                    | -                    | -                    |
|                      | 8          | -  | NT      | -      | -                    | -                    | -                    |
|                      | 9          | -  | -       | -      | -                    | -                    | -                    |
|                      | 10         | -  | -       | -      | -                    | -                    | <i>B.b.ss</i>        |
|                      | 11         | -  | NT      | -      | -                    | -                    | -                    |
|                      | 12         | -  | -       | -      | -                    | -                    | -                    |
|                      | 13         | -  | -       | -      | -                    | -                    | -                    |
|                      | 14         | -  | -       | -      | -                    | -                    | -                    |
|                      | 15         | -  | -       | -      | -                    | <i>B.g.</i>          | -                    |
|                      | 16         | -  | -       | -      | -                    | <i>B.g. + B.b.ss</i> | -                    |
|                      | 17         | -  | -       | -      | -                    | <i>B.g.</i>          | -                    |
|                      | 18         | -  | -       | -      | -                    | <i>B.g.</i>          | -                    |
|                      | 19         | -  | -       | -      | -                    | -                    | -                    |
|                      | 20         | -  | -       | -      | -                    | -                    | -                    |
|                      | 21         | -  | -       | -      | -                    | -                    | -                    |
| <i>C. glareolus</i>  | 22         | -  | -       | -      | -                    | -                    | -                    |
|                      | 23         | -  | -       | -      | -                    | -                    | -                    |
|                      | 24         | -  | NT      | -      | -                    | -                    | -                    |
|                      | 25         | -  | -       | -      | -                    | -                    | -                    |
|                      | 26         | -  | -       | -      | -                    | -                    | -                    |
|                      | 27         | -  | NT      | -      | -                    | -                    | -                    |
|                      | 28         | -  | NT      | -      | -                    | -                    | -                    |
|                      | 29         | -  | -       | -      | -                    | <i>B.g.</i>          | -                    |
|                      | 30         | -  | -       | -      | -                    | -                    | -                    |
|                      | 31         | -  | -       | -      | -                    | -                    | -                    |
|                      | 32         | -  | -       | -      | -                    | <i>B.g.</i>          | -                    |
|                      | 33         | -  | -       | -      | -                    | -                    | <i>B.b.ss</i>        |
|                      | 34         | -  | -       | -      | -                    | -                    | <i>B.b.ss</i>        |
|                      | 35         | -  | NT      | -      | -                    | -                    | -                    |
|                      | 36         | -  | -       | -      | -                    | -                    | -                    |
|                      | 37         | -  | -       | -      | -                    | -                    | -                    |
| 38                   | -          | -  | -       | -      | -                    | -                    |                      |
| 39                   | -          | NT   | -       | -      | -                    | -                    |                      |
| 40                   | -          | -  | -       | -      | -                    | -                    |                      |
| 41                   | -          | NT   | -       | -      | -                    | -                    |                      |
| 42                   | -          | -  | -       | -      | -                    | -                    |                      |
| 43                   | -          | NT   | -       | -      | -                    | -                    |                      |
| 44                   | -          | -  | -       | -      | <i>B.g. + B.b.ss</i> | <i>B.b.ss</i>        |                      |
| 45                   | -          | -  | -       | -      | -                    | -                    |                      |
| 46                   | -          | -  | -       | -      | <i>B.g.</i>          | -                    |                      |
| 47                   | -          | -  | -       | -      | <i>B.g.</i>          | -                    |                      |

<sup>a</sup> *B.b.ss*, *B. burgdorferi* sensu stricto; *B.g.*, *B. garinii*; -, negative result; NT, not tested.

pattern of infection indicates that the hosts that were feeding large numbers of nymphal ticks were selectively infective for *B. garinii* and *B. valaisiana*.

*I. ricinus* larvae feed in large numbers on mice and voles, in addition to squirrels and deer (4, 10, 17). The low level of infection with *B. burgdorferi* sensu lato in questing nymphs, as found in the present study, is consistent with the low infectivity of the trapped small rodents as assessed by larval xenodiagnosis. Compared with the data from the present study, the reservoir capacity of the rodent populations in foci in continental

Europe (10, 17) and many parts of northern North America (22) is much higher. This may be related to the particular genospecies of *B. burgdorferi* sensu lato circulating in each location; *B. afzelii*, for which rodents are particularly transmission competent, is widely distributed in continental Europe (9, 32, 33), whereas in our study site *B. afzelii* was not found. In North America, rodents, particularly rodents belonging to the genus *Peromyscus*, are highly transmission competent for *B. burgdorferi* sensu stricto (5, 22), whereas European rodent species were found to have a lower degree of reservoir competence and reservoir capacity for this genospecies (16), perhaps explaining its rarity in the present study site and throughout Eurasia (7, 24, 26). *B. garinii*, on the other hand, has the potential to persist in rodents concurrently with other genospecies (7, 26), but there is emerging evidence that it is rarely passed from rodents to ticks (9, 25, 26). This is consistent with the results of the present study, in which 19% of the small rodents were infected with *B. garinii* but none of the 771 xenodiagnostic *I. ricinus* ticks acquired *B. garinii* from these animals. Moreover, *B. garinii* infections were not detected in the skin of the rodents, but were confined to internal organs, particularly the brain. None of the rodents or the xenodiagnostic ticks fed on these rodents was found to be infected with *B. valaisiana*, despite the prevalence of this organism in questing *I. ricinus* ticks collected in the same study site. This finding, together with the fact that *B. valaisiana* has never been detected in rodent hosts, may indicate that this genospecies does not survive and persist in small mammals.

Ground-foraging birds, particularly pheasants, which may occur in the United Kingdom at densities up to 50 birds per hectare (34), constitute a major part of the tick host community in the woodland studied. Pheasants feed more than four times as many nymphs as larvae of *I. ricinus* (15). In the present study pheasants were highly infective to nymphs of *I. ricinus*. The vast majority of bird-derived infected nymphs carried *B. garinii* and *B. valaisiana*, but not *B. burgdorferi* sensu stricto. Most infraspecific variants of *B. garinii*, the most polymorphic genospecies of the *B. burgdorferi* sensu lato species complex (40), have previously been associated with ticks derived from birds (24, 28, 29). In the present study mixed infections in ticks were found only for *B. garinii* and *B. valaisiana*, suggesting that the transmission of these two genospecies is associated. This finding is consistent with the results of a recent study in Ireland on mixed infections of *B. garinii* and *B. valaisiana* in questing ticks (14), which suggested that birds are reservoirs of *B. valaisiana*. To our knowledge, the present study is the first study which provides direct evidence that there is an avian reservoir host for *B. valaisiana*.

The reason for the complete absence of *B. afzelii* in the present study is unclear, but the lack of *B. afzelii* may also be related to avian hosts; in the study site used, it is possible that a large and dense pheasant population substantially reduces the basic reproduction number of *B. afzelii*. Thus, it is possible that this genospecies is taken out of the ecosystem by means of a zooprophylactic role of such birds for *B. afzelii*, despite the presence of reservoir-competent rodents (mice, voles, and squirrels) in the study site. A lack of reservoir competence and a possible zooprophylactic role of ground-foraging birds in relation to *B. burgdorferi* sensu stricto and *B. afzelii*, respectively, would be consistent with results of previous studies on the reservoir incompetence of birds (21, 23).

Besides rodents and pheasants, other tick host species undoubtedly play a role in generating the observed pattern of species diversity of *B. burgdorferi* sensu lato. For example, the high level of infection (57%), mainly infection with *B. garinii* and/or *B. valaisiana*, in nymphs that fed on pheasants was

reduced to 16% in questing adult ticks. This was probably the result of dilution by uninfected nymphs that fed on other hosts not competent to transmit these genospecies. Apart from deer, squirrels are likely candidates for this role, because they feed large numbers of nymphs (4) and are competent to transmit *B. burgdorferi* sensu lato but apparently are not competent to transmit *B. garinii* (3).

The overall pattern which emerged from the present study is one of differential transmission and maintenance of various genospecies of *B. burgdorferi* sensu lato depending on the variable interactions between the vertebrate host species and (i) each genospecies or (ii) each developmental stage of the tick. Many hosts may be exposed to multiple tick bites, followed by the possible establishment of mixed infections (7, 26). However, there is increasing evidence that the various host species do not transmit all *B. burgdorferi* sensu lato strains to ticks with equal efficiency (5, 16, 21–25, 29). Cautions about the transfer of parameter values (e.g., transmission coefficients) from system to system for use in models (31) are supported by these results. The mechanisms underlying the apparent differential transmission of the genospecies of *B. burgdorferi* sensu lato by the various groups of mammalian and avian hosts remain to be determined.

The genetic diversity of *B. burgdorferi* sensu lato and the strain-specific interaction with each host species add additional elements to the considerable ecological diversity and thus variation in risk factors for humans of this zoonotic tick-borne disease. For example, in a site dominated by pheasants, such as the site analyzed in the present study, there is potentially a greater risk of neuroborreliosis associated with increased prevalence of *B. garinii* (38). On the other hand, the finding that many ground-foraging birds (pheasants in this study) primarily feed nymphs which, later as questing adults, are not considered as great a risk to humans as the less easily detected and more numerous nymphs may counterbalance the inflated risk of *B. garinii* infection for humans.

#### ACKNOWLEDGMENTS

This study was supported by grant GR3/09626 from the Natural Environment Research Council, United Kingdom, by grant 044488/Z/95/A from The Wellcome Trust, London, United Kingdom, and by grant EC Biomed BMHI-CT93-1183 from the Commission of the European Union.

We thank A. P. Van Dam (Amsterdam, The Netherlands) and M. M. Simon (Freiburg, Germany) for providing strains of *B. burgdorferi* sensu lato.

#### REFERENCES

- 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.
- Cogswell, F. B., C. E. Bantar, T. G. Hughes, Y. Gu, and M. T. Philipp. 1996. Host DNA can interfere with detection of *Borrelia burgdorferi* in skin biopsy specimens by PCR. *J. Clin. Microbiol.* **34**:980–982.
- Craine, N. G., P. A. Nuttall, A. C. Marriott, and S. E. Randolph. 1997. Rôle of grey squirrels and pheasants in the transmission of *Borrelia burgdorferi* sensu lato, the Lyme disease spirochaete, in the U.K. *Folia Parasitol. (Prague)* **44**:155–160.
- Craine, N. G., S. E. Randolph, and P. A. Nuttall. 1995. Seasonal variation in the rôle of grey squirrels as hosts of *Ixodes ricinus*, the tick vector of the Lyme disease spirochaete, in a British woodland. *Folia Parasitol. (Prague)* **42**: 73–80.
- 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.
- Dykuizen, D. E., D. S. Polin, J. J. Dunn, B. Wilske, V. Preac-Mursic, R. J. Dattwyler, and B. Luft. 1993. *Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development. *Proc. Natl. Acad. Sci. USA* **90**:10163–10167.
- Gorelova, B. N., E. I. Korenberg, Y. V. Kovalevskii, and S. V. Shcherbakov. 1995. Small mammals as reservoir hosts for borrelia in Russia. *Zentralbl. Bacteriol.* **282**:315–322.
- Guy, E., and G. Stanek. 1991. Detection of *Borrelia burgdorferi* in Lyme disease patients by PCR. *J. Clin. Pathol.* **44**:611–614.
- Humair, P. F., O. Peter, R. Wallich, and L. Gern. 1995. Strain variation of Lyme disease spirochetes isolated from *Ixodes ricinus* ticks and rodents collected in two endemic areas in Switzerland. *J. Med. Entomol.* **32**:433–438.
- Humair, P. F., M. N. Turrian, A. Aeschlimann, and L. Gern. 1993. *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. *Folia Parasitol. (Prague)* **40**:65–70.
- Humair, P. F., M. N. Turrian, A. Aeschlimann, and L. Gern. 1993. *Ixodes ricinus* immatures on birds in a focus of Lyme borreliosis. *Folia Parasitol. (Prague)* **40**:237–242.
- Isogai, E., S. Tanaka, I. S. Braga III, C. Itakura, H. Isogai, K. Kimura, and N. Fijii. 1994. Experimental *Borrelia garinii* infection of Japanese quail. *Infect. Immun.* **62**:3580–3582.
- 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.
- Kirstein, F., S. G. T. Rijpkema, M. Molkenboer, and J. Gray. 1997. Local variations in the distribution and prevalence of *Borrelia burgdorferi* sensu lato genospecies in *Ixodes ricinus* ticks. *Appl. Environ. Microbiol.* **63**:1102–1106.
- Kurtenbach, K., D. Carey, A. Hoodless, P. A. Nuttall, and S. E. Randolph. 1998. Competence of pheasants as reservoirs for Lyme disease spirochetes. *J. Med. Entomol.* **35**:77–81.
- Kurtenbach, K., A. Dizij, H. M. Seitz, G. Margos, S. E. Moter, M. D. Kramer, R. Wallich, U. E. Schaible, and M. M. Simon. 1994. Differential immune responses to *Borrelia burgdorferi* in European wild rodent species influence spirochete transmission to *Ixodes ricinus* L. (Acari: Ixodidae). *Infect. Immun.* **62**:5344–5352.
- 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* (Acari: Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* s.l. in German woodlands. *J. Med. Entomol.* **32**:807–817.
- Le Fleche, A., D. Postic, K. Girardet, O. Peter, and G. Baranton. 1997. Characterization of *Borrelia lusitaniae* sp. nov. by 16S ribosomal DNA sequence analysis. *Int. J. Syst. Bacteriol.* **47**:921–925.
- Liveris, D., A. Gazumyan, and I. Schwartz. 1995. Molecular typing of *Borrelia burgdorferi* sensu lato by PCR-restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* **33**:589–595.
- Livesley, M. A., D. Carey, L. Gern, and P. A. Nuttall. 1994. Problems of isolating *Borrelia burgdorferi* from ticks collected in United Kingdom foci of Lyme disease. *Med. Vet. Entomol.* **8**:172–178.
- Mather, T. N., S. R. Telford III, A. B. MacLachlan, and A. Spielman. 1989. Incompetence of catbirds as reservoirs for the Lyme disease spirochete (*Borrelia burgdorferi*). *J. Parasitol.* **75**:66–69.
- Mather, T. N., M. L. Wilson, S. I. Moore, and J. M. C. Ribeiro. 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am. J. Epidemiol.* **130**:143–150.
- Matuschka, F.-R., and A. Spielman. 1992. Loss of Lyme disease spirochetes from *Ixodes ricinus* ticks feeding on European blackbirds. *Exp. Parasitol.* **74**: 151–158.
- Nakao, M., K. Miyamoto, and M. Fukunaga. 1994. Lyme disease spirochetes in Japan: enzootic transmission cycles in birds, rodents, and *Ixodes persulcatus* ticks. *J. Infect. Dis.* **170**:878–882.
- Nakao, M., K. Miyamoto, and M. Fukunaga. 1994. *Borrelia japonica* in nature: genotypic identification of spirochetes isolated from Japanese small mammals. *Microbiol. Immunol.* **38**:805–808.
- Nakao, M., and K. Miyamoto. 1995. Mixed infection of different *Borrelia* species among *Apodemus speciosus* mice in Hokkaido, Japan. *J. Clin. Microbiol.* **33**:490–492.
- Norris, D. E., B. J. B. Johnson, J. Piesman, G. O. Maupin, J. L. Clark, and W. C. Black IV. 1997. Culturing selects for specific genotypes of *Borrelia burgdorferi* in an enzootic cycle in Colorado. *J. Clin. Microbiol.* **35**:2359–2364.
- Olsen, B., D. C. Duffy, T. G. T. Jaenson, A. Gylfe, J. Bonnedahl, and S. Bergström. 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. *J. Clin. Microbiol.* **33**:3270–3274.
- Olsen, B., T. G. T. Jaenson, L. Noppa, J. Bunikis, and S. A. Bergström. 1993. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. *Nature (London)* **362**:340–342.
- 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.
- 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.
- Rijpkema, S. G. T., and H. Bruinink. 1996. Detection of *Borrelia burgdorferi* sensu lato by PCR in questing *Ixodes ricinus* larvae from the Dutch North Sea

- island of Ameland. *Exp. Appl. Acarol.* **20**:381–385.
33. **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.
  34. **Robertson, P. A., M. I. A. Woodburn, W. Neutel, and C. E. Bealey.** 1993. Effects of land use on breeding pheasant density. *J. Appl. Ecol.* **30**:465–577.
  35. **Schwartz, L., S. Varde, R. B. Nadelman, G. P. Wormser, and D. Fish.** 1997. Inhibition of efficient polymerase chain reaction amplification of *Borrelia burgdorferi* DNA in blood-fed ticks. *Am. J. Trop. Med. Hyg.* **56**:339–342.
  36. **Stafford, K. C., III, V. C. Bladen, and L. A. Magnarelli.** 1995. Ticks (Acari: Ixodidae) infesting wild birds (Aves) and white-footed mice in Lyme, CT. *J. Med. Entomol.* **32**:453–466.
  37. **Telford, S. R., III, S. S. Urioste, and A. Spielman.** 1992. Clustering of host-seeking nymphal deer ticks (*Ixodes dammini*) infected by Lyme disease spirochetes (*Borrelia burgdorferi*). *Am. J. Trop. Med. Hyg.* **47**:55–60.
  38. **Van Dam, A. P., H. Kuiper, K. Vos, A. Widjojokusumo, L. Spanjaard, B. M. De Jongh, A. C. P. Ramselaar, M. D. Kramer, and J. Dankert.** 1993. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations in Lyme borreliosis. *Clin. Infect. Dis.* **17**:708–717.
  39. **Wang, G., A. P. Van Dam, A. Le Fleche, D. Postic, O. Peter, G. Baranton, R. de Boer, and L. Spanjaard.** 1997. Genetic and phenotypic analysis of *Borrelia valaisiana* sp. nov. (*Borrelia* genomic groups VS116 and M19). *Int. J. Syst. Bacteriol.* **47**:926–932.
  40. **Will, G., S. Jauris-Heipke, E. Schwab, U. Busch, D. Rössler, E. Soutschek, B. Wilske, and V. Preac-Mursic.** 1995. Sequence analysis of ospA genes shows homogeneity within *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* strains but reveals major subgroups within *Borrelia garinii* species. *Med. Microbiol. Immunol.* **184**:73–80.