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Substantial Rise in the Prevalence of Lyme Borreliosis Spirochetes in a Region of Western Germany over a 10-Year Period

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More than a decade after a study on the transmission cycle of *Borrelia burgdorferi* sensu lato in the Siebengebirge, a nature reserve near Bonn, Germany, questing nymphal and adult *Ixodes ricinus* ticks were collected again in three selected areas of the same low mountain range and examined for infection with *B. burgdorferi* sensu lato. Between May and October 2001, a total of 1,754 ticks were collected by blanket dragging; 374 ticks were analyzed for *B. burgdorferi* sensu lato by both an immunofluorescence assay (IFA) and at least two different PCR tests, whereas 171 ticks were analyzed by PCR only. By combining all assays, an average of 14% of the ticks tested positive for *B. burgdorferi* sensu lato, 5.5, 15.8, and 21.8% in the three collection areas. Of the nymphs and adults examined, 12.9 and 21.1%, respectively, were found to be spirochete infected. A lower total infection prevalence was obtained by IFA (14.4%) than by a nested PCR approach (16.5%), but both were higher than that obtained by a simple PCR approach (11.9%). Compared with data collected over a decade ago, the mean infection prevalence of *B. burgdorferi* sensu lato in the ticks was significantly higher for all three biotopes, whereas a similar pattern of habitat-specific infection prevalence was observed. Genotyping of *B. burgdorferi* sensu lato revealed high relative prevalences of *B. valaisiana* (identified in 43.1% of infected ticks) and *B. garinii* (32.3%), whereas *B. afzelii* (12.3%) and *B. burgdorferi* sensu stricto (1.5%) were relatively rare. We conclude that *B. burgdorferi* sensu lato infection has increased in this region over the last 15 years due to presently unknown changes in ecological conditions, perhaps related to climate change or wildlife management.

Borrelia burgdorferi sensu lato is a bacterial species complex of at least 13 genospecies and genomic groups (9) that is widely distributed in the northern hemisphere (14). The complex contains the etiologic agents of Lyme borreliosis, a multisystemic disease comprising dermatological, neurological, cardiac, and arthritic symptoms (43). The spirochetes are primarily transmitted by ticks of the *Ixodes ricinus/persulcatus* species complex (29, 42). According to present knowledge, only infections with *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and, perhaps, *B. bissettii* cause clinical symptoms in humans. The latter genospecies, formerly genomic group DN127 (36), has been isolated from various tissues of patients with diffuse clinical manifestations (35, 45).

In Europe, six genospecies of *B. burgdorferi* sensu lato have been recorded (9, 39, 44): *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. lusitaniae*, and *B. bissettii*. *B. afzelii*, *B. garinii*, and *B. valaisiana* are the most prevalent genospecies in central and western Europe, whereas *B. burgdorferi* sensu stricto, *B. lusitaniae*, and *B. bissettii* seem to be rare. In contrast, *B. burgdorferi* sensu stricto is by far the most common genospecies in North America, whereas in Asia only *B. afzelii* and *B. garinii* occur (43). In Germany, neither *B. lusitaniae* nor *B. bissettii* has so far been detected, but the other four genospecies were found at relative frequencies typical for Europe (9).

In contrast to the United States, a national reporting and surveillance system for human infection with *B. burgdorferi* sensu lato is lacking in most European countries. The number of estimated and reported cases of Lyme borreliosis varies depending on the country, province, or study area. In central and eastern Europe, annual incidence estimations are mostly below 40 cases per 100,000 inhabitants, but for Austria, for example, an average rate of about 300 per 100,000 inhabitants is given (unpublished document WHO/CDS/VPH/95.141, World Health Organization workshop on Lyme borreliosis diagnosis and surveillance, Warsaw, Poland, 20 to 22 June 1995). For Germany, an annual incidence of 50 to 100 cases per 100,000 citizens is predicted, totaling some 40,000 to 80,000 cases of Lyme borreliosis per year (32).

The infection prevalence of *B. burgdorferi* sensu lato in natural, host-seeking tick populations in Europe varies between 0 and 11% (mean, 1.9%) for larvae, 2 and 43% (mean, 10.8%) for nymphs, and 3 and 58% (mean, 17.4%) for adults (10, 16). In Germany, the values are approximately 1% for larvae, 4 to 18% for nymphs, and 10 to nearly 35% for adults (3; reviewed in reference 10).

The aims of the present study were (i) to assess whether the infection prevalence of *B. burgdorferi* sensu lato in a region of endemic Lyme borreliosis in western Germany has remained stable over the last decade, (ii) to obtain data on the distribution of the *B. burgdorferi* genospecies, and (iii) to compare different techniques for their sensitivity in detecting *B. burgdorferi* sensu lato in questing ticks.

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MATERIALS AND METHODS

Tick collection and study area. Ticks were collected in May and from August to October 2001 in three selected sylvatic ecosystems of the Siebengebirge near Bonn, Germany (50°7′N, 7°6′E). These ecosystems represent the plant commu-

nities *Fraxino-Aceretum pseudoplatani*, *Luzulo-Fagetum milietosum* (humid wing), and *Melico-Fagetum typicum* (dry wing) (see reference 25 for phytosociological details). About 10 years before this study, pronounced differences in tick abundance and infection prevalences of *B. burgdorferi* sensu lato had been found at these sites (25): the *Melico-Fagetum* biotope was characterized by low tick density and low infection prevalence, the *Luzulo-Fagetum* biotope was characterized by high tick density and a relatively high infection prevalence, and the *Fraxino-Aceretum* biotope was characterized by an intermediate tick density but the highest infection prevalence.

In this follow-up study, questing nymphs and adults were collected by blanket dragging once a week as described previously (25). Altogether, 1,754 ticks were collected, 1,549 nymphs and 205 adults (100 females and 105 males); 708 of them (658 nymphs, 20 females, and 30 males) were sampled in the *Luzulo-Fagetum* biotope, 635 (573 nymphs, 37 females, and 25 males) were sampled in the *Melico-Fagetum* biotope, and 411 (318 nymphs, 43 females, and 50 males) were sampled in the *Fraxino-Aceretum* biotope.

In the laboratory, ticks were identified to the species level (8) and cut longitudinally into two similar halves, which made it possible to analyze an individual tick by both the immunofluorescence assay (IFA) and PCR. One half was smeared onto a single field of a multitest slide in a small drop of phosphate-buffered saline (PBS) solution (PBS: 8.5 g of NaCl, 0.9 g of Na_2HPO_4, and 0.2 g of KH_2PO_4 per liter, pH 7.2). Chitinous parts and larger tissue particles were removed afterwards. The slides were air dried and fixed in ice-cold acetone for 10 min. They were wrapped in aluminum foil, hermetically sealed in plastic wrap, and stored at −20°C until examination. The other half from the bisection was added to 100 μl of 1.25% ammonia solution for the preparation of DNA.

IFA. Slides kept at −20°C were warmed to room temperature. Each antigen field was loaded with 25 μl of an anti-*B. burgdorferi* sensu lato-reactive human blood serum (immunoglobulin G [IgG] titer, 1:1,280; see reference 13 for serum source and further details) diluted 100-fold in PBS, which had shown good results for the *B. burgdorferi* sensu stricto antigen of strain B31 (Stellar Biosystems Inc.) in preliminary experiments. After incubation at 37°C for 45 min in a humid chamber, the slides were washed three times for 5 min each in PBS and air dried; 25 μl of fluorescein isothiocyanate-conjugated anti-human IgG (Fluoline G; bioMérieux) was then pipetted onto the fields. An optimal antibody dilution of 1:100 in a mixture of PBS and Evan's blue (95:4) was used. The slides were incubated again at 37°C for 45 min and washed in the dark. After drying, the slides were covered with Fluoprep (bioMérieux) and analyzed with a fluorescence microscope. As positive controls, slides coated with *B. burgdorferi* sensu stricto antigen were used (Stellar Biosystems Inc.) as well as smashed *Ixodes ricinus* nymphs that had fed in the laboratory on *B. afzelii*-positive gerbils (*Meriones unguiculatus*). *I. ricinus* nymphs from laboratory-reared tick colonies that had fed on *Borrelia*-negative white mice as larvae served as negative controls.

***B. burgdorferi* PCR.** DNA from the longitudinal halves of the ticks was prepared by tissue homogenization and boiling in 1.25% ammonia solution (4). Three different PCR techniques were applied for borrelial detection with 3 to 5 μl of the supernatant to compare nested and simple PCR approaches. A simple PCR technique for rapid screening of the ticks (30) was performed on 545 DNA samples, whereas a nested PCR (37) was conducted on 395 of the same samples for successive genotyping of the borreliae (37). In the case of contradictory results, the amplifications were repeated, and a third PCR protocol (41) was used. The nested PCR protocol of Rijpkema et al. (37) was performed as originally described, while the simple PCR protocol of Liebisch et al. (30) was amended as follows. Each reaction mixture of a total volume of 50 μl contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, a 200 μM concentration of each of the four deoxynucleoside triphosphates, 400 nM (each) primers P1 and P2, 1.5 mM $MgCl_2$, and 2.5 U of *Taq* DNA polymerase (Invitrogen). DNA amplification consisted of 40 cycles of 94°C for 1.5 min, 64°C for 2 min, and 72°C for 2 min. The third PCR was a modification of a protocol described by Schwartz et al. (41). The reaction mixtures (50 μl) contained 18 mM Tris-HCl, pH 8.3, 50 mM KCl, a 200 μM concentration of each of the four deoxynucleoside triphosphates, 500 nM (each) primers JS1 and JS2, 2.5 mM $MgCl_2$, and 1.5 U of *Taq* DNA polymerase. The thermoprofile included an initial denaturation step at 94°C for 1.5 min, 45 cycles of 94°C for 20 s, 51°C for 30 s, and 72°C for 30 s, and a final extension step at 72°C for 2 min. As positive control, 1 μl of a DNA extract prepared from a *B. afzelii* culture was used. Based on the DNA quantity, the equivalent of three bacteria was used in the positive control reactions. In preliminary experiments, with all three PCR protocols at least three *Borrelia* genomic equivalents could be detected. There, the positive-control DNA extracts were produced from noninfected *I. ricinus* nymphs supplemented with a defined number of *B. afzelii* cells from a culture in a way that again provided three *Borrelia* genomic equivalents in the volume of DNA solution added to the PCR mixture. For negative controls, only distilled water was added. PCR products were electrophoresed for about 1 h at 120 V through 1.5% agarose gels and stained with ethidium bromide (0.5 μg/ml).

***B. burgdorferi* genospecies determination by reverse line blotting.** PCR products obtained by nested PCR (37) were processed by a reverse line blot assay (26, 37). Oligonucleotide probes specific for four genospecies of the *B. burgdorferi* complex (*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana*) as well as a complex-specific probe (*B. burgdorferi* sensu lato) were linked to a membrane in order to hybridize to the complementary amplicon sequences. Hybridization was visualized with a streptavidin-peroxidase conjugate and enhanced chemiluminescence (ECL detection system; Amersham).

Statistical analysis. Differences in tick infection prevalences between sexes and localities and depending on the detection technique applied were analyzed statistically by means of a nonparametric chi-square test and *t* test for paired samples.

RESULTS

All 1,754 ticks collected were identified as *I. ricinus*. From the three collection areas, a total of 545 randomly selected nymphs and adults of both sexes were screened for *B. burgdorferi* sensu lato either by PCR only or by PCR and IFA. The overall average infection prevalence for combined results obtained with all the different techniques was 14% (Table 1); 5.5% of the ticks were positive in the *Melico-Fagetum* biotope (formerly characterized by low tick density and low infection prevalence), 15.8% were positive in the *Luzulo-Fagetum* biotope (formerly characterized by high tick density and relatively high infection prevalence), and 21.8% were positive in the *Fraxino-Aceretum* biotope (formerly characterized by intermediate tick density but the highest infection prevalence). The infection prevalences of *B. burgdorferi* sensu lato in nymphal and adult ticks calculated across all biotopes amounted to 12.9 and 21.1%, respectively (Table 1). Female ticks were more frequently infected (20.6%) than males (13.6%) ($\chi^2 = 10.563$; df = 1; $P = 0.001$).

All 545 ticks were examined by the simple PCR protocol described by Liebisch et al. (30), which gave 65 positive results (11.9%; Fig. 1); 374 of these ticks were also analyzed by IFA, resulting in a prevalence of 14.4% (54 positive results). Using the nested PCR of Rijpkema et al. (37), 65 out of 395 analyzed ticks were positive (16.5%). The results obtained by the two PCR assays for the same samples were not concordant in 22 cases (5.6%; i.e., 17 ticks which were also examined by IFA and five ticks tested by only the two PCR techniques). A comparison of 374 ticks for infection with *B. burgdorferi* sensu lato by IFA and PCR gave the following results: IFA positive, nested PCR positive, and simple PCR positive, 30 ticks; IFA positive, nested PCR positive, and simple PCR negative, 5 ticks; IFA positive, nested PCR negative, and simple PCR positive, 7 ticks; IFA positive, nested PCR negative, and simple PCR negative, 12 ticks (for a total of 24 ticks positive by IFA but negative by one or both PCRs); IFA negative, nested PCR positive, and simple PCR negative, 4 ticks; IFA negative, nested PCR negative, and simple PCR positive, 1 tick; IFA negative, nested PCR positive, and simple PCR positive, 4 ticks (for a total of 9 ticks negative by IFA but positive in one or both PCRs); and IFA negative, nested PCR negative, and simple PCR negative, 311 ticks. The IFA results differed in 27 cases (7.2%) from the nested PCR results and in 22 cases (5.9%) from the simple PCR results (Fig. 1). In 12 cases (3.2%), both PCR assays detected no *B. burgdorferi* sensu lato,

TABLE 1. Detection of *B. burgdorferi* sensu lato in *I. ricinus* nymphs and adults from three areas of the Siebengebirge[a]

Biotope studied (no. of ticks)	Stage	Total no. of ticks examined (n = 545)	No. of ticks positive for infection (no. tested)				% Positive	Overall infection rate,[d] % (no. positive/no. tested)	No. of ticks positive by simple PCR[e] (no. tested) (n = 37)
			Nested PCR[a] (n = 395)	Simple PCR[b] (n = 545)	IFA (n = 374)	Total[c] (n = 1314)			
Fraxino-Aceretum (172)	Nymphs	102	19 (102)	17 (102)	22 (101)	58 (305)	19	21.8 (89/409)	5 (9)
	Adults	70	14 (20)	13 (70)	4 (14)	31 (104)	29.8		3 (3)
Luzulo-Fagetum (181)	Nymphs	131	20 (117)	17 (131)	19 (113)	56 (361)	15.5	15.8 (69/436)	6 (8)
	Adults	50	4 (14)	7 (50)	2 (11)	13 (75)	17.3		
Melico-Fagetum (192)	Nymphs	133	5 (130)	5 (133)	6 (129)	16 (392)	4.1	5.5 (26/469)	2 (2)
	Adults	59	3 (12)	6 (59)	1 (6)	10 (77)	13		
Total (545)	Nymphs	366	44 (349)	39 (366)	47 (343)	130 (1,058)	12.9	14 (184/1314)	13 (20)
	Adults	179	21 (46)	26 (179)	7 (31)	54 (256)	21.1		3 (3)

[a] Performed as described previously (37).
[b] Performed as described previously (30).
[c] Theoretical values calculated by adding the numbers of ticks examined by different methods.
[d] Absolute values are theoretical, calculated by adding the numbers of ticks examined by different methods.
[e] Simple PCR (41); not considered in the percent positive due to nonrandom preselection of samples.

while the IFA was positive. Both PCR assays were positive and the IFA was negative in only four cases (1.1%).

By using the third PCR protocol of Schwartz et al. (41) on the 22 samples with discordant results in the other two PCRs, 16 ticks tested positive and 6 ticks tested negative for *Borrelia* infection. In 19 cases the nested PCR result was confirmed, and in three cases the result of the simple PCR described by Liebisch et al. was confirmed (30). Considering these figures (i.e., ticks are only considered positive if they either were positive when only one test was performed [n = 150] or were positive in at least two tests when two or three techniques were applied [n = 395]), an overall PCR-based infection prevalence of 13.2% (72 of 545) was calculated. With the same algorithm, 19.2% of the ticks from the *Fraxino-Aceretum* biotope (33 of 172), 14.9% from the *Luzulo-Fagetum* biotope (27 of 181), and 6.2% from the *Melico-Fagetum* biotope (12 of 192) were PCR positive. By means of the IFA, spirochetes were detected in 14.4% (54 of 374) of the ticks (nymphs and adults taken together), with 22.6% (26 of 115) of the ticks from the *Fraxino-Aceretum* biotope (21.8% of the nymphs), 16.9% (21 of 124) from the *Luzulo-Fagetum* biotope (16.8% of the nymphs), and 5.2% (7 of 135) from the *Melico-Fagetum* biotope (4.7% of the nymphs) being positive (Table 1 and Fig. 1).

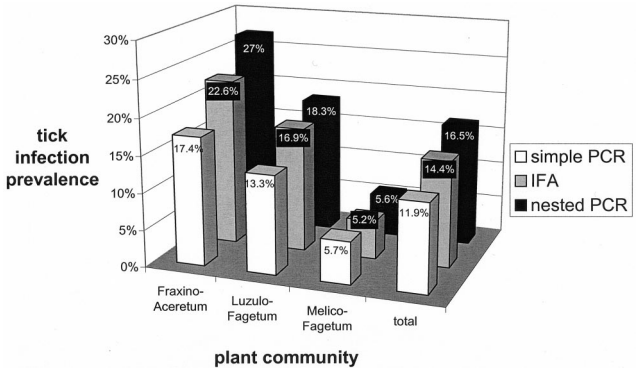

FIG. 1. Average *B. burgdorferi* sensu lato prevalences in nymphs and adults according to the detection system applied, IFA, nested PCR (37), or simple PCR (30).

If all positive test results are regarded as truly positive and all negative results as wrongly negative as long as another test on the same specimen was positive, an average infection prevalence of 16.7% (91 of 545) would result. In contrast, a significantly different infection prevalence of 9.5% (52 of 545) can be calculated if only ticks that were positive in all tests applied to them are considered positive (t = −3,291; df = 544; P = 0.001).

Genotyping of 65 *Borrelia*-positive nested PCR samples with the reverse line blot assay revealed that 28 infected ticks harbored *B. valaisiana*, 21 ticks harbored *B. garinii* and 8 ticks harbored *B. afzelii* (Table 2). Double infections were found in eight ticks, one adult and four nymphs with *B. garinii* and *B. valaisiana* combinations and one nymph each with *B. garinii* and *B. afzelii*, *B. afzelii* and *B. valaisiana*, and *B. afzelii* and *B. burgdorferi* sensu stricto. Another four PCR products reacted only with the complex-specific *B. burgdorferi* sensu lato DNA probe, and 11 samples did not show any hybridization at all. Interestingly, 17 (51.5%) of the 33 infected ticks from the *Fraxino-Aceretum* biotope were infected with *B. valaisiana*, which is equal to 60.7% of all *B. valaisiana* infections (17 of 28) found in this study.

DISCUSSION

The present study obtained two major epidemiological results: first, the average infection prevalence of *B. burgdorferi* sensu lato in questing *I. ricinus* ticks has increased considerably in the Siebengebirge over the last decade, and second, an unusually high infection prevalence was determined for *B. valaisiana*. Furthermore, it became obvious that different detection techniques may produce divergent epidemiological data.

Although at present PCR is usually the method of choice for *Borrelia* detection in ticks, in this study, apart from PCR, it was necessary to use an immunofluorescence technique to allow a

comparison of actual tick infection prevalences with data collected earlier. In fact, the results obtained in the present study show that the data may vary depending on the detection technique applied. With the exception of the *Melico-Fagetum* biotope (Fig. 1), the infection prevalences determined by IFA were lower than those obtained by the nested PCR but higher than those obtained by the simple PCR.

Nested PCR approaches are generally considered more sensitive than simple PCR approaches, but in practice they are doubtlessly more susceptible to contamination and may produce more false-positive results than a simple PCR. The detection of larger numbers of *Borrelia*-infected ticks by IFA than by PCR is a well-known phenomenon that was documented in a multilaboratory study (17). There, intralaboratory concordance between the two techniques ranged from 40 to 100%; in our study, the concordance between the IFA and the nested PCR was 92.8% and that between the IFA and the simple PCR according to Liebisch et al. was 94.1% (30). The concordance between two different (not specified) PCR approaches was 87.5% in the former study and 94.4% in ours. Kahl et al. (17) also mentioned that the IFA produced positive results with several samples that were negative in the PCR, but not a single case of samples that were negative in the IFA but positive in the PCR was observed. In our study, the majority of discordant results also belonged to the category of IFA-positive, PCR-negative results, but several samples with negative IFA and positive PCR results were registered as well (see Results).

False-positive IFA results can sometimes be traced back to misidentification of single *Borrelia*-like structures showing fluorescence (17). False-negative PCR results, on the other hand, can be explained by inhibition of DNA amplification (40), which may be a particular problem with engorged ticks due to the presence of hemoglobin (7) but which also occurs with unfed ticks because of small amounts of heme that are retained during tick molt (15). Inhibitory substances are also implicated by Wittenbrink et al. (48) with the lack of DNA amplification from tick midgut homogenates that were considered *Borrelia* positive. In comparison with dark-field microscopy, PCR sensitivity was only 66.7% in their study; i.e., a third of the samples that were positive by dark-field microscopy were not detected by PCR. On the other hand, *B. burgdorferi* sensu lato DNA was amplified from nine tick midguts that were regarded as negative by dark-field microscopy and from which no spirochetes could be cultured. As a reason for this, the authors suggest a limited distribution of borrelial cells in the tick midgut. Such a limited or uneven distribution, especially important when only a few borreliae are present, may also have led to the discrepant results in our study, particularly as the ticks' midguts were not dissected. Instead, the ticks were cut lengthwise, which does not guarantee a well-balanced distribution of organs (e.g., midgut and salivary glands).

In the study presented here, large numbers of borrelial cells were observed by fluorescence microscopy in some cases (up to 60 spirochetes per visual field), while no *Borrelia* DNA could be amplified from the corresponding tick halves by PCR even after further dilution of the DNA solution. To test for the presence of PCR inhibitors in these samples, *B. afzelii* DNA, which was used to construct the positive controls, was added to the tick DNA extracts, but still not all of these yielded PCR products.

TABLE 2. Genospecies distribution determined by reverse line blotting on nested PCR products[a]

Biotope studied	No. (%) of infections									
	B. garinii	*B. afzelii*	*B. valaisiana*	*B. garinii*/ *B. afzelii*	*B. garinii*/ *B. valaisiana*	*B. afzelii*/ *B. valaisiana*	*B. burgdorferi* sensu stricto/ *B. afzelii*	*B. burgdorferi* sensu lato	Untypeable	Total
Fraxino-Aceretum	5 (15.2)	3 (9.1)	14 (42.4)		2 (6.1)	1 (3.0)	1 (3.0)	1 (3.0)	6 (18.2)	33 (100) (14 A, 19 N)
Luzulo-Fagetum	8 (33.3)		7 (29.2)		3 (12.5)			2 (8.3)	4 (16.7)	24 (100) (4 A, 20 N)
Melico-Fagetum	2 (25)	2 (25)	1 (12.5)	1 (12.5)				1 (12.5)	1 (12.5)	8 (100) (3 A, 5 N)
Total	15 (23.1) (5 A, 10 N)	5 (7.7) (2 A, 3 N)	22 (33.8) (9 A, 13 N)	1 (1.5) (N)	5 (7.7) (1 A, 4 N)	1 (1.5) (N)	1 (1.5) (N)	4 (6.2) (3 A, 1 N)	11 (16.9) (1 A, 10 N)	65 (100) (21 A, 44 N)

[a] A, adults; N, nymphs.

About 10 years before the present study, the small-scale distribution of *I. ricinus* ticks and the zoonotic cycle of *B. burgdorferi* sensu lato were studied in the Siebengebirge (24, 25). It was found that tick density correlated mainly with soil acidity and close-to-the-ground humidity, two parameters for which certain plant species are reliable indicators. Furthermore, tick density was shown to be an important factor influencing the infection prevalence of *B. burgdorferi* sensu lato in ticks, which had been determined by using the same IFA protocol as in the present study. In general, a positive correlation between tick density and infection prevalence was observed, and it has been suggested that this correlation may be a result of increasing contacts between the vectors of the pathogen and the reservoir hosts.

The three biotopes analyzed in the present study had previously been characterized by distinct ecoepidemiological patterns of Lyme borreliosis spanning the ecological spectrum of the region (25): (i) a biotope with a very high tick density and a high *Borrelia* infection prevalence (the *Luzulo-Fagetum* biotope), (ii) a biotope with a low tick density and a low infection prevalence (the *Melico-Fagetum* biotope), and (iii) a biotope with an intermediate tick density but an unusually high prevalence of *B. burgdorferi* sensu lato infection in these ticks (the *Fraxino-Aceretum* biotope). It has been suggested that the epidemiological pattern in the last site is determined by the particular structure of the rodent cenosis, with a dominance of bank voles (*Clethrionomys glareolus*) and only few mice (25). It is known that European voles and mice differ in their role as reservoir hosts for *B. burgdorferi* sensu lato. Infected voles transmit spirochetes to ticks much more efficiently than mice do, but voles (and not mice) may acquire resistance to *I. ricinus* ticks in a density-dependent way, which in turn reduces the survival (molting) rates of the ticks feeding on them (2, 23, 25).

Of the questing nymphs and adults collected in the Siebengebirge and examined by IFA, 5.5% were found to be infected with *B. burgdorferi* sensu lato at the end of the 1980s (19, 24, 25). In the present study, the infection prevalence of *B. burgdorferi* sensu lato in nymphs and adults was 2.5-fold higher than in the earlier study. A direct comparison of the infection prevalences in questing nymphs collected then and now (Fig. 2) shows a significant increase in infection prevalence in all three biotopes (*Luzulo-Fagetum* biotope, $\chi^2 = 261.689$, df = 1, $P < 0.001$; *Melico-Fagetum* biotope, $\chi^2 = 181.175$, df = 1, $P < 0.001$; *Fraxino-Aceretum* biotope, $\chi^2 = 270.222$, df = 1, $P < 0.001$). The nymphal stage is considered the most important vector of *B. burgdorferi* sensu lato because of its high relative abundance in combination with an often substantial infection prevalence (14, 33). Therefore, an increase in the spirochetal infection prevalence in nymphs of up to fourfold (*Melico-Fagetum* biotope, 1.1% in 1988 and 4.7% in 2001; Fig. 2) suggests that the risk of Lyme borreliosis has risen substantially over the last decade in this region of Germany.

The reasons for the increase in infection prevalence remain obscure, but it is possible that an increase in the abundance of ticks may have caused a rise in the prevalence of the spirochetes. In fact, the tick populations appear to have expanded during the past few years in the Siebengebirge (unpublished observations), although exact data are presently not available. According to Rogers et al. (38), an increase in tick density may be caused both by better host availability, especially for adult

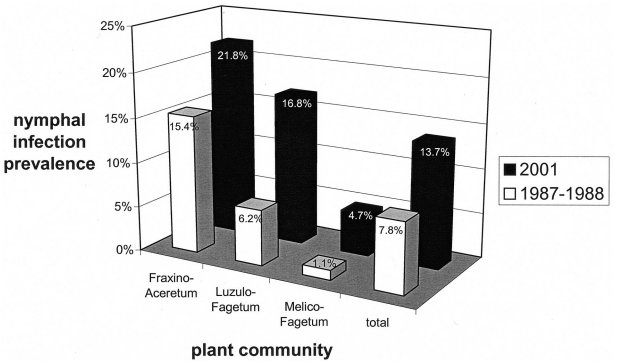

FIG. 2. Mean prevalences of infection of questing nymphs with *B. burgdorferi* sensu lato in three plant communities of the Siebengebirge as determined by IFA in 1987 to 1988 (25) and 2001. For 1987 to 1988, the total average percentage is based on the examination of 1,480 nymphs, and for 2001 the total average percentage is based on 343 nymphs.

ticks, and by climatic change. The relatively mild winters of the 1990s, for example, that led to longer seasonal tick activity periods and higher tick survival rates, are considered responsible for an increase in *I. ricinus* tick densities in Sweden (31). Whether the putative rise in the abundance of ticks in the Siebengebirge was also caused by climatic factors and/or by host availability remains to be analyzed. The vertebrate host cenosis is affected not only by changing agricultural and wildlife management but also by climatic changes (38). Micromammals, which represent the most important hosts for larvae and nymphs in deciduous and mixed woodlands, various species of birds, and even larger adult tick hosts such as roe deer and wild boar are favored by a milder climate. As far as wild boar are concerned, a tremendous rise in numbers has been recognized in the area around Bonn, including the Siebengebirge, over the last decades (1).

The finding that the overall spirochete infection prevalence has increased in all three biotopes indicates that the ecological conditions which favor the circulation of *B. burgdorferi* sensu lato must have changed across the entire region. However, the stable geographical pattern of the relative prevalence of *B. burgdorferi* sensu lato in the three biotopes suggests the operation of habitat-specific factors, such as the particular composition of the vertebrate community that is available to larvae of *I. ricinus*.

The detection of *B. garinii*, *B. afzelii*, *B. burgdorferi* sensu stricto, and *B. valaisiana* in the Siebengebirge is consistent with the results of other studies conducted in Germany (9, 21, 39). However, while the relative frequencies of the first three genospecies do not differ significantly from those determined previously (21), the high prevalence of *B. valaisiana* found in this study is quite unusual for terrestrial habitats in continental Europe (6). When double infections were regarded as two separate infections in the analysis, 38.4% of all *Borrelia* infections ($n = 73$, i.e., 65 plus another 8 for doubly infected ticks) could be assigned to *B. valaisiana*. Results obtained by Kirstein et al. (18), Humair et al. (11), Kurtenbach et al. (28), and Hanincová et al. (5) strongly suggest avian reservoirs for *B. valaisiana.* The *Fraxino-Aceretum* biotope, where more than 60% of all *B. valaisiana* tick infections were found, actually

appears to be an area more frequented by passerine birds than the other areas involved in the study.

According to Kurtenbach et al. (20), more than four times as many nymphs as larvae of *I. ricinus* feed on pheasants, resulting in higher infection prevalences of *B. valaisiana* infections in questing adults than in questing nymphs. In contrast, more larvae than nymphs feed on smaller passerine birds, which may be the predominant avian hosts in this study (12, 46; unpublished observations). A relatively high abundance of passerine birds in this study is likely to explain the high infection prevalence of *B. valaisiana* in questing nymphs. The substantially reduced infection prevalence of this genospecies in questing adult ticks may be due to the particular hosts of the different tick developmental stages. Probably, a considerable proportion of the nymphs that are infected with *B. valaisiana*, particularly in the *Fraxino-Aceretum* biotope, feed on hosts that eliminate this genospecies during the blood meal, such as rodents and large ungulates. In fact, strong experimental and epidemiological evidence suggests that the uptake of rodent blood triggers the elimination of *B. valaisiana* and *B. garinii* but not of *B. afzelii* in feeding ticks (5, 6, 22, 27, 28). Ungulates, such as deer, appear to reduce the infection prevalence of all genospecies in ticks that feed on them, a pattern that is consistent with the in vitro observation that deer complement lyses all genospecies of *B. burgdorferi* sensu lato (28, 34, 47).

This study indicates that the prevalence of *B. burgdorferi* sensu lato in the Siebengebirge has increased substantially over the last decade and that this finding is unlikely to be a technical artifact. Rather, it is probable that ecological changes in this region which favor the circulation of *B. burgdorferi* sensu lato have occurred, such as an expansion of the tick populations caused by increased availability of tick hosts.

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