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Relationships of a Novel Lyme Disease Spirochete, *Borrelia spielmani* sp. nov., with Its Hosts in Central Europe

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To determine whether the pathogenic variant of Lyme disease spirochetes, isolate A14S, is perpetuated in a particular reservoir-vector relationship, we screened vector ticks in various Central European sites for a related spirochete and determined its host association. A14S-like spirochetes infect numerous questing ticks in the Petite Camargue Alsacienne (PC). They frequently infect dormice, but no mice or voles. Garden dormice appear to be better reservoir hosts for A14S-like spirochetes than for *Borrelia afzelii*, because these spirochetes are retained longer and infect ticks more readily. Spirochetes associated with garden dormice in the PC site form a homologous entity with those isolated from a human patient in The Netherlands. Its unique biological relationship together with previous genetic characterization justifies designating this dormouse-associated genospecies as a distinct entity. Garden dormice serve as the main reservoir hosts of a novel genospecies, *Borrelia spielmani* sp. nov., one of several that cause Lyme disease in people.

At least four Lyme disease genospecies, *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia bissettii*, cause human disease in Central Europe (18, 25). A fifth pathogenic variant, designated A14S, has been isolated from a Dutch patient by culturing spirochetes from an erythema migrans lesion (28), and similar spirochetes have recently been identified in a German patient with chronic skin disease (15). The reactivity with monoclonal antibodies and the protein profile of this spirochetal variant are unique (26, 28). Sequence analysis of the *ospA*, *ospC*, 16S rRNA, and flagellin genes as well as the pattern of the restriction fragment length polymorphism of the 5S-23S rRNA intergenic spacer and of the *ospA* gene, as well as randomly amplified polymorphic DNA fingerprinting and single-strand conformation polymorphism, distinguish this isolate from other Lyme disease genospecies and justify its placement in a new genomic group (3, 15, 28, 29). This spirochete, which has been detected at least twice in patients, differs from other Lyme disease spirochetes.

Because it has been detected until now only in two human hosts and three questing ticks, the reservoir relationships of the A14S pathogen remain entirely unknown. Certain Lyme disease genospecies appear to be associated with particular kinds of reservoir hosts. Because vector ticks feeding on captured rodents frequently harbor *B. afzelii*, and because *B. garinii* and *Borrelia valaisiana* frequently are detected in bird-feeding ticks, *B. afzelii* appears to be rodent adapted and *B. garinii* and *B. valaisiana* appear to be bird adapted (9). Other observations, however, suggest that *B. afzelii* and *B. garinii* share common reservoir hosts (16, 22). Experimental evidence of differential reservoir competence might resolve this question definitively. Complement-mediated lysis appears to inhibit spirochete development in nonpermissive hosts (11). These observations led to the hypothesis of ecologic differentiation of Lyme disease spirochetes; i.e., if a spirochete variant were to comprise a distinct genospecies, it would be perpetuated in a cycle that is distinct from that of other genospecies (9). The reservoir relationships and abundance of the recently described A14S isolate have not yet been determined.

It may be that A14S spirochetes are perpetuated mainly in a peculiar reservoir relationship. Accordingly, we determined whether related spirochetes may infect vector ticks in various sites in Central Europe, chosen because diverse rodents would be present there. Where such spirochete variants proved to be most prevalent in vector ticks, we determined whether some particular rodent was infected disproportionately.

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MATERIALS AND METHODS

Our main study site was located in the Petite Camargue Alsacienne (PC), a forested region in France within the flood plain of the Rhein River, near the German and Swiss borders. In addition, we sampled questing ticks in a second French site near the town of Lembach, situated about 140 km north of PC, and in three German sites, near Maikammer and near Eberdingen, situated 40 km northeast and 90 km east of Lembach, respectively, as well as a site near Gröben, 15 km south of Berlin.

To determine whether rodents become infected by Lyme disease spirochetes, they were captured in live traps (Longworth Scientific Instruments, Abingdon, United Kingdom) baited with apple, grain, and cotton. A total of 60 traps were placed in the PC site during four successive nights each month from April through October 1995 (12). In the year 2000, 67 traps were set for three successive nights during the months of May, June, August, and October. Captured small mammals were taken to the laboratory, where they were weighed and identified. They were caged over water until all naturally attached ticks had detached. The water was inspected twice daily, and ticks were promptly removed, counted, and identified. After subsequent xenodiagnosis, the animals were released at the point of capture. Questing ticks were collected at the PC site during May, June, August, and October 2000 and in all five sites during April of 2001 by means of a flannel flag. They were confined in screened vials and stored at 10 ± 1°C until they were identified as to stage and species and examined for spirochetes.

Ticks used for xenodiagnosis were derived from laboratory-bred adult *Ixodes ricinus* ticks. Subadult and adult ticks were reared by feeding them on spirochete-free jirds, *Meriones unguiculatus*, and on rabbits, *Oryctolagus cuniculus*, respec-

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tively. These ticks were in their third generation of continuous laboratory rearing and had never been exposed to infected hosts. A portion of each larval cohort was routinely examined for the presence of spirochetes by dark-field microscopy and also by feeding them on mice. For xenodiagnosis, about 50 laboratory-reared larval ticks were brushed onto each rodent. Infested hosts were enclosed in wire mesh tubes that were wrapped in absorbent paper for 2 to 3 h. The rodents were subsequently removed and caged over water; the water was inspected twice daily, and replete ticks were removed. After detachment, engorged ticks (both laboratory reared and those naturally attached) were enclosed in screened vials and kept at 20 ± 2°C in sealed desiccator jars over supersaturated $MgSO_4$ under a light-dark (16 h:8 h) regime. Molted ticks remained in the original vials until they were tested for the presence of spirochetes by inoculating their dissected midguts into culture medium or preserved in ethanol for subsequent analysis by PCR.

To determine whether Lyme disease spirochetes infected ticks and to discriminate between certain genospecies, ticks were dissected and their midguts were tested for spirochete DNA by PCR. The body of a tick was opened, and the contained mass of soft tissue was dissected in physiologic saline and transferred to a tube containing 180 μl of lysis buffer (ATL tissue lysis buffer; QIAGEN, Hilden, Germany) and 20 μl of proteinase K (600 mAU/mg). Midguts were lysed at 56°C overnight. DNA was extracted by using the QIAamp DNA Mini kit (QIAGEN) according to the manufacturer's instructions, eluted with 50 μl of elution buffer, and stored at −20°C until PCR was performed. The presence of DNA of Lyme disease spirochetes was determined by amplifying a 400-bp segment of the gene encoding outer surface protein A (OspA), positions 191 through 589. To increase sensitivity for detecting spirochetal DNA in ticks, we used nested PCR. Aliquots of DNA suspensions (2 μl) were diluted to 50 μl by using a 200 μM concentration of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, 1.0 U of *Taq* polymerase (QIAGEN), 10 pmol of the outer primer pair, and PCR buffer supplied with the *Taq* polymerase. We used the following sequences of the OspA gene as outer primers (5): (5′ to 3′) 1A, GGTCTAAT ATTAGCCTTAATAGCATG; and 1B, TCAGCAGCTAGAGTTCCTTCAAG. The mixture was placed in a thermocycler (PTC 200; MJResearch, Biozym, Hess. Oldendorf, Germany), heated for 1 min at 94°C, and subjected to 40 cycles of 20-s denaturation at 94°C, a 20-s annealing reaction at 59°C, and a 40-s extension at 72°C, with a final extension for 2 min at 72°C. After the first amplification with the outer set of primers, 2 μl of the amplification product was transferred to a fresh tube containing 48 μl of the reaction mixture described above, except that 10 pmol of the inner primer pair was used (5′ to 3′: 2A, CATGTAAGCAAAA TGTTAGCAGCC; and 2B, CTGTGTATTCAAGTCTGGTTCC). This mixture was subjected to 40 amplification cycles using the conditions described above, except that the annealing reaction was performed at 60°C and the extension reaction lasted 20 s. DNA was extracted, reaction vials were prepared for amplification, templates were added, and electrophoresis was performed in separate rooms. In each fifth reaction mix, water was added instead of extracted DNA to serve as a negative control. PCR products were detected by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

Each PCR amplification product was purified by using a QIAquick-Spin PCR column (QIAGEN) according to the manufacturer's instructions. Amplified DNA fragments were directly sequenced in both directions, using the inner primers by the dideoxynucleotide chain-termination method on a Licor DNA4200 sequencer (Licor Biosciences, Bad Homburg, Germany). Each resulting sequence was compared by means of the AlignIR 1.1 software (Licor Biosciences) to sequences of the same gene fragment representing various spirochetal genospecies: *B. burgdorferi* sensu stricto (EMBL accession no. X80182), *B. afzelii* (EMBL accession no. X80185), various serotypes of *B. garinii* (GenBank accession no. S48323, X80256, X80257, X80186, X85441, X80251, X80252, and X80254), *B. valaisiana* (accession no. AB016979 [DDBJ] and AF095943 [GenBank]), and strain A14S (GenBank accession no. AF102057). Except for the somewhat heterogeneous serotypes constituting *B. garinii*, a complete match permitting no more than two nucleotide changes was required. Infections by more than one genospecies were recognized in the sequencing gel when simultaneous bands occurred at all signature nucleotide positions that differentiate the two coinfecting genospecies from each other.

Spirochetes were cultured from unfed nymphal ticks. Ticks were washed individually for 3 min each in distilled water, in 100% ethanol and 1% benzalkonium chloride. After three washes in 0.9% NaCl, ticks were placed in Barbour-Stoenner-Kelly-H medium (16a) (Sigma, Deisenhofen, Germany) and dissected by using sterile forceps. Each midgut was transferred to 1 ml of Barbour-Stoenner-Kelly-H medium supplemented with 6% rabbit serum (Sigma) and amphotericin (2.5 μg/ml) (Sigma). Suspensions were incubated at 33°C and checked for spirochetal growth every third day by means of dark-field microscopy. Dense cultures of spirochetes were frozen and stored at −70°C.

To describe long sequences of spirochete DNA, a 1,200-bp fragment of the 16S rRNA gene, an 800-bp fragment of the *ospA* gene, a 200-bp fragment of the 5S-23S rRNA intergenic spacer, and a 500-bp fragment of the flagellin gene were amplified from DNA of cultured spirochetes and subsequently sequenced, according to described methods (4, 6, 17, 20).

Nucleotide sequence accession numbers. For spirochete isolates PC-Eq17, PC-Eq2r, and PC-Eq2/1, sequences of virtually the entire *ospA* and 16S rRNA genes and a fragment of the 5S-23S rRNA intergenic spacer (5S-23S) and of the flagellin gene were deposited in GenBank under accession no. AY147007, AY147008, AY147009, and AY450560, respectively.

TABLE 1. Relative prevalence of *Borrelia* genospecies in questing nymphal or adult *I. ricinus* ticks sampled in various Central European sites

Site[c]	Characteristic of ticks examined			% Infected ticks with spirochete variant[a]					
	Stage	No. examined	% Infected	A14S-like	*afz*	*gar*	*bur*	*val*	Spp.[b]
GR	Nymph	131	20.6	0	100.0	0	0	0	0
	Adult	38	26.3	0	80.0	0	0	10.0	10.0
EB	Nymph	120	20.8	0	80.0	8.0	12.0	0	0
	Adult	192	36.5	0	35.1	24.3	11.4	14.3	14.3
MK	Nymph	58	22.4	0	23.1	61.5	15.4	0	0
	Adult	110	22.7	0	36.0	40.0	4.0	20.0	0
LB	Nymph	45	13.3	0	83.3	16.7	0	0	0
	Adult	200	19.0	5.3	50.0	39.5	2.6	2.6	0
PC	Nymph	159	18.9	16.7	33.3	36.7	6.7	6.7	0
	Adult	92	43.5	25.0	22.5	35.0	7.5	10.0	0

[a] *afz*, *B. afzelii*; *gar*, *B. garinii*; *bur*, *B. burgdorferi* sensu stricto; *val*, *B. valaisiana*.
[b] Variant corresponds to an unnamed genomic group of Lyme disease spirochetes described previously (2, 19).
[c] GR, Gröben; Eb, Eberdingen; MK, Maikammer; LB, Lembach; PC, Petite Camargue Alsacienne.

RESULTS

First, we examined ticks from an array of Central European sites to determine whether A14S-like spirochetes were present there. Questing nymphal and adult *I. ricinus* ticks were collected by passing a flannel flag over vegetation. They were screened by amplifying a 400-bp fragment of the *ospA* gene and sequencing the resulting products. Sequences characteristic of A14S spirochetes were detected mainly in ticks sampled in PC; only two such infected ticks were discovered in one of the other sites that were sampled, in Lembach (Table 1). Although mainly *B. afzelii* spirochetes infected ticks in Gröben, the genospecies were similarly abundant in all other sites that were sampled; *B. afzelii* and *B. garinii* predominated there. A14S-like spirochetes infect numerous ticks in one of the Central European sites that we studied; its distribution appears to be more restricted than is that of the other known spirochetes.

We then determined whether A14S-like spirochetes disproportionately infected a particular kind of rodent in our PC study site by examining nymphal ticks that developed from engorged larvae taken from animals that were sampled there. Presumably noninfected larvae feeding on rodents at the time of capture, as well as larvae from a spirochete-free laboratory colony, were used. The resulting *ospA* amplification products were sequenced. A14S-like spirochetes infected ticks that engorged on more than three-fourths of all garden dormice, *Eliomys quercinus*, that were sampled (Table 2). *B. afzelii* infected ticks that engorged on far fewer dormice but on more than a third of the mice and voles. Garden dormice infected by *B. afzelii* generally were coinfected by A14S-like spirochetes. Larvae feeding on wood mice, *Apodemus sylvaticus*, yellow-

TABLE 2. Prevalence of A14S-like spirochetes and *Borrelia afzelii* in rodents captured in the PC study site[a]

Rodent species[b] (*n*[c])	No. of derived nymphs examined	% Hosts with ≥1 derived nymph infected by spirochete		
		A14S-like alone	*B. afzelii* alone	A14S-like + *B. afzelii*
Eliomys quercinus (35)	628	57.2	8.6	22.9
Muscardinus avellanarius (4)	47	25.0	0	0
Apodemus sylvaticus (11)	48	0	45.5	0
Apodemus flavicollis (8)	45	0	25.0	0
Clethrionomys glareolus (2)	15	0	50.0	0

[a] Other Lyme disease genospecies were not detected.
[b] *E. quercinus*, *garden dormouse*; *M. avellanarius*, *hazel dormouse*; *A. sylvaticus*, *wood mouse*; *A. flavicollis*, *yellow-necked mouse*; *C. glareolus*, bank vole.
[c] *n*, number studied.

TABLE 4. Presence of infection by A14S-like spirochetes or *B. afzelii* in eight garden dormice, *E. quercinus*, that were recaptured one or more times

Host ID[a]	Infection status at:		Time interval (days)
	Initial capture	Recapture	
1	A14S-like	A14S-like	27
2	A14S-like	A14S-like	43
3	A14S-like	A14S-like	42
		A14S-like	25
4	A14S-like	A14S-like	24
		A14S-like	101
5	A14S-like	A14S-like	24
		A14S-like	53
		A14S-like	43
6	A14S-like + *B. afzelii*	A14S-like + *B. afzelii*	44
7	*B. afzelii*	A14S-like + *B. afzelii*	53
8	A14S-like + *B. afzelii*	A14S-like	67
		A14S-like	97

[a] ID, identification.

necked mice, *Apodemus flavicollis*, and bank voles, *Clethrionomys glareolus*, acquired *B. afzelii* alone. Of the four hazel dormice, *Muscardinus avellanarius*, that entered our traps, only one infected ticks with A14S-like spirochetes. No other genospecies were detected in the ticks that fed on these rodents. A14S-like spirochetes frequently infect dormice in our study site but virtually no mice or voles.

The infectivity for vector ticks by spirochete-infected rodents was evaluated by analyzing the ticks derived from those spirochete-infected rodents that were described in Table 2. Virtually all larval ticks became infected after engorging on garden dormice infected by A14S-like spirochetes (Table 3). Fewer ticks became infected when such animals were infected by *B. afzelii* ($P < 0.0001$, Fisher's exact test). In the case of coinfected garden dormice, about as many became infected by one kind of spirochete as by the other. About half as many larval ticks simultaneously acquired both kinds of spirochetes ($P < 0.003$, Fisher's exact test). *B. afzelii*-infected wood mice readily infected engorging ticks, much as did garden dormice infected by A14S-like spirochetes ($P = 0.29$, Fisher's exact test). Garden dormice appear to be better reservoir hosts for A14S-like spirochetes than for *B. afzelii* because they may more readily infect vector ticks.

We then determined whether infection by A14S-like spirochetes persisted in nature in garden dormice that were captured and serially recaptured at intervals of 1 to 3 months. These observations were derived from the infected ticks described in the previous series of observations. Their garden dormouse hosts, each of which was infected by A14S-like spirochetes, remained similarly infected throughout the course of these observations (Table 4). One of these animals infected by A14S-like spirochetes was similarly infected 9 and 22 weeks after it was captured initially. *B. afzelii* also was detected at the time of this animal's initial capture but not upon recapture. One dormouse gained infection by A14S-like spirochetes over a course of 7 weeks. Dormice appear to retain infection by A14S-like spirochetes but perhaps not by *B. afzelii*.

To define the identity of the A14S-like spirochetes detected in the course of this study, we compared their DNA sequences to those described in the published literature (3, 28). Each A14S-like sequence of the 400-bp *ospA* fragment amplified from spirochetes in questing ticks or in ticks that had engorged on the 27 garden dormice infected by A14S-like spirochetes was identical to that reported for the A14S spirochetes. Only the sequence derived from spirochetes infecting ticks that had engorged on the hazel dormouse differed by one nucleotide. Individual culture isolates were derived from 16 ticks that had engorged in the course of feeding on three garden dormice captured during June 2000; isolates were preserved at −70° after two passages, and several were cloned by limiting dilution. Three of these isolates, designated PC-Eq17, PC-Eq2r, and PC-Eq2/1, were selected for definitive characterization. We sequenced virtually the entire lengths of the *ospA* and 16S

TABLE 3. Comparison of infectivity of A14S-like spirochetes and *B. afzelii* to ticks of naturally infected dormice and of wood mice in the Petite Camargue Alsacienne study site[a]

Rodent species	Infected by spirochete:	No. of capture events	Result for derived nymphal ticks			
			No. examined	% Infected by spirochete		
				A14S-like	*B. afzelii*	A14S-like + *B. afzelii*
Eliomys quercinus[b]	A14S-like alone	32	412	89.3	0	0
	B. afzelii alone	4	41	0	61.0	0
	A14S-like + *B. afzelii*	8	132	32.6	31.1	15.2
Apodemus sylvaticus[c]	*B. afzelii* alone	5	22	0	81.8	0

[a] The observation includes dormice that were captured more than once.
[b] Garden dormouse.
[c] Wood mouse.

TABLE 5. Similarity of DNA sequences of flagellin, *ospA,* 16S rRNA, and 5S-23S rRNA intergenic spacer of A14S-like spirochetes derived from garden dormice captured in the PC study site[a] and sequences of A14S spirochetes isolated from a human patient in The Netherlands and from a Czech tick (strain I-77), as well as sequences from type strains of various *B. burgdorferi* sensu lato genospecies

Species	Strain	% Similarity (accession no.) of DNA sequences with those of dormouse-associated A14S-like spirochetes[f]			
		Flagellin	*ospA*	16S rRNA	5S-23S
Borrelia sp. nov[e]	A14S	NA[b]	99.87 (AF102057)	99.75 (AF102056)	97.85 (U76616)
	I-77	100[c] (AF497995)	NA	99.75 (AF497996)	99.28 (AF497994)
B. burgdorferi sensu stricto	B31	93.68 (X16833)	85.03 (X63412)	99.01 (AF467957)	92.09 (L30127)
B. afzelii	VS461	94.73 (D63365)	88.03 (Z29087)	99.37 (AY342034)	94.96 (L30135)
B. garinii	20047	94.20 (D82846)	85.55[d] (X60300)	99.01 (D67018)	95.68 (L30119)
B. valaisiana	VS116	94.38 (D82854)	81.00 (Y10840)	99.51 (X98232)	94.29 (L30134)
B. lusitaniae	PotiB2	94.55 (D82856)	76.21 (Y10838)	99.01 (X98228)	95.71 (L30131)
B. bissettii	DN127	93.85 (D82857)	86.45 (Y10897)	99.01 (AJ224141)	94.96 (L30126)

[a] See end of Materials and Methods for accession numbers.
[b] NA, not available.
[c] Except that four bases are missing (position 302 to 305) in the 1-77 strain.
[d] Because the type strain appears to lack the *ospA* gene (30), strain Gö2 was used for comparison.
[e] Proposed name, *B. spielmani* sp. nov. (see Discussion).
[f] Accession numbers beginning with AF, AY, U, or L are for GenBank entries; EMBL sequence accession numbers begin with AJ, X, Y, or Z; and DDBJ entries have the suffix D.

rRNA genes and a fragment of the 5S-23S rRNA intergenic spacer (5S-23S) and of the flagellin gene of these spirochetes. The *ospA* and 16S rRNA genes were virtually identical to the previously described sequences of A14S spirochetes, and the 5S-23S gene matched reasonably well (Table 5). In addition, we compared a fragment of the flagellin gene of our A14S-like spirochete with that of another A14S-like spirochete, strain I-77 (GenBank accession no. AF497995) detected in a questing Czech tick. The flagellin sequences of our isolates were identical to that of the A14S-like strain I-77, except for four nucleotides missing at the 5′end of the I-77 flagellin sequence (Table 5). The flagellin, *ospA*, and 5S-23S sequences of the A14S-like spirochetes differed strongly, however, from those of all other genospecies of Lyme disease spirochetes. We conclude that the A14S-like spirochetes associated with garden dormice in the PC site form a homologous entity with those isolated from a Dutch patient and from a Czech tick but are distinct from other Lyme disease spirochetes.

DISCUSSION

Virtually all garden dormice in our study site, but few, if any, other sympatric rodents, are infected by A14S spirochetes. The most common tick that infests any of these rodents is *I. ricinus*, the accepted vector of any other Lyme disease spirochetes endemic to Central Europe. The sole alternative tick that may serve some vector-related role for Lyme disease spirochetes would be the hedgehog tick, *Ixodes hexagonus* (7); this tick, however, feeds largely on hedgehogs, virtually never on rodents (13). If the observed reservoir specificity of A14S spirochetes compared to that of *B. afzelii* cannot be ascribed to different host-specific vectors, some physiologic property of the spirochete and its reservoir host must be invoked. A14S spirochetes would thrive in dormice but not in mice, and *B. afzelii* would thrive somewhat better in mice than in dormice. Our observations of field-derived garden dormice suggest that the main components of reservoir competence may be involved in the reservoir specificity of A14S spirochetes. Dormice appear to be more susceptible to tick-borne A14S spirochetes than to *B. afzelii*, because more dormice harbored A14S spirochetes than *B. afzelii*, despite the relative predominance of the latter variant in questing vector ticks. Whether this difference results from an increased susceptibility to acquiring A14S spirochetes or from a better ability to maintain them may only be determined experimentally. In addition, A14S-infected dormice are more infectious to ticks than are those infected by *B. afzelii*. Garden dormice, and perhaps other dormice, appear to be the main reservoir hosts for the A14S spirochete.

Although the wood tick serves as the vector for all of the variants of the Lyme disease spirochetes that coexist in Central Europe, particular genospecies appear to thrive solely in certain reservoir hosts. Such a specific host-pathogen relationship might result from an adaptation to complement-mediated lysis (9). This concept argues that certain spirochete genospecies exploit molecules of their host, protecting them from lysis by the host's alternative pathway of complement. Conversely, spirochetes not adapted to the host to which their vector tick has attached are destroyed within the tick soon after its attachment and before any contained spirochete can be transmitted to that host. According to the resulting genospecies concept, *B. garinii* and *B. valaisiana* are more closely associated to avian hosts than to rodent hosts, to which *B. afzelii* would be better adapted (10). Whereas both types of hosts permit perpetuation of *B. burgdorferi* sensu stricto, the host association of *Borrelia lusitaniae* remains entirely unknown. The host specificity of particular genospecies seems somewhat paradoxical because the vector tick feeds nonspecifically and, as a result, would inoculate spirochetes indiscriminately, including hosts that fail to support this variant. The host-genospecies association may not be exclusive, because bird-feeding larval ticks harbor rodent-associated *B. afzelii* spirochetes (16). In a previous study, the genospecies diversity detected in Norway rats and yellow-necked mice reflects that present in ticks questing in the same site (22). Garden dormice in our PC site, however, are infected by A14S spirochetes and *B. afzelii*, although questing nymphal ticks infected by *B. garinii* are as frequent as are those infected by *B. afzelii*, and both of these genospecies are more frequent in questing vector ticks than is the A14S spirochete. Garden

dormice appear to perpetuate solely two variants of Lyme disease spirochetes, A14S and *B. afzelii*.

The relationships of garden dormice with A14S spirochetes and with *B. afzelii* appear to differ. Dormice infected by A14S spirochetes are far more prevalent than are those harboring *B. afzelii*, although hosts would more frequently come in contact with *B. afzelii*-infected ticks. *B. afzelii*-infected dormice generally are coinfected by A14S spirochetes. Our observation on recaptured dormice, moreover, suggests that A14S spirochetes may persist longer in dormice than does *B. afzelii*. In addition, dormice infected by A14S spirochetes are more infectious to ticks than are those infected solely by *B. afzelii*. Ticks feeding on coinfected dormice, however, acquire either variant at the same frequency. Although this implies that the predominance of A14S spirochetes in dormice may not result from a competitive infectiousness to ticks, controlled laboratory experiments are required to examine interactions of these variants in dormice. We conclude that the A14S spirochete is more transmissible and may be more persistent in garden dormice and, as a result, better adapted to this rodent than is *B. afzelii*.

The geographical distribution of A14S spirochetes is restricted. This variant infects questing wood ticks mainly in one of our study sites. There, garden dormice appear to contribute the majority of A14S-infected ticks. The distribution of garden dormice extends from Southern Europe north into Central Europe to about 52 degrees of latitude and east into the Ural mountains (24). Generally, they prefer an ecotonal habitat where limestone surfaces or man-made stone walls offer ample crevices. Such hiding places appear more important to these ground-foraging rodents than is dense vegetation cover. In the PC site, garden dormice appear to have adapted to a somewhat different habitat; there, phragmites and other lush vegetation between humid meadows and swamps of the flood plain of the Rhein River are frequented preferentially (27). Thus, the distribution of garden dormice is associated with particular landscapes and may be somewhat more restricted than that of other small rodents. Garden dormice are abundant in all regions where A14S-like spirochetes have been detected: in The Netherlands, where A14S-spirochetes were first isolated from a patient, in the Czech Republic, where A14S-like spirochetes (strain I-77) have recently been identified in a questing *I. ricinus* nymph (3), and in the southern German cities of Munich and Constance, where questing *I. ricinus* ticks harbored A14S-like spirochetes (15, 21). The distribution of ticks infected by A14S spirochetes may correspond to that of garden dormice.

The isolate of A14S spirochetes derived from an erythema migrans of a Dutch patient is genetically identical to spirochetes infecting garden dormice in the PC site. Although this dormouse-associated variant of Lyme disease spirochetes causes at least one of the characteristic symptoms, its prevalence in people is not known. A14S-like spirochetes were isolated recently from a German patient who developed chronic dermatological symptoms of Lyme disease (15). Where these spirochetes occur, they may more frequently infect people than do *B. burgdorferi* sensu stricto spirochetes, which were less prevalent in questing ticks in our study sites. Certain amplification protocols may fail to distinguish this variant from *B. valaisiana* and *B. afzelii* (3, 28). Indeed, when using an alternative PCR protocol that amplifies a 600-bp fragment of the 16S rRNA gene, only one nucleotide substitution distinguishes the sequence of dormouse-associated spirochetes from that of *B. valaisiana* (D. Richter and D. B. Schlee, unpublished results). As a result, the identity of the dormouse-associated A14S-like spirochetes in human samples may have gone undetected, and the array of symptoms that may be caused by this variant remains unknown. Retrospective analysis of samples derived from patients residing in areas where dormice are abundant may help to elucidate the prevalence of this variant of Lyme disease spirochetes in people as well as its health implications.

The previous thorough genetic characterization of a single spirochete isolate from a human patient and additional molecular analyses on A14S-like spirochetes detected in three questing ticks demonstrate persuasively that this dormouse-associated spirochete variant is distinct from other genospecies (3, 15, 21, 28). We have demonstrated that the dormice-derived spirochetes in our PC site are identical to those in the well-characterized patient-derived isolate as well as to those detected in the Czech tick. Our observations now establish that these spirochetes comprise a population of microbes that perpetuate in a cycle involving *I. ricinus* ticks and dormice. This unique biological relationship, together with our and previous genetic characterizations (3, 15, 21, 28), justifies designating the dormouse-associated genospecies as a distinct entity that requires a distinctive name. We propose, therefore, to name this genospecies "*Borrelia spielmani*" sp. nov. in honor of Andrew Spielman, Professor of Tropical Public Health at the School of Public Health and the Center for International Development at Harvard University. Spielman and his colleagues demonstrated for the first time the life cycle and biological relationships of *B. burgdorferi* sensu lato. They determined that an *Ixodes* tick is the vector of Lyme disease, that deer serve as definitive hosts for these ticks, that the larval stage of this tick acquires infection and the nymphal stage transmits, that rodents serve as the main reservoir hosts for the Lyme disease spirochete, that this pathogen remains in the midgut of flat ticks and migrates to the salivary glands 2 days after infected nymphs begin to feed, and that it dwells mainly in the skin of infected rodents (14, 23). These and other discoveries laid much of the groundwork for our understanding of the natural history of the agent of Lyme disease. In the Petite Camargue Alsacienne region of Central Europe, garden dormice appear to be the main reservoir hosts of *B. spielmani*, one of several genospecies that cause human Lyme disease.

Description of *Borrelia spielmani* sp. nov. *Borrelia spielmani* (spiel′man.i. N.L. gen. n. *spielmani* in honor of Andrew Spielman, who described for the first time the life cycle and biological relationships of *B. burgdorferi* sensu lato [23].) Morphology as described previously for the genus (1). Cultural properties as described for *B. burgdorferi* sensu lato (8). *B. spielmani* perpetuates in a cycle involving garden dormice and *I. ricinus* ticks; its exceptionally narrow host specificity for a particular reservoir differentiates it from all other Lyme disease spirochetes described hitherto. rRNA gene restriction pattern after digestion by HindIII contains two fragments (2.7 and 1.2 kb) (26). Restriction pattern of the 5S-23S spacer after digestion by MseI contains three fragments (106, 68, and 51 bp) (29). Proteins react in immunoblots with monoclonal antibodies H3TS and LA-26, and no reactivity is observed with monoclonal

antibodies LA-31 and D6 (26). *B. spielmani* was detected in ticks feeding on garden and hazel dormice, in questing ticks, and in patients in France, Germany, The Netherlands, and the Czech Republic (3, 15, 21, 28; this study). It has pathogenic potential (15, 28). The type strain, PC-Eq17, was isolated from an *I. ricinus* tick that had engorged on a garden dormouse, *Eliomys quercinus*, in the Petite Camargue Alsacienne in France.

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