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Perpetuation of the Lyme Disease Spirochete *Borrelia lusitaniae* by Lizards

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To determine whether the Lyme disease spirochete *Borrelia lusitaniae* is associated with lizards, we compared the prevalence and genospecies of spirochetes present in rodent- and lizard-associated ticks at a site where this spirochete frequently infects questing ticks. Whereas questing nymphal *Ixodes ricinus* ticks were infected mainly by *Borrelia afzelii*, one-half of the infected adult ticks harbored *B. lusitaniae* at our study site. Lyme disease spirochetes were more prevalent in sand lizards (*Lacerta agilis*) and common wall lizards (*Podarcis muralis*) than in small rodents. Although subadult ticks feeding on rodents acquired mainly *B. afzelii*, subadult ticks feeding on lizards became infected by *B. lusitaniae*. Genetic analysis confirmed that the spirochetes isolated from ticks feeding on lizards are members of the *B. lusitaniae* genospecies and resemble type strain PotiB2. At our central European study site, lizards, which were previously considered zooprophylactic for the agent of Lyme disease, appear to perpetuate *B. lusitaniae*.

The various genospecies of the Lyme disease spirochetes (*Borrelia burgdorferi* sensu lato) that infect European vector ticks appear to be associated with particular reservoir hosts. Whereas *B. afzelii* is most frequently detected in rodent-feeding vector ticks, *B. garinii* and *B. valaisiana* appear to be associated with birds (13). Both rodents and birds are competent reservoir hosts for *B. burgdorferi* sensu stricto (27, 31). *B. spielmanii*, on the other hand, is characterized by a unique host association; only garden and hazel dormice appear to perpetuate this genospecies in nature (28, 29). The resistance of a genospecies of Lyme disease spirochetes to the bacteriolytic activities of the alternative complement pathway of various host species may determine its reservoir host association (13, 14). The local composition of genospecies in questing vector ticks depends on the composition of the reservoir hosts at the site.

Reservoir-competent rodents and birds are ubiquitous in the ecotone, and there, *B. afzelii*, *B. garinii*, and *B. valaisiana* are the most common European genospecies infecting questing ticks (30). The occurrence of *B. spielmanii* in vector ticks, however, appears to be restricted to areas where dormice are abundant (28). Another Lyme disease spirochete, *B. lusitaniae*, is distributed focally. In countries of the Mediterranean basin, such as Portugal, Morocco, and Tunisia, this spirochete appears to infect vector ticks more frequently than do other genospecies (1, 5, 32, 37). *B. lusitaniae* infected three-quarters of all questing adult ticks at a site in Portugal (5). *B. lusitaniae* is found as far east as Slovakia, Moldovia, the Czech Republic, and Ukraine (8, 25), but its prevalence appears to be low and restricted to only a few sites. At an Alsacian site, where one-quarter of all infected ticks harbored *B. lusitaniae* (30), lizards were abundant. Although lizards are frequent hosts of ticks in the Mediterranean coastal region, they are generally considered incompetent for Lyme disease spirochetes (15, 19, 20). A vertebrate host that serves as a natural reservoir for *B. lusitaniae*, explaining its focal distribution, has not been identified.

It may be that *B. lusitaniae* is perpetuated in nature by lizards. To determine whether lizards are associated with this genospecies of Lyme disease spirochetes, although they are generally considered zooprophylactic, we examined ticks feeding on lizards and rodents at a site at which this spirochete frequently infects questing ticks. In particular, we determined whether and how frequently these vertebrate hosts infected ticks feeding on them and compared the genospecies of spirochetes present in lizard- and rodent-associated ticks. In addition, we obtained isolates of these spirochetes and characterized them genetically.

MATERIALS AND METHODS

Our study site was located at the ridge of Ranzenberg Mountain about 300 m above sea level, near the town of Heilbronn in the state of Baden-Württemberg, Germany. The southern slope of this mountain is used as a vineyard. Lizards, rodents, and ticks were obtained at the edge of the forest which was separated from the vineyard by an agricultural road.

Lizards were captured by hand in the early morning hours in April, June, and August 2005. They were identified to species and inspected by using a magnifying glass. Any ticks infesting them were carefully removed with forceps and identified to stage and species. The lizards were released promptly after ticks were removed. Rodents were captured in live traps (Longworth Scientific Instruments, Abingdon, United Kingdom) baited with apple, grain, and cotton. A total of 40 traps were placed during three consecutive nights in April and June 2005. Captured small rodents were taken to the laboratory, where they were identified and caged over water until all attached ticks had detached. The water was inspected twice daily, and the ticks were removed, identified, and counted. Ticks derived from hosts were confined in screened vials and stored at 22°C, until they were examined for spirochetes. Questing ticks were collected at the site in April 2005 by using a flannel flag, identified to stage and species, and preserved in 80% ethanol.

To detect and identify the various spirochetes that were present in questing and host-associated ticks, the opisthosoma of each tick was opened, and the mass of soft tissue was dissected in physiological saline, transferred to a tube containing 180 μl lysis buffer (ATL tissue lysis buffer; QIAGEN, Hilden, Germany) and 20 μl proteinase K (600 milli-activity units/mg), and lysed at 56°C overnight. DNA was extracted using a QIAamp DNA mini kit (QIAGEN) according to the manufacturer's instructions, and DNAs of nymphal and adult ticks were eluted with 50 and 75 μl of elution buffer, respectively, and stored at −20°C until PCR was performed.

Borrelia genospecies were characterized by amplifying and sequencing a 600-nucleotide fragment of the gene encoding the 16S rRNA. To increase the

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TABLE 1. Prevalence of spirochetes in questing *I. ricinus* ticks at the Ranzenberg study site

Ticks			% of *Borrelia* genospecies in infected ticks					
Stage	No. examined	% Infected	*B. lusitaniae*	*B. afzelii*	*B. garinii*	*B. valaisiana*	*B. spielmanii*	*B. miyamotoi*
Nymph	60	16.7	0	70.0	0	20.0	10.0	0
Adult	135	39.3	49.1	13.2	15.1	17.0[a]	3.8	3.8

[a] Including a tick coinfected with *B. garinii*.

sensitivity for detection of spirochetal DNA in ticks, we used nested PCR. Aliquots of DNA suspensions (2 μl) were added to 48-μl mixtures containing each deoxynucleoside triphosphate at a concentration of 200 μM, 1.5 mM $MgCl_2$, 1 U *Taq* polymerase (QIAGEN), and 15 pmol of the outer primer pair and the PCR buffer supplied with the *Taq* polymerase. We used the following sequences of the 16S rRNA gene as outer primers (30) (5′-3′): CTA ACG CTG GCA GTG CGT CTT AAG C (16S1A) and AGC GTC AGT CTT GAC CCA GAA GTT C (16S1B) (positions 36 to 757). The mixture was placed in a thermocycler (PTC 200; MJ Research, Biozym, Germany), heated for 1 min at 94°C, and subjected to 30 cycles of 20 s of denaturation at 94°C, 20 s of annealing at 63°C, and 40 s of extension at 72°C, followed by 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 2.5 mM $MgCl_2$ and 20 pmol of the following inner primer pair (5′-3′) were used: AGT CAA ACG GGA TGT AGC AAT ACA (16S2A) and GGT ATT CTT TCT GAT ATC AAC AG (16S2B) (positions 66 to 720). This mixture was subjected to 35 amplification cycles using the cycle conditions described above, except that the annealing reaction was performed at 56°C and the extension reaction lasted 30 s. DNA was extracted, reaction vials were prepared for amplification, templates were added, and products were electrophoresed in separate rooms. As an additional precaution, the reaction mixtures were prepared in a designated PCR workstation (Labcaire Systems, North Somerset, United Kingdom) and templates were added to the mixtures in a second PCR workstation. Benches and equipment were wiped down with a DNA decontamination solution (DNA erase; MP Biomedicals, Eschwege, Germany) after each use. To each sixth reaction mixture, water was added instead of extracted DNA 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 with a Licor DNA4200 sequencer (Licor Biosciences, Bad Homburg, Germany). Each resulting sequence was compared with sequences of the same gene fragment from various spirochete genospecies. The following sequences (identified by the accession numbers under which they were deposited) were used for comparison: X85196 and X85203 for *B. burgdorferi* sensu stricto; X85190, X85192, and X85194 for *B. afzelii*; X85193, X85199, and M64311 for *B. garinii*; X98228 and X98229 for *B. lusitaniae*; X98232 and X98233 for *B. valaisiana*; AY147008 for *B. spielmanii*; and AY253149 for *B. miyamotoi*. A complete match (no more than two nucleotide changes) was required for identification.

Spirochetes were cultured from nymphal or adult ticks that had derived from larvae or nymphs, respectively, feeding on lizards. The ticks were washed individually for 3 min in distilled water, for 3 min in 100% ethanol, and for 3 min in 1% benzalkonium chloride. After three washes in 0.9% NaCl, ticks were placed in Barbour-Stoenner-Kelly H medium (Sigma, Deisenhofen, Germany) and dissected using sterile forceps. Each midgut was transferred to 1 ml of Barbour-Stoenner-Kelly H medium supplemented with 6% heat-inactivated rabbit serum (Sigma) and amphotericin (2.5 μg/ml; Sigma). The suspensions were incubated at 32°C and checked for spirochete growth every third day by dark-field microscopy. Dense cultures of spirochetes were frozen and stored at −70°C.

To confirm the identities of the *B. lusitaniae* isolates, fragments of the *ospA*, flagellin, *hbb*, *groEL*, *recA*, 16S rRNA, and *rrf-rrl* intergenic spacer genes were amplified from DNA of cultured spirochetes and sequenced by using previously described methods (3, 6, 7, 23, 24, 35).

RESULTS

Prevalence of spirochetes in questing ticks. First, we estimated the prevalence of spirochetes in questing *I. ricinus* ticks at our Ranzenberg study site. We sampled nymphal and adult ticks by flagging the vegetation and determined the presence and identity of spirochetal DNA. One-sixth of all nymphs were infected by spirochetes, and most of these ticks harbored *B. afzelii*, whereas a few ticks were infected by *B. valaisiana* or *B. spielmanii* (Table 1). More than one-third of the adult ticks contained spirochetes; *B. lusitaniae* was found in almost one-half of these ticks. *B. afzelii*, *B. valaisiana*, and *B. garinii* each were found in only about one-sixth of all infected adults. *B. burgdorferi* sensu stricto seemed to be absent in our sample. Whereas every tenth nymphal tick harbored *B. afzelii* spirochetes in our study site, every fifth adult tick harbored *B. lusitaniae*.

Density of ticks on lizards and rodents. The densities of subadult *I. ricinus* ticks infesting lizards and rodents captured at the Ranzenberg site were determined. Common wall lizards (*Podarcis muralis*), sand lizards (*Lacerta agilis*), and slow worms (*Anguis fragilis*) were collected by hand, whereas yellow-necked mice (*Apodemus flavicollis*), wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), and common voles (*Microtus arvalis*) were live trapped. Larvae parasitized virtually all lizards and yellow-necked mice but somewhat fewer wood mice and bank voles (Table 2). The two slow worms collected were not infested with ticks, and only two of the three common voles captured were parasitized (data not shown). Yellow-necked mice, the rodent most heavily infested with larvae, supported four and eight times more larvae than did common wall lizards and sand lizards, respectively. Nymphal ticks were detected on more than one-half of the lizards, on somewhat fewer yellow-necked mice, and on only one-quarter and one-fifth of the wood mice and bank voles, respectively. About two nymphs fed on each lizard. As a result, lizards supported at least twice as many nymphs as did yellow-necked mice and seven times more nymphs than did wood mice and bank voles. Although small rodents fed more larvae

TABLE 2. Density of subadult *I. ricinus* ticks on lizards, including common wall lizards (*P. muralis*) and sand lizards (*L. agilis*), and rodents, including yellow-necked mice (*A. flavicollis*), wood mice (*A. sylvaticus*), and bank voles (*C. glareolus*), at the Ranzenberg study site

Host		Larvae		Nymphs		Larva/nymph ratio
Species	No.	% of hosts infested	Mean no./host	% of hosts infested	Mean no./host	
P. muralis	18	94.4	4.4	50.0	1.9	2.3
L. agilis	6	100	2.2	66.7	2.5	0.9
A. flavicollis	11	100	18.3	45.5	0.9	20.1
A. sylvaticus	15	86.7	13.1	26.7	0.3	49.3
C. glareolus	34	79.4	6.2	20.6	0.3	21.0

TABLE 3. Prevalence of spirochetes in lizards, including common wall lizards (*P. muralis*) and sand lizards (*L. agilis*), and rodents, including yellow-necked mice (*A. flavicollis*), wood mice (*A. sylvaticus*), and bank voles (*C. glareolus*), and their infectivity for larval *I. ricinus* ticks that attached naturally to them at the study site

Host			Infection in fed larvae or derived nymphs[a]				
					% of *Borrelia* genospecies in infected ticks		
Kind	No.	% with ≥1 fed larva or derived nymph infected	No. examined	% with *Borrelia* spp.	*B. lusitaniae*	*B. afzelii*	*B. miyamotoi*
P. muralis	17	76.5	139	31.7	100	0	0
L. agilis	6	66.7	57	21.1	100	0	0
A. flavicollis	11	63.6	55	41.8	0	91.3	8.7
A. sylvaticus	12	33.3	50	26.0	0	100	0
C. glareolus	21	33.3	81	28.4	0	100	4.3[b]

[a] Fed larvae were obtained mainly from lizards, and derived nymphs were obtained from rodents.
[b] Tick coinfected with *B. afzelii*.

than did sand lizards and common wall lizards, these reptiles harbored more nymphal ticks.

Prevalence of spirochetes in lizards and rodents. We then compared the prevalence of spirochetes in lizards to the prevalence of spirochetes in rodents and determined their infectivity for ticks by examining larvae that were engorging on these hosts at the time of capture or nymphs resulting from these larvae. Spirochetes infected three-quarters of the common wall lizards and two-thirds of the sand lizards (Table 3). Whereas two-thirds of the yellow-necked mice infected ticks feeding on them, only one-third of the wood mice and bank voles were infected. Common wall lizards infected one-third and sand lizards infected about one-fifth of the larvae feeding on them. All infected larvae obtained from lizards had acquired *B. lusitaniae*. Almost one-half of the nymphs that had fed as larvae on yellow-necked mice contained spirochetes. Wood mice and bank voles infected more than one-quarter of their larval ticks. Virtually all infected rodent-feeding ticks harbored *B. afzelii*, but a tick from a yellow-necked mouse was infected by *B. miyamotoi* and a tick derived from a bank vole was coinfected by *B. afzelii* and *B. miyamotoi*. Lyme disease spirochetes infected about one-half of the rodents but considerably more than one-half of the lizards; larvae feeding on rodents acquired mainly *B. afzelii*, whereas larvae feeding on lizards became infected by *B. lusitaniae*.

Infectivity of lizards and rodents for nymphal ticks. We determined the spirochetal genospecies present in nymphal ticks infesting lizards or rodents at our Ranzenberg site. About one-third of the nymphs obtained from lizards were infected by *B. lusitaniae* (Table 4). No other genospecies were detected in nymphal ticks from sand lizards, but *B. valaisiana* and *B. miyamotoi* each infected a single nymph obtained from common wall lizards. More than three-quarters of the nymphal ticks feeding on yellow-necked mice or bank voles and somewhat less than one-half of the nymphs feeding on wood mice harbored spirochetes. Except for a single nymph infected by *B. garinii*, all nymphs derived from rodents were infected by *B. afzelii*. Although infected nymphal ticks obtained while they were feeding on lizards or rodents may have also acquired an infection during their larval blood meal from a previous host, the majority of nymphs feeding on lizards were infected by *B. lusitaniae* and those feeding on rodents were infected by *B. afzelii*.

Genetic identities of lizard-associated spirochetes. To confirm the identities of the spirochetes infecting vector ticks feeding on lizards, we isolated spirochetes from nymphs that had fed as larvae on lizards and sequenced fragments of several of their genes. We examined one spirochete isolate from each kind of lizard: RB-Pm2N6 from a common wall lizard and RB-La1N1 from a sand lizard. All seven gene fragments sequenced were identical for the isolates. An approximately 470-bp fragment of the 16S rRNA gene (GenBank accession numbers DQ379484 and DQ379485) was identical to sequences found in representatives of the PotiB2 group of *B. lusitaniae*. The 222-bp fragment of the *rrf-rrl* intergenic spacer (GenBank accession numbers DQ379492 and DQ379493) was identical to *B. lusitaniae* sequences derived from a Portuguese patient isolate and several Turkish tick isolates (4, 9). The sequence of the 282-bp fragment of the *groEL* gene (GenBank accession numbers DQ379488 and DQ379489) differed at one base from the sequence of type strain PotiB2. Whereas the 565-bp fragment of the flagellin gene (GenBank

TABLE 4. Genospecies infecting nymphal *I. ricinus* ticks that had attached naturally to common wall lizards (*P. muralis*), sand lizards (*L. agilis*), yellow-necked mice (*A. flavicollis*), wood mice (*A. sylvaticus*), or bank voles (*C. glareolus*)

Host		Infection of feeding nymphs					
			% of *Borrelia* genospecies				
Species	No.	No. tested	*B. lusitaniae*	*B. afzelii*	*B. garinii*	*B. valaisiana*	*B. miyamotoi*
P. muralis	9	33	33.3	0	0	3.0	3.0
L. agilis	4	15	46.7	0	0	0	0
A. flavicollis	5	11	0	72.7	9.1	0	0
A. sylvaticus	5	5	0	40.0	0	0	0
C. glareolus	4	8	0	87.5	0	0	0

TABLE 5. Prevalence of *B. lusitaniae* in questing *I. ricinus* ticks at various European and North African sites

Country (area)[a]	Ticks			% *B. lusitaniae* in infected ticks	Method[b]	Reference
	Stage(s)	No. examined	% Infected with *B. burgdorferi* sensu lato			
Germany (Württemberg)	Nymph	60	16.7	0	PCR, seq	This study[d]
	Adult	135	40.0	48.1	PCR, seq	This study[d]
France (Vosges)	Nymph	95	22.1	19.0	PCR, seq	Richter and Matuschka, submitted
	Adult	236	40.3	18.9	PCR, seq	Richter and Matuschka, submitted
Switzerland (Tincino)	Nymph and adult	460	10.9	15.0	Culture, PCR, RFLP	11
Poland	ND[c]	ND	ND	5.9	PCR, RFLP	36
Slovakia (Martinsky)	Adult	114	49.1	3.6	Culture, PCR, RFLP	8
Turkey (Trakya)	Nymph and adult	299	4.0	40.0	Culture, PCR, seq	9
Morocco	ND	218	47.8	92.7	Culture, PCR, RFLP	32
Tunisia	Nymph	60	33.3	100.0	Pooled culture, PCR, RFLP	37
	Adult	81	49.4	97.5	Pooled culture, PCR, RFLP	37
Portugal (Lisbon)	Adult	55	75.0	100.0	PCR, RLB	5
Portugal (Mafra)	Adult	1363	10.5	0.6	PCR, RFLP, RLB, seq	1
Portugal (Grandola)	Adult	104	62.5	60.0	PCR, RFLP, RLB, seq	1

[a] Where no area is given, ticks were collected at various sites in the country.
[b] seq, sequencing; RLB, reverse line blot; RFLP, restriction fragment length polymorphism.
[c] ND, not described.
[d] See Table 1.

accession numbers DQ379486 and DQ379487) varied at only one base from the sequence of a Polish tick-derived *B. lusitaniae* isolate, three bases were different from bases in the sequence of PotiB2. Compared with the type strain, four base changes were observed for the 162-bp fragment of the *recA* gene (GenBank accession numbers DQ379496 and DQ379497) and six base changes were observed for the 327-bp fragment of the *hbb* gene (GenBank accession numbers DQ379490 and DQ379491). Although the *ospA* gene of our isolates (GenBank accession numbers DQ379494 and DQ379495) most closely resembled the *ospA* gene of the PotiB2 strain, this gene fragment was more heterogeneous than any of the other fragments analyzed. Including several deletions and insertions of nucleotides, about 13% of the 620-bp fragment of *ospA* differed from the PotiB2 fragment. Our genetic analysis confirmed that the spirochetal isolates derived from ticks feeding on lizards at the Ranzenberg study site are members of the *B. lusitaniae* genospecies and resemble type strain PotiB2.

DISCUSSION

The geographic distribution of *B. lusitaniae* differs from that of most other Lyme disease spirochetes. This species is most prevalent in Mediterranean countries, such as Portugal, Tunisia, and Morocco, where it generally constitutes the sole or major genospecies infecting vector ticks (Table 5) (1, 5, 32, 37). The first isolates of *B. lusitaniae* were obtained from *I. ricinus* ticks collected in Portugal, and the species name reflects this origin (16, 22). In more northern or eastern countries, *B. lusitaniae* has been detected at only a few sites (30), at which it infects ticks less frequently than it does on the Mediterranean coast (8, 11, 36; Richter and Matuschka, submitted for publication). On the Turkish coast of the Black Sea, where infected ticks are rare, almost one-half of the infected ticks harbored *B. lusitaniae* (9). Whereas *B. lusitaniae* appears to be focally distributed and is rare compared to other genospecies in central or eastern Europe, which has a continental climate, it appears to be the predominant genospecies and to be widespread in countries with a Mediterranean climate.

At our Ranzenberg study site, subadult vector ticks feeding on sand lizards and common wall lizards acquired virtually only *B. lusitaniae* spirochetes. *B. lusitaniae* infection in these lizards was more prevalent than *B. afzelii* infection in rodents. Generally, the smaller the larva-nymph ratio of ticks feeding on a competent host, the more frequently this host is exposed to infection. Indeed, spirochetal infection is more frequent in larger rodents, such as rats or dormice, than in mice and voles, because they feed more nymphal ticks (17, 18). Although we detected no questing nymphal ticks infected by *B. lusitaniae* in our sample, lizards may be readily infected with this genospecies, because they fed up to eight times more nymphal ticks than did rodents. In addition, lizards live longer than mice and voles, and thus, spirochetal infection may accumulate throughout their life. The observation that these lizards appeared to contribute mainly adult ticks infected by *B. lusitaniae* at our study site may have been affected by seasonal variation; no questing ticks were obtained in the summer and fall. At another central European study site, Lembach, about 4 of 100 questing nymphs harbored *B. lusitaniae* (Richter and Matuschka, submitted). Also, every third nymph was infected by this genospecies at a Tunisian site (37), where different lizards may serve as hosts for vector ticks and *B. lusitaniae*. At our German study site, *B. lusitaniae* spirochetes appear to be perpetuated by at least two kinds of lizards.

Larval ticks attaching to hosts in nature may have inherited spirochetes from their mothers. Whereas *B. miyamotoi* spirochetes appear to be transmitted transovarially (33), this route of transmission has not been proven for any Lyme disease spirochete and, indeed, this possibility has been excluded for *B. afzelii* (21). However, even if *B. lusitaniae* were transovarially transmitted, our field observations strongly suggest that lizards, but not rodents, permit survival in feeding ticks and subsequent transstadial transmission of the spirochetes. To further examine the reservoir competence of lizards for *B. lusitaniae* spirochetes, the susceptibility, intrinsic incubation period, and degree and duration of infectivity of lizards have to be analyzed experimentally in the laboratory.

Particular reservoir hosts may contribute differentially to the prevalence of diverse spirochetal genospecies at a site. *B. spielmanii*, for example, appears to be prevalent at sites at which garden dormice are abundant (29). At our Ranzenberg study site, virtually all subadult ticks that acquired spirochetes from lizards harbored *B. lusitaniae*, whereas the subadult ticks that became infected while feeding on rodents harbored *B. afzelii*. The lizard- and rodent-associated spirochetes mutually excluded each other when ticks were feeding on their permissive hosts. Interestingly, the genospecies detected in a tick following its nymphal blood meal reflected the latest host association. Spirochetes belonging to other genospecies to which a tick may have been exposed while feeding in its larval stage on another kind of host were rarely detected. Our observation that spirochetes from the latest host association appear to prevail in the tick supports the suggestion that the reservoir competence of a host for a particular genospecies results from the spirochete's resistance to serum complement of that host (13, 14). Any spirochete that is not adapted to the host on which the vector feeds may thus be eliminated from the tick during the blood meal. Lizards appear to contribute *B. lusitaniae* spirochetes to the genospecies composition at our Ranzenberg study site.

In areas where the climate is continental, the distribution of lizards is focal and their home range (2) may not allow *B. lusitaniae* to spread from one lizard habitat to another if this spirochete is exclusively associated with reptiles. Recently, an association of *B. lusitaniae* with birds was suggested, because numerous larval ticks obtained from birds captured during migration harbored this genospecies (26). If birds were regular reservoir hosts for *B. lusitaniae*, as they are for *B. valaisiana* and *B. garinii*, *B. lusitaniae* should be as widespread as these bird-associated spirochetes. The reservoir competence of birds and lizards for *B. lusitaniae*, however, needs to be determined experimentally. Because the distribution of *B. lusitaniae* in areas with a continental climate is more focal than its distribution in areas with a Mediterranean climate, it may be that migratory birds help spread this spirochete by seeding foci of transmission where both lizards and vector ticks are present. The stress of migration may induce temporary reservoir competence for *B. lusitaniae*. Indeed, latent spirochetes may become reactivated in birds by migratory stress (10). Comparing the genetic diversity of *B. lusitaniae* isolates derived from central and eastern European foci with the genetic diversity of *B. lusitaniae* isolates of Mediterranean origin may help elucidate this suggestion. It appears that *B. lusitaniae* is perpetuated by reptiles, but it may be distributed by avian hosts.

B. lusitaniae is not perpetuated at all sites where lizards and vector ticks are present. The abundance of sand lizards at a study site in Berlin resulted in an overall low prevalence of Lyme disease spirochetes in questing ticks, as determined by dark-field microscopy, compared to the prevalence at other sites where lizards were not present (19). Sand lizards that were experimentally exposed to infected ticks failed to become infectious for xenodiagnostic ticks. The spirochetes used in that study had been derived originally from a Berlin study site, had been perpetuated in rodents in the laboratory, and thus most likely were *B. afzelii* and/or *B. burgdorferi* sensu stricto (20). On the Pacific coast of the United States, western fence lizards (*Sceloporus occidentalis*) and southern alligator lizards (*Elgaria multicarinata*) exert a similar zooprophylactic effect (15). Both spirochetal genospecies that are transmitted by the vector tick *Ixodes pacificus*, *B. burgdorferi* sensu stricto and *B. bissettii*, are sensitive to bacteriolysis by the alternative complement pathway of these reptiles (12, 34). In a tick-permissive habitat that supports lizards, the overall prevalence of Lyme disease spirochetes in questing ticks may be low, as long as *B. lusitaniae* spirochetes are absent.

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REFERENCES

1. **Baptista, S., A. Quaresma, T. Aires, K. Kurtenbach, M. Santos-Reis, M. Nicholson, and M. Collares-Pereira.** 2004. Lyme borreliosis spirochetes in questing ticks from mainland Portugal. Int. J. Med. Microbiol. **293S:**109–116.
2. **Bischoff, W.** 1984. *Lacerta agilis* Linnaeus 1758—Zauneidechse, p. 23–68. *In* W. Böhme (ed.), Handbuch der Reptilien und Amphibien Europas, II/1. Aula-Verlag, Wiesbaden, Germany.
3. **Casati, S., M. V. Bernasconi, L. Gern, and J.-C. Piffaretti.** 2004. Diversity within *Borrelia burgdorferi* sensu lato genospecies in Switzerland by *recA* gene sequence. FEMS Microbiol. Lett. **238:**115–123.
4. **Collares-Pereira, M., S. Couceiro, I. Franca, K. Kurtenbach, S. M. Schäfer, L. Vitorino, L. Goncales, S. Baptista, M. L. Vieira, and C. Cunha.** 2004. First isolation of *Borrelia lusitaniae* from a human patient. J. Clin. Microbiol. **42:**1316–1318.
5. **De Michelis, S., H.-S. Sewell, M. Collares-Pereira, M. Santos-Reis, L. M. Schouls, V. Benes, E. C. Holmes, and K. Kurtenbach.** 2000. Genetic diversity of *Borrelia burgdorferi* sensu lato in ticks from mainland Portugal. J. Clin. Microbiol. **38:**2128–2133.
6. **Dykhuizen, D. E., D. S. Polin, J. J. Dunn, B. Wilske, V. Preac-Mursic, R. J. Dattwyler, and B. J. Luft.** 1993. *Borrelia burgdorferi* is clonal: implications for taxonomy and vaccine development. Proc. Natl. Acad. Sci. USA **90:**10163–10167.
7. **Fukunaga, M., K. Okada, M. Nakao, T. Konishi, and Y. Sato.** 1996. Phylogenetic analysis of *Borrelia* species based on flagellin sequences and its application for molecular typing of Lyme disease borreliae. Int. J. Syst. Bacteriol. **46:**898–905.
8. **Gern, L., C. M. Hu, E. Kocianova, V. Vyrostekova, and J. Rehacek.** 1999. Genetic diversity of *Borrelia burgdorferi* sensu lato isolates obtained from *Ixodes ricinus* ticks collected in Slovakia. Eur. J. Epidemiol. **15:**665–669.
9. **Güner, E. S., N. Hashimoto, N. Takada, K. Kaneda, Y. Imai, and T. Masuzawa.** 2003. First isolation and characterization of *Borrelia burgdorferi* sensu lato strains from *Ixodes ricinus* ticks in Turkey. J. Med. Microbiol. **52:**807–813.
10. **Gylfe, A., S. Bergström, J. Lundström, and B. Olsen.** 2000. Reactivation of *Borrelia* infection in birds. Nature **403:**724–725.
11. **Jouda, F., M. Crippa, J.-L. Perret, and L. Gern.** 2003. Distribution and prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks of canton Ticino (Switzerland). Eur. J. Epidemiol. **18:**907–913.
12. **Kuo, M. M., R. S. Lane, and P. C. Giclas.** 2000. A comparative study of mammalian and reptilian alternative pathway of complement-mediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*). J. Parasitol. **86:**1223–1228.
13. **Kurtenbach, K., S. De Michelis, S. Etti, S. M. Schäfer, H.-S. Sewell, V. Brade, and P. Kraiczy.** 2002. Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. Trends Microbiol. **10:**74–79.

14. **Kurtenbach, K., H.-S. Sewell, N. H. Ogden, S. E. Randolph, and P. A. Nuttall.** 1998. Serum complement sensitivity as a key factor in Lyme disease ecology. Infect. Immun. **66:**1248–1251.
15. **Lane, R. S., and J. E. Loye.** 1989. Lyme disease in California: interrelationship of *Ixodes pacificus* (Acari: Ixodidae), the western fence lizard (*Sceloporus occidentalis*), and *Borrelia burgdorferi*. J. Med. Entomol. **26:**272–278.
16. **Le Fleche, A., D. Postic, K. Giradet, 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.
17. **Matuschka, F.-R., R. Allgöwer, A. Spielman, and D. Richter.** 1999. Characteristics of garden dormice that contribute to their capacity as reservoirs for Lyme disease spirochetes. Appl. Environ. Microbiol. **65:**707–711.
18. **Matuschka, F.-R., S. Endepols, D. Richter, A. Ohlenbusch, H. Eiffert, and A. Spielman.** 1996. Risk of urban Lyme disease enhanced by the presence of rats. J. Infect. Dis. **174:**1108–1111.
19. **Matuschka, F.-R., P. Fischer, M. Heiler, S. Blümcke, and A. Spielman.** 1992. Stage-associated risk of transmission of the Lyme disease spirochete by European *Ixodes* ticks. Parasitol. Res. **78:**695–698.
20. **Matuschka, F.-R., P. Fischer, M. Heiler, D. Richter, and A. Spielman.** 1992. Capacity of European animals as reservoir hosts for the Lyme disease spirochete. J. Infect. Dis. **165:**479–483.
21. **Matuschka, F.-R., T. W. Schinkel, B. Klug, A. Spielman, and D. Richter.** 1998. Failure of *Ixodes* ticks to inherit *Borrelia afzelii* infection. Appl. Environ. Microbiol. **64:**3089–3091.
22. **Nuncio, M. S., O. Peter, M. J. Alves, F. Bacellar, and A. R. Filipe.** 1993. Isolamento e characterizacao de borrelias de *Ixodes ricinus* L. em Portugal. Rev. Port. Doencas Infect. **16:**175–179.
23. **Park, H.-S., J. H. Lee, E. J. Jeong, S. E. Koh, T. K. Park, W. J. Jang, K. H. Park, B. J. Kim, Y. H. Kook, and S. H. Lee.** 2004. Evaluation of *groEL* gene analysis for identification of *Borrelia burgdorferi* sensu lato. J. Clin. Microbiol. **42:**1270–1273.
24. **Postic, D., M. V. 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.
25. **Postic, D., E. Korenberg, N. Gorelova, Y. V. Kovalevski, E. Bellenger, and G. Baranton.** 1997. *Borrelia burgdorferi* sensu lato in Russia and neighbouring countries: high incidence of mixed isolates. Res. Microbiol. **148:**691–702.
26. **Poupon, M.-A., E. Lommano, P.-F. Humair, V. Douet, O. Rais, M. Schaad, L. Jenni, and L. Gern.** 2006. Prevalence of *Borrelia burgdorferi* sensu lato in ticks collected from migratory birds in Switzerland. Appl. Environ. Microbiol. **72:**976–979.
27. **Richter, D., B. Klug, A. Spielman, and F.-R. Matuschka.** 2004. Adaptation of diverse Lyme disease spirochetes in a natural rodent reservoir host. Infect. Immun. **72:**2442–2444.
28. **Richter, D., D. Postic, N. Sertour, I. Livey, F.-R. Matuschka, and G. Baranton.** 2006. Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. Int. J. Syst. Evol. Microbiol. **56:**873–881.
29. **Richter, D., D. B. Schlee, R. Allgöwer, and F.-R. Matuschka.** 2004. Relationships of a novel Lyme disease spirochete, *Borrelia spielmani* sp. nov., with its hosts in central Europe. Appl. Environ. Microbiol. **70:**6414–6419.
30. **Richter, D., D. B. Schlee, and F.-R. Matuschka.** 2003. Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent. Emerg. Infect. Dis. **9:**697–701.
31. **Richter, D., A. Spielman, N. Komar, and F.-R. Matuschka.** 2000. Competence of American robins as reservoir hosts for Lyme disease spirochetes. Emerg. Infect. Dis. **6:**133–138.
32. **Sarih, M., F. Jouda, L. Gern, and D. Postic.** 2003. First isolation of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks in Morocco. Vector-borne Zoonotic Dis. **3:**133–139.
33. **Scoles, G. A., M. Papero, L. Beati, and D. Fish.** 2001. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. Vector Borne Zoonot. Dis. **1:**21–34.
34. **Ullmann, A. J., R. S. Lane, K. Kurtenbach, M. Miller, M. E. Schriefer, N. Zeidner, and J. Piesman.** 2003. Bacteriolytic activity of selected vertebrate sera for *Borrelia burgdorferi* sensu stricto and *Borrelia bissettii*. J. Parasitol. **89:**1256–1257.
35. **Valsangiacomo, C., T. Balmelli, and J.-C. Piffaretti.** 1997. A phylogenetic analysis of *Borrelia burgdorferi* sensu lato based on sequence information from the *hbb* gene, coding for a histone-like protein. Int. J. Syst. Bacteriol. **47:**1–10.
36. **Wodecka, B., and B. Skotarczak.** 2005. First isolation of *Borrelia lusitaniae* DNA from *Ixodes ricinus* ticks in Poland. Scand. J. Infect. Dis. **37:**27–34.
37. **Younsi, H., D. Postic, G. Baranton, and A. Bouattour.** 2001. High prevalence of *Borrelia lusitaniae* in *Ixodes ricinus* ticks in Tunisia. Eur. J. Epidemiol. **17:**53–56.