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The role of the sand lizard (*Lacerta agilis*) in the transmission cycle of *Borrelia burgdorferi* sensu lato

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Abstract

In order to examine the role of *Lacerta agilis* in the transmission cycle of *Borrelia burgdorferi* sensu lato, lizards were captured in three different localities in Slovakia, Poland, and Romania. Skin biopsy specimens from collar scales and ticks feeding on the lizards at the time of capture were collected. In total, 87 individuals (11 in Slovakia, 48 in Poland, 28 in Romania) of *L. agilis* were captured. Altogether, 245 (74, 74, 97) larvae and 191 (78, 113, 0) nymphs were removed from captured lizards. Borrelial infection was detected by PCR amplifying a fragment of the 5S–23S rDNA intergenic spacer and genotyping by restriction fragment length polymorphism (RFLP). When examining the presence of borreliae in biopsy specimens, striking differences between separate populations were observed. Whilst none of the biopsy specimens from *L. agilis* from Poland were positive for *B. burgdorferi* s.l., 45% of the sand lizards from Slovakia and 57% from Romania were positive. *B. lusitaniae* was confirmed in all positive biopsy specimens. The prevalence of borreliae in ticks that had fed on lizards was 6% in Poland, 21% in Slovakia, and 13% in Romania. While *B. lusitaniae* was the only genospecies in ticks from Slovakia (except for 2 larvae infected with *B. afzelii*) and Romania, it represented 64% and *B. valaisiana* 27% of the borrelia infections in ticks from lizards captured in Poland. The highest probability of ticks to get infected expressed as specific infectivity of lizards was recorded in Slovakia (0.102) and the lowest in Poland (0.003). Our findings underline the importance of the sand lizard in the transmission cycle of *B. lusitaniae*.

Keywords: *Borrelia lusitaniae*; Sand lizard; *Lacerta agilis*; *Ixodes ricinus*; *Borrelia burgdorferi* sensu lato; Slovakia; Poland; Romania

Introduction

Lyme borreliosis (LB) is the most widespread vector-borne disease in the northern hemisphere. The causative agents of LB are spirochetes from the *Borrelia burgdorferi*

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sensu lato (s.l.) complex which currently comprises at least 12 genospecies. The main European vector of LB is *Ixodes ricinus*, the most common tick in Europe. Currently, *B. burgdorferi* s.s., *B. garinii*, *B. afzelii*, and *B. spielmanii* are clearly established as pathogenic to humans (van Dam et al., 1993; Wang et al., 1999; Földvári et al., 2005). *B. valaisiana* and *B. lusitaniae* previously considered as non-pathogenic might cause disease as well (Collares-Pereira et al., 2004; Diza et al., 2004). The geographic distribution of *B. burgdorferi* s.l. genospecies in Europe is very variable and can vary even in relatively small areas as well as over time in a given area (Derdáková and Lenčáková, 2005). In endemic areas of Europe, at least 6 *Borrelia* genospecies may circulate between vertebrate hosts and ticks. *B. afzelii* and *B. garinii* are the most frequent and the most widely distributed genospecies and occur over the whole of Europe (Hubálek and Halouzka, 1997) followed by *B. valaisiana* and *B. burgdorferi* s.s. (Derdáková et al., 2003). *B. spielmanii* and *B. lusitaniae* belong to the less abundant genospecies in Europe. Records of *B. spielmanii* in *I. ricinus*, in reservoir hosts, and in human patients come from the Netherlands (Wang et al., 1999), the Czech Republic (Derdáková et al., 2003), Germany (Richter et al., 2004), and Hungary (Földvári et al., 2005). The prevalence of *B. lusitaniae* varies in different parts of Europe. While in the Mediterranean basin it represents the dominant or the only genospecies present in *I. ricinus* ticks, reports from other parts of Europe are rather rare (Postic et al., 1997; Gern et al., 1999; De Michelis et al., 2000; Younsi et al., 2001; Sarih et al., 2003; Wodecka and Skotarczak, 2005; Poupon et al., 2006; Amore et al., 2007). Particular *Borrelia* genospecies differ in their transmission cycles with different vertebrate taxons as reservoir hosts. Birds are competent reservoir hosts of *B. garinii* and *B. valaisiana* (Humair et al., 1998; Hanincová et al., 2003b). Rodents serve as reservoir hosts for *B. afzelii* (Humair et al., 1999; Hanincová et al., 2003a) and *B. garinii* belonging to the OspA type 4 (Postic et al., 1997). The newly described genospecies *B. spielmanii* is maintained in natural foci by the edible dormouse (*Eliomys quercinus*) and the fat dormouse (*Glis glis*) (Matuschka et al., 1994; Richter et al., 2004). Several lizard species were proved to possess a complement with borreliacidal activity (Lane and Loye, 1989; Wright et al., 1998), and therefore the role of lizards in the circulation of *B. burgdorferi* s.l. has been underestimated. Recent studies suggest associations of lizards with *B. lusitaniae* circulation. The reservoir competence of *Psammodromus algirus* was proven recently (Dsouli et al., 2006). *B. lusitaniae* was detected in skin biopsy specimens and blood samples and in larvae and nymphs of *I. ricinus* removed from green lizards (*Lacerta viridis*) (Majláthová et al., 2006) and wall lizards (*Podarcis muralis*) (Amore et al., 2007). Richter and Matuschka (2006) detected a high prevalence of *B. lusitaniae* in ticks feeding on *L. agilis* and *Podarcis muralis*.

The aim of this study was to demonstrate that the sand lizard (*L. agilis*) contributes to the maintenance cycle of *B. burgdorferi* s.l. and to compare 3 distinct populations of *L. agilis* in Europe.

Materials and methods

Study area

The study was carried out during summer of 2006 in 3 distinct localities. The first locality was chosen in Martinské Hole Mountains, Slovakia (49°05′N, 18°49′E). The dominant vegetation consisted of European beech (*Fagus sylvatica*) and silver fir (*Abies alba*). Beech is mostly mixed with silver fir, Norway spruce (*Picea abies*), and sycamore (*Acer pseudoplatanus*). The climate is cold with high humidity. Lizards were sampled on the forest edges and clearings. The second sampling area was located near the town Odolanow, Poland (51°34′N, 17°40′E). This study area was characterised by intensively farmed land with a mosaic of arable fields, meadows, and small woodlots and scattered trees and shrubs of different age, with dominance of white willow (*Salix fragilis*), silver birch (*Betula pendula*), black poplar (*Populus nigra*), and pine (*Pinus sylvestris*). The third locality in the Delta Danube, Romania, was situated on the Sfântu Gheorghe arm of Danube directly at the Black Sea shore (44°54′N, 29°36′E). Local habitats are sandy, covered with feather grass (*Stipa* spp.), *Juncus* spp., and other steppe species. Elevated mounds support stands of white willow (*Salix alba*), black poplar (*Populus nigra*), alder (*Alnus* spp.), ash tree (*Fraxinus* spp.), and oak (*Quercus* spp.).

Lizard species

L. agilis is a short-legged, rather robust, small to medium-sized lizard (up to 90 mm snout-to-vent length) from the family Lacertidae. The sand lizard is a ground-dwelling and strongly diurnal species with one of the widest distribution ranges of all reptiles in Europe (Bischoff, 1984). Sand lizards are largely insectivorous, actively chasing and consuming a range of spiders and insects (Corbett and Tamarind, 1979). In Slovakia and Poland, *L. agilis agilis*, the subspecies of sand lizard abundant in north-western Europe, was captured in May 2006, and in Romania, *L. agilis chersonensis*, an east European subspecies preferring dry sandy habitats such as sand dunes, heaths, and meadows with sandy soils, was caught in July 2006.

Tick and lizard collection

Sand lizards were captured using landing fishnets, by hand, or by noosing, where a loop made from fishing

nylon was attached to the end of a wooden stick and dangled in front of a lizard, who would be captured upon walking through the loop. Animals were sexed, aged (adult, subadult, juvenile), and examined for the presence of ticks. Ticks were removed with forceps immediately after capture and stored in 70% ethanol.

Tissue samples were taken from each individual. A skin biopsy from collar scales (1 × 1.5 mm) was taken with sterile scissors and put in separate vials with 70% ethanol. Ticks were identified to the species, sexed, and only *I. ricinus* ticks were further examined for the presence of *B. burgdorferi* s.l. Questing ticks were collected by flagging the vegetation and stored in 70% ethanol.

DNA isolation

Immediately prior to extraction, ticks and tissues were dried for 30 min to evaporate the ethanol. Each sample was cut with a disposable sterile scalpel.

Genomic DNA from lizard's scales and ticks was isolated by alkaline hydrolysis (Guy and Stanek, 1991). Incubation time was extended from 5 to 30 min. Isolated DNA was stored at −20 °C.

PCR

PCR amplification was performed in a total of 25-μl reaction mixture of a MasterTaq DNA polymerase kit (Eppendorf AG, Hamburg, Germany) containing 10.4 μl of deionized water, 5 μl of 5 × TaqMaster PCR Enhancer, 2.5 μl of 10 × Taq buffer (with 15 mM Mg^{2+}), 1.5 μl of a 25-mM solution of $Mg(OAc)_2$, 0.1 μl of Taq DNA polymerase (5 U/μl), 0.5 μl of dNTP-mix (10 mM) (Fermentas, Vilnius, Lithuania), 1.25 μl of each primer (10 pmole/μl) (Invitrogen, Paisley, Scotland), and 2.5 μl of DNA template. In order to verify that DNA had been successfully isolated from each tick, primers for the fragment of the tick's mitochondrial cytochrome *b* gene (620 bp) were used (Black and Roehrdanz, 1998). Negative samples were excluded from further analysis. Positive samples were examined for the presence of *B. burgdorferi* s.l. by amplifying a portion of the 5S (*rrfA*)–23S (*rrlB*) rDNA intergenic spacer (Derdáková et al., 2003). The PCR products were electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized with a UV transilluminator.

Restriction fragment length polymorphism (RFLP) analysis

The positive PCR products of the 5S–23S rDNA intergenic spacer regions were further analyzed by RFLP. Previously extracted DNA of *B. afzelii*, *B. garinii*, *B. valaisiana*, *B. burgdorferi* s.s., and *B. lusitaniae* were used as positive controls. For each positive sample, 13 μl of amplified DNA was digested at 65 °C overnight in a solution containing 5 U of *TruI* I (300 U/ml) and 1 × Buffer R (Fermentas). Electrophoresis was carried out in 16% polyacrylamide gel at 150 V for 3 h. The gels were stained with SYBR Gold nucleic acid gel stain (Molecular Probes, Leiden, The Netherlands) for 20 min, and bands were visualized with a UV transilluminator (Derdáková et al., 2003).

All procedures, DNA isolation, PCR, and electrophoresis were performed in separate rooms using different pipettes and racks, wearing separate coats and disposable gloves in each laboratory to prevent carry-over contamination and to avoid false-positive results. PCR mixture was prepared in a sterile PCR box. All liquid handling procedures were performed using disposable sterile filter tips. In each DNA isolation and PCR reaction, the negative control (water) was included.

Data analysis and statistics

To estimate the probability of a tick becoming infected after engorging on a green lizard and to measure the degree of infectiousness of infected animals, the specific infectivity I_s (Mather et al., 1989) and the transmission coefficient β_{H-T} (Hanincová et al., 2003a) were calculated. Individual infectivity (i) is defined as the proportion of infected larvae derived from an individual lizard ($i = l_i/l_h$; l_i is the number of larvae that become infected and l_h is the total number of larvae derived from that host). The specific infectivity (I_S) of a reservoir host species is defined as the sum of individual infectivities and the number of individuals sampled ($I_S = \Sigma i_S/n_S$; n is the number of individuals captured). The host-to-tick transmission coefficient (β_{H-T}) is defined as the portion of the sum of individual infectivities and the number of lizards that infected at least one larva ($\beta_{H-T} = \Sigma i_S/n_{iS}$ (n_i is the number of individual hosts that gave rise to at least one infected tick). Differences in the prevalence of borreliae in ticks and skin biopsies were evaluated statistically using the chi square test (χ^2) and a value of $p \leqslant 0.05$ was considered as significant.

Results

Captured lizards and infestation with *Ixodes ricinus* ticks

During the study, 87 sand lizards were captured at 3 localities in Europe and examined for the presence of *I. ricinus* ticks.

In Slovakia, 11 (6 females, 2 males, and 3 juveniles) sand lizards were captured. Each lizard was infested by ticks. Altogether, 152 *I. ricinus* (74 larvae, 78 nymphs) were removed (Table 1). The mean infestation was 13.8

ticks per lizard. The highest number of ticks collected from a single lizard, an adult female, was 29.

Totally, 48 sand lizards (16 females, 16 males, 16 juveniles) were captured in Poland, 32 of which (11 females, 9 males, 12 juveniles) were infested with ticks (prevalence of infestation: 67%). The mean infestation was 5.8 ticks per lizard. Altogether, 187 *I. ricinus* ticks (74 larvae, 113 nymphs) were collected (Table 2). The highest number of ticks collected from a single lizard, an adult female, was 46.

A total of 28 individuals of *L. agilis* (20 females, 8 males) were captured in Romania, and 50% of them were infested with ticks. The mean infestation was 6.9 ticks per lizard. Altogether, 97 *I. ricinus* ticks – only larvae – were collected. The highest number of ticks collected from a single lizard, an adult female, was 29.

Prevalence of *Borrelia burgdorferi* sensu lato in lizards and feeding ticks

Altogether, 436 *I. ricinus* ticks and 87 skin biopsy specimens from collar scales were collected from lizards at three selected localities and further analyzed for the presence of *B. burgdorferi* s.l.

Out of 152 ticks that had fed on *L. agilis* in Slovakia, 32 (21%) were found to be infected with *B. burgdorferi* s.l. A higher prevalence of borreliae was detected in the nymphs (35%) than in the larvae (7%) ($\chi^2 = 17.33$, $df = 1$, $p \leqslant 0.001$). The only detected genospecies in nymphs was *B. lusitaniae*. The same genospecies was found in 3 out of 5 positive larvae, the remaining 2 infections were caused by *B. afzelii* (Table 1). Out of 11 skin biopsies, 5 tested positive for borreliae. RFLP genotyping showed *B. lusitaniae* profile in all 5 samples.

Out of 187 examined ticks removed from sand lizards in Poland, infection prevalence reached 6%. *B. lusitaniae* was the dominant genospecies and was present in 7 of the positive samples and 3 others belonged to *B. valaisiana* (Table 2). The infection prevalence between nymphs and larvae differed significantly ($\chi^2 = 4.45$, $df = 1$, $p = 0.03$). In total, 48 skin biopsy specimens were obtained and examined for the presence of *B. burgdorferi* s.l. None of the skin biopsies revealed a positive result.

In Romania, only larvae were feeding on sand lizards at the time of capture. Altogether, 13 out of 97 removed *I. ricinus* larvae tested positive. The only detected genospecies was *B. lusitaniae*. Additionally, 28 skin biopsies from collar scales were examined. The PCR product was present in 16 samples and again only *B. lusitaniae* was detected.

When comparing the 3 selected localities, the prevalence of *B. burgdorferi* infection in ticks feeding on lizards was significantly higher in larvae from Romania than from Poland ($\chi^2 = 8.11$, $df = 1$, $p \leqslant 0.004$). The differences were not significant when comparing Slovakia with Poland ($\chi^2 = 2.78$, $df = 1$, $p = 0.095$) or Romania ($\chi^2 = 1.97$, $df = 1$, $p = 0.161$). Differences in the prevalence of borreliae between nymphs removed from lizards in Slovakia and Poland were also significant.

Prevalence of *Borrelia burgdorferi* s.l. in questing ticks

A total of 241 ticks (198 nymphs, 25 females, and 18 males) was collected in the Polish locality and examined for *B. burgdorferi* s.l. The total number of *Borrelia*-infected ticks was 57 (23.7%) (Table 3). The highest prevalence was found in females (36%), followed by the

Table 1. *Borrelia burgdorferi* s.l. detected in *Ixodes ricinus* ticks collected from 11 sand lizards (*Lacerta agilis*) in Slovakia

Stage	No. of ticks examined	No. of positive ticks (%)	No. of ticks positive (% of *Borrelia*-positive ticks)				
			B-lus	*B-afz*	*B-gar*	*Bb-ss*	B-val
Larvae	74	5 (7)	3 (60)	2 (40)	0	0	0
Nymphs	78	27 (35)	27 (100)	0	0	0	0
Total	152	32 (21)	30 (94)	2 (6)	0	0	0

B-lus: B. lusitaniae; B-afz: B. afzelii; B-gar: B. garinii; Bb-ss: B. burgdorferi sensu stricto; B-val: B. valaisiana.

Table 2. *Borrelia burgdorferi* s.l. detected in *Ixodes ricinus* ticks collected from 48 sand lizards in Poland

Stage	No. of ticks examined	No. of positive ticks (%)	No. of ticks positive (% of *Borrelia*-positive ticks)				
			B-lus	*B-afz*	*B-gar*	*Bb-ss*	*B-val*
Larvae	74	1 (1)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Nymphs	113	10 (9)	6 (60)	0 (0)	1 (10)	0 (0)	3 (30)
Total	187	11 (6)	7 (64)	0 (0)	1 (9)	0 (0)	3 (27)

B-lus: B. lusitaniae; B-afz: B. afzelii; B-gar: B. garinii; Bb-ss: B. burgdorferi sensu stricto; B-val: B. valaisiana.

Table 3. Prevalences of different genospecies of *Borrelia burgdorferi* s.l. in questing *Ixodes ricinus* ticks in Poland

Stage	No. of ticks examined	No. of positive ticks (%)	No. of ticks positive (% of *Borrelia*-positive ticks)						
			B-afz	*B-gar*	*B-val*	*Bb-ss*	B-lus	*B-val+B-gar*	*B-lus+*
Nymphs	198	44 (22)	16 (36)	10 (23)	5 (11)	2 (5)	9 (20)	1 (2)	0 (0)
Females	25	9 (36)	4 (44)	0 (0)	1 (11)	1 (11)	2 (22)	0 (0)	1 (11)
Males	18	4 (22)	3 (75)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)
Total	241	57 (23.7)	23 (40)	10 (18)	6 (11)	3 (5)	12 (21)	1 (2)	1 (2)

B-lus: B. lusitaniae; B-afz: B. afzelii; B-gar: B. garinii; Bb-ss: B. burgdorferi sensu stricto; B-val: B. valaisiana; B-val+B-gar: coinfection of *B. valaisiana* and *B. garinii, B-lus+:* coinfection of *B. lusitaniae* with an undetermined *borrelia*.

males and nymphs (both 22%). The dominant genospecies was *B. afzelii* present in 23 (40%) of all *Borrelia*-positive ticks. The second most prevalent genospecies was *B. lusitaniae*, found in 12 (21%) ticks. *B. garinii* was found in 10 (18%) ticks.

Specific infectivity and transmission coefficient

The specific infectivity I_S and the transmission coefficient β_{H-T} as measures of the probability of a larva of becoming infected with *B. lusitaniae* after engorging on sand lizards were calculated. The relation between the transmission coefficient and specific infectivity reflects the ratio of lizards that gives rise to at least one infected larva (β_{H-T}) versus all captured lizards (I_S). The comparison of specific infectivities and the transmission coefficient of different populations of *L. agilis* sampled in different localities revealed discrepancies (Table 4).

Discussion

The implication and the role of lizards in the maintenance cycle of *B. burgdorferi* s.l. in the field are currently studied intensively. Specific bonds between *B. valaisiana* as well as *B. garinii* and birds, and *B. afzelii* and rodents were described; however, the specific reservoir host for *B. lusitaniae* was unknown. Recently, *Psammodromus algirus*, a small lacertid lizard and the dominant host for immature *I. ricinus* ticks in Tunisia, was proved to be a reservoir host for *B. lusitaniae* (Dsouli et al., 2006). Moreover, 3 other lizard species (*P. muralis*, *L. viridis*, and *L. agilis*) were shown to be implicated in the transmission of *B. lusitaniae* (Majláthová et al., 2006; Richter and Matuschka, 2006; Amore et al., 2007). In our study, we captured sand lizards in Slovakia, Poland, and Romania and examined the removed ticks from these hosts and skin biopsy specimens from collar scales for the presence of *B. burgdorferi* s.l. Additionally, questing ticks were flagged in the concerning locality in Poland and examined for borrelia infection as well.

Table 4. Specific infectivity (I_S) and host-to-tick transmission coefficient (β_{H-T}) for *Borrelia lusitaniae* in sand lizards captured in different geographic areas

Genospecies	Males		Females		Totals	
	I_S	β_{H-T}	I_S	β_{H-T}	I_S	β_{H-T}
L. agilis (SR)	0	0	0.19	0.56	0.102	0.56
L. agilis (PL)	0.015	0.166	0	0	0.003	0.166
L. agilis (RO)	0.14	0.38	0.07	0.5	0.09	0.44

SR: Slovakia; PL: Poland; RO: Romania; for further explanation see Materials and methods.

In general, lizards serve as hosts for ticks in localities where both ticks and lizards are present (Eisen et al., 2001; Scali et al., 2001; Dsouli et al., 2006; Majláthová et al., 2006). The intensity of infestation is dependent on host as well as tick activity (Scali et al., 2001), on host gender, and is influenced by the season (Eisen et al., 2001). Male lizards generally carry higher tick loads than females in the spring (Lane and Loye, 1989; Tälleklint-Eisen and Eisen, 1999). This phenomenon is probably related to behavioral differences and mating activity (Scali et al., 2001). Additionally, home ranges of male individuals are larger than those of females and differ between lizard species (Bauwens et al., 1983; Eisen et al., 2001). We captured lizards at the end of the mating period in Poland and Slovakia and after the mating period in Romania. The prevalence of infestation was higher in males only in Romania, in Slovakia all captured lizards were infested, and in Poland subadults were infested most frequently followed by males. Interestingly, the mean infestation was the highest in females in all localities. Removed ticks were examined for the presence of *B. burgdorferi* s.l. As *B. lusitaniae* was indicated to circulate between lizards and ticks in natural foci, we focused on that genospecies. The geographic distribution of *B. lusitaniae* was considered to be restricted to the Mediterranean region with rare focal occurrence in other parts of Europe. De Michelis et al. (2000) hypothesized that the distribution of *B. lusitaniae* is associated with a specific reservoir host abundant especially in that region. This fits to lizards

which prefer warm habitats in southern Europe and are less abundant in northern latitudes. Several studies dealing with the heterogeneity of *B. burgdorferi* s.l. in various localities in Europe revealed that *B. lusitaniae* is not that rare as it had been thought previously. *B. lusitaniae* was reported from small numbers of ticks in a few localities in western Slovakia (Gern et al., 1999; Hanincová et al., 2003b). Koči (2006) detected a fraction of 21% of *Borrelia*-positive *I. ricinus* nymphs and adult ticks infected with *B. lusitaniae* (corresponding to an infection rate of 2.2%) in western Slovakia. Recently, a focus with *B. lusitaniae* was found in northern Slovakia montane (above 650 m a.s.l.) (Cíglerová, unpublished). In Poland, *B. lusitaniae* was detected by Wodecka and Skotarczak (2005). Our study led to the detection of a new *B. lusitaniae* focus in Wielkopolska region in Poland and to the first detection of *B. lusitaniae* in Romania.

In all three investigated localities, we found *B. lusitaniae* in larvae and nymphs of *I. ricinus* feeding on sand lizards and with the exception of lizards from Poland also in skin biopsy specimens from collar scales. The prevalence of *B. lusitaniae* in sand lizards was 45% in Slovakia and 57% in Romania. In Romania, *B. lusitaniae* was the only genospecies found in larvae that had fed on lizards. According to the life cycle of ticks and borreliae, larvae acquire the spirochetes by feeding on an infected reservoir host. Transovarial transmission seems rather rare (Piesman et al., 1986). However, even if *B. lusitaniae* were transovarially transmitted, field observations of Richter and Matuschka (2006) strongly suggest that lizards, but not rodents, permit their survival in feeding ticks and subsequent transstadial transmission to the nymphal stage. In Slovakia, nymphal *I. ricinus* removed from lizards were infected solely with *B. lusitaniae*. PCR examination of removed larvae revealed *B. afzelii* infection in two cases. Both larvae were feeding in the close proximity on the same lizard. We assume that co-feeding transmission from infected nymphs was the way of infection, as also incompetent host species (e.g. lizards for *B. afzelii*) may contribute to the circulation of *B. burgdorferi* s.l. in nature (Ogden et al., 1997). Of great interest is the finding of the relatively high prevalence (21%) of *B. lusitaniae* among *Borrelia*-positive questing ticks at the locality in Poland when compared to the prevalence of *B. lusitaniae* in ticks that had fed on lizards. Possibly also another animal taxon is involved in the transmission of *B. lusitaniae* as pointed out by Poupon et al. (2006).

In conclusion, we found *B. lusitaniae* in skin biopsies and larvae and nymphs of *I. ricinus* ticks feeding on sand lizards in Slovakia, Poland, and Romania. These findings give support to the hypothesized reservoir role of the sand lizard in the transmission cycle of *B. lusitaniae*. We found differences among distinct populations of the same lizard species, *L. agilis*. The reasons for these differences are currently unknown.

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