Association of *Borrelia afzelii* with rodents in Europe

K. HANINCOVÁ[1,2], S. M. SCHÄFER[1,4,5], S. ETTI[1], H.-S. SEWELL[1,5], V. TARAGELOVÁ[2], D. ZIAK[3], M. LABUDA[2] *and* K. KURTENBACH[1]*

[1] *Department of Infectious Disease Epidemiology, Imperial College of Science, Technology and Medicine, Norfolk Place, London W2 1PG, UK*
[2] *Institute of Zoology, Slovak Academy of Science, Dubravská cesta 9, 81364 Bratislava, Slovakia*
[3] *Department of Zoology, Comenius University, Mlynska dolina, 84215 Bratislava, Slovakia*
[4] *NERC Centre for Ecology and Hydrology, Mansfield Road, Oxford OX1 3SR, UK*
[5] *Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*

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SUMMARY

Borrelia burgdorferi sensu lato (s.l.) is maintained in nature by complex zoonotic transmission cycles, involving a large variety of vertebrates as hosts and hard ticks of the genus *Ixodes* as vectors. Recent studies suggest that the genospecies of *B. burgdorferi* s.l. and sometimes their subtypes are propagated by different spectra of hosts, mainly birds and rodents. In order to test the concept of host-association, we analysed the relationships between *Borrelia* genospecies, rodent hosts and *I. ricinus* ticks in an endemic focus of Lyme borreliosis in western Slovakia. Rodents and questing ticks were collected at a forested lowland locality near Bratislava. Tick infestation levels on rodents were determined, and spirochaete infections in ticks and in ear punch biopsies were analysed by PCR followed by genotyping. Mice were more heavily infested with ticks than bank voles, and a higher proportion of mice was infected with spirochaetes than voles. However, the infectivity of voles was much higher than that of mice. The vast majority of infections detected in the skin and in ticks feeding on the rodents represented *B. afzelii*. In contrast, more than half of all infections in questing ticks collected in the same region of Slovakia were identified as *B. valaisiana* and *B. garinii*. In conclusion, whilst the study reveals that mice and voles play different quantitative roles in the ecology of Lyme borreliosis, it demonstrates that *B. afzelii* is specifically maintained by European rodents, validating the concept of host-association of *B. burgdorferi* s.l.

Key words: *Borrelia burgdorferi* sensu lato, *B. afzelii*, rodents, host-association.

INTRODUCTION

Lyme borreliosis is a multi-facetted chronic bacterial disease in humans of the northern hemisphere. It is caused by *Borrelia burgdorferi* sensu lato (s.l.) which is transmitted by hard ticks of the genus *Ixodes* Latreille, 1795. The wider taxon forms a species complex that now comprises 11 named genospecies (Postic *et al*. 1994, 1998; Le Fleche *et al*. 1997; Masuzawa *et al*. 1997, 2001). In Europe, *B. afzelii*, *B. garinii* and *B. valaisiana* are the most frequent genospecies (Gern *et al*. 1999; Kurtenbach *et al*. 2001). *I. ricinus* (Linnaeus, 1758), the principal vector of Lyme borreliosis in Europe, has a broad host range (Milne, 1949; Randolph & Craine, 1995), and about 50 vertebrate species have been described to serve as reservoir hosts of *B. burgdorferi* s.l. (Gern *et al*. 1998). For this reason, the zoonotic transmission cycles of *B. burgdorferi* s.l. are highly complex and interwoven. From various experimental and epidemiological studies undertaken since the delineation of genospecies, it can be inferred that *B. burgdorferi* s.l. is host-associated.

* Corresponding author: Department of Infectious Disease Epidemiology, Imperial College of Science, Technology and Medicine, St Mary's Campus, Norfolk Place, London W2 1PG, UK. Tel: 0044 20 759 43629. Fax: 0044 20 759 43693. E-mail: k.kurtenbach@ic.ac.uk

For example, *B. afzelii* seems to be preferentially transmitted by rodents (Humair *et al*. 1995; Hu *et al*. 1997; Humair & Gern, 1998; Humair, Rais & Gern, 1999), whilst *B. valaisiana* and many variants of *B. garinii* appear to be associated with birds (Olsen *et al*. 1993; Olsen Jaenson & Bergström, 1995; Humair *et al*. 1998; Kurtenbach *et al*. 1998*a*, *b*; Gylfe *et al*. 1999, 2000). Recently, we have proposed a testable biological model to explain the host-association of *B. burgdorferi* s.l., which predicts that spirochaetes are selected in the midgut of feeding ticks by host complement (Kurtenbach *et al*. 2002*a*).

The aim of this study was to test the concept of host-association of *B. burgdorferi* s.l. rigorously in the field. The field work was undertaken in an endemic focus of Lyme borreliosis in western Slovakia. The region was chosen because several genospecies of *B. burgdorferi* s.l. are prevalent in questing ticks and even co-circulate in the same micro-habitats (Gern *et al*. 1999; Kurtenbach *et al*. 2001). Animals in such habitats must, therefore, encounter ticks that are infected with any of these spirochaetal genospecies. We tested the prediction that, if a vertebrate host species displays an association with a particular genospecies, the genetic diversity of *B. burgdorferi* s.l. in the host and in ticks engorged on such a host should differ from that observed in the questing ticks.

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MATERIALS AND METHODS

Study site

The study was conducted in Western Slovakia within a focus of Lyme borreliosis near Zahorska Ves (16°53′W, 48°22′N), 45 km north-west of Bratislava. The tree layer consists mainly of *Quercus robur*, *Pinus sylvestris* and *Robinia pseudoacacia*. The undergrowth is dominated by *Sambucus nigra*, *Ligustrum vulgare*, *Calamagrostis epigeios*, *Crataegus monogyna*, *Dactylus glomerata* and *Robinia pseudoacacia*.

Capturing and handling of rodents

Rodents were live-trapped for 2 consecutive days every 2 weeks from March until November 2000 using Swedish bridge metal traps baited with oat flakes. Traps were placed in two 100 m lines 900 m apart. On each line 20 traps were set at intervals of 5 m. Captured rodents were identified to species, weighed, measured, and sexed. Their age was estimated based on the body weight, and the populations were divided into juveniles, subadults and adults (Gliwicz, 1988). After obtaining ear punch biopsies and feeding *I. ricinus* ticks, the rodents were released exactly at the same spot where trapped. Feeding ticks and biopsies were preserved in 70% ethanol.

Questing ticks

Nymphal and adult *I. ricinus* ticks were collected from the vegetation by blanket dragging at the same time and place where the rodents were captured. The 1 m^2 blanket was dragged along the entire length of the 100 m transects and periodically checked for questing ticks. Field-derived ticks were preserved in 70% ethanol until analysis for the presence of *B. burgdorferi* s.l. by PCR.

PCR and genotyping

Genomic DNA was extracted from ticks and rodent skin biopsies by alkaline hydrolysis (Guy & Stanek, 1991). Nested PCR was performed targeting the *rrf* (5S)–*rrl* (23S) intergenic spacer of *B. burgdorferi* s.l. as described previously (Rijpkema *et al.* 1995; Kurtenbach *et al.* 1998*b*). All steps of the PCR were performed under strictly aseptic conditions and separated temporally and spatially, and negative controls were included at a ratio of approximately 2:3. Serial dilutions of cultured *B. andersonii*, a genospecies confined to America, were used as positive controls. All samples that contained fragments of approximately 380 bp and/or 230 bp were analysed by the reverse line blot assay, a DNA–DNA hybridization method (Rijpkema *et al.* 1995; Kurtenbach *et al.* 1998*b*). Probes specific for *B. burgdorferi* s.l., *B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. afzelii* and *B. valaisiana* were used. Amplicons that were untypeable using these DNA probes were subjected to dye terminator cycle sequencing.

Table 1. Species, number and relative proportion of captured rodents

(Calculated as the percentage of total captures represented by each species. Two *C. glareolus* and *A. flavicollis* escaped and could not be assessed for ticks (see Table 4).)

Species	Number of hosts	Relative proportion of hosts
Clethrionomys glareolus (Schreber, 1780)	111	42·21
Apodemus flavicollis (Melchior, 1834)	96	36·50
Microtus arvalis (Pallas, 1779)	33	12·55
Apodemus sylvaticus (Linnaeus, 1758)	21	7·98
Micromys minutus (Pallas, 1771)	2	0·76

Data analysis and statistics

Estimates of host infestation with *I. ricinus* ticks were obtained using the following parasitological indices (Margolis *et al.* 1982): *prevalence of infestation* – percentage of hosts carrying ticks; *abundance of infestation* – average number of ticks per host considering the entire host population sampled, and *mean intensity of infestation* – average number of ticks per tick-infested host.

Estimates of *B. burgdorferi* s.l. infections were measured as *infection prevalence* – percentage of ticks or hosts infected, *specific infectivity* (I_s) – the sum of individual infectivities (i.e. number of infected larvae divided by the total number of larvae derived from a host) divided by the number of hosts sampled (Mather *et al.* 1989) and *transmission coefficient* ($\beta_{\text{H-T}}$) – a measure of the degree of infectiousness of infected animals as outlined in the results section.

Parameter values were analysed statistically by means of Pearson's χ^2 test with Yates' continuity correction and Mann-Whitney U-Test.

RESULTS

Rodents and infestation with ticks

During 1360 trap-nights, 263 rodents representing 5 species of 2 families (Muridae and Cricetidae) were captured. The yellow-necked mouse (*Apodemus flavicollis*) and the bank vole (*Clethrionomys glareolus*) constituted 79% of all captures (Table 1). Therefore, a detailed analysis is presented only for these 2 rodent species.

I. ricinus (744 larvae and 30 nymphs) represented 99% of the feeding ticks obtained from these hosts. Seven larvae and 3 nymphs of *Dermacentor reticulatus*

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Table 2. Infestation of *Apodemus flavicollis* and *Clethrionomys glareolus* with *Ixodes ricinus*

	Larvae				Nymphs			
Host species	*n**	*P* (%)†	$A \pm s_x$‡	$M \pm s_x$§	*n*	*P* (%)	$A \pm s_x$	$M \pm s_x$
A. flavicollis	482	73	5·07 ± 0·83	6·99 ± 1·05	15	14	0·16 ± 0·04	1·15 ± 0·10
C. glareolus	262	62	2·40 ± 0·36	3·85 ± 0·51	15	11	0·14 ± 0·04	1·25 ± 0·13

* Number of ticks.
† Prevalence of animals infested by ticks.
‡ Abundance of infestation ± standard error.
§ Mean intensity of infestation ± standard error.

Table 3. Prevalence of infected hosts in different age categories

	Apodemus flavicollis		*Clethrionomys glareolus*	
Age category	Tested animals	Infected animals*	Tested animals	Infected animals*
Juvenile	21	3 (14%)	15	0
Subadult	30	10 (33%)	35	4 (11%)
Adult	25	9 (36%)	26	10 (38%)

* Based on PCR-positive skin biopsies.

(Fabricius, 1794) found on *C. glareolus* were excluded from the following analysis. The overall prevalence of infestation with *I. ricinus* larvae was 73% (69/95) for *A. flavicollis* and 62% (68/109) for *C. glareolus*. Abundance and mean intensity of larval infestation were significantly higher on *A. flavicollis* than on *C. glareolus* (Mann-Whitney U-Test, $P < 0·05$; Table 2), but not so for nymphs. For both species, maxima of tick infestation levels were observed in late May.

In addition, 28 out of the 30 engorged nymphs were found on animals that were also infested with larvae. The animals were generally parasitized by a single nymph, however, a few individuals harboured 2 nymphs each (data not shown).

Animal biopsies

Ear punch biopsies were taken from 76 *A. flavicollis* and 76 *C. glareolus*. Spirochaetes were found in 29% of the mice and in 18% of the voles. More adult rodents than juveniles of both species were infected (Table 3). All positive biopsies of *C. glareolus* and 20 out of 22 of those derived from *A. flavicollis* carried *B. afzelii* (Fig. 1). Of the other 2 biopsies from *A. flavicollis*, 1 was *B. garinii* and 1 was untypeable by the reverse line blot assay, and its nucleotide sequence could not be determined.

B. burgdorferi *s.l. infection in* I. ricinus *ticks feeding on rodents*

The infection prevalence of *B. burgdorferi* s.l. in larvae feeding on *C. glareolus* (31%) was significantly higher than in those taken from *A. flavicollis* (12%; χ^2 test, $P < 0·01$). The infection prevalence in engorged nymphs was 47% for both rodent species (Fig. 1). Approximately one quarter of the tick-infested rodents carried at least 1 *B. burgdorferi* s.l.-infected tick larva. Thus, about 18% of all rodents were infested with infected ticks. On average, infectious rodents yielded between 3 and 4 infected *I. ricinus* larvae (Table 4). The prevalence of rodents that carried spirochaete-infected larvae rose with age class, such that almost half of the adult rodents that were tick-infested were parasitized by at least 1 infected tick (Table 5).

When the animals were divided into tick-infested and non-infested groups, significantly fewer biopsy infections were detected in non-infested *C. glareolus* than in those infested with ticks (χ^2 test, $P < 0·05$; Table 6). No such differences were observed for *A. flavicollis* (χ^2 test, $P > 0·05$; Table 6). When the animals were divided into carriers and non-carriers of *B. burgdorferi* s.l., the majority of non-infected rodents of both species were infested with non-infected ticks, and the majority of carriers were parasitized by *Borrelia*-infected ticks (χ^2 test, $P < 0·05$; Table 7). These differences were more pronounced in voles than in mice.

Genotyping of *B. burgdorferi* s.l. infections in larvae and nymphs which had fed on the rodents showed that 100% of the infected ticks derived from *C. glareolus* and around 90% of larvae and 100% of nymphs derived from *A. flavicollis* harboured *B. afzelii* (Fig. 1). The specific infectivity from hosts to larval ticks was found to be highest for *B. afzelii*, and approximately twice as high for *C. glareolus* as for *A. flavicollis* (Table 8).

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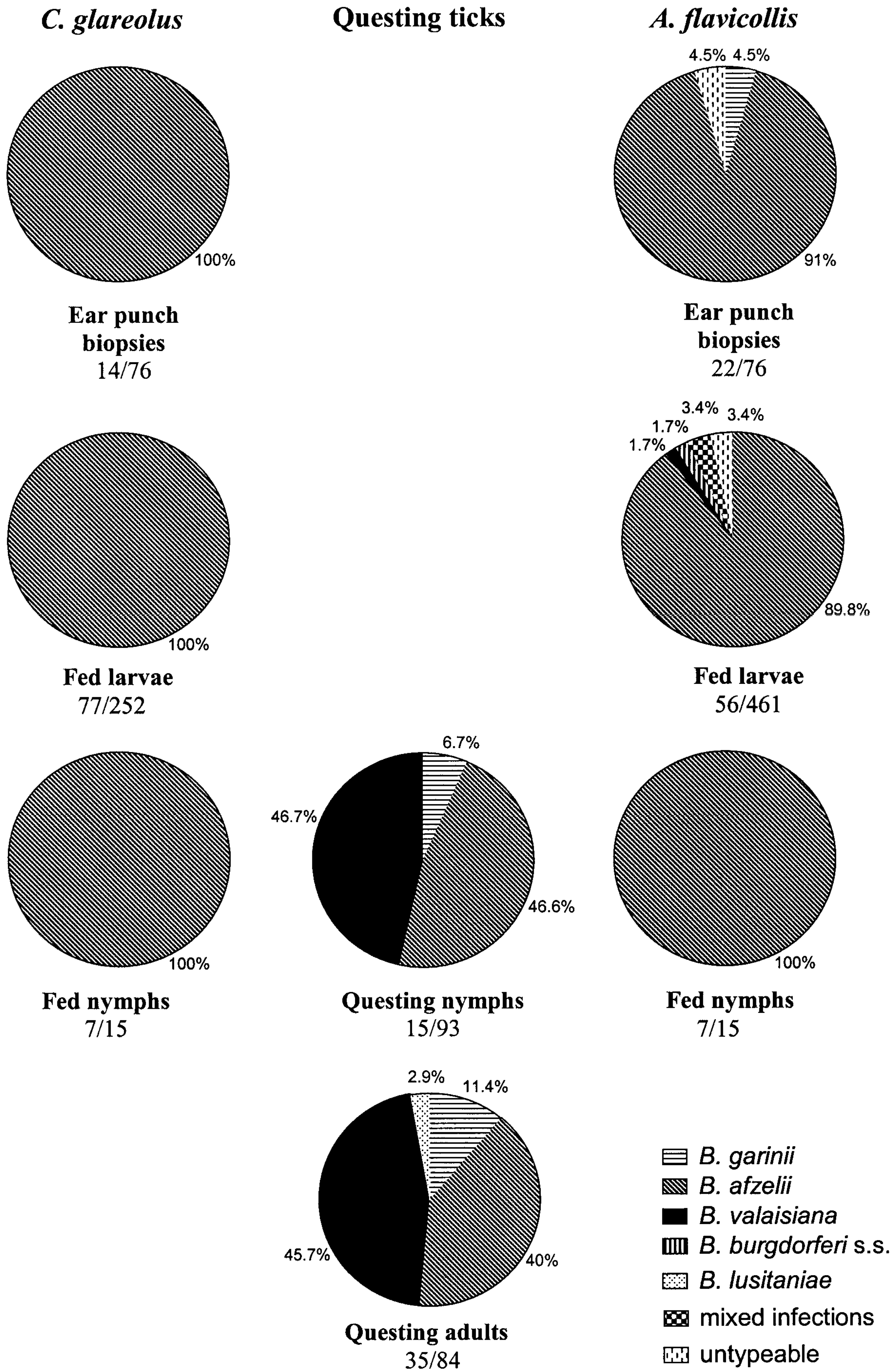

Fig. 1. Pie charts illustrating the genospecies diversity of *Borrelia burgdorferi* s.l. in ear punch biopsies and in fed larval ticks derived from *Clethrionomys glareolus* and *Apodemus flavicollis*, as well as in questing nymphal and adult ticks collected in the same site near Bratislava, Slovakia.

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Table 4. Prevalence of hosts infested by infected ticks and mean number of infected ticks at these animals

	No. of hosts infested by ticks*		No. of hosts infested by infected ticks		No. of infected ticks		Mean no. of infected ticks per host ± S.E.	
Host species	Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs
Apodemus flavicollis	69/95	13/95	17 (25%)	6 (46%)	56	7	3·29±0·7	1·17±0·2
Clethrionomys glareolus	68/109	12/109	19 (28%)	5 (42%)	77	7	4·05±1·3	1·40±0·3

* Number of infested hosts/number of hosts sampled.

Table 5. Prevalence of tick-infested hosts carrying *Borrelia burgdorferi* s.l.-infected *Ixodes ricinus* larvae in different age categories

	Apodemus flavicollis		*Clethrionomys glareolus*	
Age category	No. of hosts infested by larvae	No. of hosts infested by infected larvae	No. of hosts infested by larvae	No. of hosts infested by infected larvae
Juvenile	23	1 (4%)	11	2 (18%)
Subadult	20	4 (20%)	29	5 (17%)
Adult	26	12 (46%)	28	12 (43%)

Table 6. Prevalence of *Borrelia burgdorferi* s.l. infection in animals infested by *Ixodes ricinus* ticks and animals non-infested by ticks

	Apodemus flavicollis		*Clethrionomys glareolus*	
	Tested animals	Infected animals*	Tested animals	Infected animals*
Hosts infested by ticks	54	15 (28%)	48	13 (27%)
Hosts non-infested by ticks	22	7 (32%)	28	1 (4%)

* Based on PCR-positive skin biopsies.

Specific infectivity is calculated by considering the entire host population sampled and, thus, includes larval ticks from individual hosts that are not infectious (Mather *et al.* 1989). Therefore, in order to measure the degree of infectiousness of hosts that are infectious, this index was modified as follows: $I_s = \sum_s i_s/n_s$, where n_s is the number of individual hosts that gave rise to at least 1 infected tick. Assuming a low rate of transovarial transmission of spirochaetes from engorged female ticks to questing larvae (Piesman *et al.* 1986), this parameter value will approximate to the host-to-larval transmission coefficient ($\beta_{\text{H-T}}$) of infectious individuals (Randolph & Craine, 1995; Randolph, 1998) and therefore this parameter is designated as such in this paper. Again, a higher value was calculated for *C. glareolus* than for *A. flavicollis* (Table 8).

B. burgdorferi *s.l. infection in questing* I. ricinus *ticks*

B. burgdorferi s.l. infections were analysed in questing nymphs and adult *I. ricinus* ticks collected at the same time and site where the rodents were captured. The infection prevalence was 16% in questing nymphs and 42% in questing adult ticks. The most frequent genospecies in both developmental stages of the tick were *B. valaisiana* and *B. afzelii*, followed by *B. garinii*. Taken together, *B. garinii* and *B. valaisiana* represented approximately half of all infections in questing ticks. One adult tick was found to be infected with *B. lusitaniae* as inferred from the nucleotide sequence. The genospecies diversity of *B. burgdorferi* s.l. questing ticks, thus, differed significantly from that observed in ticks engorged on rodents (Fig. 1).

DISCUSSION

The present study shows that *B. afzelii* is closely associated with rodents in Europe. Furthermore, the data show that mice and voles play different quantitative roles in the ecology of Lyme borreliosis.

The study was performed using direct PCR amplification of *B. burgdorferi* s.l. DNA from ticks and biopsy material. This protocol is not allele-specific and allows the detection of all genospecies of *B.*

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Table 7. Number of hosts infested by *Borrelia burgdorferi* s.l.-infected ticks amongst infected and non-infected animals

	Apodemus flavicollis		*Clethrionomys glareolus*	
	Infected larvae	Non-infected larvae	Infected larvae	Non-infected larvae
Infected hosts*	9 (60%)	6 (40%)	11 (92%)	1 (8%)
Non-infected hosts*	6 (15%)	33 (85%)	2 (6%)	34 (94%)

* Based on PCR-positive or -negative skin biopsies.

Table 8. Specific infectivity (I_s) and host-to-tick transmission coefficient ($\beta_{H\text{-}T}$)*

	Apodemus flavicollis		*Clethrionomys glareolus*	
Genospecies	I_s	$\beta_{H\text{-}T}$	I_s	$\beta_{H\text{-}T}$
Borrelia burgdorferi s.l.	0·0883	0·3547	0·1845	0·5981
B. afzelii	0·0851	0·3954	0·1845	0·5981
B. valaisiana	0·0047	N.C.	0·0000	0·0000
B. burgdorferi s.s.	0·0001	N.C.	0·0000	0·0000
B. garinii	0·0001	N.C.	0·0000	0·0000

* Based on tick larvae; N.C., not calculated.

burgdorferi s.l. with the same sensitivity. The data of this study are, therefore, likely to reflect the true diversity patterns of *B. burgdorferi* s.l. On the other hand, PCR amplification of spirochaetal DNA does not *per se* prove the presence of viable bacteria in a given sample. However, our PCR-based study is consistent with a study from Switzerland which was based on culturing of *B. burgdorferi* s.l. (Humair, Rais & Gern, 1999), indicating that both PCR and isolation are appropriate methods in the analysis of the diversity of Lyme borreliosis spirochaetes.

The most frequently captured rodent species were *C. glareolus* and *A. flavicollis*, known to be the most abundant rodents in sylvatic habitats in western Slovakia (Dudich, Lysý & Štollman, 1985; Labuda, Lysý & Krippel, 1989; Labuda, Lysý & Kozuch, 1991; Kozuch *et al.* 1995; Krištofik, 1999). In line with previous studies from Europe, *A. flavicollis* was more heavily infested with *I. ricinus* ticks than *C. glareolus* (Labuda *et al.* 1989, 1991; Matuschka *et al.* 1992; Tälleklint & Jaenson, 1994; Kurtenbach *et al.* 1995; Humair *et al.* 1999; Randolph *et al.* 1999), which could in part be attributed to resistance to ticks acquired by *C. glareolus*. Anti-tick resistance reduces the feeding time of ticks on such hosts, thereby reducing the infestation levels (Dizij & Kurtenbach, 1995). Behavioural and physiological differences between the two rodent species could further contribute to different tick burdens (Bergstedt, 1966; Zejda & Pelikán, 1969; Labuda *et al.* 1989, 1991).

At the time of capture, not all individual rodents were infested with ticks. For individual animals that were captured once, no conclusion about the history of tick bites can be drawn. It is, however, possible that those individual hosts were generally less infested with ticks. If that were the case, the infection prevalence of *B. burgdorferi* s.l. in those animals should be lower compared to those heavily infested at the time of trapping. Most interestingly, a significant difference was observed for *C. glareolus*, but not for *A. flavicollis*. This suggests that the subpopulation of bank voles that was not infested with ticks at the time of trapping had a history of fewer encounters with infected vector ticks. It is possible that this fraction of the voles displayed some degree of resistance to *I. ricinus*, reducing the efficiency of spirochaete transmission (Dizij & Kurtenbach, 1995; Kurtenbach *et al.* 1995).

When biopsy-positive animals were compared with biopsy-negative animals, more spirochaetemic rodents yielded *B. burgdorferi* s.l.-infected ticks than biopsy-negative animals. However, this correlation was not absolute as a few animals whose biopsies were negative were parasitized by positive ticks and *vice versa*. Possible explanations of this finding could be that the spirochaete burden in vertebrate hosts is generally very low and that spirochaetes may lodge in deeper organs (Kurtenbach *et al.* 2002*b*). As a consequence, the failure to detect *Borrelia* DNA in a single ear punch biopsy may not prove that an animal is spirochaete-free. Alternatively, or in addition, co-feeding transmission might explain why biopsy-negative animals carry positive ticks (Gern & Rais, 1996; Randolph, Gern & Nuttall, 1996). Another possibility is that spirochaete-negative rodents are

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occasionally infested with transovarially infected larvae (Piesman *et al*. 1986).

In this study *C. glareolus* was found to be more efficient in transmitting spirochaetes to ticks than *A. flavicollis*. Although *C. glareolus* was infested with fewer ticks than *A. flavicollis*, voles gave rise to more infected larvae than mice. This is consistent with data from previous field studies in Europe (Kurtenbach *et al*. 1995; Humair *et al*. 1999). From these findings it might be concluded that voles are more efficient reservoir hosts for *B. burgdorferi* s.l. than mice. However, this is unlikely always to be the case as voles acquire resistance to *I. ricinus*, whereas *A. flavicollis* remain tolerant to repeated tick bites. Resistance to ticks has not only been shown to reduce the attachment time, but also to impair the moulting success, thereby reducing survival rates of ticks (Randolph, 1994; Dizij & Kurtenbach, 1995; Humair *et al*. 1999). For this reason, mathematical models calculating the basic reproduction rate, R_0 (Anderson & May, 1991), of *B. burgdorferi* s.l. must incorporate the differential survival rates of ticks feeding on different host species (Randolph & Craine, 1995; Randolph, 1998).

The data from this study suggest that yellow-necked mice control the infection with *B. burgdorferi* s.l. more effectively than bank voles. A previous experimental study has shown that this is correlated with the humoral immune response mounted to spirochaetes (Kurtenbach *et al*. 1994). On the other hand, voles respond more efficiently to repeated tick bites, which reduces their capacity to produce infected nymphs that serve as vectors of Lyme borreliosis. We have, therefore, suggested that the 2 rodent species have adopted different immunological strategies to cope with tick-borne pathogens: mice are high responders to the microparasite, whereas voles are high responders to the vector (Kurtenbach *et al*. 1994; Dizij & Kurtenbach, 1995).

Genotyping of spirochaetes detected in biopsies and feeding ticks derived from both rodent species revealed that the vast majority of the infections were caused by *B. afzelii*. A similar finding has been reported in a previous study in Switzerland (Humair *et al*. 1999). Because more than 50% of the infected questing nymphs collected in the present study harboured *B. valaisiana* and *B. garinii*, the rodents must have been exposed to ticks infected with these 2 genospecies. The contrasting diversity patterns found in questing nymphs, fed larvae and in skin biopsies allow the conclusion that rodents are highly susceptible and transmission competent for *B. afzelii*. In contrast, the lack of *B. valaisiana* and *B. garinii* in larvae fed on voles and the single incidence in ticks fed on mice indicate that rodents do not, or only occasionally, transmit these genospecies in western Slovakia. Furthermore, and most interestingly, the nymphs engorged on the rodents were infected solely with *B. afzelii*. In addition, the infection prevalence of *B. afzelii* increased from 7% in questing nymphs to 47% in nymphs engorged on the rodents. This strongly suggests that *B. valaisiana* and *B. garinii* are negatively selected in ticks feeding on rodents, whilst *B. afzelii* is amplified.

The virtual absence of *B. garinii* and *B. valaisiana* in rodent-derived larvae, but the presence of the genospecies in questing nymphs collected in the same sites, indicate that animals other than the 2 rodent species investigated are the main reservoir hosts for these genospecies in this part of Europe. There is increasing evidence from experimental and epidemiological studies from Europe of a close association of *B. valaisiana* and *B. garinii* with birds (Olsen *et al*. 1993, 1995; Humair *et al*. 1998; Gylfe *et al*. 1999, 2000; Kurtenbach *et al*. 1998*a*, *b*, 2002*c*). However, the picture for *B. garinii* is more complex. This genospecies is highly diverse and has been divided into 6 OspA serotypes (Marconi *et al*. 1999; Hu *et al*. 2001; Escudero *et al*. 2000). Most OspA serotypes of *B. garinii* appear to be associated with birds. In contrast, OspA serotype 4 strains have never been identified in ticks derived from birds, but were experimentally found to be transmissible through laboratory mice to ticks (Hu *et al*. 2001). It remains to be determined whether natural rodents serve as reservoir for this hyperinvasive clone. Furthermore, diverse ribotypes of *B. garinii* from Asia seem to be associated with wild rodents, rather than with birds (Nakao, Miyamoto & Fukunaga, 1994; Masuzawa *et al*. 1997, 2001). These findings indicate that *B. garinii* comprises at least 2 distinct 'ecotypes'.

The data of the present study are consistent with our recent model of transmission of *B. burgdorferi* s.l. It proposes that spirochaetes which are sensitive to host complement are negatively selected in the midgut of feeding ticks, whereas complement-resistant strains survive the bloodmeal of the tick and are transmitted horizontally and transstadially (Kurtenbach *et al*. 2002*a*). The model is based on *in vitro* studies that have shown that the genospecies of *B. burgdorferi* s.l. and sometimes their subtypes are differentially resistant/sensitive to the alternative pathway of the complement system of different animal species, matching the known transmission patterns (Kurtenbach *et al*. 1998*c*; Lane & Quistad, 1998, Kuo *et al*. 2000; Nelson *et al*. 2000). Strains of *B. burgdorferi* s.s. appear to be much less specialized than strains of the other genospecies, as they are relatively resistant to both rodent and avian complement (Kurtenbach *et al*. 1998*c*). This finding is consistent with observations that identical strains of *B. burgdorferi* s.s. such as the ZS 7 or the N 40 strain, are transmissible to ticks through both rodents and birds (Donahue, Piesman & Spielman, 1987; Kurtenbach *et al*. 1994, 1998*a*; Rand *et al*. 1998; Richter *et al*. 2000). Our transmission model is further supported by a recent experimental study using the pheasant (*Phasianus colchicus*) as an avian model of Lyme borreliosis. There, it was observed that *B. afzelii* was eliminated from

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feeding nymphs, whereas *B. garinii*, *B. valaisiana* and *B. burgdorferi* s.s. survived the bloodmeal in ticks (Kurtenbach *et al.* 2002*c*).

Resistance to complement is now known to be mediated by outer surface protein E and related proteins (Erps) of *B. burgdorferi* s.l. that bind host-derived complement control proteins in a specific manner (Kraiczy *et al.* 2001; Stevenson *et al.* 2002). It has, therefore, been hypothesized that the 'ecotype' of a *B. burgdorferi* s.l. strain is determined by its *erp* gene repertoire (Kurtenbach *et al.* 2002*a*, *b*, *c*; Stevenson *et al.* 2002). In conclusion, whilst the present study reveals that mice and voles play different quantitative roles in the ecology of Lyme borreliosis in Europe, it demonstrates that *B. afzelii* is specifically maintained by European rodents, validating the concept of host-association of *B. burgdorferi* s.l.

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