



***Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks and rodents in a recreational park in south-western Ireland**

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Abstract. *Ixodes ricinus* ticks infected with *Borrelia burgdorferi* sensu lato were numerous on the edges of paths and roads in a recreational park in south-western Ireland. The abundance of ticks at different sites was related to the presence of deer, but a negative relationship was shown between tick abundance and tick infection rates. This is thought to be due to the deposition of large numbers of uninfected ticks by deer, which are apparently not good reservoir hosts of *B. burgdorferi* s.l. Blood meal analysis only detected deer DNA in uninfected nymphs. Reservoir competent rodents, *Apodemus sylvaticus* and *Clethrionomys glareolus*, were abundant at all sites and a high proportion of captured specimens were infested with larval ticks. However, very few rodents were infected with *B. burgdorferi* s.l. and none of the unfed infected nymphs analysed for the identity of their larval blood meal had fed on rodents. The spirochaetes detected in *I. ricinus* in the study area may be poorly adapted to rodents or are not transmitted readily because of the absence of nymphal infestation. The majority of spirochaetes in these ticks were apparently acquired from non-rodent hosts, such as birds.

Key words: *Ixodes ricinus*, ticks, *Borrelia burgdorferi* s.l., genospecies, rodents, blood meal analysis

Introduction

Lyme borreliosis (LB), caused by the spirochaete *Borrelia burgdorferi* s.l., is the most prevalent arthropod-transmitted zoonosis in Europe and the distribution of the disease is closely associated with that of the vector tick, *Ixodes ricinus* L. (Acari: Ixodidae). *I. ricinus* is common and widespread in Ireland and *B. burgdorferi* s.l. occurs throughout the country (Gray *et al.*, 1992, 1995; Kirstein *et al.*, 1997a), but only about 30 human cases of LB are identified each year (Gray *et al.*, 1996). The risk factors associated with this disease are evidently complex and in addition to exposure to tick-bites, the distribution of the main reservoir hosts must be taken into account. For example, in a previous study in Ireland it was found that infected ticks were mainly associated with habitats where *Apodemus sylvaticus* was relatively abundant (Gray *et al.*, 1995). *A. sylvaticus* and other rodents such as the bank vole,

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Clethrionomys glareolus, are important reservoir hosts in Europe (Jaenson, 1991; de Boer *et al.*, 1993; Kurtenbach *et al.*, 1995) and heterogeneous woodlands in which these rodents and *I. ricinus* ticks are abundant apparently pose the highest risk for LB (Gray *et al.*, 1998). More detailed studies are required to identify the factors that are responsible for the occurrence of infected ticks in a particular habitat in order to further understand the ecology of the disease and to develop methods of LB risk assessment for the subsequent mapping of high risk habitats. In the present study tick abundance and tick infection rates with *B. burgdorferi* s.l. were examined in an area where the rodents *A. sylvaticus* and *Cl. glareolus* and the deer species, *Cervus elaphus* (red deer) and *Ce. nippon* (sika deer) occur. Blood meal analysis was used for the first time to identify the hosts that field-caught unfed nymphal ticks had fed on as larvae.

Materials and methods

Period of study

The study was conducted from 1994–1996 and most of the sampling was carried out in mid-May to early June of each year, a period when questing *I. ricinus* larvae, nymphs and adults are all abundant (Gray, 1991). The majority of the rodents captured at this time will have been born in the previous year and thus more likely to have been exposed to an infectious tick bite. Furthermore, numbers of visitors to the park increase in early summer, so that the data collected on infected ticks are relevant to risk of LB. Some sites were sampled in September 1994 and 1995 to determine whether a significant risk of tick bite occurred at that time of year.

Study areas

Ticks and small mammals were sampled at six sites in Killarney National Park, Co. Kerry, Ireland (52°N 9°30'W). Four sites (1, 2, 3, 6) were within a parkland area of 4000 hectares, 5 km south of Killarney town, and the remaining two (4, 5) in a forested area of 1000 hectares, just to the west of the town and partly within its boundaries.

Rainfall in this area is high, averaging 1400 mm per year. Yearly maximum and minimum temperatures average 19.0 °C and 3.5 °C respectively. During the sampling periods in late May of each year it was warmer and drier in 1994 than in 1995 or 1996.

The large animal species thought to maintain the tick populations in the parkland were mainly sika deer (*Ce. nippon*), red deer (*Ce. elaphus*) and a small number of wild goats (*Capra hircus*). In the forested area the only significant large animals were red deer. Medium-sized tick hosts in the two areas included hares (*Lepus timidus*),

hedgehogs (*Erinaceus europaeus*), red squirrels (*Sciurus vulgaris*) and rats (*Rattus norvegicus*). These animals were scarce in the areas studied with the possible exception of hedgehogs, which are nocturnal and difficult to observe. Rabbits (*Oryctolagus cuniculus*) were common in some parts of the parkland but did not inhabit the tick-infested regions and are not thought to be significant hosts of *I. ricinus* (Gern *et al.*, 1998). Badgers (*Meles meles*) and foxes (*Vulpes vulpes*) were present in the area, but were not abundant and do not feed large numbers of *I. ricinus* (Milne, 1949). Amongst the small mammals the most abundant tick hosts were the woodmouse (*A. sylvaticus*) and the bank vole (*Cl. glareolus*). The only shrew species present, the pygmy shrew (*Sorex minutus*), was relatively uncommon and has rarely been found to be infested with ticks in Ireland. Ground feeding birds such as blackbirds (*Turdus merula*) and robins (*Erithacus rubecula*), which are important tick hosts, were common. Pheasants (*Phasianus colchicus*) were occasionally heard in the general area, but were never seen near the sites.

Sampling was mostly confined to the edges of roads or pathways where members of the public are readily exposed to questing ticks. All six sites were sampled in May 1994, thereafter attention was confined to the three sites (2, 5, 6), at which infected ticks were most abundant.

Sites 1, 2a, 3 and 4 were the edges of roads and pathways passing through mixed woodland consisting of silver birch (*Betula pendula*), beech (*Fagus sylvatica*), oak (*Quercus robur*), ash (*Fraxinus excelsior*) and spruce (*Picea* spp.). The secondary vegetation included hazel (*Corylus avellana*), holly (*Ilex aquifolium*) and alder (*Alnus glutinosa*) and the ground vegetation consisted mainly of grasses, ivy (*Hedera helix*), bracken fern (*Pteridium aquilinum*) and bramble (*Rubus fruticosus*), with bilberry (*Vaccinia myrtillus*) in some areas. A deep leaf litter or debris from fallen wood occurred in all areas and moss growth was much in evidence, indicating the high humidity in the woods. At site 2 ticks were also collected on two occasions (May 1995, 1996) from within the woodland (site 2b) bordering the roadside.

Site 5 was in the north-western portion of the forested area west of Killarney town and consisted of a roadside to the south of a 4 m wide stream which ran for about 2 km westward from the town. The area sampled was the northern edge of the roadside consisting of short variable vegetation bordered by a thick hedge of mainly beech, birch, willow and alder, with the composition varying considerably at different points. The red deer referred to above had access to this area and were most in evidence at the western end, farthest from the town.

Site 6 was a section of the arboretum adjacent to the main house in the parkland and consisted of an area of about 1.5 hectares containing a variety of garden shrubs and trees. The area was much intersected with broad footpaths and was enclosed by a 2 m high deer fence. The area outside the deer fence was mixed deciduous and coniferous woodland dominated by spruce. The undergrowth throughout site 6 was highly variable, but a thick litter of dead vegetation and debris from fallen and rotting

wood was present. Sampling site 6a was inside the arboretum and sampling site 6b was outside the enclosing deer fence.

Tick sampling

Blanket dragging was used to estimate tick abundance. White blankets (1.0 m²) were dragged across the vegetation and attached nymphs and adults collected into vials. The presence of larvae was recorded, but they were not collected. Ticks were usually collected between 14.00 and 18.00 h by four persons at each site on one occasion during each field trip. Relative tick abundance was estimated by determining the average number of ticks of each stage per person per hour. This sampling approach can result in variations related to operator efficiency, so efforts were made to standardize sampling procedures and when possible tick abundance was also determined per 10 m² (average of ten 10-metre drags). The ticks were kept in sealed vials containing moistened paper towel and transported to the laboratory for determination of *B. burgdorferi* infection rates.

Rodent trapping

Longworth traps (Watkins and Doncaster, Hawkhurst, Kent, UK) were used for the live-capture of rodents. The traps were put down approximately 10 m apart in transects between 17.00 and 20.00 h, baited with a muesli/oats mixture and left overnight. They were examined the following morning between 9.00 and 12.00 h and captured rodents identified, sexed, approximately aged and any attached ticks counted. Tissue samples (2 mm diameter) were taken from each ear and placed in 70% ethanol for detection of *B. burgdorferi* DNA by polymerase chain reaction (PCR). The ear punch used for sampling was flamed and quenched in 70% ethanol to avoid cross contamination. Captured rodents were released at the place of capture.

On two occasions (September 1995 and May 1996) captured rodents (67 in total) were not examined for ticks, but were infested with approximately 50 laboratory-reared uninfected *I. ricinus* larvae and caged for up to five days over water for subsequent collection of engorged ticks. The rodents were then released.

Detection of B. burgdorferi in ticks and rodents

Unfed nymphal and adult ticks from each site were examined individually for *B. burgdorferi* by indirect immunofluorescence assay (IFA) and PCR. Xenodiagnostic larvae fed on rodents were allowed to develop to nymphs at 18 °C and 90% RH and then 10 from each rodent were examined for infection by IFA.

IFA

Nymphs were triturated in phosphate buffered saline (PBS) on multi-well slides. Adults were processed by cutting off the posterior tip of the abdomen, expressing the

internal organs and triturating in PBS. Slides were air dried, and fixed in acetone at -20°C . The IFA was performed using a rabbit polyclonal antiserum to *B. burgdorferi* s. s. B31 at a dilution of 1:100 (Gray *et al.*, 1992).

PCR

B. burgdorferi s.l. DNA in unfed ethanol-stored ticks was detected by nested PCR (Kirstein *et al.*, 1997a) and genospecies were identified by reverse line blot analysis of PCR products (Rijpkema *et al.*, 1995). Strains of *B. burgdorferi* s.s., *B. afzelii*, *B. garinii* and *B. valaisiana* were amplified in tandem as positive controls and laboratory-reared uninfected *I. ricinus* ticks were included in each batch as negative controls. In order to check for PCR-inhibiting substances in tick homogenates a selection of negative samples were spiked with 100 fg of B31 strain *B. burgdorferi* s.s. DNA and the sample re-amplified.

PCR on ear biopsies from mice and voles

Hofmeister *et al.* (1992) and Hofmeister and Childs (1995) showed that *B. burgdorferi* s.l. DNA can be detected in rodent ear biopsies by nested PCR and concluded that this method is an efficient and practical alternative to xenodiagnosis or tissue culture. The same general method of analysis was used as for the ticks, except for a longer period of DNA extraction (60 min in boiling ammonium hydroxide compared with 15 min). Negative and positive control ear biopsies were included in the tests and were obtained from six *A. sylvaticus* captured in a tick-free city area. Negative control samples were obtained at capture and the mice were then infested with five *B. afzelii*-infected nymphal *I. ricinus* under laboratory conditions. Positive control biopsy samples were obtained two weeks after tick detachment. The detached ticks were allowed to moult to adults and all were found to be positive for borreliae by IFA. Borrelia genospecies in ear biopsies were identified by reverse line blot analysis of PCR products (Rijpkema *et al.*, 1995).

Blood meal analysis

Unfed nymphal ticks captured at site 2 were analysed to determine the origin of their larval blood meal remnants. Briefly, degenerate PCR primers were designed based on alignments of published cytochrome b sequences for the animal species *Mus musculus*, *Ovis aries*, *Dama dama* and *Bos taurus* and a nested PCR used to obtain 95 bp products from blood meal remnants in nymphal ticks as described by Kirstein and Gray (1996). The PCR products were analysed by reverse line blot using oligonucleotide probes specific for *A. sylvaticus*, *Cl. glareolus*, *Ce. nippon* and *Ce. elaphus* (Kirstein, 1996).

DNA extracts from the same ticks were analysed by PCR for the presence of *B. burgdorferi* s.l. as described above.

Results

Tick abundance

Nymphal ticks were very numerous on the edges of roadsides in the early summer of all three years of the study (1994–1996), ranging from 43.0–198.5 nymphs per hour (Table 1). Adults were less numerous (1.5–22.7 per hour) and larvae were never captured from roadsides, but were occasionally captured in the woodland. At site 2 the numbers of nymphal and adult ticks captured on the roadside (2a) were not significantly different from those captured within the adjacent wood (2b) (ticks per 10 m², t-test, nymphs $P = 0.6781$, adults $P = 0.3095$). At site 6 no larvae were caught in the arboretum (6a), but larvae were very numerous just outside the arboretum fence (6b) where there was much evidence of the presence of deer (e.g., deer droppings). Consistently more nymphs and adults were captured outside the fence (6b) than within it (6a), (ticks per hour over three years, paired t-test, nymphs $t = -7.153$, $P = 0.0008$, adults $t = -3.53$, $P = 0.0167$).

Table 1. Unfed *Ixodes ricinus* abundance at selected sites in Killarney National Park, Co. Kerry, 1994–1996

Date	Site	Nymphs per hour	Nymphs per 10 m ²	Adults per hour	Adults per 10 m ²	Presence of larvae
May 1994	1	130.3	ND ^a	22.7	ND	–
	2a	115.3	ND	14.6	ND	–
	3	121.5	ND	13.0	ND	–
	4	198.5	ND	10.0	ND	–
	5	98.0	ND	9.5	ND	–
	6a	88.0	ND	4.0	ND	–
	6b	130.0	ND	6.0	ND	+
Sept 1994	2a	2.0	0.08	3.0	0.13	–
	5	0.0	0.0	0.0	0.0	–
	6a	17.0	1.4	1.0	0.08	–
	6b	11.0	0.85	2.0	0.15	+
May 1995	2a	59.6	2.9	7.6	0.43	–
	2b	81.3	2.4	7.8	0.24	+
	5	49.0	1.8	1.5	0.15	–
	6a	81.0	2.6	4.0	0.12	–
	6b	94.2	2.9	9.0	0.28	+
May 1996	2b	117.0	5.8	7.8	0.45	+
	5	110.8	4.4	2.8	0.20	–
	6a	43.0	2.1	5.0	0.25	–
	6b	127.0	5.0	21.0	0.6	+

^a ND, not done.

Table 2. Tick infection rates with *B. burgdorferi* s.l. for three selected sites sampled May 1994–1996

Site	% nymphs infected			% adults infected		
	n ^a	mean	range	n ^a	mean	range
2a	488	12.0	2.0–19.0	67	17.3	14.2–20.5
5	467	12.2	10.7–17.0	40	2.1	0–4.2
6a	393	17.5	7.0–23.0	26	21.4	0–42.9
6b	358	7.0	2.0–14.0	68	5.0	0–11.8

^a pooled from 3 samples.

Very few ticks (0–17 nymphs, 1–3 adults per hour) were captured at sites 2, 5 and 6 in September 1994 and 1995, although weather conditions were suitable for tick activity.

Tick infection with B. burgdorferi s.l.

Both IFA and PCR were used to determine infection rates in 100 randomly selected nymphs from each of five of the 28 tick samples examined and there were no statistically significant differences between the results obtained with the two methods (infection rate range 10–28%, Chi² test $P > 0.05$). For the remaining 23 samples infection rates were determined by either IFA or PCR.

Infection rates of nymphal and adult ticks varied considerably both between sites within sampling periods and within sites from year to year. In 1994 infection rates for sites 1, 3 and 4 were 7.0, 7.0 and 6.0% respectively. At sites 2, 5, 6a and 6b infection rates were 19.0, 17.0 7.0 and 2.0% respectively and data from these sites were collected for a further two years (Table 2). Higher infection rates in adults than in nymphs were found at sites 2 and 6a, but the reverse was found for sites 5 and 6b.

A statistically significant negative relationship was observed between tick infection rates and tick abundance when the data from all six sites were pooled over three years (Fig. 1). This negative relationship can also be seen in changes that occurred in sites from year to year. For example, in site 2 increased numbers of ticks were captured in 1996 compared to previous years (Table 1), but the proportion of infected ticks decreased from 19% to 2%. As a consequence of this relationship there was no correlation between the density of infected nymphs and the overall density of nymphs ($r^2 = 0.13$ $P = 0.178$).

Rodent abundance, tick infestations and infection with B. burgdorferi s.l.

Trapping rates (percentage traps occupied) were used as a measure of relative rodent abundance. Trapping rates varied from year to year and season to season (overall range 22–89%), but no consistent differences between sites were observed. The

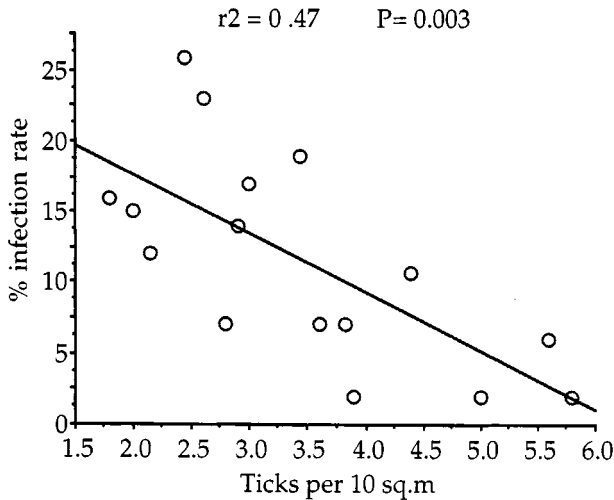


Figure 1. Relationship between density of nymphal *Ixodes ricinus* and *B. burgdorferi*-nymphal infection rates determined by pooled data obtained from all six sites over three years (1993–1995)

majority of the 484 rodents captured in 914 trap nights over the period May 1994 to May 1996 were *A. sylvaticus* (73.1%, range 47–90%), the remaining being *Cl. glareolus* (26.9%, range 10–53%). Larval *I. ricinus* were found on both *A. sylvaticus* and *Cl. glareolus*, but the frequency and intensity of infestation were higher for *A. sylvaticus* than for *Cl. glareolus* (Table 3). A total of 189 out of the 417 (45.3%) rodents examined were infested with larvae, but only one attached nymph was found (on *A. sylvaticus* at site 4, May 1994).

Infection rates of rodents determined by PCR and xenodiagnosis were very low (Table 4). One *A. sylvaticus* and one *Cl. glareolus* positive by PCR were negative by xenodiagnosis and the two *A. sylvaticus* positive by xenodiagnosis were negative by PCR. Reverse line blot analysis performed on ear biopsy PCR products detected *B. burgdorferi* s.s. in one *A. sylvaticus* and *B. afzelii* in one *Cl. glareolus*.

Table 3. Frequency and intensity of infestation of *Apodemus sylvaticus* and *Clethrionomys glareolus* with *Ixodes ricinus* larvae

	n	Frequency			Intensity		
		% infestation	Chi ²	P	mean larvae/rodent	t-test	P
<i>A. sylvaticus</i>	306	49.0	5.789	0.0161	2.124	2.289	0.0226
<i>C. glareolus</i>	111	35.1			0.946		

Table 4. *B. burgdorferi* s.l.-infected rodents determined by PCR and xenodiagnosis

	% PCR positive		% xenodiagnosis*		% PCR or xenodiagnosis positive	
<i>A. sylvaticus</i>	2.6	(6/226)	5.0	(2/40)	3.5	(8/226)
<i>Cl. glareolus</i>	1.5	(1/67)	0.0	(0/10)	1.5	(1/67)
All rodents	2.4	(7/293)	4.0	(2/50)	3.1	(9/293)

* All rodents examined by xenodiagnosis were also examined by PCR.

Blood meal analysis

A total of 80 unfed nymphs, 40 of which had tested positive for *B. burgdorferi* s.l. by PCR, were tested to identify the host source of spirochaetes detected in ticks captured at site 2 in 1995. Nested 95 bp cytochrome b PCR products were amplified from a total of 50/80 nymphs: 19/50 were infected with *B. burgdorferi* s.l. and 31 were uninfected. Analysis of the PCR products by reverse line blot using oligonucleotide probes for the rodents *A. sylvaticus* (woodmouse) and *Cl. glareolus* (bank vole) and the deer species *Ce. elaphus* (red deer) and *Ce. nippon* (sika deer) showed that 23/31 uninfected nymphs contained DNA from *Ce. nippon*, 2 from *Cl. glareolus*, 2 from both *Ce. nippon* and *Ce. glareolus* and 4 of the products were unidentifiable. None of the PCR products from the infected ticks could be identified with the particular oligonucleotide probes used.

Discussion

The study showed that in this location considerable risk of tick bite exists for people using the recreational park in early summer. Ticks were mainly sampled from the sides of paths and roadways and nymphs and adults were found to be as numerous in these locations as in the centre of adjoining woodland. Larvae were never collected from roadsides, but were found wherever there was evidence of deer. Very few ticks were captured in autumn, probably because deer, the most important tick maintenance host in the area, leave the lowland woods for the hills in summer and do not return in numbers until mid-winter.

Tick infection rates in this study were inversely related to tick abundance. Inverse relationships between tick density and tick infection rates have been observed previously in partitioned habitats in Ireland involving deer (Gray *et al.*, 1992) and sheep and cattle (Gray *et al.*, 1995) and also for *I. scapularis* in the U.S.A. (Mather and Ginsberg, 1994). However, a positive relationship between tick density and infection rates at lower tick densities in Swedish woodland was reported by Tälleklint and Jaenson (1996). In the present study inverse relationships were seen in comparisons between sites in particular years, within sites in different years and also within partitioned sites, such as site 6. The presence of a deer-proof fence at site 6

caused an imbalance in both the overall abundance of ticks and in the proportions of infected ticks, with tick abundance being higher outside the fence but infection rates higher within the fence. This phenomenon is interpreted as resulting from the relative reservoir incompetence of deer for *B. burgdorferi*-, so that deer increase the number of uninfected nymphs and adults in areas to which they have access. Whereas unfed larvae were abundant outside the fence, no larvae were captured inside the fence, which strongly suggests that the observed differences are due to the absence of deer within the fence. The low reservoir competence of deer probably also contributes to the lower adult than nymph infection rates at site 6b, because it is likely that most nymphs there fed on deer. The presence of large numbers of deer, despite the absence of adjacent woodland, is probably responsible for the same situation at site 5.

This study is the first time that blood meal analysis of unfed nymphal ticks has been used to identify the hosts they fed on as larvae. The finding that all the nymphs containing deer DNA were free of *B. burgdorferi* s.l. further supports the view that deer are not good reservoir hosts. Although rodent DNA was detected in some uninfected ticks none was found in infected ticks, suggesting that rodents were not important reservoir hosts at that particular site. DNA from both *Cl. glareolus* and *Ce. nippon* was identified in two uninfected nymphs. Semi-engorged larvae are occasionally collected by blanket dragging and the blood meal analysis data suggest that individual larvae are capable of feeding on more than one host.

Most of the rodents captured were *A. sylvaticus* and this species was also more frequently and more heavily infested with tick larvae than was *Cl. glareolus*, which supports the observations of Kurtenbach *et al.* (1995). Spirochaetes were found in a relatively high proportion of the nymphs collected from the sites (10–19%) and, although rodent abundance was high, very few rodents were infected as determined by PCR and/or xenodiagnosis. This suggests that rodents are not the main source of the spirochaetes in the ticks and supports the conclusions from the blood meal analysis data. *Borrelia* genospecies were not identified in the blood meal analysis nymphs, but in a subsequent study on ticks from the same site (Kirstein *et al.*, 1997b) the majority were infected with *B. valaisiana* (41%) and *B. garinii* (33%). There is increasing evidence that these genospecies are mainly associated with birds (Humair *et al.*, 1993; Olsén *et al.*, 1995; Hubálek *et al.*, 1996; Kirstein *et al.*, 1997a; Humair *et al.*, 1998). *B. burgdorferi* s.s., which occurs in both birds and rodents, was also present (20%) but the rodent-associated *B. afzelii* (Humair *et al.*, 1995; Hu *et al.*, 1997) was uncommon (6%) in this particular site. It is interesting to note that the only two genospecies detected in rodents in the present study were *B. afzelii* and *B. burgdorferi* s.s., which supports the view that these genospecies may be particularly associated with rodents.

Similar findings on the limited participation of small rodents in the circulation of *B. burgdorferi* s.l. were reported by Kurtenbach *et al.* (1998b) for a site in the UK. In that study although 27% (13/47) rodents were infected with *B. burgdorferi* s.l., most of these were carrying *B. garinii* infections of internal organs and only four

(8.5%) of them infected xenodiagnostic larvae, all with *B. burgdorferi* s.s. The fact that most infected unfed nymphs collected from the area were infected with *B. garinii* and/or *B. valaisiana* and less than one per cent with *B. burgdorferi* s.s. was explained by the high density of pheasants (*Phasianus colchicus*), which are probably reservoir competent for both *B. garinii* and *B. valaisiana* (Kurtenbach *et al.*, 1998a, 1998b). Although these two genospecies were found in the majority of the infected ticks in the area of the present study (Kirstein *et al.*, 1997b), pheasants were uncommon and a more natural avifauna may be responsible for the infection in the ticks.

The identity of the host DNA detected in the unfed infected nymphs was not determined in the present study because of the limited availability of different host species-specific oligonucleotide probes for the reverse line blot. However, in view of the borrelia genospecies present in ticks from this site it is likely that birds are the source for most of these spirochaetes. In addition to *A. sylvaticus* and *Cl. glareolus*, other reservoir competent mammalian species that may have been present at this site include the hedgehog (*Erinaceus europaeus*), the red squirrel (*Sciurus vulgaris*) and the brown rat (*Rattus norvegicus*). Some of these species are known to be efficient hosts for *B. afzelii* (Gern *et al.*, 1997; Humair and Gern, 1998) and hedgehogs can also be infected by other genospecies (Gern *et al.*, 1997).

Only one nymphal *I. ricinus* was seen on the 417 rodents examined in this study compared with 755 larvae. The rarity of rodent infection with *B. burgdorferi* s.l. suggests that neither larval nor nymphal *I. ricinus* are significant sources of infection, but in other studies the low rodent nymphal infestation rate has been taken as evidence that larvae may have a role in the infection of small rodents (de Boer *et al.*, 1993; Randolph and Craine, 1995). This may be true for study sites in which a large proportion of rodents have been unequivocally shown to be infected, but where such data are absent, or depend solely on PCR analysis, the possibility that rodents are not the main reservoir hosts for the spirochaetes detected in the ticks should be considered. The present study has shown that rodent involvement in the local circulation of *B. burgdorferi* s.l. may be low even though rodents are numerous, rodent-associated *B. burgdorferi* genospecies (e.g. *B. burgdorferi* s.s., *B. afzelii*) are present in the ticks and there is no obvious large population of other reservoir hosts (such as pheasants). The reasons for the low proportion of ticks carrying *B. afzelii* in an area inhabited by numerous tick-infested rodents remain to be elucidated, but may be related to the rarity of rodent nymphal infestations.

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