Published in Acta Tropica 69, issue 3 : 213-227, 1998,
which should be used for any reference to this work

Relationship between *Borrelia burgdorferi* sensu lato species, red squirrels (*Sciurus vulgaris*) and *Ixodes ricinus* in enzootic areas in Switzerland

Pierre-François Humair, Lise Gern *

Département de Parasitologie, Institut de Zoologie, Université de Neuchâtel, Rue Emile Argand 11, 2007 Neuchâtel, Switzerland

Received 6 September 1997; accepted 17 October 1997

Abstract

The infection and reservoir status of red squirrels (*Sciurus vulgaris*) for *Borrelia burgdorferi* sensu lato were studied in Switzerland. *B. burgdorferi* sensu lato was isolated from 15 skin samples from 4/6 dead red squirrels, victims of road traffic. Isolates were identified using restriction fragment length polymorphism (RFLP): *B. burgdorferi* sensu stricto was present in 14 culture tubes containing skin samples and *B. afzelii* in two other tubes. A mixed infection was revealed in one case. A total of 227 ticks attached to squirrels were cultivated in BSKII medium and 90 isolates were obtained. Genotypic identification by RFLP showed that *B. afzelii* (59%) and *B. burgdorferi* sensu stricto (46%) dominated in ticks feeding on red squirrels. Data collected from one particular animal, highly infested with *Ixodes ricinus* and harbouring numerous *Borrelia*-infected *Ixodes ricinus* ticks, showed that transmission of *B. burgdorferi* sensu lato occurred from *S. vulgaris* to feeding ticks. More precisely, *B. burgdorferi* sensu stricto and *B. afzelii* were mainly transmitted from *S. vulgaris* to ticks. The present data emphasized the results obtained previously from small rodents and birds in Japan and in Switzerland, showing the occurrence of specific associations between host species and *Borrelia* genospecies.

Keywords: Lyme borreliosis; Ecology; *Sciurus vulgaris*; Host; *Ixodes ricinus*; *Borrelia burgdorferi* sensu lato

* Corresponding author. Tel.: + 41 32 7182111; fax + 41 32 7183001; e-mail: lise.gern@zool.unine.ch

1. Introduction

Lyme borreliosis is a tick-borne disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato (sl) complex. In Europe, this complex includes three genospecies pathogenic for humans, *B. burgdorferi* sensu stricto (ss), *B. afzelii* and *B. garinii* (Johnson et al., 1984; Baranton et al., 1992; Canica et al., 1993) and two newly described genomic groups apparently non-pathogenic for humans, group VS116 and group PotiB2 (Péter and Bretz, 1992; Postic et al., 1994). These borreliae are commonly vectored by *Ixodes ricinus* in European endemic areas. Lyme borreliosis spirochetes are mainly maintained in nature through a transmission cycle involving ticks and vertebrates that act as reservoirs. Ecological studies on Lyme borreliosis conducted in Europe have shown that some mammal and bird species frequently infested by *I. ricinus* transmit infection to ticks. Mammals, the most extensively studied, are small rodents, especially *Apodemus* mice and *Clethrionomys* voles (Aeschlimann et al., 1986; Matuschka et al., 1992; De Boer et al., 1993; Humair et al., 1993; Tälleklint and Jaenson, 1994; Humair et al., 1995; Kurtenbach et al., 1995). Medium-sized rodents, like edible dormice (*Glis glis*) (Matuschka et al., 1994), rats (*Rattus norvegicus*) (Matuschka et al., 1996) and grey squirrels (*Sciurus carolinensis*) (Craine et al., 1997) have been determined to be reservoirs as well. Insectivores like *Neomys* and *Sorex* shrews (Tälleklint and Jaenson, 1994) and hedgehogs (*Erinaceus europaeus*) (Gray et al., 1994; Gern et al., in prep), as well as lagomorphs (*Lepus europaeus* and *L. timidus*) (Tälleklint and Jaenson, 1994) are also implicated. Besides mammals, some bird species like pheasants (*Phasianus colchicus*) and *Turdus* sp. passerines and migrating birds may act as reservoirs for *B. burgdorferi* sl (Olsén et al., 1995; Craine et al., 1997; Humair et al., in press; Kurtenbach et al., in press).

In this study, we tried to evaluate the infection and reservoir status of the European indigenous red squirrel, *Sciurus vulgaris*, in endemic areas in Switzerland. For this purpose, *B. burgdorferi* sl was isolated from these hosts and from squirrel-feeding ticks. Isolates were identified using restriction fragment length polymorphism (RFLP) analysis to demonstrate if red squirrels were infected by a particular genospecies.

2. Materials and methods

2.1. Collection of squirrels and feeding ticks

Dead red squirrels (*S. vulgaris*), victims of road traffic, were collected at different locations near Neuchâtel, Switzerland (Canton of Neuchâtel and Canton of Jura, at various altitudes, Table 1), and were brought to the laboratory. They were examined for ticks and skin samples were taken for spirochete isolation. Ticks were removed by forceps, counted, identified to species, stage and sex and were maintained in labelled vials at room temperature and 95% humidity until use for cultivation. Some ticks, which were fully engorged, were held in the conditions

mentioned above until moult was completed and were inoculated into BSKII medium for isolation of spirochetes.

2.2. Collection of free-living ticks

Host-seeking *I. ricinus* nymphs and adults were collected by flagging the vegetation at the site of collection of squirrel No. 1 (Neuchâtel) during June 1995. Ticks were identified to species, stage and sex and were maintained as mentioned (Section 2.1) until use for spirochete isolation. Flagging sessions were not planned at each squirrel collection site, since the three most important genospecies were shown to be present in enzootic areas in Switzerland (Hu et al., 1994; Humair et al., 1995; Péter et al., 1995; Gern et al., in prep).

2.3. Isolation of spirochetes

Skin necropsies were collected from each squirrel to isolate spirochetes. Skin samples were systematically removed from left and right ears and occasionally from chin, throat and axillae. Skin samples were taken with sterilized small sharp scissors after shaving and cleaning the skin with 70% ethanol. Needle aspiration of skin described by Piesman et al. (1991) was also used in one case (squirrel No. 2). Blood, urine, synovial fluid and tissue samples from liver, heart, spleen, urinary bladder and kidney were also removed in another case (squirrel No. 1). Skin necropsies, aspirate and tissue samples were immediately inoculated into tubes containing BSKII medium supplemented according to Sinsky and Piesman (1989). Partially engorged, fully engorged and moulted ticks collected from squirrels and free-living

Table 1
Ixodes ricinus ticks on red squirrels (*Sciurus vulgaris*) collected near Neuchâtel, Switzerland

Animal number	Sex	Age	Collection			No. I. *ricinus* ticks		
			Locality	Altitude (m)	Date	Larvae	Nymphs	Females
1	M	Ad	Neuchâtel[a]	620	March 1994	1	46	0
2	M	Ad	Neuchâtel[b]	540	April 1996	6	87	0
3	M		Cerneux-Veusil	1020	April 1996	0	0	0
4	F		Rochefort	840	June 1996	31	50	0
5	M	Juv	Neuchâtel[b]	640	June 1996	369	377	1
6	M	Ad	Hauts-Geneveys	1045	June 1996	2	10	1
Total						409	570	2

F, female; M, male; Ad, adult; Juv, juvenile.
[a] Bois de l'Hopital.
[b] Voens.

ticks were briefly soaked in 70% ethanol and squashed with sterilized forceps in tubes containing BSKII medium modified by Sinsky and Piesman (1989).

Inoculated cultures were screened by dark-field microscopy for the presence of spirochetes after 10 days and 3, 4, 6 and 8 weeks of incubation at 34°C. Spirochetes in culture tubes were analyzed by SDS-PAGE, Western blot and RFLP.

2.4. SDS-PAGE and Western blot

After 2–5 subcultures, squirrel and tick isolates were analyzed by SDS-PAGE and Western blotting as described elsewhere (Humair et al., in press). For Western blots, monoclonal antibodies (MAbs) were used for specific identification of borreliae: MAb H3TS recognizes the OspA (31 kDa) of *B. burgdorferi* ss (Barbour et al., 1985), MAb I 17.3 reacts with the OspB (35 kDa) of *B. afzelii* (Canica et al., 1993), and MAb D6 identifies a 12 kDa protein of *B. garinii* (Péter and Bretz, 1992).

2.5. RFLP analysis

The variable intergenic spacer between repeated 23S (*rrl*)—5S (*rrf*) ribosomal genes of *B. burgdorferi* sl was used as a template for polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analysis with *Mse*I endonuclease, as described by Postic et al. (1994). Two ml of initial cultures were used to detect *Borrelia* DNA in positive cultures, the whole culture volume (4 ml) was used to detect *Borrelia* DNA in culture tubes negative after 8 week incubation at 34°C.

3. Results

3.1. Tick infestation of squirrels

Immature and adult stages of *I. ricinus* were removed from five out of six squirrels (Table 1). Immatures were far more numerous than adults on these medium-sized rodents, since a total of 409 larvae (41.7%), 570 nymphs (58.1%) and only two females (0.2%) were collected. One squirrel (No. 5), harbouring a total of 747 *I. ricinus* ticks (369 larvae, 377 nymphs and 1 female) showed that *S. vulgaris* could be highly infested. Two squirrels collected at higher altitude (>1000 m) showed fewer ticks (No. 6) or no tick at all (No. 3). Squirrels may get infested by ticks early in the year (mid-March) as observed for squirrel No. 1. *I. ricinus* ticks were found attached on the ears, chin and throat and also in the axillae in the case of the highly infested squirrel (No. 5). On this animal, feeding ticks were distributed in clusters and red skin lesions were observed around clusters, suggesting that an immunologic response to tick infestation and/or to spirochete infection occurred.

Table 2
Isolation and DNA detection of *Borrelia* genospecies in BSK medium inoculated with skin samples of *Sciurus vulgaris*

Animal number	Skin origin[a]	Isolate number	PCR	Species[b]
1	Necropsy/ear L	NE 63	+	Bb
	Necropsy/ear R	NE 59	+	Bb
2	Necropsy/ear L	NE 260	+	Bb[c]
	Necropsy/ear R	NE 261	+	Bb
	Necropsy/throat	NE 262	+	Ba
	Aspirate/ear	RE 263	+	Bb[c]
	Aspirate/throat		−	
3	Necropsy/ear L		+	Bg
	Necropsy/ear R		−	
4	Necropsy/ear L_1		−	
	Necropsy/ear L_2		−	
	Necropsy/ear R_1		−	
	Necropsy/ear R_2		−	
	Necropsy/throat		−	
5	Necropsy/ear L	NE 264	+	Bb
	Necropsy/ear R_1	NE 265	+	Bb
	Necropsy/ear R_2		+	Ba
	Necropsy/chin	NE 266	+	Bb and Ba
	Necropsy/throat	NE 267	+	Bb
	Necropsy/axilla	NE 268	+	Bb
6	Necropsy/ear L_1	NE 269	+	Bb
	Necropsy/ear R	NE 270	+	Bb
	Necropsy/chin	NE 271	+	Bb[c]
	Necropsy/ear L_2	NE 272	+	Bb

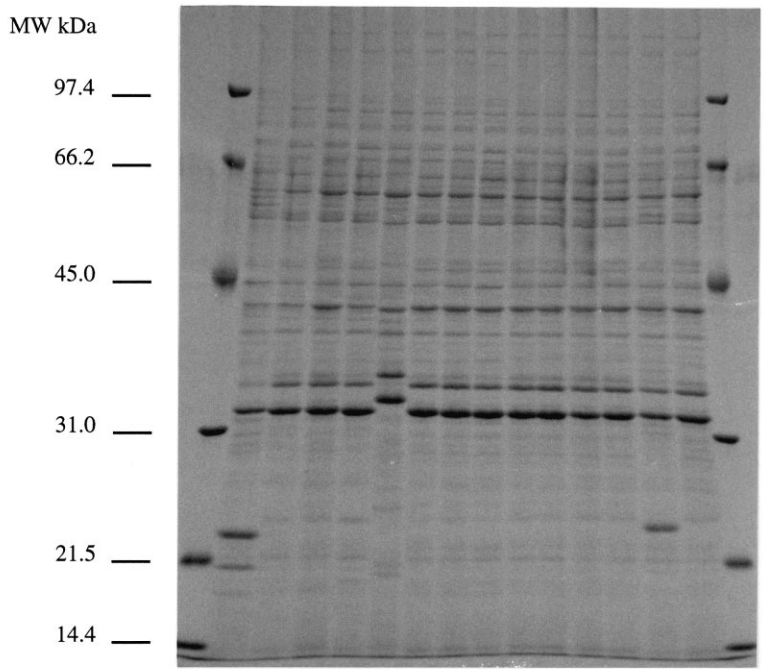
[a]L, left-hand side; R, right-hand side.
[b]Species identified by RFLP: Ba, *Borrelia afzelii*; Bb, *B. burgdorferi* sensu stricto.
[c]Atypical RFLP pattern.

3.2. Isolation and DNA detection of B. burgdorferi sl from squirrel skin samples

A total of 15 isolates were obtained from cultivation of 24 squirrel skin samples taken from six animals (Table 2). Spirochetes were isolated from ears, chin, throat and axillae. Isolates were named according to our laboratory denomination (NE). These isolates were identified by RFLP as *B. burgdorferi* ss ($n = 13$), *B. afzelii* ($n = 1$) and one isolate presented a mixed infection with *B. burgdorferi* ss and *B. afzelii* (Table 2). Three *B. burgdorferi* ss isolates exhibited an atypical DNA pattern with a fragment of 40 bp instead of 38 bp. No spirochetes were detected in culture tubes containing samples of blood, urine, synovial fluid, liver, heart, spleen, urinary bladder and kidney from squirrel No. 1, although ear skin necropsies from this host were positive. An important growth of contaminants—probably preventing successful cultivation of spirochetes—was observed in culture tubes from squirrel No.

4. This could be due to the late delivery of this animal into our laboratory (> 24 h after collection).

Protein profiles and Western blots using species-specific MAbs of squirrel isolates confirmed the identification obtained by RFLP (Fig. 1). Squirrel isolates showed usual protein profiles for *B. burgdorferi* ss (OspA of 31 kDa and OspB of 34 kDa)



Lanes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Reactivity with MAbs																
H3TS		+	+	+	+	-	+	+	+	+	+	+	+	+	+	
I 17.3		-	-	-	-	+	-	-	-	-	-	-	-	-	-	
D6		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
RFLP		b	b	b	b	a	b	ab	b	b	b	b	b	b	b	

Fig. 1. Protein profiles, Western blotting and RFLP identification of *Borrelia* isolates from *Sciurus vulgaris*. Immunoblotting: reactivity with monoclonal antibodies H3TS (anti-*B. burgdorferi* sensu stricto), I 17.3 (anti-*B. afzelii*), and D6 (anti-*B. garinii*). Lanes A and P, molecular weight standard; lane B, isolate NE 63 from squirrel No. l; lanes C–F, isolates NE 260, NE 261, NE 263 and NE 262 from squirrel No. 2; lanes G–K, isolates NE 264–268 from squirrel No. 5; lanes L–O, isolates NE 269–272 from squirrel No. 6. +, positive reaction; −, negative reaction. a, *B. afzelii*; b, *B. burgdorferi* sensu stricto.

and for *B. afzelii* (OspA of 32 kDa and OspB of 35 kDa). Proteins in the OspC region exhibited strong expression in two cases only (lanes B and N). Mixed infection detected by RFLP in isolate NE266 (No. 5) was not revealed, neither by SDS electrophoresis nor by Western blot, suggesting that subcultures needed for these two latter methods selected one of the genospecies (*B. burgdorferi* ss, lane H).

Cultures of skin samples from animals Nos. 2, 3, 4 and 5, which remained negative after 8 week incubation at 34°C, were screened by PCR and *Borrelia* DNA was amplified in two tubes. RFLP analysis revealed *B. garinii* and *B. afzelii* in culture tubes containing skin samples from squirrels Nos. 3 and 5, respectively (Table 2).

In summary, *B. burgdorferi* sl was isolated or PCR detected in skin samples from 5/6 (83%) *S. vulgaris*, and *B. burgdorferi* ss dominated in squirrel skin cultures followed by *B. afzelii*.

3.3. Isolation of B. burgdorferi sl from ticks fed on squirrels

A total of 227 partially engorged or moulted *I. ricinus* ticks (16 larvae and 211 nymphs), fed on five *S. vulgaris*, were inoculated into BSK medium and 90 isolates (39.6%) were obtained (Table 3). A total of 81 isolates were cultivated from 211 nymphs (38.4%) and nine isolates were obtained from 16 larvae (56.2%). The isolation rate from squirrel-feeding ticks varied from 3.3 to 69.5%. A high isolation rate of spirochetes was obtained from *I. ricinus* larvae (64.3%) and nymphs (69.5%) from squirrel No. 5. The genotypic identification by RFLP of the 90 isolates showed that *B. afzelii* ($n = 43$) and *B. burgdorferi* ss ($n = 31$) were the two genospecies most frequently found in ticks fed on squirrels. *B. garinii* was rare ($n = 4$). Mixed infection concerned only *B. afzelii* and *B. burgdorferi* ss ($n = 10$). Considering mixed infections, *B. afzelii* was found in 58.9% of infected ticks and *B. burgdorferi* ss in 45.6% of infected ticks.

Phenotyping results using SDS-PAGE and Western blot with species-specific MAbs corroborated genotyping findings (Fig. 2). Most *B. afzelii* isolates ($n = 11$) showed a typical pattern with an OspA of 32 kDa and an OspB of 35 kDa. However, we observed an electrophoretic mobility of OspB at 35.5 kDa for three *B. afzelii* isolates (lanes F, J and U). *B. garinii* isolates tested by SDS-PAGE and Western blot showed an atypical electrophoretic mobility of OspA (lanes K and V). Independent of the genospecies, proteins in the OspC region were present or not.

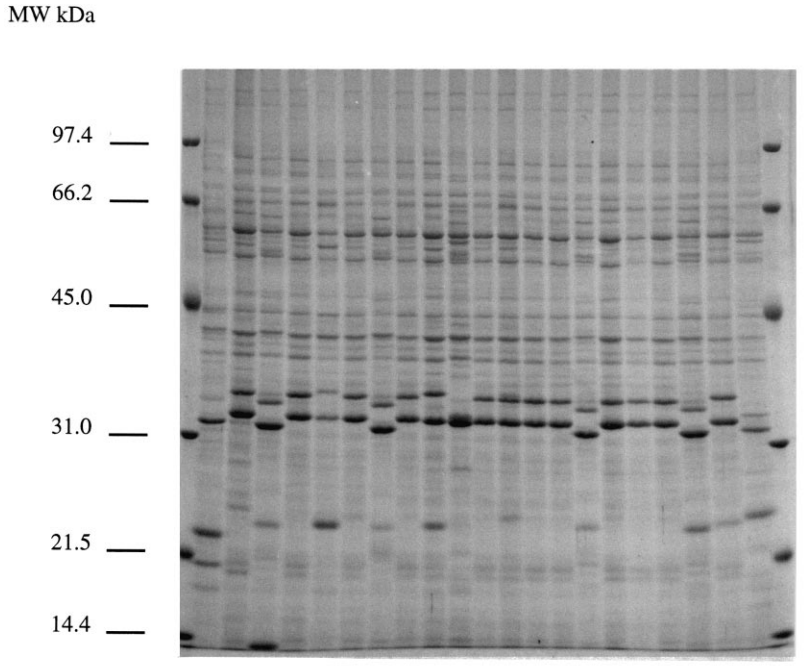
3.4. Isolation of B. burgdorferi sl from free-living ticks

The cultivation of 118 free-living *I. ricinus* ticks (90 nymphs, 17 females, 11 males) from Neuchâtel collection site yielded 29 isolates: 21 from 90 nymphs (23.3%), seven from 17 females (41.2%) and one from 11 males (9.1%). RFLP analysis of these isolates showed that *B. afzelii* was obtained from 11 nymphs and two females, *B. burgdorferi* ss from two nymphs and three females, *B. garinii* from seven nymphs and two females, VS116 from one nymph. A mixed infection with *B. afzelii* and *B. garinii* was observed in the only male isolate.

Table 3
Isolation of *Borrelia* genospecies in BSK medium inoculated with *Ixodes ricinus* ticks fed on *Sciurus vulgaris*

Animal number	Tick stages infesting hosts	Isolation rate (%)	RFLP identification				
			Bb	Ba	Bg	Bb and Ba	Unidentified
1	N	1/30 (3.3)	1				
2	N	20/67 (29.9)	3[c]	16	1		
	L	0/1					
4	N	3/31 (9.7)	1[c]	1	1		
	L	0/1					
5	N	57/82[a] (69.5)	23[c]	22	2	9	1
	L	9/14[b] (64.3)	3	4		1	1
6	N	0/1					
Total		90/227 (39.6)	31	43	4	10	2

L, larvae; N, nymphs; Ba, *Borrelia afzelii*; Bb, *B. burgdorferi* sensu stricto; Bg, *B. garinii*.
[a]3 Isolates were obtained from moulted nymphs.
[b]5 Isolates were obtained from moulted larvae.
[c]Atypical RFLP pattern observed in 3, 1 and 1 isolates from squirrels No. 2, 4 and 5, respectively.



Lanes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Reactivity with MAbs																							
H3TS	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-		
I 17.3	-	+	-	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	-	+	-		
D6	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+		
RFLP	b	a	b	a	a	a	b	a	a	g	a	a	a	a	b	a	a	a	b	a	g		

Fig. 2. Protein profiles, Western blotting and RFLP identification of *Borrelia* isolates from field-derived ticks feeding on *Sciurus vulgaris*. Immunoblotting: reactivity with monoclonal antibodies H3TS (anti-*B. burgdorferi* sensu stricto), I 17.3 (anti-*B. afzelii*), and D6 (anti-*B. garinii*). Lanes A and W, molecular weight standard; lane B, isolate NE 64 from *Ixodes ricinus* fed on squirrel No. l; lanes C–S, isolates NE 273–275 and NE 278–291 from *I. ricinus* fed on squirrel No. 2; lanes T–V, isolates NE 292–294 from *I. ricinus* fed on squirrel No. 4. +, positive reaction; −, negative reaction. a, *B. afzelii;* b, *B. burgdorferi* sensu stricto; g, *B. garinii*.

Species-specific MAbs confirmed the identification by RFLP (Fig. 3A,B). Protein profiles showed atypical electrophoretic mobility of Osps for some isolates: OspB at 35.5 kDa for eight *B. afzelii* isolates (Fig. 3A: lanes B, E, H, M, N, P and Q; Fig. 3B: lane D) and OspA for eight *B. garinii* varying between 31 and 33 kDa (Fig. 3A: lanes D, G, K, L, O, T; Fig. 3B: lanes F and H). Proteins in the OspC area were always present.

4. Discussion

B. burgdorferi sl is maintained in nature through a transmission cycle involving ticks and hosts. Although transovarial transmission exists (Zhioua et al., 1994; Bellet-Edimo, 1997), Lyme borreliosis spirochetes need hosts and transstadial

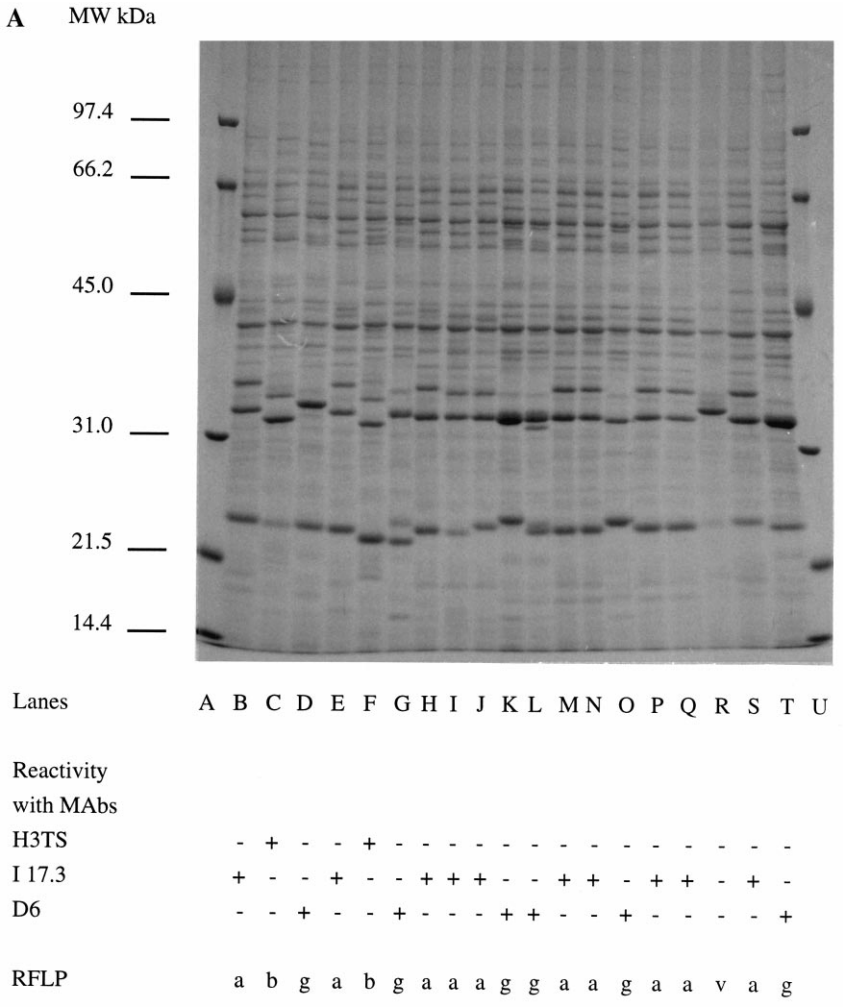


Fig. 3. (A and B) Protein profiles, Western blotting and RFLP identification of *Borrelia* isolates from free-living *Ixodes ricinus* ticks. Immunoblotting: reactivity with monoclonal antibodies H3TS (anti-*B. burgdorferi* sensu stricto), I 17.3 (anti-*B. afzelii*) and D6 (anti-*B. garinii*). (A): Lanes A and U, molecular weight standard; lane B–T, isolates NE 125–130 and NE 158–170 from *I. ricinus* nymphs. (B): Lane A, molecular weight standard; lane B to G, isolates NE 172, NE 205–206, NE 232, NE 208–209 from *I. ricinus* females; lane H, isolate NE 213 from *I. ricinus* male. +, positive reaction; −, negative reaction. a, *B. afzelii;* b, *B. burgdorferi* sensu stricto; g, *B. garinii;* v, *Borrelia* VS116.

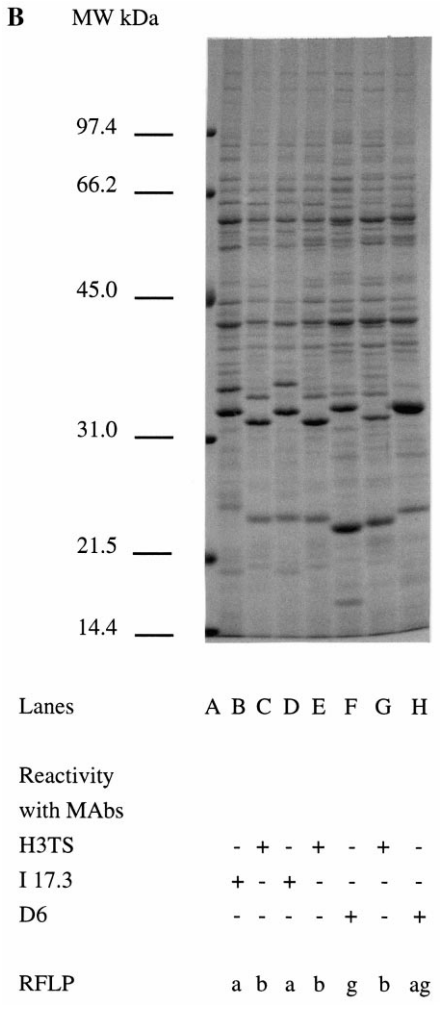


Fig. 3. (*Continued*)

transmission to be maintained efficiently in nature. Vertebrate hosts defined as reservoirs or amplifiers are infected by *B. burgdorferi* sl and act as sources of infection for *I. ricinus* vectors. Practically, the reservoir/amplifier status of a host species should be evaluated using tick xenodiagnosis. This method has been mainly used with animal species which can be easily captured and maintained under laboratory conditions, like *Apodemus* and *Clethrionomys* rodents (Aeschlimann et al., 1986; De Boer et al., 1993; Humair et al., 1993; Kurtenbach et al., 1995). An alternative method consists in comparing infection rate of host-feeding larvae with infection rate of free-living larvae. Initially, the present study aimed to test red squirrels by tick xenodiagnosis. Trappings of squirrels were done but were given up after several attempts in various sites near Neuchâtel, where these animals were

observed. Consequently, we decided to focus on the analysis of dead individuals and attached ticks.

The isolation of *B. burgdorferi* sl from squirrel skin necropsies demonstrates that this animal species is exposed to spirochetal infection as grey squirrels (*S. carolinensis*) in the UK (Craine et al., 1997). In the present study, living spirochetes were obtained from the skin of 4/6 *S. vulgaris*, showing a relatively high prevalence of infection (67%). Borreliae were also present in one squirrel living at high altitude where *I. ricinus* is less frequently found (> 1000 m) (Aeschlimann, 1972), suggesting that a low level of tick population is not a limiting factor for *B. burgdorferi* sl survival.

The prevalence of infection in ticks attached to squirrels varied from 3.3 to 69%. The low number of larvae inoculated in BSKII medium (due to a high mortality rate among larvae between collection and examination) does not allow us to determine the reservoir status of each squirrel, except for squirrel No. 5. Prevalences of infection in *I. ricinus* larvae (64.3%) and nymphs (69.5%), feeding on this host, were far higher than those usually observed in free-living larvae (0–3%, Zhioua et al., 1994) and nymphs (5–34%, Aeschlimann et al., 1986) in Switzerland. Thus, *B. burgdorferi* sl transmission occurs from *S. vulgaris* to feeding ticks. However, we are not able to elucidate whether *S. vulgaris* is a real amplifying/reservoir host or if squirrel-feeding ticks acquired infection via co-feeding transmission (Kimura et al., 1995; Gern and Rais, 1996; Randolph et al., 1996; Sato and Nakao, 1997). This is particularly relevant for animal No. 5 on which ticks were distributed in clusters. However, the demonstration made by Craine et al. (1997) of reservoir competence of *S. carolinensis*, a host species closely related to *S. vulgaris*, led to the supposition that borrelial transmission other than cofeeding transmission also occurs between *S. vulgaris* and attached ticks.

Considering the high tick infestation ($n = 747$) of squirrel No. 5 and the high prevalence of infection in feeding ticks (69%), we can estimate that this individual would have released about 500 infected *I. ricinus* (237 infected larvae and 262 infected nymphs) in its habitat. Even if these data are time-limited data, they show that a single squirrel may transmit *B. burgdorferi* sl to a large number of ticks, particularly during larval and nymphal peak activity. The reservoir competence of a host species is actually defined by three main factors: high infectivity to the vector, high infestation with the vector and host abundance (Mather et al., 1989). Although our data do not allow to evaluate the reservoir competence of squirrels (host abundance unknown), they show that red squirrels can contribute to the maintenance of *B. burgdorferi* sl in nature, considering the high infectivity to ticks and the high infestation by ticks.

B. burgdorferi ss, *B. afzelii*, *B. garinii* and *Borrelia* VS116 are ubiquitous in host-seeking tick populations on the Swiss Plateau (Hu et al., 1994; Leuba-Garcia et al., 1994; Humair et al., 1995; Humair et al., in press). Our data concerning infection in host-seeking ticks presented herein confirmed these observations. That means that in endemic areas in Switzerland animal hosts are in contact with ticks infected by various genospecies. Three genospecies were identified in ticks feeding on squirrels. *B. burgdorferi* ss and *B. afzelii* clearly dominated, whereas *B. garinii*

was less frequently observed. The infection in ticks collected from hosts may come: (1) From a previous infectious blood meal; or (2) from the current blood meal. In the endemic area where squirrels Nos. 1, 2 and 5 were collected, *B. garinii* was often isolated from field-collected ticks (in the present study; Hu et al., 1994). Nevertheless, the high prevalence of *B. burgdorferi* and *B. afzelii* infection in squirrel-feeding ticks suggests that these two genospecies are transmitted from squirrels to feeding ticks rather than the other way round. This is confirmed by the fact that only two genospecies, *B. burgdorferi* ss and *B. afzelii* were isolated from skin samples of squirrels, whereas *B. garinii* was only PCR detected, suggesting that *B. garinii* may have difficulty in establishing infection in squirrels. We suggest that squirrels act as filters and are reservoirs for *B. burgdorferi* ss and *B. afzelii*. Interestingly, this is in accordance with results obtained with grey squirrels in the UK, where *B. afzelii* was observed in the skin of *S. carolinensis* and where successful infection of grey squirrel by NE550 (*B. burgdorferi* ss, Hu et al., 1994) was experimentally obtained (Craine et al., 1997). These observations emphasized the hypothesis made by Nakao et al. (1994) and Humair et al. (1995), who suggested possible associations between *Borrelia* species and host species. Such associations between hosts and genospecies have been shown in Switzerland, where *B. afzelii* was associated with *Apodemus* and *Clethrionomys* rodents (Humair et al., 1995; Hu et al., 1997), and *B. garinii* and *Borrelia* VS116 with *Turdus* passerines (Humair et al., in press). Thus, in the ecology of Lyme borreliosis, vertebrate reservoirs act as biological filters by selecting *Borrelia* species compatible with host physiological environment and then transmit this (these) genospecies to following feeding ticks.

This paper represents a further step towards a better understanding of tick-host–*Borrelia* species relationships by showing an additional association between *Borrelia* genospecies and host species, in this case *B. burgdorferi* ss, *B. afzelii* and red squirrels.

Acknowledgements

This research was supported by the Swiss National Science Foundation (No. 32-29964-90), the Swiss Federal Office of Education and Science (No. 93.0363), the British Council/Swiss National Foundation Joint Research Programme and the European Union Biomedical and Health Research (Biomed I) Programme (No. BMH-CT93-1183). We thank Noel Craine for introducing the squirrel trappings, Katy Sell, Sandrine Meyer and Martine Siegenthaler for providing some of the squirrels, Raquel Martinez and Olivier Rais for their excellent laboratory assistance, Guy Baranton, Alan Barbour and Olivier Péter for the monoclonal antibodies. This work is part of the PhD dissertation of P.F.H.

References

Aeschlimann, A., 1972. *Ixodes ricinus*, Linné, 1758 (Ixodoidea: Ixodidae). Essai préliminaire de synthése sur la biologié de cette espéce en Suisse. Acta Trop. 29, 321–340.

Aeschlimann, A., Chamot, E., Gigon, F., Jeanneret, J.P., Kesseler, D., Walther, C., 1986. *B. burgdorferi* in Switzerland. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. 263, 450–458.

Baranton, G., Postic, D., Saint Girons, I., Boerlin, P., Piffaretti, J.-C., Assous, M., Grimont, P.A.D., 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. 42, 378–383.

Barbour, A.G., Heiland, R.A., Howe, T.R., 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. 152, 478–484.

Bellet-Edimo, R., 1997. Importance de la transmission transstadiale et transovarienne du spirochete *Borrelia burgdorferi* chez la tique *Ixodes ricinus* dans l'épidemiologie de la borréliose de Lyme. PhD Thesis. Université de Neuchâtel, Neuchâtel, Switzerland, 123 pp.

Canica, M.M., Nato, F., du Merle, L., Mazie, J.C., Baranton, G., Postic, D., 1993. Monoclonal antibodies for identification of *Borrelia afzelli* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand. J. Infect. Dis. 25, 441–448.

Craine, N.G., Nuttall, P.A., Marriott, A.C., Randolph, S.E., 1997. Role of grey squirrels and pheasants in the transmission of *Borrelia burgdorferi* sensu lato, the Lyme disease spirochaete, in the UK. Folia Parasitol. 44, 155–160.

De Boer, R., Hovius, K.E., Nohlmans, M.K.E., Gray, J.S., 1993. The woodmouse (*Apodemus sylvaticus*) as a reservoir of tick-transmitted spirochetes (*Borrelia burgdorferi*) in the Netherlands. Zentralbl. Bakteriol. 279, 404–416.

Gern, L., Rais, O., 1996. Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). J. Med. Entomol. 33, 189–192.

Gern, L., Rouvinez, E., Toutoungi, L.N., Godfroid, E. Transmission cycles of *Borrelia burgdorferi* involving *Ixodes ricinus* and/or *I. hexagonus* ticks and the European hedgehog, *Erinaceus europaeus*, in suburban and urban areas in Switzerland. Folia Parasitol., in press.

Gray, J.S., Kahl, O., Janetzki-Mittman, C., Stein, J., Guy, E., 1994. Acquisition of *Borrelia burgdorferi* by *Ixodes ricinus* ticks fed on the European hedgehog *Erinaceus europaeus* L. Exp. Appl. Acarol. 18, 485–491.

Hu, C.M., Leuba-Garcia, S., Kramer, M.D., Aeschlimann, A., Gern, L., 1994. Comparison in the immunological properties of *Borrelia burgdorferi* isolates from *Ixodes ricinus* derived from three endemic areas in Switzerland. Epidemiol. Infect. 112, 533–542.

Hu, C.M., Humair, P.-F., Wallich, R., Gern, L., 1997. *Apodemus* sp. rodents, reservoir hosts for *Borrelia afzelii* in an endemic area in Switzerland. Zentralbl. Bakteriol. 285, 558–564.

Humair, P.-F., Turrian, N., Aeschlimann, A., Gern, L., 1993. *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. Folia Parasitol. 40, 65–70.

Humair, P.-F., Péter, O., Wallich, R., Gern, L., 1995. Strain variation of Lyme disease spirochetes isolated from *Ixodes ricinus* ticks and rodents collected in two endemic areas in Switzerland. J. Med. Entomol. 32, 433–438.

Humair, P.-F., Postic, D., Wallich, R., Gern, L. An avian reservoir (*Turdus merula*) of the Lyme disease spirochetes. Zentralbl. Bakteriol., in press.

Johnson, R.C., Schmid, G.P., Hyde, F.W., Steigerwalt, A.G., Brenner, D.J., 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. 34, 496–497.

Kimura, K., Isogai, E., Isogai, H., Kamewaka, Y., Nishikawa, T., Ishii, N., Fujii, N., 1995. Detection of Lyme disease spirochetes in the skin of naturally infected wild sika deer (*Cervus nippon yeosoensis*) by PCR. Appl. Environ. Microbiol. 61, 1641–1642.

Kurtenbach, K., Kampen, H., Dizij, A., Arndt, S., Seitz, H.M., Schaible, U.E., Simon, M.M., 1995. Infestation of rodents with larval *Ixodes ricinus* (Acari: Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* s.l. in German woodlands. J. Med. Entomol. 32, 807–817.

Kurtenbach, K., Carey, D., Hoodless, A., Nuttall, P. A., Randolph, S.E., submitted. Game birds as wildlife reservoirs of Lyme disease spirochetes. J. Med. Entomol., in press.

Leuba-Garcia, S., Kramer, M.D., Wallich, R., Gern, L., 1994. Characterization of *Borrelia burgdorferi* isolated from different organs of *Ixodes ricinus* ticks collected in nature. Zentralbl. Bakteriol. Hyg. 280, 468–475.

Mather, T.N., Wilson, M.L., Moore, S.I., Ribeiro, J.M.C., Spielman, A., 1989. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). Am. J. Epidemiol. 130, 143–150.

Matuschka, F.-R., Fischer, P., Heiler, M., Richter, D., Spielman, A., 1992. Capacity of European animals as reservoir hosts for the Lyme disease spirochete. J. Infect. Dis. 165, 479–483.

Matuschka, F.-R., Eiffert, H., Ohlenbusch, A., Spielman, A., 1994. Amplifying role of edible dormice in Lyme disease transmission in Central Europe. J. Infect. Dis. 170, 122–127.

Matuschka, F.-R., Endepols, S., Richter, D., Ohlenbusch, A., Eiffert, H., Spielman, A., 1996. Risk of urban Lyme disease enhanced by presence of rats. J. Infect. Dis. 174, 1108–1111.

Nakao, M., Miyamoto, K., Fukunaga, M., 1994. Lyme disease spirochetes in Japan: enzootic transmission cycles in birds, rodents and *Ixodes persulcatus* ticks. J. Infect. Dis. 170, 878–882.

Olsén, B., Jaenson, T.G.T., Bergström, S., 1995. Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. Appl. Environ. Microbiol. 61, 3082–3087.

Péter, O., Bretz, A.-G., 1992. Polymorphism of outer surface proteins of *Borrelia burgdorferi* as a tool for the classification. Zentralbl. Bakteriol. 277, 28–33.

Péter, O., Bretz, A.-G., Bee, D., 1995. Occurrence of different genospecies of *Borrelia burgdorferi* sensu lato in ixodid ticks of Valais, Switzerland. Eur. J. Epidemiol. 11, 463–467.

Piesman, J., Maupin, G.O., Campos, E.G., Happ, C.M., 1991. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi*, with description of a needle aspiration isolation method. J. Infect. Dis. 163, 895–897.

Postic, D., Assous, M.V., Grimont, P.A.D., Baranton, G., 1994. Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of *rrf* (SS)-*rrl* (23S) intergenic spacer amplicons. Int. J. Syst. Bacteriol. 44, 743–752.

Randolph, S.E., Gern, L., Nuttall, P.A., 1996. Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parasitol. Today 12, 472–479.

Sato, Y., Nakao, M., 1997. Transmission of the Lyme disease spirochete; *Borrelia garinii*, between infected and uninfected immature *Ixodes persulcatus* during cofeeding on mice. J. Parasitol. 83, 547–550.

Sinsky, R.J., Piesman, J., 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. J. Clin. Microbiol. 27, 1723–1727.

Tälleklint, L., Jaenson, T.G.T., 1994. Transmission of *Borrelia burgdorferi* s.l. from mammal reservoirs to the primary vector of Lyme borreliosis, *Ixodes ricinus* (Acari: Ixodidae), in Sweden. J. Med. Entomol. 31, 880–886.

Zhioua, E., Aeschlimann, A., Gern, L., 1994. Infection of field-collected *Ixodes ricinus* (Acari: Ixodidae) larvae with *Borrelia burgdorferi* in Switzerland. J. Med. Entomol. 31, 763–766.