Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance

P. F. HUMAIR, O. RAIS *and* L. GERN*

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

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SUMMARY

This study deals with the ecology of Lyme borreliosis in Europe. The relationships between *Borrelia burgdorferi sensu lato* spirochetes, *Clethrionomys* and *Apodemus* rodent reservoirs and the *Ixodes ricinus* tick vector were investigated during 16 consecutive months in an enzootic area in Switzerland. Cultivation of ear skin biopsies was used to isolate spirochetes from *C. glareolus*, *A. sylvaticus*, *A. flavicollis* and *Glis glis*. *Borrelia* infection was more frequently observed in *Clethrionomys* than in *Apodemus*. Tick xenodiagnosis was used to determine the infectivity of rodents. The infection rate in ticks fed on *Clethrionomys* was higher than that in ticks fed on *Apodemus*, but *Apodemus* yielded more infected ticks than *Clethrionomys* because of a better tick moulting success. Xenodiagnostic ticks were placed into BSK medium to obtain isolates. Isolates from rodents and rodent-feeding ticks were all identified as *B. afzelii*. The follow-up of the infectivity status of repeatedly recaptured rodents clearly demonstrated that these hosts remained infective for ticks during winter till the following spring. Comparing *C. glareolus* and *A. sylvaticus*, each rodent species showed different host infection, different host infectivity and contributed differently to the moulting success of feeding ticks. These factors influence differentially the pattern of transmission of *B. afzelii* from *Clethrionomys* voles and *Apodemus* mice to *I. ricinus* ticks.

Key words: Lyme borreliosis, ecology, reservoirs, rodents, *Borrelia afzelii*, overwintering.

INTRODUCTION

Lyme borreliosis is a tick-borne disease caused by the spirochete *Borrelia burgdorferi sensu lato* (s.l.) which is commonly transmitted in Europe by the bite of *Ixodes ricinus* ticks. Studies on the ecology of Lyme borreliosis have demonstrated that the efficient persistence of *Borrelia* pathogens in endemic areas requires the involvement of reservoir hosts. Small-sized mammals, *Apodemus* mice and *Clethrionomys* voles in particular, have been studied in various enzootic areas in Europe (Aeschlimann *et al*. 1986; Matuschka *et al*. 1992; de Boer *et al*. 1993; Humair *et al*. 1993; Tälleklint & Jaenson, 1994; Kurtenbach *et al*. 1995). Competent reservoirs were also found among medium-sized mammals – dormice, hedgehogs, rats, squirrels, hares (Gray *et al*. 1994; Matuschka *et al*. 1994, 1997; Tälleklint & Jaenson, 1994; Craine *et al*. 1997; Gern *et al*. 1997; Humair & Gern, 1998) – and ground-frequenting birds – passerines and pheasant (Olsén, Jaenson & Bergström, 1995; Humair *et al*. 1998; Kurtenbach *et al*. 1998*a*). On the other hand, large-sized mammals – moose and deer – are apparently incompetent as reservoirs (Gray *et al*. 1992, 1995; Jaenson & Tälleklint, 1992; Tälleklint & Jaenson, 1994).

The heterogeneity of European *Borrelia* isolates, widely documented since 1986 in free-living ticks of various enzootic areas (Wilske *et al*. 1986) has led to the splitting of *B. burgdorferi* into diverse genospecies. In Europe, 3 pathogenic genospecies belong to this complex: *B. burgdorferi sensu stricto* (s.s.), *B. afzelii* and *B. garinii* (Johnson *et al*. 1984; Baranton *et al*. 1992; Canica *et al*. 1993). Two newly named species apparently non-pathogenic for humans are also included in this complex: *B. valaisiana* (previously group VS116) and *B. lusitaniae* (previously group PotiB2) (Le Fleche *et al*. 1997; Wang *et al*. 1997). Different genospecies generally co-exist in tick populations in Switzerland (Hu *et al*. 1994; Péter, Bretz & Bee, 1995). Which *Borrelia* species infects which host species and the ticks feeding on them remained enigmatic for a long time, since very few isolates were obtained from hosts (Hovmark *et al*. 1988) and none were obtained from host-feeding ticks until recently (Hu *et al*. 1997). However, specific associations between hosts, ticks and *Borrelia* species were observed: *B. afzelii* and small rodents in Switzerland (Humair *et al*. 1995; Hu *et al*. 1997), *B. burgdorferi* s.s. and small rodents in the UK (Kurtenbach *et al*. 1998*a*), *B. garinii* and

* Corresponding author: Département de Parasitologie, Institut de Zoologie, Université de Neuchâtel, Rue Emile Argand 11, 2007 Neuchâtel, Switzerland. Tel.: +41 32 718 21 11. Fax +41 32 718 30 01.
E-mail: lise.gern@zool.unine.ch

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B. valaisiana and birds (Humair *et al*. 1998; Kurtenbach *et al*. 1998*a*), *B. afzelii* and grey squirrels in the UK (Craine *et al*. 1997) and *B. burgdorferi* s.s. and *B. afzelii* and red squirrels in Switzerland (Humair & Gern, 1998).

In this study, we investigated the maintenance of *B. burgdorferi* s.l. in *Clethrionomys* and *Apodemus* rodents over 16 months in an enzootic area in Switzerland. For this purpose, *B. burgdorferi* s.l. was isolated from hosts and from rodent-feeding ticks, and tick xenodiagnosis was used to assess host infectivity for ticks. The identification of *Borrelia* species allowed precise investigation of the association between *Borrelia* and rodent reservoirs.

MATERIALS AND METHODS

Study site

Small mammals and questing ticks were collected at Glütschbachtal near Thun (Canton of Berne, Switzerland, altitude 589 m), at the same enzootic site described previously (Humair *et al*. 1995).

Investigation of small mammals and infesting ticks

Small mammals were trapped alive for 2 successive nights each month from May 1993 to August 1994. One hundred wooden box traps were placed in a grid of 4 lines of 25 traps with identical location for each capture session. Traps were baited with grains, sunflower seeds and pieces of apple. Each small mammal, except shrews, was brought into the laboratory and caged individually over a pan of water until the feeding ticks dropped off. Engorged ticks were collected daily and maintained at relative humidity close to saturation (RH $> 95\%$) and at room temperature until the moult was completed. After about 1 week of captivity, each rodent was anaesthetized, identified to species, sexed, aged (juvenile or adult) and marked with numbered ear-tag. An ear skin biopsy was taken for isolation of *B. burgdorferi* s.l. Tick xenodiagnosis was performed to study infectivity of rodents. Finally, rodents were released at the exact trapping site, generally 14 days after capture. Retrapped animals were identified by marks and were re-examined. Attached ticks were removed by forceps from the single *Glis glis*, an ear skin biopsy was taken, and the individual was released the same day at the capture site.

Tick xenodiagnosis

Xenodiagnosis was performed 1–2 days after all field-derived ticks were detached from hosts. About 40 xenodiagnostic *I. ricinus* larvae, derived from a laboratory colony free of spirochetal infection, were placed on the head of rodents and were allowed to engorge. Replete xenodiagnostic ticks were collected daily from each host into a pan of water and maintained in the same conditions as described for field-derived ticks, until they moulted.

Collection of free-living ticks

Host-seeking *I. ricinus* nymphs and adults were collected by flagging the vegetation at the site of collection of small mammals and in the close vicinity once a month from August to October 1993 and from March to June 1994. Ticks were identified to species, stage and sex, and were maintained as described above for engorged ticks until processed for spirochete isolation.

Isolation of spirochetes

Ear skin biopsies were collected from anaesthetized rodents and from 4 dead shrews to isolate spirochetes. Skin samples (1–2 mm^2) were taken with sterilized scissors after cleaning the ear with 70% ethanol and were immediately placed into tubes containing supplemented BSKII medium as described by Sinsky & Piesman (1989). Tissue samples from liver, heart, spleen, kidneys and brain were removed from 1 *C. glareolus* (No. 168) and also placed into supplemented BSKII medium (Sinsky & Piesman, 1989).

Ticks collected on rodents and free-living ticks were briefly soaked in 70% ethanol and individually squashed with sterilized forceps in tubes containing BSKII medium modified by Sinsky & Piesman (1989).

The above cultures were screened by dark-field microscopy for the presence of spirochetes after 10 days, and 3, 6, and 8 weeks of incubation at 34 °C. Cultivable spirochetes were subcultured to allow analyses by SDS–PAGE and Western blot. Spirochetes in original culture tubes were identified by PCR/RFLP.

SDS–PAGE and Western blot

After 2–4 subcultures, rodent and tick isolates were analysed by SDS–PAGE and Western blots using species-specific monoclonal antibodies allowing *Borrelia* identification, as described elsewhere (Humair *et al*. 1998).

RFLP analysis

Polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) described by Postic *et al*. (1994) were used to identify the *Borrelia* species. The variable intergenic spacer between repeated 23S (*rrl*) – 5S (*rrf*) ribosomal genes of *B. burgdorferi* s.l. was used as a template for PCR and RFLP analysis using *Mse* I endonuclease.

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Table 1. Infestation of rodents with larval and nymphal *Ixodes ricinus* (Glütschbachtal, Switzerland, May 1993–August 1994)

Scientific name Common name	No. of hosts examined	No. of hosts infested with larvae* (%)	Larvae		No. of hosts infested with nymphs (%)	Nymphs	
			Number	Mean no. per host ± S.D.		Number	Mean no. per host ± S.D.
Apodemus flavicollis Yellow necked mouse	26†	24 (92·3)	143	5·5 ± 4·7	3 (11·5)	5	0·2 ± 0·6
Apodemus sylvaticus Wood mouse	60	37 (61·7)	362	6·0 ± 11·2	8 (13·3)	19	0·3 ± 1·3
Apodemus sp.	3	2 (66·7)	2	0·7 ± 0·6	0 (0)	0	
Clethrionomys glareolus Bank vole	107	79 (73·8)	770	7·2 ± 10·1	13 (12·1)	19	0·2 ± 0·6
Microtus agrestis Field vole	3‡	0 (0)					
Glis glis Edible dormouse	1	1 (100)	5		1 (100)	1	
Total	200	143 (71·5)	1282	6·4 ± 9·8	25 (12·7)	44	0·2 ± 0·8

* Corresponds to the number of hosts with *I. ricinus* subadults, since nymphs were always attached with larvae.
† Captured during tick activity period.
‡ Captured in January and February.

Borrelia DNA was amplified by PCR directly from initial cultures to identify the genospecies present in the original materials and to detect mixed infections. For this procedure, 2 ml were used from cultures that contained spirochetes as observed by dark-field microscopy, whereas the whole volume (4 ml) was used for those cultures that were negative by dark-field microscopy after 8 weeks incubation at 34 °C.

Detection of spirochetes by immunofluorescence

For rodents from which no ticks were cultivated or from which tick cultivation and PCR detection were negative, xenodiagnostic rodent-feeding ticks were also examined by direct immunofluorescence (IF), as described previously (Humair *et al.* 1993).

Statistical analysis

The Fisher's exact test was used to compare the proportions. Probabilities of $P < 0{\cdot}05$ were considered statistically significant.

RESULTS

Investigation of small mammals

Over 3200 trap nights, a total of 214 micromammals of 6 different species were captured at Glütschbachtal from May 1993 to August 1994: 107 *C. glareolus*, 60 *A. sylvaticus*, 26 *A. flavicollis*, 3 *Apodemus* sp., 14 *Sorex araneus*, 3 *Microtus agrestis* and 1 *G. glis*. Thirty-three animals (20 *C. glareolus*, 9 *A. sylvaticus*, 3 *A. flavicollis*, 1 *M. agrestis*) were recaptured once or several times (up to 10 times for 1 individual) during the study period.

Tick infestation of rodents and tick moulting success

Between May 1993 and August 1994, 200 rodents were examined for ticks (shrews were not examined) and 1372 ticks were obtained. *I. ricinus* was the dominant tick species ($n = 1326$, 96·6 %), while the remaining 3·4 % comprised 46 *I. trianguliceps* (36 larvae, 9 nymphs, 1 female) and 1 larva of *I. hexagonus*. Of the attached *I. ricinus*, larvae were far more numerous ($n = 1282$, 96·7 %) than nymphs ($n = 44$, 3·3 %) (Table 1). Mean numbers of ticks per rodent reached 6·4 for larvae and 0·2 for nymphs (Table 1).

Attached *I. ricinus* ticks were found on rodents from May to November 1993 and from April to August 1994, except for 1 larva found on a wood mouse in January 1994 (Fig. 1). During these periods, the prevalence of infested rodents reached more than 90 % for the 3 most abundant rodent species. The monthly median number of larvae per rodent showed a bimodal seasonal pattern in 1993 (Fig. 1), with peaks in May and in August/September. In 1994, the seasonal pattern is incomplete but the spring peak of infestation was also present in May.

The moulting success of field-derived *I. ricinus* ticks fed on *A. sylvaticus*, *A. flavicollis* and *C. glareolus* reached 33·3 % (127/381), 14·9 % (22/148) and 6·2 % (49/789), respectively. The same was observed for xenodiagnostic ticks fed on *A. sylvaticus* (34·2 %, 515/1506), *A. flavicollis* (31·5 %, 115/365) and *C. glareolus* (18·3 %, 234/1280). Interestingly, the proportion of hosts with ticks which moult successfully was higher for *A. sylvaticus* (51/58, 87·9 %) than for *A. flavicollis* (15/25, 60·0 %, $P = 0{\cdot}006$) or for *C. glareolus* (41/94, 43·6 %, $P < 0{\cdot}001$).

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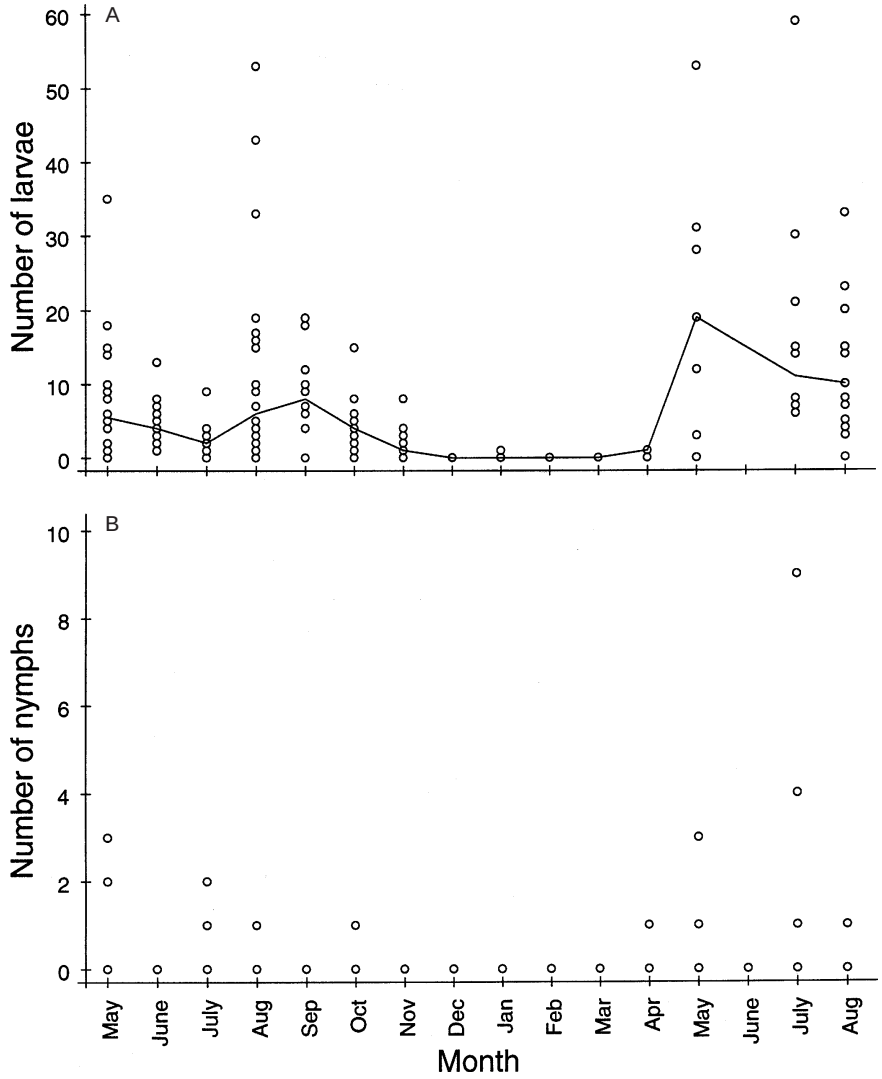

Fig. 1. Seasonal distribution of *Ixodes ricinus* larvae (A) and nymphs (B) on rodents (*Apodemus* and *Clethrionomys*). Seasonal distribution of the monthly median number of larvae per rodent (—). (Glütschbachtal, Switzerland, May 1993–August 1994).

Table 2. Isolation and DNA detection of *Borrelia burgdorferi sensu lato* from rodent ear biopsies (Glütschbachtal, Switzerland, May 1993–August 1994)

Species	No. of hosts examined	Isolation rate (%)	DNA detection rate in negative cultures (%)	Host infection rate* (both methods) (%)
A. flavicollis	26	1/29† (3·4)	0/6§ (0)	1/26 (3·8)
A. sylvaticus	60	4/63† (6·3)	1/13¶ (7·7)	5/60 (8·3)
Apodemus sp.	3	0/3 (0)	0/1 (0)	0/3 (0)
C. glareolus	98	25/106‡ (23·6)	5/15‖ (33·3)	27/98 (27·6)
M. agrestis	3	0/3 (0)	—	0/3 (0)
G. glis	1	1/1 (100)	—	1/1 (100)
S. araneus	4	0/4 (0)	—	0/4 (0)
Total	195	31/209 (14·8)	6/35 (17·1)	34/195 (17·4)

* No. of infected hosts/No. of hosts examined.
† For 3 individuals, 2 skin biopsies were taken.
‡ For 8 individuals, 2 skin biopsies were taken.
§ From 3 individuals.
¶ From 10 individuals.
‖ From 12 individuals.

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Table 3. Success of tick xenodiagnosis on field-collected rodents evaluated through immunofluorescence, isolation and DNA detection of *Borrelia burgdorferi sensu lato* in cultures of xenodiagnostic *Ixodes ricinus* ticks

	Hosts	Xenodiagnostic ticks			
Species	No. of hosts with positive xenodiagnosis* (%)	Isolation rate (%)	DNA detection in negative culture tubes (%)	Detection rate by immunofluorescence (%)	Tick infection rate (all methods) (%)
A. flavicollis	1/10 (10·0)	0/61 (0)	2/55 (3·6)	0/31 (0)	2/92 (2·2)
A. sylvaticus	22/50 (44·0)	22/244 (9·0)	45/182 (24·7)	9/75 (12·0)	76/319 (23·8)
Apodemus sp.	0/2 (0)	0/3 (0)	0/3 (0)	0/1 (0)	0/4 (0)
C. glareolus	19/37 (51·4)	6/110 (5·5)	29/92 (31·5)	15/39 (38·5)	50/149 (33·6)
M. agrestis	0/2 (0)	0/11 (0)	0/11 (0)	0/8 (0)	0/119 (0)
Total	42/101 (41·6)	28/429 (6·5)	76/343 (22·2)	24/154 (15·6)	128/683 (18·7)

* Obtained by all methods.

Isolation and DNA detection of B. burgdorferi *s.l. from mammal tissues*

A total of 209 ear skin biopsies were removed from 195 small mammals (for 14 individuals, 2 skin biopsies were taken) for the isolation of spirochetes (Table 2). Thirty-one isolates were obtained from 29 animals of 4 rodent species (*C. glareolus*, *A. sylvaticus*, *A. flavicollis* and *G. glis*). One additional isolate was obtained from a heart sample from 1 *C. glareolus*, from which no spirochete was isolated from left or right ear biopsies. All isolates, except 2 which could not be amplified by PCR, were analysed by RFLP and identified as *B. afzelii*. Protein profiles of 21 isolates, including the heart isolate, presented a protein profile typical of *B. afzelii* with an OspA of 32 kDa and an OspB of 35 kDa, and all isolates reacted to MAb I.17.3 specific for *B. afzelii*.

In addition, 35 initial culture tubes containing ear biopsies of 26 small mammals trapped from April to August 1994 and which remained negative under dark-field microscopy, were screened by PCR (Table 2). *Borrelia* DNA was detected in 6 culture tubes of 5 additional rodents (4 *C. glareolus* and 1 *A. sylvaticus*). *B. afzelii* was detected in 5 cases and 1 mixed infection of *B. afzelii* and *B. burgdorferi* s.s. was observed in 1 *C. glareolus* trapped in August 1994.

The infection rate of *C. glareolus*, evaluated by isolation and DNA detection of *B. burgdorferi* s.l. from mammal tissues, reached 27·6 % (Table 2). This rate was significantly higher than those of *A. sylvaticus* (8·3 %, $P = 0{\cdot}004$) and *A. flavicollis* (3·8 %, $P = 0{\cdot}015$).

Detection of B. burgdorferi *s.l. in field-derived rodent-feeding ticks*

The cultivation of 77 *I. ricinus* ticks (70 nymphs and 7 adults) – attached on captured rodents as larvae and nymphs – yielded 17 isolates: 13 from nymphs (*A. sylvaticus*: 12/37, *A. flavicollis*: 1/9, *C. glareolus*: 0/22) and 4 from adults (*A. sylvaticus*: 3/6, *C. glareolus*: 1/3). RFLP, SDS–PAGE and Western blot revealed that all isolates belonged to *B. afzelii*. Three out of 4 *I. ricinus* larvae partly-engorged on *G. glis* were found to be positive by IF.

Detection of B. burgdorferi *s.l. in xenodiagnostic ticks* (*isolation*, *DNA detection and IF*).

A total of 683 xenodiagnostic ticks collected from 101 rodents were examined either by *Borrelia* isolation, DNA detection or IF. The cultivation of 429 xenodiagnostic *I. ricinus* nymphs fed on 79 rodents as larvae yielded a total of 28 isolates (Table 3). All these isolates, except 2 which could not be amplified by PCR, were identified as *B. afzelii* by RFLP analysis. Isolates tested by SDS–PAGE and Western blot showed complete correspondence with RFLP results.

A PCR screening was used to detect *Borrelia* DNA in 343 initial culture tubes which remained negative in dark-field microscopy. *Borrelia* DNA was detected in 76 culture tubes containing xenodiagnostic ticks, mostly those that had fed on *C. glareolus* and *A. sylvaticus* (Table 3). DNA of *B. afzelii* ($n = 73$) and *B. burgdorferi* s.s. ($n = 2$) was detected. One mixed infection (*B. afzelii*/*B. burgdorferi* s.s.) was observed.

Using IF, infected ticks (24/154) were obtained from 6 additional *C. glareolus* and 4 additional *A. sylvaticus* (Table 3).

To sum up, positive xenodiagnoses (isolation, DNA detection and IF) were obtained from *C. glareolus*, *A. sylvaticus* and *A. flavicollis* (Table 3). Many more *C. glareolus* (51·4 %) and *A. sylvaticus* (44·0 %) were infective for ticks than *A. flavicollis* (10·0 %, $P = 0{\cdot}029$ and $P = 0{\cdot}073$, respectively). The infection rate in ticks fed on *C. glareolus* (33·6 %) was higher than those in ticks fed on *A. sylvaticus*

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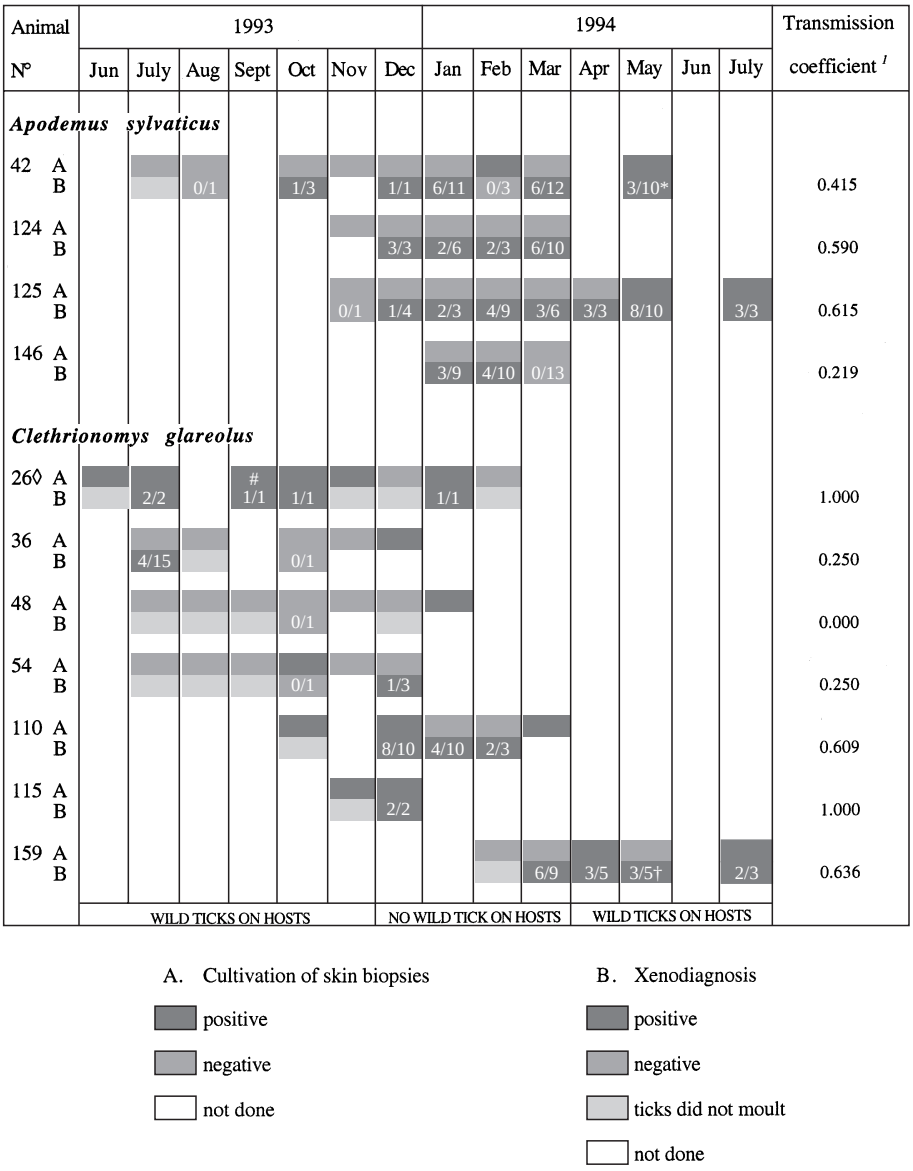

Fig. 2. Follow-up of host infection using skin biopsy cultivation and host infectivity using tick xenodiagnosis of infected *Apodemus sylvaticus* mice and *Clethrionomys glareolus* voles repeatedly recaptured during winter (November 1993–March 1994). [1] Transmission coefficient is the product of the probability of an infected host passing the infection to a tick and the probability of the infection being maintained transstadially from engorged larva to unfed nymph (Randolph & Craine 1995). * One xenodiagnostic tick presented mixed infection with *B. afzelii* and *B. burgdorferi sensu stricto*. ◇ Animal No. 26 was kept in captivity after its first recapture in August 1993. Since then, the animal had no contact with potentially infective ticks and remained infective for ticks. # Positive blood culture and negative skin biopsy culture. † One xenodiagnostic tick was infected by *B. burgdorferi sensu stricto*.

(23·8 %, $P = 0{\cdot}033$) and on *A. flavicollis* (2·2 %, $P < 0{\cdot}001$). Numerically, however, *A. sylvaticus* yielded more infected ticks than *C. glareolus* because of a better tick moulting success after feeding on *Apodemus* mice. Tick xenodiagnosis was a more efficient method of revealing borrelial infection in *C. glareolus* (51·4 %, $P = 0{\cdot}014$) and in *A. sylvaticus* (44·0 %, $P < 0{\cdot}001$) than was cultivation of ear biopsy (27·6 % and 8·3 %, respectively).

Comparing the infectivity of rodents to xenodiagnostic ticks with their infection status (using *Borrelia* isolation), we observed that only very few *A. sylvaticus* infective for ticks also yielded spirochetes isolated by skin biopsy cultivation (4/22, 18 %), whereas about half the infective *C. glareolus* gave positive skin spirochetal infection (10/19, 53 %).

From our data, the transmission coefficient from vertebrate hosts to ticks (Randolph & Craine, 1995) can be measured to assess the infectivity of each rodent species. The transmission coefficient reached 0·653 for *C. glareolus*, 0·542 for *A. sylvaticus* and 0·217 for *A. flavicollis*. The differential tick survival after feeding on specific hosts may, however, modify the relative contribution of each rodent species as reservoirs (see the Discussion below).

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Isolation of B. burgdorferi *s.l. from free-living ticks*

From 235 free-living *I. ricinus* ticks (93 nymphs, 69 females, 73 males) collected by flagging, a total of 45 isolates were obtained: 8 from nymphs, 19 from females and 18 from males. The RFLP identification revealed 18 *B. burgdorferi* s.s., 11 *B. afzelii*, 7 *B. garinii* and 2 unidentified *Borrelia*. Two double (*B. burgdorferi* s.s. and *B. afzelii*) and 1 triple (*B. burgdorferi* s.s., *B. afzelii* and *B. garinii*) mixed infections were observed, but only in adult ticks. Ten isolates (9 *B. burgdorferi* s.s and 1 *B. afzelii*) presented atypical RFLP profiles. DNA of 4 isolates were not amplified. Isolates analysed by SDS–PAGE showed typical protein profiles, except for some isolates which presented atypical electrophoretic mobilities of OspA and OspB. Complete correspondence was observed between reaction to MAbs and respective species identification by RFLP.

Repeated observations of infection and infectivity of rodents captured during winter

The changing infection status, using *Borrelia* isolation, and the infectivity status, using tick xenodiagnosis, of infected rodents repeatedly recaptured during winter (November to March) is presented in Fig. 2. *A. sylvaticus* and *C. glareolus* remained infective for ticks during winter till the following spring and the individual infectivity (Mather *et al.* 1989) or coefficient of transmission (Randolph & Craine, 1995) of rodents varied between 0·219 and 1·000 (Fig. 2). Infection using *Borrelia* isolation from skin biopsy is more easily detected in *C. glareolus* than in *A. sylvaticus* as already observed above. Fig. 2 clearly shows the 'negative cultivation/positive xenodiagnosis' pattern characterizing *A. sylvaticus*, and the 'positive cultivation/positive xenodiagnosis' pattern as well as the 'positive cultivation/infectivity not determined' pattern due to failure of tick moult characteristic of *C. glareolus*.

DISCUSSION

This study supports previous reports on the importance of *Apodemus* mice and *Clethrionomys* voles in the ecology of *I ricinus* subadults and of *B. burgdorferi* s.l., and it highlights the differential transmission pattern of *B. burgdorferi* s.l. between these hosts and ticks.

The tick infestation of *C. glareolus*, *A. sylvaticus* and *A. flavicollis* observed at Glütschbachtal (May–October 1993) was different from that observed at Staatswald (May–October 1988) 50 km away (Humair *et al.* 1993). The prevalence of tick infestation on *C. glareolus* was higher at Glütschbachtal (90·0 %) than at Staatswald (77·6 %, $P = 0{\cdot}048$) as was the mean number of larvae per *C. glareolus* ($P = 0{\cdot}054$). Using tick xenodiagnosis, the infectivity of *C. glareolus* was twice as high at Glütschbachtal (51·4 %) as at Staatswald (20·5 %, $P = 0{\cdot}005$). The dominance of *C. glareolus* as the primary reservoir of *B. burgdorferi* s.l. at Glütschbachtal in 1993–94 corresponds to the situations observed in foci in Sweden in 1991 and 1992 (Tälleklint & Jaenson, 1994) and in Germany in 1988 and 1990 (Kurtenbach *et al.* 1995), but contrasts with the situation observed in 1988 at Staatswald, where *Apodemus* mice played the dominant reservoir role (Humair *et al.* 1993). In Europe, the respective contribution of *C. glareolus*, *A. sylvaticus* and *A. flavicollis* as hosts for ticks and as reservoirs for the Lyme borreliosis spirochetes varies geographically and temporally (seasonally and from year to year). The ratio of *I. trianguliceps*/*I. ricinus* ticks attached to rodents was greater at Glütschbachtal in 1980–82 (1:4, Gern & Aeschlimann, 1986) than in 1993–94 (1:29, $P < 0{\cdot}001$) showing that the proportion of *I. trianguliceps*/*I. ricinus* ticks can change markedly in a biotope in a decade.

In the studies of Tälleklint & Jaenson (1994) and Kurtenbach *et al.* (1995), demonstrating the importance of *C. glareolus* in Sweden and in Germany, the relative contribution of rodents was assessed according to the reservoir potential developed by Mather *et al.* (1989). The reservoir potential (Mather *et al.* 1989) is defined by the following formula: $R_s = I_s L_s D_s / \Sigma_s (I_s L_s D_s)$, where I_s is the proportion of larvae that become infected after feeding on a host species (s), L_s is the average number of larvae infesting that host species, and D_s is the local host density. In the present study, the reservoir potential reached 0·745 for *C. glareolus* ($I_s = 0{\cdot}336$, $L_s = 7{\cdot}2$, $D_s = 7{\cdot}3$), 0·247 for *A. sylvaticus* ($I_s = 0{\cdot}238$, $L_s = 6{\cdot}0$, $D_s = 4{\cdot}1$) and 0·008 for *A. flavicollis* ($I_s = 0{\cdot}022$, $L_s = 5{\cdot}5$, $D_s = 1{\cdot}6$). This clearly demonstrated as well the dominance of *C. glareolus* as the main rodent reservoir. But this formula does not take into account the tick moulting success, which is dependent on the biology of the host species on which ticks feed. Dizij & Kurtenbach (1995) have shown, however, in an experimental study in the laboratory that the moulting success of ticks fed on *Clethrionomys* was lower than that of ticks fed on *Apodemus*. In the present study, we observed the same phenomenon with ticks fed on wild hosts, both for field-derived ticks and for xenodiagnostic ticks. This point should be taken into account to evaluate as accurately as possible the relative contribution of a host species as reservoir for *B. burgdorferi* s.l. in a particular enzootic site. The tick survival of xenodiagnostic ticks fed on *C. glareolus*, *A. sylvaticus* and *A. flavicollis* reached 0·183, 0·342 and 0·315, respectively. The framework developed by Randolph & Craine (1995) takes into account this parameter. The

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product of the vector/host ratio, the vertebrate-to-tick transmission coefficient, and the tick's interstadial proportional survival give an estimate of the relative R_0 index for each host species. This gave a value of 0·860 for *C. glareolus*, 1·112 for *A. sylvaticus* and 0·376 for *A. flavicollis*. To obtain the full R_0 indices, the duration of infectivity and the transmission coefficient from ticks to vertebrate hosts should also be considered, as exposed by Randolph, Gern & Nuttall (1996), but more data concerning these parameters should be obtained to have better estimates. Anyway, considering the moulting success of feeding ticks, the relative contribution of *A. sylvaticus* as reservoirs is higher than that of *C. glareolus*, despite the high infection rate of *C. glareolus*.

We observed that the borrelial infection was more easily detectable from *Clethrionomys* voles than from *Apodemus* mice using cultivation of ear skin biopsy, whereas the prevalence of infection was almost equivalent for *C. glareolus* and *A. sylvaticus* using tick xenodiagnosis. This discrepancy might be caused by the number of spirochetes present in the skin samples and this phenomenon may be related to the stronger immune response to *B. burgdorferi* s.l. in *Apodemus* mice than in *Clethrionomys* voles (Kurtenbach *et al*. 1995). Thus, the inability to isolate *Borrelia* from *Apodemus* skin could be explained either by a control of the number of spirochetes by the host immune response or by the fact that they invaded other organs than skin. The immunosuppressive properties of tick saliva (Ribeiro *et al*. 1985) acting locally could help the ingestion of spirochetes by the feeding ticks. It is known that the continuous exposure of infected rodents to tick bites enhances the transmission of spirochetes from rodents to ticks (Gern *et al*. 1994).

The overwintering maintenance of *B. burgdorferi* s.l. has been ascribed to ticks rather than rodent reservoirs (de Boer *et al*. 1993; Tälleklint & Jaenson, 1995). It is known that infected ticks maintain the pathogen during starvation. However, our observations clearly show that *C. glareolus* and *A. sylvaticus* also act as overwintering reservoirs of *B. afzelii* since they remain infective to ticks during winter. Once infected, small rodents remain infective to ticks for life (Gern *et al*. 1994), although infectivity may vary temporally. In our view, ticks and rodents contribute in their own way to the maintenance of *B. burgdorferi* s.l. from one year to the next in enzootic areas. Overwintering infected rodents are infective to ticks directly at the onset of tick activity. On the other hand, overwintering infected ticks will infect hosts at once, before infected ticks produced later in the season by the overwintering infected rodents. This phenomenon probably permits a more efficient maintenance of the pathogen in the focus, yielding infected ticks and hosts throughout the season of tick activity.

Our results provide yet more strong evidence of the infection of *Clethrionomys* voles and *Apodemus* mice with *B. afzelii* (Humair *et al*. 1995) and the transmission of *B. afzelii* from rodents to ticks (Hu *et al*. 1997), in a second Swiss enzootic focus where various *Borrelia* genospecies occur. Neither *B. burgdorferi* s.s. nor *B. garinii* nor *B. valaisiana* were ever isolated from rodent skin or from rodent-feeding ticks. Only DNA of *B. burgdorferi* s.s. was amplified by PCR in 1 rodent skin and in 3 feeding ticks but this *Borrelia* species was never isolated, suggesting that the infection with this genospecies may occur in the rodents but certainly at too low a level to be efficiently transmissible to ticks. Moreover, the fact that *B. burgdorferi* s.s. was observed only during the period of tick activity on hosts, but not during winter, suggests that *B. burgdorferi* s.s. infection is short-lived in mice and voles. The association between mice and voles and *B. afzelii* is very specific and is not the consequence of a selection process due to the cultivation, since similar associations involving passerines, *B. valaisiana* and *B. garinii*, or squirrels, *B. burgdorferi* s.s. and *B. afzelii* have also been demonstrated using the same methods (Humair & Gern, 1998; Humair *et al*. 1998).

The immune system of vertebrate hosts living in a focus of Lyme borreliosis is confronted with repeated tick bites and with *Borrelia*. Comparing *Apodemus* and *Clethrionomys* immunity, each host species seems to have developed a different strategy towards tick infestation and *Borrelia* infection. *Apodemus* mice do not acquire a resistance to ticks but develop a stronger immune response to *Borrelia* infection (Kurtenbach *et al*. 1994; Dizij & Kurtenbach, 1995). *Clethrionomys* voles react immunologically the other way round. Our observations suggest that, through their immune system, *Apodemus* maintains the borrelial infection at a low level, rarely detectable by skin cultivation, but enough to infect ticks efficiently. On the other hand, *Clethrionomys* voles develop an immune response primarily to ticks, while allowing borreliae to multiply in the host skin. Spirochetes are easily detectable in *Clethrionomys* and are transmitted to ticks. Vole-fed ticks, however, do not engorge or moult successfully because of the acquired resistance of *Clethrionomys* voles to ticks (Dizij & Kurtenbach, 1995; our own data). This indicates that the reservoir competence of a host is modulated by its immune response to the pathogen and to the vector. Similarly, the host immune system probably reacts differentially to the different *Borrelia* genospecies and this could explain the host–*Borrelia* associations already observed (Kurtenbach *et al*. 1998*b*).

The immunological aspects of reservoir hosts should be particularly investigated in the future to understand how reservoirs work, why associations exist between some groups of hosts and *Borrelia*

genospecies, why hedgehogs can be infected by several genospecies (Gern *et al*. 1997) and why large-sized mammals like deer are incompetent as reservoirs (Jaenson & Tälleklint, 1992).

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