Identification of Host Bloodmeal Source and *Borrelia burgdorferi* Sensu Lato in Field-Collected *Ixodes ricinus* Ticks in Chaumont (Switzerland)

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ABSTRACT To evaluate the importance of vertebrate species as tick hosts and as reservoir hosts in two endemic areas for Lyme borreliosis in Switzerland, we applied molecular methods for the analysis of bloodmeal source and *Borrelia* infection in questing *Ixodes ricinus* L. ticks. In total, 1,326 questing ticks were simultaneously analyzed for *Borrelia* and for blood meal remnants by using reverse line blot. An overall infection prevalence of 19.0% was recorded for *Borrelia* sp., with similar rates in both sites. Using a newly developed method for the analysis of bloodmeal targeting the 12S rDNA mitochondrial gene, identification of host DNA from field-collected ticks was possible in 43.6% of cases. Success of host identification at the genus and species level reached 72%. In one site, host identification success reached its maximum in spring (93% in May), decreasing in summer (20% in July) and rising in autumn (73% in October). In the other site, identification rate in ticks remained low from April to July and increased in autumn reaching 68% in October and November. The most prevalent identified host DNA was artiodactyls in both sites. Red squirrel DNA was significantly more frequently detected in ticks collected in one site, whereas insectivore DNA was more frequent in ticks in the other site. DNA from more than one vertebrate host was detected in 19.5% of nymphs and 18.9% of adults. Host DNA was identified in 48.4% of the *Borrelia* infected ticks. Although DNA from all *Borrelia* species was found in at least some ticks with DNA from mammals and some ticks with DNA from birds, our results confirm a general association of *B. afzelii* and *B. burgdorferi* sensu stricto with rodents, and *B. valaisiana* and *B. garinii* with birds.

KEY WORDS *Borrelia* infected ticks, bloodmeal, host DNA identification

The tick *Ixodes ricinus* L. is widespread in Europe where a range of vertebrate species, including mammals, birds, and reptiles play important roles for tick cycle maintenance. This tick species is also the main vector of *Borrelia burgdorferi* sensu lato (s.l.), the etiologic agent of Lyme borreliosis. Seven different *Borrelia* genospecies have been found associated with *I. ricinus: B. burgdorferi* sensu stricto (s.s.), *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. bissettii*, and *B. spielmanii* (Rauter and Hartung 2005, Richter et al. 2006). *Borrelia* spirochetes are maintained in nature in transmission cycles involving ticks and some of their vertebrate hosts (Gern and Humair 2002). Identification of tick hosts and reservoir hosts is a difficult task, because it requires animal trapping and even, if xenodiagnosis is applied, their maintenance in the laboratory. Application of molecular methods for the analysis of bloodmeal remnants in questing ticks to identify host DNA permits evaluation of the importance of vertebrate species as tick hosts as well as reservoir hosts, even those rarely trapped (Tobolewski et al. 1992; Kirstein and Gray 1996; Pichon et al. 2003, 2005, 2006; Estrada-Peña et al. 2005). The main drawbacks to this approach are the small quantities of bloodmeal remnants in questing tick guts and time passed since the last bloodmeal.

Reverse line blotting (RLB) is a reliable technique for the detection and identification of tick-borne pathogens. A RLB was recently developed to identify host DNA at the genus and species levels in questing *I. ricinus* ticks targeting the 12S rDNA mitochondrial gene (Humair et al. 2007). RLB was applied here to identify host DNA that remains in tick guts since the previous bloodmeal (Humair et al. 2007), as well as to detect *Borrelia* DNA (Burri et al. 2007).

The current study is part of a 3-yr study (2003–2005) on *I. ricinus* tick phenology and *Borrelia* infection prevalence along two altitudinal gradients on the north- and south-facing slopes of a mountain (Chaumont, Switzerland) (Morán Cadenas et al. 2007). The aims of this study were to validate the bloodmeal analysis method targeting the 12S rDNA mitochondrial gene and to concomitantly identify *Borrelia* infection in ticks. Therefore, we applied the newly developed technique allowing identification of host

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DNA in ticks at the species level to a large number of ticks (Humair et al. 2007) collected in 2005 in two sites showing different *Borrelia* infection and tick population patterns (Morán Cadenas et al. 2007). The final goal of identifying *Borrelia* genospecies and vertebrate DNA together in the same tick is to obtain information on both tick host and reservoir host diversity in different biotopes that helps comprehension of pathogen circulation within the natural cycle between invertebrate vector and vertebrate host.

Materials and Methods

Collection of Ticks. This study was carried out in a mixed forest dominated by deciduous trees on the north- and south facing-slopes of Chaumont Mountain (Neuchâtel, Switzerland) (Jouda et al. 2004, Morán Cadenas et al. 2007). Questing ticks were collected monthly from March to November 2005. A 1-m^2 cotton flag was dragged across low vegetation over a variable distance ranging between 100 and 150 m (Morán Cadenas et al. 2007). Tick density was expressed as the number of ticks collected per 100 m^2. The flag was inspected every 25 m, and nymphs and adults were placed separately in vials containing fresh grass and kept alive at the laboratory for several days (at room temperature and relative humidity close to 95%) until detections of *Borrelia* sp. infections and analyses of bloodmeal remnants for host identification were performed.

DNA Extraction from Ticks and from Vertebrate Hosts. Before DNA isolation, ticks were soaked in 70% ethanol and air-dried. Isolation of DNA was achieved using ammonium hydroxide (NH_4OH) as described previously (Guy and Stanek 1991, Rijpkema et al. 1996) and slightly modified (Humair et al. 2007). Briefly, entire ticks were individually boiled for 15 min at 100°C in 100 μl of 0.7 M NH_4OH, cooled quickly and boiled again for 15 min in open vials to evaporate the ammonia (10- or 20-μl samples were retained for polymerase chain reaction (PCR) analysis, according to *Borrelia* or host DNA identification, respectively). To check for cross-contamination, negative controls were included during each DNA extraction procedure from ticks, which consisted of 100 μl of 0.7 M NH_4OH without ticks.

To obtain positive controls for host DNA identification, DNA was extracted from muscle, skin, or liver tissues of vertebrates using a DNeasy tissue kit (QIAGEN, Basel, Switzerland) according to the manufacturer protocol. DNA was eluted in 200 μl of elution buffer (QIAGEN), the DNA concentration was measured with a spectrophotometer, and DNA extracts were stored at −20°C until further use.

Identification of *Borrelia* sp. by PCR and RLB. *I. ricinus* ticks were analyzed for detection of *B. burgdorferi* s.l. genospecies by PCR and RLB hybridization. DNA amplification was performed in a reaction volume of 50 μl containing 10 μl of DNA samples. Primers B5S-Bor and 23S-Bor were used to amplify the variable spacer region between two repeated copies of the 23*S* and 5*S* ribosomal genes described in Alekseev et al. (2001). PCR amplifications were run in a Tgradient Thermocycler 96 (Whatman Biometra, Göttingen, Germany) by using a touchdown PCR program modified from Schouls et al. (1999) and described in Burri et al. (2007). Negative and positive controls were included in each PCR. Isolates of *B. burgdorferi* s.s. (B31), *B. garinii* (NE11), *B. afzelii* (NE632), *B. lusitaniae* (PotiB1, PotiB2, and PotiB3), and *B. valaisiana* (VS116) were used as positive controls.

For *Borrelia* identification by RLB, PCR products were hybridized to seven different oligonucleotide probes (75 pmol) (Rijpkema et al. 1995, Poupon et al. 2006) blotted in lines on an activated Biodyne C membrane (Pall Europe Ltd., Portsmouth, United Kingdom) by using a Miniblotter 45 (Immunetic, Cambridge, MA). Hybridization was visualized by incubating the membrane with enhanced chemiluminescence detection liquid (GE Healthcare, Otelfingen, Switzerland) and exposing the membrane to X-ray film (Hyperfilm; GE Healthcare).

Identification of Host DNA by PCR and RLB. Vertebrate DNA remaining in tick guts was amplified by a touchdown PCR by using primers targeting the 12S rDNA mitochondrial gene: 12S-6F (CAAACTGGGATTAGATACC) and B-12S-9R (5′biotin-AGAACAGGCTCCTCTAG), followed by a RLB hybridization carried out to identify host DNA as described in Humair et al. (2007). DNA extraction from field-collected ticks was achieved using ammonium hydroxide (see above), and 20 μl of tick lysates used as template. For each PCR reaction, negative and positive controls were included. Positive controls were 100 ng of vertebrate DNA.

For host DNA identification by RLB, PCR products were hybridized to a set of 35 different oligonucleotide probes (Table 1) (100 pmol; except for lizard, Sylvia and S. araneus probes: 500 pmol) that were used to identify the bloodmeal at the genus or species level within the major groups of vertebrates (small-, medium- and large-mammals, birds, and lizards) (Humair et al. 2007). Hybridization was performed as described above for *Borrelia.* To prevent contamination owing to the sensitivity of this technique, DNA extraction, PCR setup, and sample addition were completed in separate rooms under UV-hoods dedicated to host DNA identification.

Statistical Analysis. All statistics were calculated with S-Plus 7.0 for Windows (Insightful, Seattle, WA). The chi-square test was used for *Borrelia* infection comparisons between stages and over field sites (north- and south-facing slopes) and to compare host identification rates by exposure. The Fisher exact test was used to compare frequency of associations between *Borrelia* genospecies and particular host sources in infected ticks.

Results

In total, 1,326 ticks were analyzed for both host DNA identification and *Borrelia* genospecies: 414 collected on the north-facing slope and 912 on the south-facing slope.

Table 1. Probes used in RLB assays and target vertebrates

Probe name	Target organism
Small rodent	Muroidea (Muridae, Cricetidae)
Artiodactyl	Bovidae, Cervidae, Suidae
Bird	Birds
Lizard	Lizards
Clethrionomys	*Clethrionomys glareolus* (bank vole)
Apodemus	*Apodemus sylvaticus* (wood mouse), *A. flavicollis* (yellow-necked mouse)
M. agrestis/ Micromys	*Microtus agrestis* (field vole), *Micromys minutus* (harvest mouse)
M. arvalis	*Microtus arvalis* (common vole)
M. minutus	*Micromys minutus* (harvest mouse)
R. norvegicus	*Rattus norvegicus* (brown rat)
R. rattus	*Rattus rattus* (black rat)
Sciurus	*Sciurus vulgaris* (red squirrel)
Glis	*Myoxus glis* (fat dormouse)
Lepus	*Lepus europaeus* (European hare)
Erinaceus	*Erinaceus europaeus* (European hedgehog)
S. araneus	*Sorex araneus* (common shrew)
Neomys sp.	*Neomys anomalus* (Miller's water shrew), *N. fodiens* (Eurasian water shrew)
N. anomalus	*Neomys anomalus* (Miller's water shrew)
T. europaea	*Talpa europaea* (European mole)
Vulpes	*Vulpes vulpes* (red fox)
Meles	*Meles meles* (Eurasian badger)
M. erminea	*Mustela erminea* (stoat/ermine)
M. putorius	*Mustela putorius* (European polecat)
Capreolus	*Capreolus capreolus* (roe deer)
Sus	*Sus scrofa* (wild boar)
Turdus/Parus	*Turdus merula* (black bird), *T. iliacus* (redwing), *T. philomelos* (song thrush), *T. pilaris* (fieldfare), *Parus major* (great tit), *P. caeruleus* (blue tit)
Erithacus	*Erithacus rubecula* (European robin)
Parus	*Parus major* (great tit), *P. caeruleus* (blue tit)
P. ater	*Parus ater* (coal tit)
Fringilla/ Pyrrhula	*Fringilla coelebs* (chaffinch), *F. montifringilla* (brambling), *Pyrrhula pyrrhula* (Eurasian bullfinch)
Prunella	*Prunella modularis* (dunnock/hedge accentor)
Sitta	*Sitta europaea* (Eurasian nuthatch)
Sylvia	*Sylvia atricapilla* (blackcap)
Troglodytes	*Troglodytes troglodytes* (wren)
Garrulus	*Garrulus glandarius* (Eurasian jay)

***Borrelia* Identification and Infection Prevalence.** Overall infection prevalence of *Borrelia* spp. was 19.0% (252/1,326), with similar rates on both slopes of the mountain, 19.8% (82/414) on the north-facing slope of Chaumont and 18.6% (170/912) on the south-facing slope. Details are presented in Table 2. Among these 252 infected ticks, 23 ticks (16 nymphs and seven adults) carried multiple *Borrelia* infection. Therefore, 276 *Borrelia* identifications were achieved clustered into four *Borrelia* species: *B. afzelii* ($n = 122$; 44.2%), *B. garinii* ($n = 42$; 15.2%), *B. burgdorferi* s.s. ($n = 51$; 18.5%), and *B. valaisiana* ($n = 34$; 12.3%). Twenty-seven (9.8%) *Borrelia* could not be typed at the species level by the panel of probes used in this study. *B. afzelii* was widely predominant in nymphs followed by *B. garinii*, whereas *B. burgdorferi* s.s. and *B. afzelii* had a fairly similar prevalence in adults (Table 2). Mixed infections were *B. afzelii* and *B. burgdorferi* ss in 10 ticks, *B. garinii* and *B. valaisiana* in nine ticks, *B. garinii* and *B. afzelii* in three ticks, and one triple infection with *B. afzelii*, *B. garinii*, and *B. burgdorferi* ss.

Tick Host DNA Identification. Identification of tick host DNA from field-collected ticks by using RLB was possible in 578 of the 1,326 analyzed ticks (43.6%). Figure 1 shows the RLB pattern obtained with some field-collected ticks. Identification success was significantly higher in adults (214/429, 49.9%) than in nymphs (364/897, 40.6%) ($P = 0.002$; χ^2 test). Furthermore, identification rate varied between sites, so it was significantly higher on the north-facing slope (204/414; 49.3%) than on the south-facing slope of Chaumont (374/912; 41.0%) ($P = 0.006$; χ^2 test). Identification success varied greatly among months. On the north-facing slope, host DNA identification success reached its maximum in spring, 93% (75/81) in May, decreasing to 20% (8/40) in July, and it was high again in autumn (58%, 35/60 in September; and 73%, 22/30 in October) (Fig. 2A and B). On the south-facing slope, identification rate in ticks remained low from April to July and increased in autumn reaching 68% in October and November (58/85 and 48/71, respectively) (Fig. 2A and B). In 2005, questing tick densities showed a bimodal distribution on both slopes of the mountain. Tick density was higher on the south-facing slope than on the north-facing slope, in 2005 and during previous years (Morán Cadenas et al. 2007).

DNA from more than one host was detected in 111/578 ticks (71 nymphs and 40 adults) in which host DNA was detected (south-facing slope: 80/374, 21.4%;

Table 2. *Borrelia burgdorferi* s.l. infection in questing *I. ricinus* ticks collected on the north- and south-facing slopes of Chaumont Mountain

Site	Nymphs						Adults					
	No. (%)	*Borrelia* identification					No. (%)	*Borrelia* identification				
		af	ga	ss	vs	sl		af	ga	ss	vs	sl
N1	32/116 (27.6)	22	6	4	2	1	3/40 (7.5)	0	1	2	0	0
N2	11/101 (10.9)	6	2	3	0	1	11/39 (28.2)	6	1	5	1	0
N3	21/90 (23.3)	9	6	4	3	3	4/28 (14.3)	1	0	1	1	1
Total N	64/307 (20.8)	37 (51.4)	14 (19.4)	11 (15.3)	5 (6.9)	5 (6.9)	18/107 (16.8)	7 (35.0)	2 (10.0)	8 (40.0)	2 (10.0)	1 (5.0)
S1	25/123 (20.3)	4	4	6	9	2	17/69 (24.6)	3	3	7	3	2
S2	23/149 (15.4)	18	3	2	3	1	25/94 (26.6)	12	2	9	2	2
S3	16/164 (9.8)	9	4	1	4	1	25/137 (18.2)	6	3	5	6	7
S4	36/154 (23.4)	26	5	2	0	5	3/22 (13.6)	0	2	0	0	1
Total S	100/590 (16.9)	57 (52.3)	16 (14.7)	11 (10.1)	16 (14.7)	9 (8.3)	70/322 (21.7)	21 (28.0)	10 (13.3)	21 (28.0)	11 (14.7)	12 (16.0)
N & S	164/897 (18.3)	94 (51.9)	30 (16.6)	22 (12.2)	21 (11.6)	14 (7.7)	88/429 (20.5)	28 (29.5)	12 (12.6)	29 (30.5)	13 (13.7)	13 (13.7)

Mixed infections were included in single columns. N1 to N3, sampling sites on the north-facing slope. S1 to S4, sampling sites on the south-facing slope. af, *B. afzelii;* ga, *B. garinii;* ss, *B. burgdorferi* ss; vs, *B. valaisiana;* s1, untypeable *Borrelia.*

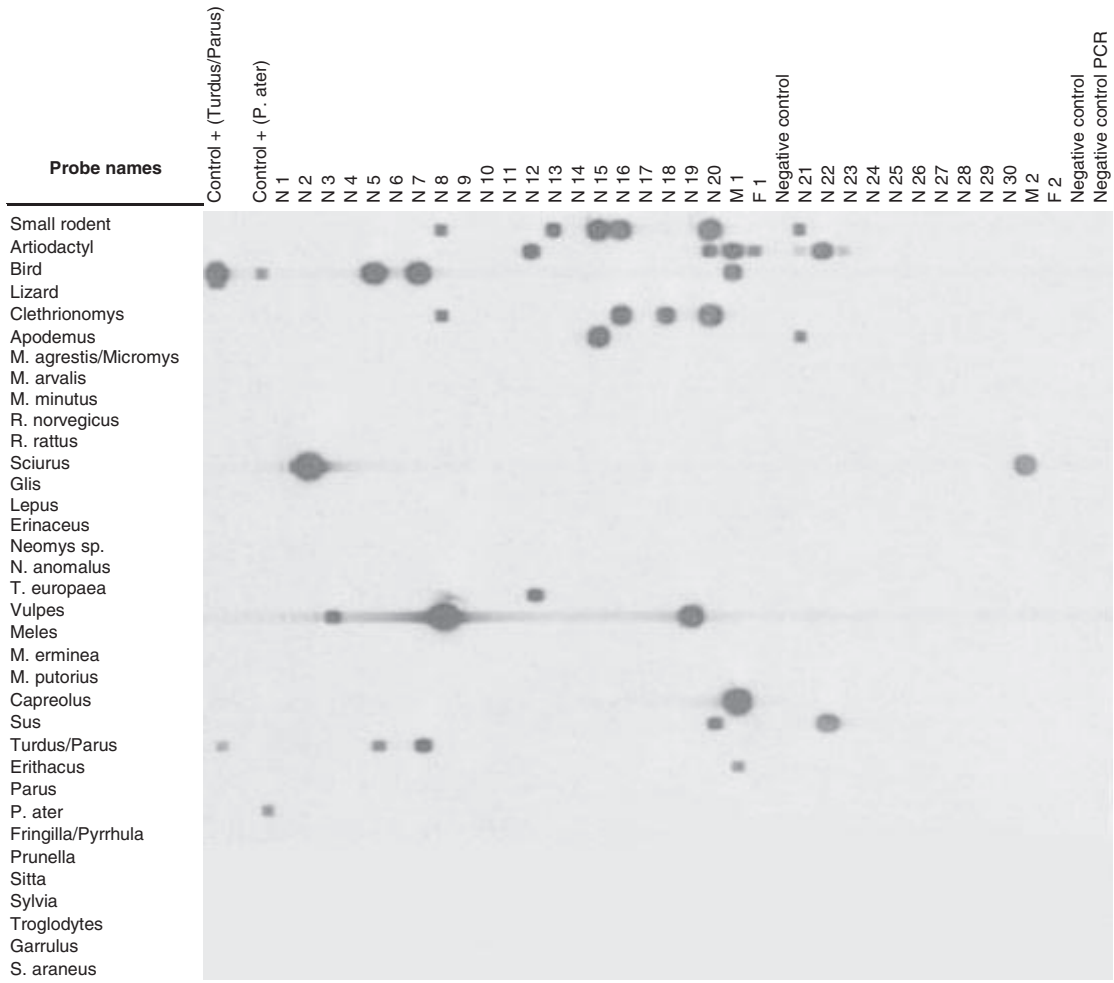


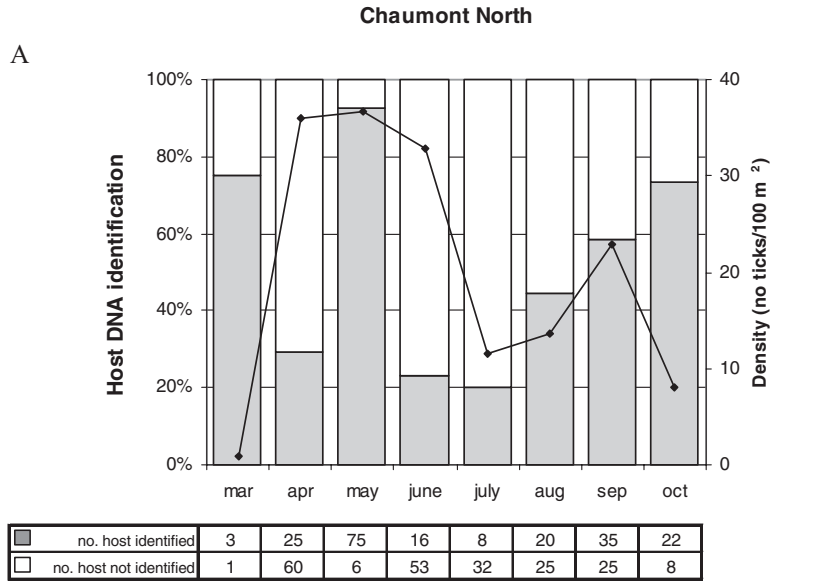
Fig. 1. RLB results for host DNA identification in field-collected ticks by using 12S rDNA as genetic marker (Humair et al. 2007). Probe names are listed on the left. N, nymphs. M, males. F, females.

north-facing slope: 31/204, 15.2%) ($P = 0.09$; χ^2 test). Therefore, 712 host identifications were achieved from 578 ticks (Table 3). Multiple host DNA was detected as frequently in nymphs (71/364; 19.5%) as in adults (40/214; 18.7%) ($P = 0.89$; χ^2 test). In 62.6% (446/712) host identification was possible at the species level, in 9.4% (67/712) at the genus level and in 27.9% (199/712) at the group level only. Rodent DNA and squirrel DNA were more frequently identified in nymphs than in adults ($P = 0$ and $P = 0.04$, respectively; χ^2 test), and roe deer DNA more frequently in adults than in nymphs $P = 0$; χ^2 test) (Table 3). For other host DNA, no significant differences were observed between tick stages.

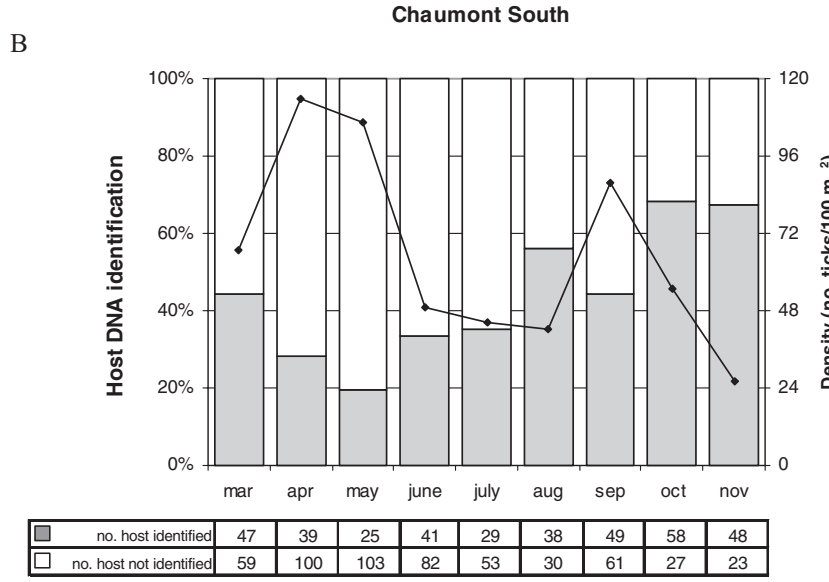
On both slopes of Chaumont Mountain, the most commonly identified host DNA was from artiodactyls (north- facing slope: 47%, 112/239; south-facing slope: 40%, 190/473) (Fig. 3). For artiodactyls, small rodents, birds, and carnivores, no significant difference was observed between both slopes. However, red squirrel DNA was significantly more frequently detected in ticks collected on the south-facing slope (100/473; 21%) ($P = 0.0028$; χ^2 test), whereas insectivore DNA was significantly more frequently detected in those from the north-facing slope (8/239; 3%) ($P = 0.0052$; χ^2 test).

Reservoir Hosts for *Borrelia* spp. Host DNA identification was possible in 47.6% (39/82) and 48.8% (83/170) of ticks infected with *B. burgdorferi* s.l. on the north- and south-facing slopes, respectively. Bloodmeal analyses in infected questing ticks are summarized in Table 4.

From previous reports, it is known that *B. afzelii* and *B. burgdorferi* s.s. are associated with rodents (small mammals and squirrels) and that *B. garinii* and *B. valaisiana* are associated with birds (Hu et al. 1997; Humair and Gern 1998; Humair et al. 1998, 1999; Kurtenbach et al. 1998a; Hanincová et al. 2003a,b). To examine host-*Borrelia* associations thoroughly, we excluded ticks in which more than one host source was identified and those infected with untypeable *Borrelia.* Moreover, since *B. burgdorferi* is known to be



	mar	apr	may	june	july	aug	sep	oct
no. host identified	3	25	75	16	8	20	35	22
no. host not identified	1	60	6	53	32	25	25	8



	mar	apr	may	june	july	aug	sep	oct	nov
no. host identified	47	39	25	41	29	38	49	58	48
no. host not identified	59	100	103	82	53	30	61	27	23

Fig. 2. Monthly host identification success in field-ticks collected on both slopes of Chaumont Mountain (left scale). Questing tick densities (expressed as the number of ticks collected per 100 m^2) showed a bimodal distribution (right scale). (A) North-facing slope of Chaumont. (B) South-facing slope of Chaumont. Percentages of identified and not identified hosts are shown by filled bars and by open bars, respectively. Numbers are presented on the table.

transstadially maintained, only nymphs were considered in the analysis of host–*Borrelia* association. So, among *Borrelia* species identified in nymphs fed on small rodents and on squirrels, 4/4 (100%) and 12/14 (86%), respectively, were *B. afzelii* and *B. burgdorferi* s.s. In infected ticks fed on birds, 7/15 (47%) carried *B. garinii* and *B. valaisiana*. *B. afzelii* and *B. burgdorferi* s.s. were more frequently identified in ticks fed on rodents than on birds; likewise, *B. garinii* and *B. valaisiana* were more frequently identified in ticks fed on birds than on rodents ($P = 0.047$; Fisher exact test).

Discussion

In the current study, molecular analysis of bloodmeals in *I. ricinus* ticks allowed assessment of the importance of host species for *I. ricinus* on a large number of ticks collected in two sites, on the north- and south-facing slopes of Chaumont Mountain. Host DNA detection success significantly varied in both studied biotopes between 49.3% and 41.0%. Temperatures on the south-facing slope are higher than on the north-facing slope (Morán Cadenas et

Table 3. Identification of host origin of the bloodmeal remnants in ticks (nymphs and adults) collected on the north- and south-facing slopes of Chaumont Mountain

Host DNA identification	Nymphs			Adults			Total		
	North	South	Total	North	South	Total	North	South	Total
Small rodents	1	5	6		1	1	1	6	7
Apodemus sp.	5	19	24	1		1	6	19	25
Clethrionomys glareolus	14	12	26	1	4	5	15	16	31
Sciurus vulgaris	18	53	71	10	47	57	28	100	128
Artiodactyls	40	58	98	13	32	45	53	90	143
Capreolus capreolus	5	10	15	17	15	32	22	25	47
Sus scrofa	30	38	68	7	37	44	37	75	112
Birds	13	17	30	6	13	19	19	30	49
Erithacus rubecula	1	1	2	1	1	2	2	2	4
Fringilla/Pyrrhula	1	6	7				1	6	7
Garrulus glandarius		1	1		1	1		2	2
Parus ater		1	1					1	1
Parus sp.	1	1	2				1	1	2
Sylvia atricapilla		11	11		3	3		14	14
Turdus sp.	6	15	21	2	8	10	8	23	31
Vulpes vulpes	9	27	36	1	23	24	10	50	60
Meles meles	1	4	5		6	6	1	10	11
Mustela erminea		1	1	1		1	1	1	2
M. putorius	19		19	7		7	26		26
Neomys anomalus		1	1					1	1
Neomys sp.	2		2				2		2
Sorex araneus			0		1	1		1	1
Talpa europaea	6		6				6		6
Total	172	281	453	67	192	259	239	473	712

al. 2007), and they may induce a faster digestion of blood, explaining the lower success of DNA detection in ticks collected on the south-facing slope. The ability to detect host DNA in adult ticks was significantly higher than in nymphs, probably due to the higher quantity of blood ingested by ticks during their previous bloodmeals as nymphs and as larvae. Success of host identification at the genus and species levels reached 72% among ticks with identified host DNA. Previous studies on host identification in ticks by using 18S rRNA and 12S rRNA were unable to identify host DNA at the species level (Pichon et al. 2003, 2005, 2006; Estrada-Peña et al. 2005). Overall, the most frequent bloodmeal source detected in field-collected ticks was artiodactyls when identification was possible only at the group level, and wild boar, red squirrel, and red fox when identification succeeded at the species level. These results are in line with reports from the Swiss Federal Office of Environment, OFEV, www.wildtier.ch/stat-chasse. In fact, artiodactyls (deer and chamois) populations increased in Neuchâtel in 2004 and decreased slightly in 2005 due to the harsh winter, whereas wild boar population increased in 2002–2005. Other hosts are differently represented according to the site. Red squirrel DNA was more frequently detected in ticks collected on the south-facing slope, whereas insectivore DNA was further detected in ticks from the north-facing slope, suggesting that host distribution differed in the two biotopes.

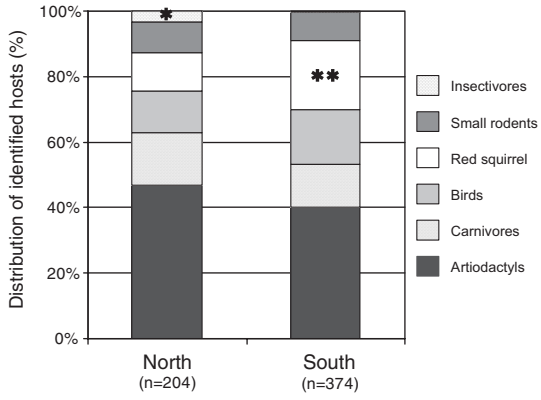


Fig. 3. Distribution of host DNA identification in *I. ricinus* ticks collected on the north- and south-facing slopes of Chaumont Mountain. *, insectivores, more abundant on the north-facing slope of the mountain ($P < 0.05$). **, red squirrels, more abundant on the south-facing slope of the mountain ($P < 0.05$).

In almost 20% of ticks in which host DNA was identified, DNA from multiple vertebrate hosts was detected. This observation suggests that interrupted feeding may occur more frequently than expected in *I. ricinus* as formerly observed in *I. scapularis* (Piesman 1991). Alternately, external DNA contamination of ticks due to contacts with hosts without success of attachment is also possible. Nevertheless, this information is useful because it indicates the diversity of host fauna in a biotope.

In 2005, a clear bimodal seasonal pattern of tick density occurred on both slopes of Chaumont. On the south-facing slope, clearly host DNA detection success was higher in autumn than in spring, reaching 68% in October and November. Pichon et al. (2005) reported similar improvement of host source identification in nymphs collected in October in Ireland. Sensitivity of host DNA detection in ticks is dependent on

Table 4. Host origin of the bloodmeal remnants in infected ticks (nymphs and adults) collected on the north- and south-facing slopes of Chaumont Mountain

Chaumont Mountain	Host DNA	*Borrelia* identification											
		Nymphs (n = 31)						Adults (n = 8)					
		af	ss	ga	vs	sl	Total	af	ss	ga	vs	sl	Total
North	*Apodemus* sp.	1	1				2						
	Clethrionomys glareolus	2					2						
	Sciurus vulgaris	4	1			1	6						
	Artiodactyls	3	1	2	2		8		1	1			2
	Sus scrofa	3	1				4	2	1			1	4
	Vulpes vulpes	2					2						
	Mustela putorius								2				2
	Birds	2	1				3						
	Turdus sp.			1		2	3						
	Mixture of DNA	5		2			7	1					1
	Total N	22	5	5	2	3	37	3	4	1	0	1	9
		Nymphs (n = 44)						Adults (n = 39)					
South	Small rodents					1	1					1	1
	Sciurus vulgaris	4	3	1	1		9	1	7			1	9
	Artiodactyls	5	2			1	8		2	1		1	4
	Capreolus capreolus							1					1
	Sus scrofa	5	1			1	7	1		2		3	6
	Vulpes vulpes	5	1	1			7	1		2		2	5
	Meles meles							1					1
	Birds	1			2		3				1	1	2
	Turdus sp.	2		2	2		6				1	1	2
	Sylvia atricapilla	2					2	1					1
	Mixture of DNA	5	1		1		7	2		1	4		7
	Total S	29	8	4	6	3	50	8	9	6	6	10	39
	Total N & S	51	13	9	8	6	87	11	13	7	6	11	48

Mixed infections were included in single columns. af, *B. afzelii;* ga, *B. garinii;* ss, *B. burgdorferi* ss; vs, *B. valaisiana;* sl, untypeable *Borrelia.*

the interval from molting until the next questing activity (i.e., the time when ticks are collected and analyzed). According to Randolph et al. (2002), a single cohort of each stage of ticks emerges each year in the autumn. This is based on the fact that in various sites in United Kingdom, ticks with high fat content appeared each year in autumn and that a temperature-dependent development model predicted also the mean emergence of ticks in autumn. That detection success of host DNA was higher in autumn than in spring on the south-facing slope supports the interpretation of *I. ricinus* population dynamics evidenced by Randolph et al. (2002) in the United Kingdom and supported by Jensen and Kaufmann (2003) in Denmark. However, the situation on the north-facing slope seems different because two peaks in host DNA detection success emerged, one peak in May with host identification in 93% of ticks, suggesting that ticks molted in spring, and a second peak in October with host identification of 73%. Although these results concern only 1 yr, they give reason to question whether local *I. ricinus* population dynamics may be different from a dynamic with a single cohort in autumn and whether this might be due to very specific climatic conditions.

The capacity of several vertebrates to serve as reservoir hosts for Lyme disease spirochetes has been more extensively evaluated for rodents, birds, and deer. Little is known concerning the status of other potential hosts, such as carnivores, foxes, or edible dormice (Matuschka et al. 1994, Kahl and Geue 1998, Liebisch et al. 1998). We present here field evidence that confirms the reservoir status of foxes (Kahl and Geue 1998, Liebisch et al. 1998) and that sheds light on the possible reservoir roles of some hosts hitherto not studied, such as badger, polecat, and wild boar. For example, we identified *B. afzelii* in ticks in which wild boar DNA was detected. A similar association between *B. afzelii* and wild boar DNA was recently reported in Spain by Estrada-Peña et al. (2005) targeting the same gene. Additional similar studies in other areas may help us better understand the reservoir status of these hosts, which are difficult to capture and maintain in the laboratory.

One important finding in Lyme borreliosis ecology in the past 10 yr is the observation of an association between different *Borrelia* genospecies or strains with different vertebrate host species (Kurtenbach et al. 1998b). *B. afzelii* was the most frequent genospecies identified in ticks collected on both slopes of the mountain. High prevalence of *B. afzelii* in infected nymphs suggested that small rodents, such as bank vole, woodmouse, and yellow-necked mouse, might be relatively important as reservoir hosts for feeding larvae in our region (Humair et al. 1995, 1999; Hu et al. 1997). Unexpectedly, molecular analysis of bloodmeal remnants in nymphs showed that small rodents were relatively scarce as reservoir hosts of *Borrelia* spp. and as hosts for ticks in our study sites. The implications of these observations are not clear, and they are possibly related to reduced populations of mice and voles

during the study period. Moreover, previous studies in this area showed the importance of small mammals as tick hosts and as reservoirs for *Borrelia* (unpublished data). Most probably other rodents, such as red squirrels, acted more successfully as reservoir hosts for *Borrelia* spirochetes than did mice during the study period. Red squirrels have been shown to be heavily infested by *I. ricinus* larvae and nymphs and to transmit *B. afzelii* and *B. burgdorferi* ss to ticks (Humair and Gern 1998).

As expected, we observed that *B. burgdorferi* s.s. and *B. afzelii* were associated with small mammals and red squirrels, and *B. valaisiana* and *B. garinii* with birds (Gern and Humair 2002), although these *Borrelia* species were not entirely confined to rodents or birds. The absence of a strict association between *Borrelia* spp. and particular hosts is not surprising. Similar results were obtained in Switzerland and France, showing no restricted specificity between *Borrelia* species and some host species, such as badgers (unpublished data), hedgehogs, and deer (Gern et al. 1997, Pichon et al. 2000). Other studies on bloodmeal analysis similarly showed strict and loose associations between *Borrelia* spp. and hosts (Pichon et al. 2003, 2005, 2006; Estrada-Peña et al. 2005). This implies that some host species that are not generally considered as reservoir hosts could in fact serve as reservoirs, such as artiodactyls (particularly Cervidae and Bovidae). Alternative explanations for this absence of specificity could be cofeeding transmission of *Borrelia* without systemic infections (Gern and Rais 1996, Hu et al. 2003) as already described for artiodactyls, for sika deer in Japan, and for sheep in the United Kingdom (Kimura et al. 1995, Ogden et al. 1997). Additionally, transovarially transmitted spirochetes (Bellet-Edimo et al. 2005), interrupted feeding (Piesman 1991) on various infected hosts or external DNA contamination due to unsuccessful attachment to a host might be responsible for the variety of *Borrelia* in the different hosts.

In this study, we were able to validate our method for host DNA identification in a large number of field-collected ticks, to corroborate the association between *Borrelia* species and birds and rodents, to confirm the role of red foxes as reservoir hosts and to provide evidence for the role of wild boar as reservoir host for *B. afzelii.* Interestingly, the presence of multiple host DNA in ticks and the loose association between some hosts and *Borrelia* genospecies were rather frequent. This suggests that interrupted feeding of ticks is a common phenomenon in nature, that reservoir hosts for *Borrelia* are more diverse that previously thought and that some *Borrelia* species or strains are not confined to some host species. Cofeeding transmission may also be an explanation, adding to the range of reservoir hosts additional animal species that do not develop systemic infection, and yet might contribute significantly to the transmission of the pathogens (Randolph et al. 1996).

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