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Prevalence of *Borrelia burgdorferi* sensu lato in the tick *Ixodes ricinus* in the Styrian mountains of Austria

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Summary. A total of 691 *Ixodes ricinus* (22 male, 39 female, 501 nymphs and 129 larvae), the tick vector of Lyme borreliosis, were collected by flagging from vegetation in 11 areas at altitudes between 789 m and 1350 m above sea level in mixed woodland with pasture land (cattle) in the province of Styria in Austria. The ticks were individually examined for presence of *Borrelia burgdorferi* sensu lato by dark-field microscopy and 107 of them by real-time PCR. Attempts to cultivate borreliae were made in BSK-H medium. The overall positivity rate of all collected ticks (excepting larvae) was 10.9%: 9.1% in males, 17.9% in females and 10.4% in nymphs. The 129 larvae examined showed no presence of *B. burgdorferi* s.l. The mean infection rate of *I. ricinus* collected at the highest altitude in this study, Gaberl at 1350 m a.s.l. – and at the same time the highest one reported in Europe – was 6.4%: 1/9 males, 2/18 females and 6/114 (5.3%) nymphs were positive. Culture attempts were positive in 12 cases and species identification showed eight isolates were *B. afzelii* and four *B. garinii*. Three additional positive results found by PCR (negative by culture) were identified twice as *B. afzelii* and once as *B. garinii*. This study shows that the risk of acquiring Lyme borreliosis in habitats at higher altitudes is limited, because of the lower density of *I. ricinus* and its lesser infection rate than at lower altitudes in central Europe, but nevertheless the risk does exist.

Key words: Borrelia burgdorferi, ixodid ticks.

Introduction

Ixodes ricinus, the principal tick vector of Lyme borreliosis in western and central Europe, has been shown to harbor *Borrelia burgdorferi* sensu lato at an infection rate of up to 25% in Styria, a province of Austria [1]. Some areas of Styria, located between 360 m and 800 m above sea level, are also known as natural foci of tick-borne encephalitis [2]. Very few data have been published on the borrelial infection rate of ticks at altitudes above 800 m a.s.l. [3–6]. The aim of this study was to find out whether *I. ricinus* infected with *B. burgdorferi s.l.* are present at higher altitudes in Styria.

Materials and methods

Collection of ticks and investigation for borreliae

A total of 691 *I. ricinus* (22 males, 39 females, 501 nymphs and 129 larvae) were collected by flagging from vegetation in 11 different locations in Eastern and Western Styria between 750 m and 1350 m a.s.l. in mixed woodland with cattle pastures (Fig.1) in 1999 and 2002.

All ticks collected were placed in tubes and stored at 8 °C until examination for the presence of spirochetes. For this purpose, midgut tissues of dissected ticks were examined by dark-field microscopy (400 ×). Tissues containing motile and/or more than 50 spirochetes were transferred to small tubes containing 2.5 ml BSK-H medium (SIGMA) with rifampicin (50 μg/ml).

All 107 nymphal ticks collected from the Gaberl 1 area (1350 m a.s.l.) in June 2002 were examined by dark-field microscopy and PCR following a real-time LightCycler protocol.

Spirochetal species identification

To differentiate the *Borrelia burgdorferi* s.l. genomic species, LightCycler real-time PCR was used for fluorescence (SYBR Green I) melting curve analysis of borrelial *recA* PCR

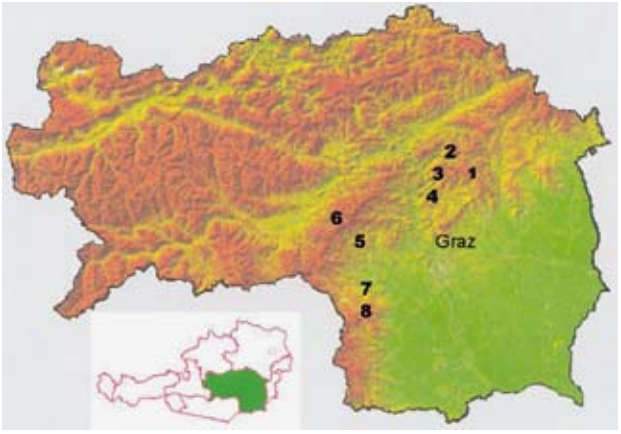

Fig. 1. Tick collection areas (Styria, Austria): *1* Kulm, *2* Teichalpe, Arzberg, *3* Gollersattel, *4* Breitenfeld, *5* Salla, *6* Gaberl, *7* Trahütten, *8* Schwanberg

Table 1. The number of collected and infected *Ixodes ricinus* in high areas of Styria

Locality, elevation, and no. examined ticks (n)	Tick stage	No. examined	No. microscopy pos.	Number of spirochetes in positive ticks	Culture positive	PCR positive	Borrelia genomospecies
Teichalpe 1, 1300 m							
N: 103	M	3	0	–	n.d.	n.d.	–
	F	4	1	90	0/1	1/1	1 afzelii
	N	36	5	17, 69, 102, 4, 73	3/3	3/3	2 afzelii,1 garinii
	L	60	0				
Teichalpe 2, 950 m							
n: 39	F	3	1	180	1/1	1/1	1 afzelii
	N	35	3	9, 36, 17	n.d.	n.d.	–
	L	1	0				
Gollersattel, 789 m							
n: 103	M	1	0	–	n.d.	n.d.	–
	F	4	1	>250	1/1	1/1	1 garinii
	N	84	7	24, 26, 11, 3, 2, n.d., n.d.	n.d.	n.d.	–
	L	14	0				
Breitenfeld, 825 m							
n: 11	M	1	0	–	n.d.	n.d.	–
	F	1	0	–	n.d.	n.d.	–
	N	9	1	6	n.d.	n.d.	–
Sallagraben, 865 m							
n: 36	F	1	0	–	n.d.	n.d.	–
	N	25	5	85, 6, 23, 17, 21	n.d.	n.d.	–
	L	10	0				
Gaberl 1, 1350 m (1999)							
n: 16	F	2	0	–	n.d.	n.d.	–
	N	7	1	>250	1/1	n.d.	1 afzelii
	L	7	0				
Gaberl 1, 1350 m (2002)							
n: 132	M	9	1	23	0/1	1/1	1 garinii
	F	16	2	61, 32	1/1	2/2	1 afzelii, 1 garinii
	N	107	5	25, 125, 4, 32, 12	1/3	5/5	4 afzelii,1 garinii
Gaberl 2, 1210 m (1999)							
n: 71	M	3	0	–	n.d.	n.d.	–
	F	4	1	380	0/1	n.d.	–
	N	40	5	52, 22, 4, >250, 105	1/2	n.d.	1 afzelii
	L	24	0	–	n.d.	n.d.	–
Trahütten, 980 m							
n: 8	N	8	0	–	n.d.	n.d.	–
Schwanberg, 865 m							
n: 36	N	23	1	5	n.d.	n.d.	–
	L	13	0				
Kulm 1, 970 m							
n: 74	M	5	1	20	1/1	1/1	1 garinii
	F	2	0	–	n.d.	n.d.	–
	N	67	12	138, 34, 11, 600, 53, 28, 26, 48, 76, 47, 7, 1600	1/3	n.d.	n.d.
Kulm 2, 810 m							
n: 62	F	2	1	1900	1/1	1/1	1 afzelii
	N	60	7	27, 11, 5, 21,13, 7,15	n.d.	n.d.	n.d.
TOTAL	M	22	2				2 garinii
n: 691	F	39	7				4 afzelii, 2 garinii
	N	501	52				8 afzelii, 2 garinii
	L	129	0				–

products, according to Pietilä et al. [7]. Isolate genospecies were identified using PCR-RFLP analysis with species-specific primers to amplify the variable spacer rrf-rrl as described by Postic et al. [8]. *Mse*I endonuclease was used for cleaving PCR products.

Results

Sixty-one out of 691 (8.8%) *I. ricinus* ticks examined were positive by dark-field microscopy and/or PCR. The overall infection rate with borreliae was 18.4% for females (7/38 specimens tested), 9.1% for males (2/22), and 9.3% for nymphs (52/561); all 129 tested larvae were negative. The number of spirochetes counted by dark-field microscopy in midgut specimens ranged from 4 to 1900 per tick.

In the area with the highest altitude, Gaberl at 1350 m, 1/9 (11%) males, 2/18 (11%) females and 6/114 (5.3%) nymphs tested positive. In the first study in this area in June 1999, the team only managed to collect 16 *I. ricinus* (2 females, 7 nymphs and 7 larvae), and one of the nymphs was infected with *B. afzelii.* An additional collection was carried out in 2002: all 132 collected ticks (9 males, 16 females and 107 nymphs) were examined with real-time PCR and showed a positivity rate of 6.1% (8/132 specimens). *B. afzelii* was demonstrated in one female and in four nymphs; *B. garinii* was found in one male, one female and in one nymph. Another study site in the Gaberl area at an altitude of 1210 m a.s.l. showed a tick infection rate of 12.5%, with 5/40 nymphs harboring spirochetes.

In the Teichalpe area (1300 m a.s.l.), 1/4 females and 5/36 (13.9%) nymphs tested positive. Species identification revealed three strains of *B. afzelii* (in 1 female and 2 nymphs) and one strain of *B. garinii* (in 1 nymph). A second study site in the Teichalpe area (Arzberg, 950 m a.s.l.) found 1/3 females positive for *B. afzelii.*

Of the spirochete-positive ticks from the remaining locations (Table 1), a strain of *B. garinii* was isolated from one male (Kulm 1; 970 m a.s.l.) and one female specimen (Gollersattel, 789 m a.s.l.). *B. afzelii* was also cultivated from a female tick in the area Kulm 2 at 810 m a.s.l. (Table 1).

Discussion

Comparison of species identification in the two methods used, PCR-RFLP analysis and real-time PCR, showed that the results were identical.

The overall average infection rate of 'mountain' *I. ricinus* was 8.8%, lower than that of ticks collected at lower altitudes in Styria, where the mean infection rate is 25% [1]. The present study demonstrates that borrelial-infected *I. ricinus* occur in Austria at elevations as high as 1350 m a.s.l. and confirms that *B. burgdorferi* s.l. occurs in all European populations of *I. ricinus* [9]. These spirochetes may be regarded as commensal microorganisms of this tick species. The borrelial infection rate of ticks decreased with increasing altitude. There have been four studies on 'mountain' borreliae in Switzerland. Aeschlimann et al. [3] found borrelial-infected ticks in Switzerland at altitudes as high as 1250 m and stated that the infection rate of the vector decreased with increasing altitude. Miserez et al. [4] collected ticks at altitudes between 300 m and 1080 m in a Swiss province (Canton Tessin) and reported infected *I. ricinus* only at altitudes not higher than 650 m. A recent study by Jouda et al. [5] on *I. ricinus* density and prevalence of *B. burgdorferi* s.l. infections at 620 m, 740 m and 900 m showed a 21% infection rate in nymphs and 30% rate in adults. Burri et al. [6] found infected *I. ricinus* in the Swiss Alps at 1020 m a.s.l.: at elevations between 750 m and 1020 m, the genomic species detected were *B. afzelii, B. garinii, B. valaisiana* and *B. burgdorferi* s.s.

In the last decade, the vertical distribution of *I. ricinus*, including those infected with tick-borne encephalitis virus, has moved towards higher altitudes in central Europe, as reported from the Czech Republic [10–13].

References

1. Stünzner D, Hubálek Z, Halouzka J, Postic D, Pierer K, Marth E (1998) Prevalence of *Borrelia burgdorferi* s.l. in *Ixodes ricinus* ticks from Styria (Austria) and species identification by PCR-RFLP analysis. Zbl Bakt 288: 471–478
2. Labuda M, Stünzner D, Kozuch O, Sixl W, Kocianova E, Schaffler R, Vyrostekova V (1993) Tick-borne encephalitis virus activity in Styria, Austria. Acta Virol 37: 187–190
3. Aeschlimann A, Chamot E, Gigon F, Jeanneret J-P, Kesseler D, Walther C (1986) *B. burgdorferi* in Switzerland. Zbl Bakt Hyg A 263: 450–458
4. Miserez V, Gern L, Aeschlimann A (1990) *Borrelia burgdorferi* in ticks of the Canton Tessin (Switzerland). Parassitologia 32: 293–299
5. Jouda F, Perret JL, Gern L (2004) *Ixodes ricinus* density, and distribution and prevalence of *Borrelia burgdorferi* sensu lato infection along an altitudinal gradient. J Med Entomol 41: 162–169
6. Burri C, Morán F, Douet V, Humair P-F, Moret J, Gern L (2005) *Ixodes ricinus* density and infection prevalence of *Borrelia burgdorferi* sensu lato along a north facing altitudinal gradient in the Swiss Alps (Rhone valley, Valais, Switzerland). 5th International Conference on Ticks and Tick-borne Pathogens, Neuchatel, 29.8.–2.9.2005, Abstracts: 19
7. Pietilä J, He QS, Oksi J, Viljanen MK 2000: Rapid differentiation of *Borrelia garinii* from *Borrelia afzelii* and *Borrelia burgdorferi* sensu stricto by light Cycler fluorescence melting curve analysis of a PCR product of the recA gene. J Clin Microbiol 38: 2756–2759
8. Postic D, Assous MV, Grimont P, Baranton G (1994) Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction-fragment-length-polymorphism of rrf (5S) rrl (23S) intergenic spacer amplicons. Int J Syst Bact 44: 743–752
9. Hubálek Z, Halouzka J (1998) Prevalence rates of *Borrelia burgdorferi* sensu lato in host-seeking *Ixodes ricinus* ticks in Europe. Parasitol Res 84: 167–172
10. Daniel M, Danielová V, Kríž B, Jirsa A, Nožicka J (2003) Shift of the tick *Ixodes ricinus* and tick-borne encephalitis to higher altitudes in Central Europe. Eur J Clin Microbiol Infect Dis 22: 327–328
11. Daniel M, Danielová V, Kríž B, Kott I 2004 An attempt to elucidate the increased incidence of tick-borne encephalitis and its spread to higher altitudes in the Czech Republic. Int J Med Microbiol 293 [Suppl 37]: 55–62

12. Zeman P, Beneš Č (2004) A tick-borne encephalitis ceiling in Central Europe has moved upwards during the last 30 years: possible impact of global warming? Int J Med Microbiol 293 [Suppl 37]: 48–54
13. Materna J, Daniel M, Danielová V (2005) Altitudinal distribution limit of the tick *Ixodes ricinus* shifted considerably towards higher altitudes in Central Europe: results of three years monitoring in the Krkonoše Mts. (Czech Republic). Centr Eur J Publ Hlth 13: 105–122

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