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VERTICAL DISTRIBUTION OF THE TICK *IXODES RICINUS* AND TICK-BORNE PATHOGENS IN THE NORTHERN MORAVIAN MOUNTAINS CORRELATED WITH CLIMATE WARMING (JESENÍKY MTS., CZECH REPUBLIC)

Milan Daniel[1], Jan Materna[2], Václav Hönig[3,4], Ladislav Metelka[5], Vlasta Danielová[1], Josef Harčarik[2], Stanislava Kliegrová[5], Libor Grubhoffer[3,4]

[1]National Institute of Public Health, Prague, Czech Republic
[2]Krkonoše National Park Authority, Krkonoše Museum, Vrchlabí, Czech Republic
[3]Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic
[4]Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
[5]Czech Hydrometeorological Institute, Hradec Králové, Czech Republic

SUMMARY

A study of the vertical distribution of the common tick *Ixodes ricinus* and tick-borne pathogens – tick-borne encephalitis virus (TBEV) and genospecies of *Borrelia burgdorferi* s.l. – was performed in the highest part of the Jeseníky mountain area (the Hrubý Jeseník Mts. with the highest summit Praděd, 1,491 m above see level). Altogether 1,253 specimens of all tick stages (607 larvae, 614 nymphs, 8 females and 24 males) were collected at the altitude 990–1,300 m above sea level on 12 collection sites by the flagging method. Altogether 1,207 ticks (8 females, 24 males, 568 nymphs and 607 larvae) were examined for the presence of tick-borne encephalitis virus and *B. burgdorferi* s.l. None of the samples contained TBEV, 35 samples (6% of adult ticks, 5% of nymphs, 0.7% of larvae) were positive for *B. burgdorferi* s.l. The most prevalent genospecies were *B. afzelii* (44%), *B. garinii* (28%), less frequent were *B. burgdorferi* sensu stricto (5%), *B. valaisiana* (3%). The rather large number of ticks (in absolute numbers as well as recounted to the index: average number of nymphs/worker/collection hour) and the presence of all developmental stages clearly demonstrate that there are viable local tick populations in all the sites, and that recorded ticks were not randomly individuals brought into higher altitudes by birds or game animals. The results are compared with the long-term (2002–2007) monitoring of the tick altitudinal distribution in the Krkonoše Mts. and the conditions, which allow ticks to establish local populations up to the timberline in both mountain areas, are discussed. Simultaneously, changes in climatic conditions (especially the air temperature) monitored at 3 meteorological stations in the area of the Jeseníky Mts. were compared with the records from another 8 stations in other mountain areas in the Czech Republic. A very similar statistically significant trend of increasing mean air temperatures during the last three decades is found at all analyzed stations. The trend is most pronounced in the spring and summer months with the highest activity of *I. ricinus* ticks.

Key words: Ixodes ricinus, *Borrelia burgdorferi* genospecies, tick-borne encephalitis virus, vertical distribution, climate warming

Address for correspondence: M. Daniel, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic. E-mail: midaniel@seznam.cz

INTRODUCTION

The problem of the *Ixodes ricinus* ticks infiltration into higher altitudes in Central Europe became important at the beginning of the 1990s, together with the increasing occurrence of tick-borne infections in new areas. In the Czech Republic, the first such recorded manifestation was the infection by tick-borne encephalitis virus (TBEV) following verified infestation by the tick *I. ricinus* at an altitude of 900 m above sea level (a.s.l.) in the Šumava Mts. in 1996 and 2001 (1). Consequently, the investigation of the changes in *I. ricinus* vertical distribution was first focused on the area of the Šumava Mts. where *I. ricinus* was recorded at an altitude of 1,020 m in 2002. The comparison with the historical unpublished field and experimental data concerning the vertical distribution of *I. ricinus* in these mountains in 1959 and in 1960s (1) confirmed that its vertical distribution limit had considerably changed since that time.

The subsequent investigation was aimed at the highest mountain area in the Czech Republic, the Krkonoše Mts., where detailed historical data (based on the field experiment in 1981–1983) on *I. ricinus* vertical distribution up to 700–750 m were available (2, 3). Recently (2002–2007), ticks were regularly recorded up to 1,100 m and rarely up to 1,270 m a.s.l. (thus up to the timber line) during regular monitoring of ticks along two vertical transects in the central and eastern parts of the Krkonoše Mts. (4, 5). Simultaneously, the TBEV was detected in collected ticks up to 1,140 m (6), borreliae (*Borrelia valaisiana*) up to 1,270 m (7 and unpublished data). The long term (2004–2007) field experiment on the development of individual *I. ricinus* stages along an altitudinal transect in the central part of the Krkonoše Mts. (650–1,550 m) allowed to correlate the shift in its vertical distribution with general temperature increase in spring and summer periods in the Krkonoše Mts. during the last 5 decades and also with present local microclimatic conditions (5).

The above mentioned results led to the aim to verify the degree of general validity of obtained conclusions in other mountain areas in the Czech Republic, foremost in the Jeseníky Mts. as the second highest mountain range in the Czech Republic after the Krkonoše Mts. Our aim was moreover also supported by the fact that the Northern Moravian Region (particularly the districts Šumperk and Bruntál) has long exhibited a high occurrence of TBE human cases, frequently in localities close to mountain areas. For example, in the 1970–2000 period, TBE cases were recorded sporadically at altitudes above 600 m (localities Lázně Jeseník and Branná) and even at 780 m (Karlova Studánka) (8).

No systematic attention has been yet devoted to ticks in the northern Moravian mountain areas. The former Northern Moravian Region (districts Olomouc, Bruntál, Šumperk and Opava) was represented only by localities at an altitude of 263–390 m in an extensive study of the phenology of *I. ricinus* performed simultaneously in seven regions in Bohemia and Moravia by Černý et al. in 1960–1962 (9). Kašpar (10) studied the occurrence and seasonal dynamics of *I. ricinus* at 7 northern Moravian localities at an altitude of 260–556 m in the districts of Olomouc and Opava. He concluded that "ticks were less numerous in the colder locations close to 600 m and the seasonal dynamic curves differed from the course found at lower altitudes". Chmela (11) carried out the field experiment concerning the ecology of *I. ricinus* in the northern Moravian natural focus of TBE in the foothills of the Nízký Jeseník Mts. at an altitude of 325 m. In the same area, Ašmera et al. (12) collected *I. ricinus* ticks up to 600 m. Rosický (13) mentioned the Jeseníky Mts. (without specification of the locality) as an example of the occurrence of *I. ricinus* in mountain areas. He found ticks within the study of ectoparasites of small terrestrial mammals and we suppose that tick individuals found were probably confused with *Ixodes trianguliceps*, which is a specific nest species of small terrestrial mammals, common in submountain and mountain areas. Reliable distinction of immature stages of these two ticks species was published only in 1958 (14) when, however, the material of ticks collected by Rosický from the Jeseníky Mts. was no more available for verification.

We are attempting to fill in the gap in knowledge of the distribution of *I. ricinus* in the highest mountains in Northern Moravia and compare the results obtained with experience gained in the Krkonoše area, especially in relation to climatic conditions and their potential impact on circulation of tick-borne pathogens under natural conditions in these mountains.

MATERIALS AND METHODS

Study Area

The investigation was performed in the territory of the Hrubý Jeseník Mts. (with the highest summit Praděd, 1491 m), which is the central and highest part of the Jeseníky Protected Landscape Area. This is a very cold area (average annual temperature on Praděd Mt. is 0.9 °C). Approximately 1,200–1,300 mm of precipitation falls on the ridges annually and 800–1,000 mm in the foothills; this is reduced to 700 mm on the eastern side. The timber line lies at 1,300–1,350 m, above which mountain meadows are situated. A natural dwarf pine zone is not developed and this species has been only artificially planted in some places.

Altogether 12 sites were selected for the tick collection at the altitude 990–1,300 m, both in planted and autochthonous spruce (*Picea abies*) forest stands. Special attention was paid to the forest edges (ecotones) and to open canopy parts of young forests with dense grass and herb undergrowth, where the probability of occurrence of ticks would be highest. Also good accessibility of the examined sites and the frequency of tourist visits was taken into consideration.

Collection of Host-seeking Ticks

Ticks were collected by the flagging method in the period 16–20 June 2008, i.e. at the time of the highest expected *I. ricinus* host-seeking activity. The ground-level temperature during collection at the individual sites varied from 10.6 to 17.9 °C, and the relative air humidity from 64 to 99%. Flagging was performed simultaneously by four workers at one place for at least one working hour. For comparison of tick density on the individual sites, the numbers of collected ticks were recounted as the average number of ticks (nymphs) per worker / hour (*Ir*N/worker/h). Following species determination, the collected ticks were stored at -80 °C for further laboratory examination.

Detection of Tick-borne Encephalitis Virus and *Borrelia burgdorferi* s.l.

Tick samples were prepared by the Chelex method according to Rudenko et al. (15). For the detection of TBEV pools of 10 samples were prepared, for the detection of *B. burgdorferi* s.l. only larvae were pooled nymphs and adults were tested individually.

The final concentrations in the RT-PCR reaction were: 1x M-MLV reaction buffer (50 mM Tris-HCl (pH 8.3), 75 mM $MgCl_2$), 10 mM DTT, 0.5 mM dGTP, 0.5 mM dATP, 0.5 mM dTTP, 0.5 mM dCTP (Top-Bio, Czech Republic); 1 μM primer E (R) (CCGTTGGAAGGTGTTCCACT) (Generi-Biotech, Czech Republic); 50 U M-MLV reverse transcriptase (Top-Bio, Czech Republic); 40 U RNase inhibitor (RiboLock, Fermentas) + 8 μl of the sample. The cDNA synthesis was conducted 1 hour at 37 °C. Positive and negative controls were included.

PCR mixture contained: 1x PPP Master Mix (75 mM Tris-HCl, pH 8,8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 2.5 U Taq purple DNA polymerase), 0.4 μM of each primer + 6.5 μl H_2O + 4 μl of cDNA. Amplification was conducted as follows: 95 °C 5 min. followed by 30 cycles of 94 °C 30 s, 55 °C (50 °C for EF/ER primer pair, 52 °C for P8/P9), 1min. 72 °C followed by final extension at 72 °C 5 min. Positive and negative controls were included.

For the detection of TBEV primer pair EF (GGGGACYACGAGGGTYACCT), ER (CCGTTGGAAGG-TGTTCCACT) was used. *Borrelia burgdorferi* sensu lato was detected by the primer pair SL1, SL2 (16). Positive samples were subjected to genospecies-specific PCR according to Demaerschalck et al. (16). For the detection of *B. valaisiana* primers P8, P9 were used (17).

Table 1. Vertical distribution of Ixodes ricinus tick in the Hrubý Jeseník Mts

Locality	Habitat	Coordinates	Altitude m a.s.l.	Date of collection	Number of collected ticks*				*Ir*N/ worker/h**
					L	N	F	M	
Červenohorské sedlo I	forest edges along ski pistes	50°07'52"N 17°09'32"E	990	18.6.2008	42	75	0	0	25.0
Červenohorské sedlo II	forest edge along footpath	50°07'52"N 17°09'32"E	1,040-1,050	18.6.2008	122	40	0	2	13.3
Červenohorské sedlo III	young spruce forest	50°07'52"N 17°09'32"E	1,020-1,040	18.6.2008	68	53	1	1	10.4
Vidly – Karlova Studánka	edge of young spruce forest	50°05'00"N 17°17'42"E	1,004	17.6.2008	0	78	4	6	13.0
Volská louka	edge of young spruce forest	50°09'35"N 17°04'45"E	1,000-1,010	18.6.2008	26	86	1	3	21.5
Videlské sedlo	edge of young spruce forest	50°07'27"N 17°14'58"E	1,007	19.6.2008	76	98	0	3	19.6
Orlík Mt. (Kristovo loučení)	forest edge along footpath	50°10'34"N 17°17'24"E	1,054	19.6.2008	1	12	0	0	3.0
Černý vrch Mt. southern slope	young open spruce forest	50°08'27"N 17°16'36"E	1,140-1,150	19.6.2008	159	97	0	3	24.3
Kamzičí vrch Mt. – southern slope I	old autochthonous mixed forest	50°05'53"N 17°14'54"E	1,070-1,100	19.6.2008	0	17	0	3	8.5
Kamzičí vrch Mt. – southern slope II	young open spruce forest	50°05'53"N 17°14'54"E	1,070-1,100	19.6.2008	30	55	2	3	27.5
Vysoká hole Mt. – eastern slope	spruce groups on alpine medows	50°03'43"N 17°14'49"E	1,300	20.6.2008	83	3	0	0	1.5
Vysoká hole Mt. – north- eastern slope	solitary old spruces at timber line	50°03'43"N 17°14'49"E	1,350-1,370	20.6.2008	0	0	0	0	0.0
Total					607	614	8	24	

* L – larvae; N – nymphs; F – females; M – males;
** Average number of nymphs per 1 worker per 1 hour

Analysis of Climate Development in the Jeseníky Mts. (1979–2007) and its Comparison with other Czech and Moravian Mountains

Long-term meteorological data (1979–2007) from 2 meteorological stations of the Czech Hydrometeorological Institute (CHMI) from the Jeseníky Mts. (Karlova Studánka [50°04'23"N, 17°18'21"E, 780 m], Praděd [50°04'58"N, 17°13'48"E, 1490 m – measurements were terminated in 1997]) and one from their foothills (Staré Město pod Sněžníkem [50°11'34"N, 16°56'31"E, 658 m]) were analysed to gain the information about climate development (air temperature, precipitation) in the studied area.

To compare and generalize the results regarding climate development and *I. ricinus* vertical distribution in the Jeseníky Mts. and the Krkonoše Mts. (5) data (1979–2007) from another 8 meteorological stations from 7 Czech and Moravian mountain areas were included in the analysis: the Beskydy Mts. (Lysá hora [49°32'46"N, 18°26'52"E, 1322 m]), the Orlické hory Mts. (Rokytnice v Orlických horách [50°09'52"N, 16°27'37"E, 572 m]), the Krkonoše Mts. (Labská bouda [50°45'39"N, 15°33'02"E, 1300 m], Harrachov [50°46'20"N, 15°26'10"E, 670 m]), the České středohoří Highland (Milešovka [50°33'17"N, 13°55'53"E, 837 m]), the Šumava Mts. (Churáňov [49°04'06"N, 13°36'54"E, 1118 m], the Český Les Mts. (Přimda [49°40'10"N, 12°40'41"E, 742 m]) and the Českomoravská vysočina Highland (Svratouch [49°44'06"N, 16°02'01"E, 737 m]). Five professional stations of the CHMI (Churáňov, Svratouch, Přimda, Lysá hora, Praděd), 1 professional station of the Institute of Atmospheric Physics (Milešovka) and 5 meteorological stations of the CHMI (Labská bouda, Rokytnice v Orlických horách, Harrachov, Karlova Studánka a Staré Město pod Sněžníkem) were included. The missing data at each station were before processing filled in on a monthly principle. Monthly, seasonal and annual temperatures and amount of precipitations were then calculated for filled series of observations. Verified data were evaluated by STATISTICA 7 software (www.statistica.com).

RESULTS

Vertical Distribution of *Ixodes ricinus*

In the altitude 990–1,300 m, altogether 1,253 specimens of all *I. ricinus* stages (607 larvae, 614 nymphs, 8 females and 24 males) were collected (Table 1). The relatively high number of ticks (in absolute numbers as well as recounted to the comparative index *Ir*N/worker/h) and the presence of all developmental stages clearly demonstrated that there are local tick populations at all investigated sites, and that recorded ticks were not individuals randomly brought into higher altitudes by birds or game animals.

A particular attention was paid to the area of the saddle Červenohorské sedlo, which is well-known sport and tourist resort because of the easy access by car and public transport and as a starting point for many marked tourist paths. Typical habitats were investigated at altitudes 990–1,050 m at this locality: edges of the spruce forest aisles created for ski pistes, edges of marked footpath; young open canopy spruce plantations and young mixed (*Salix caprea, Sorbus aucuparius, Fagus sylvatica*) forest stands (all with dense herb undergrowth). The edges of the ski pistes with substantially altered soil surface (piled-up mounds of soil) covered by very dense herb vegetation showed the twice more abundant tick populations (25 *Ir*N/worker/h) than the other habitats (10.4–13.3 *Ir*N/worker/h).

The comparison of tick densities between old autochthonous mixed forest and adjacent young planted spruce forest stands on the southern slope of the Kamzičí vrch Mt. (1,070–1,100 m) brought very interesting results. Whereas tick density in an open canopy old beech and spruce mixed forest stand with well-developed herbaceous layer reached 8.5 *Ir*N/worker/h, it was 27.5 *Ir*N/worker/h in planted open canopy young spruce stand, also with well developed herbaceous layer. This example is in agreement with the general results of our observation, that the tick *I. ricinus* was most abundant in open canopy young spruce forests with rich herbaceous undergrowth, especially along their edges (ecotones) (Table 1).

The highest finding of *I. ricinus* at an altitude of 1,300 m in a mosaic of groups of spruce trees and alpine meadows close to the timber line was recorded on the slope of the Vysoká hole Mt. (Table 1). It should be emphasized that, in addition to nymphs, a considerable number of larvae were collected at this habitat. It indicates that the whole tick developmental cycle have to proceed there.

Tick-borne Encephalitis Virus and *Borrelia burgdorferi* s.l. Investigation

A total of 1,207 ticks (8 females, 24 males, 568 nymphs and 607 larvae) were examined for the presence of tick-borne encephalitis virus and *Borrelia burgdorferi* s.l. All the samples were negative for TBEV, 35 samples (including four pools of larvae) were positive for *B. burgdorferi* s.l. The prevalence rate reached 6.3% in adults (2 positive/32 examined), 5.1% for nymphs (29/541) and the minimum infection rate in larvae reached 0.7% (4/607). The results are summarized in Table 2.

The genospecies of *B. burgdorferi* s.l. were identified by means of species-specific PCR. *B. afzelii* and *B. garinii* were the most frequently detected species (43.6 % and 28.2% respectively), *B. burgdorferi* s.s. (5.1%) and *B. valaisiana* (2.5%) were less frequent. A relatively high portion (20.5%) of positive samples was not identified as one of the above mentioned species. Co-infection with *B. afzelii* and *B. garinii* occurred in 4 samples.

Ticks positive for borreliae were found in samples from all the localities through the whole range of the altitudes (990–1,300 m). All samples positive for *B. garinii* except of one were collected at altitudes above 1,040 m whereas 12 of 17 samples positive for *B. afzelii* originated from altitudes not higher than 1,010 m.

Climate Development in the Jeseníky Mts. (1979–2007) and its Comparison with Other Czech and Moravian Mountains

A statistically significant rise in the mean annual air temperature by 0.5°C/10 years was recorded at all analysed stations from

Table 2. *Borrelia burgdorferi genospecies in Ixodes ricinus ticks in Hrubý Jeseník Mts*

Locality	Altitude m a.s.l.	Adults positive/exam.	*B. burgdorferi* genospecies	Nymphs positive/ exam. (prevalence rate)	*B. burgdorferi* genospecies	Larvae positive/ exam. (prevalence rate)	*B. burgdorferi* genospecies
Červenohorské sedlo	990	0/0		2/66 (3.0%)	1 *B. afzelii* 1 undetermined	0/42	
	1,040–1,050	0/2		2/42 (4.8%)	1 *B. garinii* 1 undetermined	1/122 (0.8%)	*B. burgdorferi* s.s.
	1,020–1,040	0/2		1/49 (2.0%)	*B. afzelii*	1/68 (1.5%)	*B. valasiana*
Vidly - Karlova Studánka	1,004	0/10		2/71 (2.8%)	1 *B. afzelii* 1 undetermined	0/0	
Volská louka	1,000–1,010	0/4		7/80 (8.8%)	3 *B. afzelii* 4 undetermined	0/26	
Videlské sedlo	1,007	1/3	*B. afzelii*	6/90 (6.7%)	5 *B. afzelii* 1 *B. afzelii + garinii*	0/76	
Sedlo Orlíku	1,054	0/0		0/12 (0.0%)		0/1	
Černý vrch	1,140–1,150	0/3		4/63 (6.3%)	1 *B. afzelii* 1 *B. garinii* 1 *B. afzelii + garinii* 1 undetermined	1/159 (0.6%)	*B. garinii*
Kamzičí vrch	1,070–1,100	1/8	*B. garinii*	4/65 (7.7%)	2 *B. garinii* 2 *B afzelii + garinii*	0/30	
Vysoká hole	1,300	0/0		1/3 (33.3%)	*B. burgdorferi* s.s.	1/83 (1.2%)	*B. garinii*
Total	990–1,300	2/32 (6.3%)		29/541 (5.4%)		4/607 (0.7%)	

Table 3. *Linear trends (ºC/10 years) of annual and seasonal mean air temperature courses for the period 1979–2007 at 11 selected meteorological stations from different mountain areas of the Czech republic and its statistical significance*

Meteorological station	Altitude (m a.s.l.)	Year		Spring (III.–V.)		Summer (VI.–VIII.)		Autumn (IX.–XI.)		Winter (XII.–II.)	
Rokytnice v Orlických horách	564	+ 0.34	*	+ 0.28		+ 0.56		+ 0.30		+ 0.14	
Staré Město pod Smrkem	658	+ 0.49	**	+ 0.42		+ 0.88	**	+ 0.42		+ 0.14	
Harrachov	670	+ 0.45	**	+ 0.36		+ 0.68	**	+ 0.38		+ 0.29	
Svratouch	737	+ 0.49	**	+ 0.51	*	+ 0.76	**	+ 0.37		+ 0.22	
Přimda	742	+ 0.57	**	+ 0.62	**	+ 0.77	**	+ 0.39		+ 0.44	
Karlova Studánka	775	+ 0.38	*	+ 0.37		+ 0.54	**	+ 0.24		+ 0.32	
Milešovka	833	+ 0.52	**	+ 0.58	*	+ 0.75	**	+ 0.33		+ 0.34	
Churáňov	1,118	+ 0.55	**	+ 0.59	*	+ 0.74	**	+ 0.36		+ 0.41	
Labská Bouda	1,315	+ 0.56	*	+ 0.51	*	+ 0.73	*	+ 0.44		+ 0.60	
Lysá hora	1,322	+ 0.51	**	+ 0.47		+ 0.83	**	+ 0.37		+ 0.28	
Praděd	1,490	+ 0.48	**	+ 0.39		+ 0.68	**	+ 0.39		+ 0.36	

** – significant at the 1% level, * – significant at the 5% level

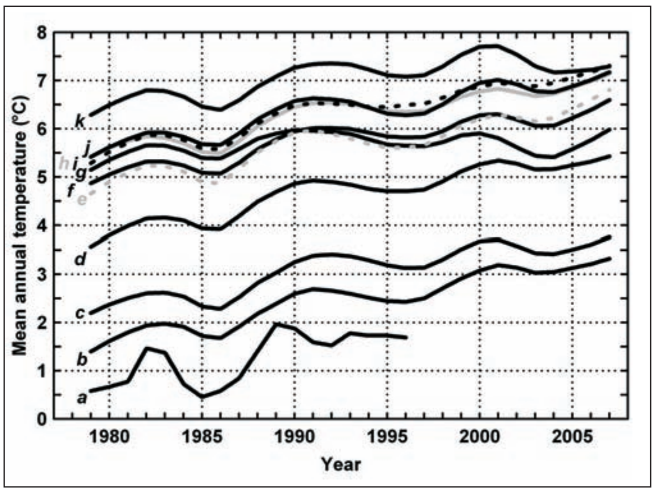

Fig. 1. *The mean annual air temperatures 1979–2007 at 11 selected meteorological stations from different mountain areas of the Czech Republic: a – Praděd, b – Labská bouda, c – Lysá hora, d – Churáňov, e (gray dashed curve) – Milešovka, f – Harrachov, g – Karlova Studánka, h (gray curve) – Svratouch, i – (black dashed curve) – Přimda, j – Staré Město pod Sněžníkem, k – Rokytnice v Orlických horách. The values were smoothed by robust locally weighted regression. For meteorological station details see Materials and methods and Table 2.*

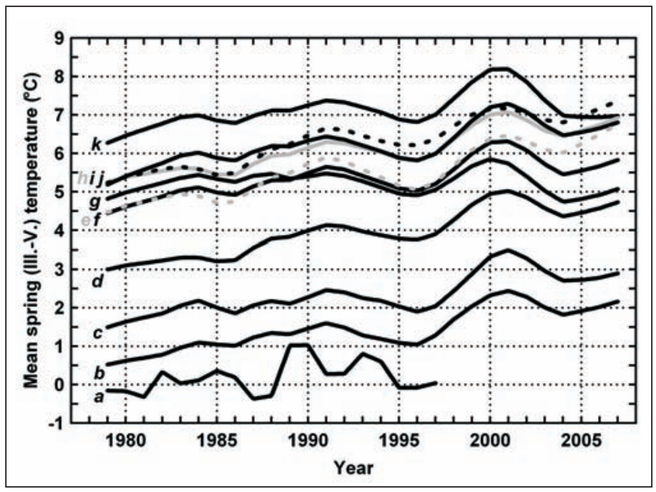

Fig. 2. *The mean spring (March–May) air temperatures 1979–2007 at 11 selected meteorological stations from different mountain areas of the Czech Republic. The values were smoothed by robust locally weighted regression. For the legend see Fig. 1.*

1979 to 2007 (Table 3). The trend of increasing annual temperature is parallel for all the stations (Fig. 1). The warming differs among individual seasons. The most obvious and statistically significant trends in increasing air temperature were observed at half of evaluated stations in spring (0.5–0.6 °C/10 years) and at all the stations except one in summer (0.5–0.9 °C/10 years). The trends are not significant in autumn and winter. The course of seasonal temperatures is similar at all studied stations (Fig. 2, 3).

Fluctuations of the average amount of precipitation are without any long-term trend, but data sets reveal similar course at all stations during 1979–2007. Linear trends of precipitation amounts were not calculated because of considerable year-to-year variability and weak trend component. Large inter-annual variability without long-term trend is dominant also for seasonal precipitation amounts.

DISCUSSION

The assumption that the change in the vertical distribution of *I. ricinus* ticks observed in the Krkonoše Mts. has more general validity was confirmed. The present *I. ricinus* altitudinal distribution limit up to the timber line (approximately 1,250 m) in the

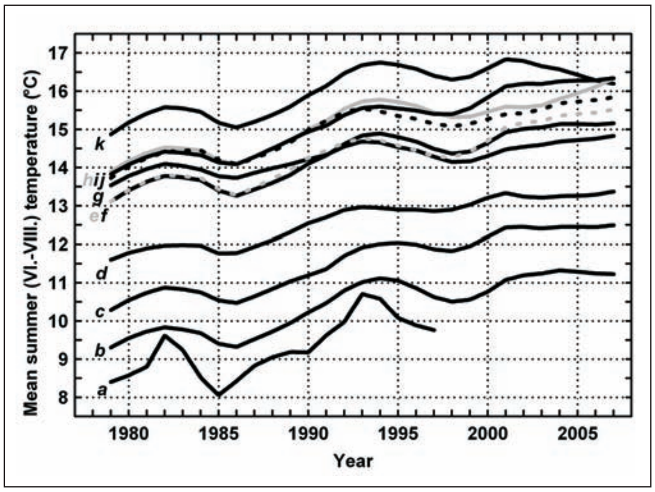

Fig. 3. *The mean summer (June–August) air temperatures 1979–2007 at 11 selected meteorological stations from different mountain areas of the Czech Republic. The values were smoothed by robust locally weighted regression. For the legend see Fig. 1.*

Krkonoše Mts. (5) was verified in the present study in the Hrubý Jeseník Mts. Comparison of the climatic conditions development in 1997–2007 (Fig. 1–3) and linear temperature trends (Table 3) recorded in the Krkonoše Mts. and those observed in other Czech mountain areas, including the Jeseníky Mts., can be used for evaluating the factors which have enabled shift in *I. ricinus* vertical distribution. The trends in climate development and obvious warming in spring and mainly summer seasons in past twenty years are very similar in all the mountain areas. It indicates the prevailing influence of large-scale processes on climate warming in the individual mountain areas of the Czech Republic.

If the observed linear trends in the mountain areas are compared with the published values on global warming in the Czech Republic as a whole, an average temperature increase over the past 100 years is 0.74 ^{o}C (18) (while the increase recorded over the past 240 years at the Prague Klementinum station is only 0.8 °C), it is obvious that the temperature increase is clearly higher at mountain areas. The observed warming in spring and summer seasons, in the period of high tick activity, forms suitable conditions for *I. ricinus* shift into higher altitudes in all the mountain areas in the Czech Republic.

The high occurrence of *I. ricinus* found in the Hrubý Jeseník Mts. at the altitude of 1,000 m upwards is noteworthy, not only in the total number of collected specimens, but especially in the density of local populations expressed as the index *Ir*N/worker/h (Table 1). These data are compared with those recorded during long-term monitoring (2002–2007) at similar altitudes in the Krkonoše Mts. in May to July period. The values from the Krkonoše Mts. (maximum value of 5 nymphs/worker/hour in the central part and 16.3 nymphs/worker/hour in the eastern part) did not attain the values in the Hrubý Jeseník Mts. Two control collections in the Krkonoše Mts. in June 2008, i.e. at the same time of the research in the Hrubý Jeseník Mts., are much closer (21.3 *Ir*N/worker/h at 980–1,040 m and 14.3 *Ir*N/worker/h at 1,040–1,050 m) to the values from the Hrubý Jeseník Mts.

Suitable climatic factors are an important, but not the only one factor conditioned the existence of tick local populations. However the entire biocenosis, of which ticks are an integral part, changes in dependence on modifications in the climate; this includes also the tick animal hosts and the vegetation cover, which have another crucial influence on the existence of tick populations. The differences in these conditions are evident when the Krkonoše results are compared on vertical transects in the central part and in the eastern part of the mountains (4, 5). Without detailed botanical analysis, it is thus possible to accept the hypothesis that the differences in the vegetation conditions in the Hrubý Jeseník Mts. and the Krkonoše Mts. could form the basis for the higher occurrence of *I. ricinus.* We suppose that higher situated timber line (for approximately 100 m compared with the Krkonoše Mts.) and missing dwarf pine zone (montane spruce forest zone is directly followed by alpine meadows) in the Hrubý Jeseník Mts. may substantially influence abundance of *I. ricinus* hosts species (especially small mammals as host for tick larvae and nymphs), which may led to higher tick density observed in the Hrubý Jeseník Mts. compared with those from the Krkonoše Mts. As far as the principal hosts of adult ticks *I. ricinus* are concerned, the recent population of hoofed game in the Landscape Protected Area Jeseníky (740 km^2) has been regulated significantly in accordance with the local ecosystem since 1993. An extremely high number of red deer was lowered from 1,514 to 1,044 animals between 1994 and 2000. Simultaneously, roe deer were reduced from 2,182 to 1,811 animals. A five degree scale (19) indicates the contemporary size of red deer to be high (5), that of roe deer being well-balanced (4), and that of wild boars to be low (3).

Concerning the tick-borne pathogens in ticks, no TBEV-positive tick was detected. Data from other mountain areas of Czech Republic show that TBEV can be present in ticks as high as 1,140 m (6). Nevertheless the prevalence of TBEV in ticks may be very low and therefore we can not fully exclude the possibility of its presence in the studied area. Ticks positive for borreliae were found in samples from all the collection sites except of one, where the total number of ticks was very low (n=13). The prevalence of *B. burgdorferi* s.l. was found to be lower than the European average – 10.8% in nymphs and 17.4% in adults (20). B. burgdorferi positive ticks were found as high as 1,300 m. Previously borrelia-positive tick was reported at an altitude of 1,270 m in the Czech Republic (7). Borreliae were detected in the pools of larvae indicating transovarial transmission. Borrelia-positive larvae were previously reported also by other authors (e.g. 21).

The dominance of *B. afzelii* and *B. garinii* among the positive samples is concordant with the data collected throughout Europe (20). The portion of *B. burgdorferi* s. s. is slightly lower but according to the review (20) the prevalence of this genospecies is declining towards the east in Europe. High number of unidentified positive samples can be explained by the presence of other genospecies than detected. Our study focused on the main pathogenic genospecies. Interestingly, the distribution of *B. garinii* and *B. afzelii* was not equal throughout the altitudinal gradient. *B. afzelii* prevailed in samples collected at lower altitudes (900–1,010 m), whereas *B. garinii* clearly dominated at higher altitudes (1,040–1,300 m). As *B. garinii* is known to be associated mainly with birds as hosts (22) this finding could indicate an important role of birds as hosts of ticks and source of borreliae at higher altitudes. This conclusion as well as the findings concerning the *B. burgdorferi* genospecies distribution is similar to that found in the Krkonoše Mts. (6, 23).

In conclusion, it should be emphasized that the most numerous populations of *I. ricinus* ticks were found in young spruce planted stands predominantly at their edges with dense herbaceous undergrowth along foot and biking forest paths, picnic and resting places, frequently visited by visitors of the Jeseníky Landscape Protected area. It emphasizes the necessity of further public education of holidaymakers and nature visitors, leading to effective prevention of tick-borne diseases.

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