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SHORT COMMUNICATION

Tick-borne encephalitis virus expansion to higher altitudes correlated with climate warming

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Abstract

Since 2002, the expansion of *Ixodes ricinus* ticks and tick-borne infection agents have been studied in the Krkonoše Mts., Czech Republic. Tick-borne encephalitis virus was detected by means of RT-PCR. In 2003, it was detected in 2 out of 491 ticks at 620 and 710–720 m a.s.l., respectively, and in 3 out of 939 ticks at 600 m a.s.l. at the same locality in 2004. In 2005, tick-borne encephalitis virus was detected in 5 out of 295 ticks at 900–1100 m a.s.l., which is above the formerly known altitudinal limit of *I. ricinus* distribution. The reason for that could be found in the changing climate. Based on the meteorological data collected in the Krkonoše Mts., 1961–2005, there was a significant increase in the mean annual temperature (1.3–1.4 °C) over that period, namely by 2–3.5 °C in May through August. Thus, with respect to the average vertical temperature gradient in summer of about 0.6 °C/100 m, 2 °C correspond to 300–350 m in altitude, and accordingly 3.5 °C correspond to a shift in altitude of approximately 550–600 m, that being in accordance with environmental conditions of the former *I. ricinus* altitudinal limit confirmed in the Krkonoše Mts. 20 years ago.
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Keywords: Tick-borne encephalitis virus; *Ixodes ricinus*; Altitude; Mountains; Climate

Introduction

The occurrence of tick-borne encephalitis virus (TBEV) in Central Europe is strictly correlated with the occurrence of *Ixodes ricinus* ticks. This species is distributed throughout Europe, but its altitudinal limit of distribution differs with geographical latitude, being near the sea level in Scandinavia and reaching up to 3000 m a.s.l. in North Africa (Filippova, 1977). In the Czech Republic, the altitudinal limit was studied as early as the 1950s, and it was found to be at 700 m (Černý et al., 1965). It was confirmed again in the early 1980s in a field experiment in the Krkonoše Mountains (Daniel, 1993). Therefore, it is now possible to document the spread of *I. ricinus* to higher altitudes. This is very important from an epidemiological point of view, as Czech mountain areas over 750 m a.s.l. were considered to be tick-free and consequently free of TBEV. In the Czech register of mandatorily reported infections of the National Institute of Public Health in Prague (database EPIDAT), only two cases of human TBE infection from the mountains can be found, cases acquired in Šumava at the end of the 1990s (Daniel et al., 2003).

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Because the altitudinal expansion of *I. ricinus* in the Krkonoše Mts. has been proven to be beyond 1200 m a.s.l. (Materna et al., 2005), the question arises whether tick-borne pathogens occur in the ticks in those high altitudes. Since 2002, *I. ricinus* ticks were collected in the Krkonoše Mts. and investigated for the presence of TBEV and *Borrelia burgdorferi* sensu lato. Borreliae were already found in ticks at >1000 m a.s.l. since 2002 (Danielová et al., 2006). Records of TBEV in altitudes extending the former known altitudinal limit of *I. ricinus* (Černý et al., 1965; Daniel, 1993) in the Krkonoše Mts. are presented here. Data from the first phase of our study in the Krkonoše Mts. are included for clear demonstration of the gradual infiltration of TBEV into montane biocenosis. Over the past 50 years at least, no notable changes in land-use or demography, which could have influenced the occurrence of ticks and tick-borne agents, were observed and therefore for an explanation of our findings an evaluation of the climatic situation and trends was done.

Materials and methods

Ticks

I. ricinus were collected by flagging on two vertical transects on forest-meadow ecotones at altitudinal distances of approximately 100 m and at several individual places (980–1060 m a.s.l.). Six localities at 620, 700–765, 800–850, 900–920, 1030–1100, and 1160–1270 m a.s.l. were selected on the transect in the central and highest part of the Krkonoše Mts. reaching 1602 m a.s.l., where higher altitudes have subalpine (1250–1400 m) and alpine (>1400 m) (Obenberger, 1952) characteristics with a very harsh climate (Materna et al., 2005). Five localities (600–620, 710–750, 780–820, 870–930, and 960–1020 m a.s.l.) were chosen on the transect in the eastern part of the Krkonoše Mts. reaching the top of the main ridge covered with forest (for details see Materna et al., 2005). The area of tick collection ranged from 50°37′25″N to 50°42′38″N and from 15°37′30″E to 15°51′18″E. In 2002–2005, ticks were collected in late spring (May to June, depending on the snow cover in high altitudes), summer, and early autumn. In 2002, only ticks collected in the summer or early autumn were tested.

Climatic data

Climatic data from the database of the Czech Hydrometeorological Institute in Hradec Králové were used. The data were obtained from 5 meteorological stations in the Krkonoše Mts. in different altitudes (650–1315 m) in the years 1961–2005. They were evaluated for possible climate changes by standard statistical methods used in climatology. For the evaluation of the average monthly and annual temperature the least-squares multiple linear regression was used (Von Storch and Zwiers, 1999). The following climatological data were evaluated: monthly, seasonal, annual mean temperatures and precipitation, relative air humidity, snow cover, and sunny days. Evaluation of temperature and precipitation trends in the period of potential tick activity with daily mean temperature >5 °C and evaluation of the number of days with daily mean temperatures exceeding 5, 10, and 15 °C in the period without snow cover were done.

Tick processing and RT-PCR

All the collected nymphs and adults but only a part of the collected larvae were virologically tested (Table 1). Ticks collected in 2002 were pooled in groups of 1–39 according to stage, locality, and season. Those collected at low altitudes (600–620 m a.s.l.) in 2005 were pooled as follows: 39 larvae, 20–24 nymphs, and up to 6 adults according to locality and season formed one pool, respectively. They were tested for TBEV by intracerebral and subcutaneous inoculation of suckling mice (one litter/one pool). Suckling mice in those isolation experiments were observed for clinical signs of TBE, Uukuniemi, and Eyach virus infection for 3 weeks. All other ticks were investigated individually for TBEV by RT-PCR according to Rudenko et al. (2004). The tick samples were prepared with Chelex® 100 resin (Sigma) involving no RNA or DNA purification. The final supernatant (up to 12.5 μl) was used as template for RT-PCR. RT-PCR was carried out with SuperScript™ II RNase H⁻ RT using the SuperScript™ One Step RT-PCR system (Invitrogen). The reaction volume consisted of 10 μl Reaction Mix (final concentration 1×), 0.5 μl RT/Taq Mix, 1 μl (final concentration 1 μM) of each specific primer 1 and 2 according to Ramelow et al. (1993), and the chelex-buffered sample up to a total volume of 20 μl. The primers were synthesized by Generi-Biotech (Czech Republic).

Results

In 2002–2005, we investigated 298, 664, 888, and 1212 *I. ricinus* ticks for TBEV, respectively. For conciseness, ticks collected in 15 sites on both transects and additional places at 11 altitudinal zones in central and eastern Krkonoše are grouped in 4 altitudinal zones in Table 1 where TBEV prevalence is shown. Ticks (1 larva, 294 nymphs, 3 males) collected at 600–1180 m a.s.l. in 2002 were all tested negative for TBEV, Uukuniemi, and Eyach viruses. In 2003, TBEV was

Table 1. Occurrence of tick-borne encephalitis virus (TBEV) in *Ixodes ricinus* ticks in four altitudinal zones in the Krkonoše Mts., Czech Republic, 2002–2005

Year	Altitude in m a.s.l.	Ticks examined					TBE virus-positive
		Life stages[a]				Total	
		La	Ny	FF	MM		
2002	600–620	1	*125*			*126*	
2003	600–620	15	299	2	1	317	1 N
2004	600–620	2	311	1		314	3 N
2005	600–620	*39*	*397*	*2*	*7*	*445*	
Total	600–620	57	1132	5	8	1202	4 N
2002	710–850		*121*		*3*	*124*	
2003	700–850	1	248		1	250	1N
2004	700–850	3	373	5	5	386	
2005	700–850	28	435	1	8	472	1 N
Total	700–850	35	1177	6	17	1232	2 N
2002	900–1050		*47*			*47*	
2003	870–1070	3	86			89	
2004	870–1020	3	166		3	172	
2005	870–1070	62	204		1	267	4 N
Total	870–1070	68	503		4	575	4 N
2002	1180		1			1	
2003	1080–1270		8			8	
2004	1080–1270	1	15			16	
2005	1080–1270		27		1	28	1 N
Total	1080–1270	1	51		1	53	1 N

For further details see Materials and methods. (Ticks examined in pools are italicized.)
[a]La: larvae, Ny: nymphs, FF: females, MM: males.

detected in 2 nymphs (out of 19 larvae, 641 nymphs, 2 females, 2 males processed), one from 620 m a.s.l. in the central part of Krkonoše (Končiny, September) and another one from 710–720 m a.s.l. in the eastern part of Krkonoše (Zlatý potok, July). In 2004, TBEV was detected in 3 nymphs (out of 9 larvae, 865 nymphs, 6 females, and 8 males tested) at 600 m a.s.l., all collected in the same locality in eastern Krkonoše, one in June and two in September. The infected ticks were collected at the borders of mixed and beech forests, respectively, with meadows in the neighbourhood.

TBEV was not detected above the level formerly known as the altitudinal limit of *I. ricinus* distribution in the Krkonoše Mts. before 2005 (700 m a.s.l. after Černý et al., 1965; Daniel, 1993). In 2005, the virus was found in 6 *I. ricinus* nymphs, two of them collected in eastern Krkonoše near the ridge top at 960–1000 m a.s.l. (localities Rýchorská Bouda and Sněžné Domky, June) and four others in central Krkonoše at 765 m (Vápenice, July), 900–920 m (Husí Boudy, July), 1040–1050 m (Tetřeví Boudy, June), and 1080–1100 m a.s.l. (Přední Renerovky, July) (Fig. 1 and Table 1). Ticks were collected at the border of mixed forest in the locality Vápenice. In all other TBEV-positive localities, ticks were collected at the border of spruce forest with meadows in the neighbourhood. For more details about the area under study see Materna et al. (2005). No coinfection of TBEV with borreliae was found.

Of meteorological factors evaluated, we focus on air temperature data on the one hand revealing the most significant changes and a clear rising trend, and on the other, being a factor highly influencing tick biology and arbovirus circulation. Precipitation does not reveal any clear trend, only low statistically non-significant changes. The annual average temperature increased from 3.25 °C in 1961 to 4.65 °C in 2005 at 1000 m a.s.l. This significant increase ($p \leqslant 0.05$ except June) in the annual mean temperature by 1.3–1.4 °C is most distinct in late spring and in the summer months, i.e. in the period of major tick activity (by 3.5 °C in May, by 2 °C in June and July, and by 2.5 °C in August). Thus, with respect to the average vertical temperature gradient in summer of about 0.6 °C/100 m, a systematic change of 2 °C would correspond to 300–350 m in altitude, and a temperature change of 3.5 °C would correspond to a shift in altitude of approximately 550–600 m. Thus, the average monthly temperatures at 1100 m a.s.l. in 2005 corresponded with those at 750–800 m a.s.l. (June, July)

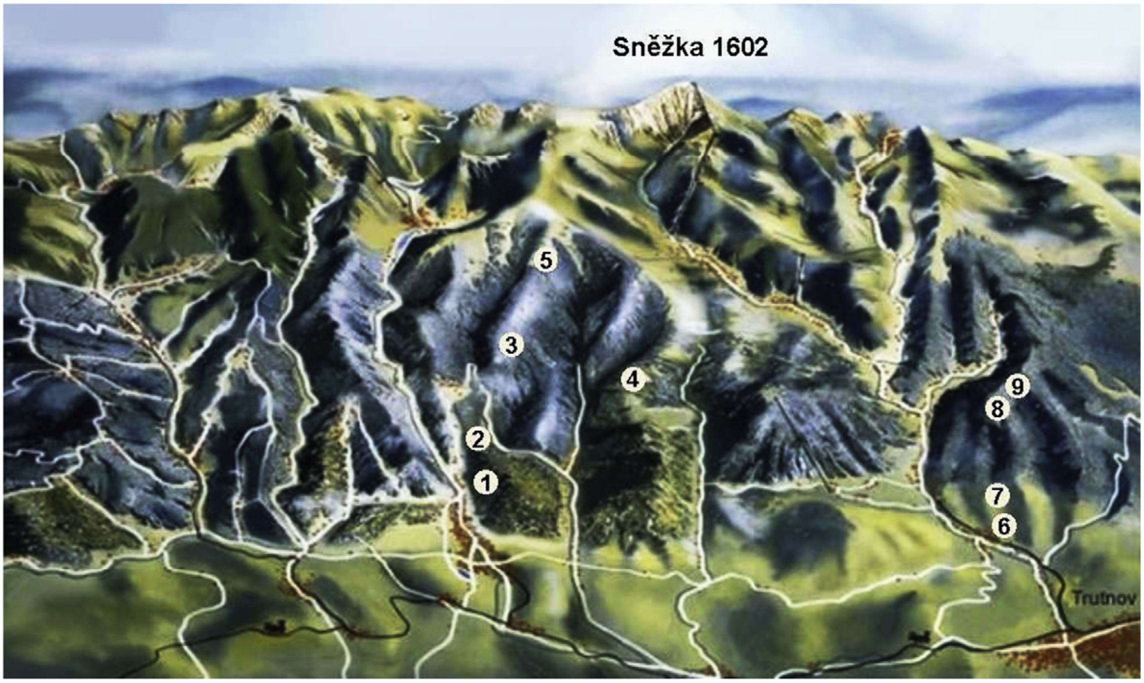

Fig. 1. Relief map of the Krkonoše Mts. with indicated places of tick-borne encephalitis virus detection in *Ixodes ricinus* ticks. View from the south. In the central part of Krkonoše: (1) Končiny (620 m a.s.l.): 1 TBEV-positive nymph in 2003; (2) Vápenice (700–765 m a.s.l.): 1 TBEV-positive nymph in 2005; (3) Husí Boudy (900–920 m a.s.l.): 1 TBEV-positive nymph in 2005; (4) Tetřeví Boudy (1040–1050 m a.s.l.): 1 TBEV-positive nymph in 2005; (5) Přední Renerovky (1080–1100 m a.s.l.): 1 TBEV-positive nymph in 2005. In the eastern part of Krkonoše: (6) Zlatý potok (600 m a.s.l.): 3 TBEV-positive nymphs in 2004; (7) Zlatý potok (710–720 m a.s.l.): 1 TBEV-positive nymph in 2003;(8) Rýchorská Bouda (960–1000 m a.s.l.): 1 TBEV-positive nymph in 2005; (9) Sněžné Domky (960–1000 m a.s.l.): 1 TBEV-positive nymph in 2005.

and with those at 550–500 m a.s.l. (May, August) in 1961, respectively.

Discussion

Two methods for TBEV detection were used. Most ticks were processed individually by PCR because the ticks were also investigated for *B. burgdorferi* s.l., known to occur in higher prevalences than TBEV in ticks. Tick infection rates with *B. burgdorferi* s.l. in different altitudes in the Krkonoše Mts. in 2002 and 2003 were already reported (Danielová et al., 2006). In 2002 and 2005, it was not possible to investigate all collected ticks individually by RT-PCR for a technical reason. As a consequence, some ticks collected in 2002 and those collected at 600–620 m a.s.l. in 2005 were investigated by an isolation test in suckling mice, another proven sensitive method. A comparison of sensitivity of both used methods was not done as yet. It is well known that TBEV infection rates in host-seeking ticks are generally very low. Because larvae and adults were collected in very limited numbers, the virus was detected in nymphs only.

The results presented are an example of how the TBEV and its vector become gradually integrated into the local ecosystems that were not colonized by that vector previously. Of the climatological data evaluated, the monthly and annual mean air temperatures reveal the most distinct changes and therefore, we put them into a causal correlation with expanding not only of ticks but also of tick-borne agents. Former data (Daniel, 1993) and a more detailed climatological analysis in the Krkonoše Mts.(Danielová et al., 2008) showed that the increase in temperature was not gradual, but that the steep rise in temperature, mainly in summer months, started in the first half of the 1990s. These data are a good predictor for the gradual expansion of *I. ricinus* over the former altitudinal limit of distribution beginning in the second half of the1990s. Before 2002, no virological and bacteriological investigation on ticks was done in that area. The detection of TBEV in higher altitudes does not mean that a focus is already formed; nevertheless, if systematic changes in meteorological conditions continue it will be formed.

The above findings accord with our hypothesis that *B. burgdorferi* s.l. occupies territories newly colonized by ticks, including higher altitudes, more quickly than the TBEV, which needs a longer time for natural focus formation. *B. burgdorferi* s.l. was found at 1000 m a.s.l. as early as in 2002 and 2003 (Danielová et al., 2006) and up to 1270 m a.s.l. in 2004 (our unpublished data) in the Krkonoše Mts. The reason might be the wider reservoir-host range of borreliae, e.g. birds, resulting in the rather frequent occurrence of *B. garinii* and that of *B. valaisiana* at the highest altitudes of *I. ricinus* occurrence on the one hand, and the lifelong bacteriaemia in reservoir hosts on the other.

Currently, the above-mentioned situation can be applied to other areas where in the year 2006 a steep rise in morbidity appeared in districts located at higher altitudes in which the disease was not observed previously or only sporadically, whereas, in 2006 it became an epidemiological problem (Daniel et al., 2008). In the district of Trutnov where the Krkonoše area under study is situated, four human cases of TBE were registered in the foothills in 2006, while over the preceding 10 years only one case (2003) had been noted. In districts in the highlands at 400–650 m a.s.l., the increase of human TBE cases was even more significant in the last decade (Danielová et al., 2008).

The presence of TBEV in ticks in the Krkonoše Mts. poses the risk of human infection, now slight. However, if the climatic situation is maintained or the trend continues, tick abundance will increase together with the number of infected ticks and thus the danger of TBE infection would increase.

The tick population in higher altitudes has been rather low as yet. Nevertheless, attacks on humans were repeatedly observed in the residents and members of the staff of the National Park. Unfortunately, no data about tick bites in visitors are available. Moreover, the Krkonoše National Park belongs to the most frequently visited recreational area (2.3 million visitors in June through September according to estimates of the National Park authorities).

Conclusions

The results show that the Krkonoše Mts., free of TBE in the past, appears more dangerous now. These findings can be extremely important for the public health service. Recent climatic changes, namely in air temperature, may have caused an increase in the number of infected ticks up to the level of epidemiological importance in the montane zone (up to 1100 m a.s.l.).

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