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)

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SUMMARY

The aim of the study was to monitor present status of the *Ixodes ricinus* tick vertical distribution in the Krkonoše Mts. (Czech Republic) and evaluate its potential changes. Two methods were used: monitoring of tick presence on dogs in 2001–2002 and direct monitoring of host-seeking ticks by flagging on two vertical transects reaching from 620 to 1270 m above sea level (a. s. l.) and from 600 to 1020 m in 2002–2003. Moreover ticks were monitored by flagging at another 13 localities situated in 800–1299 m a. s. l. in 2003. Both monitoring methods revealed an obvious shift in altitudinal distribution limit of the tick *I. ricinus* in the Krkonoše Mts. They both showed that ticks recently penetrate even up to a timberline (approximately 1250 m a. s. l.). The number of recorded host-seeking ticks rapidly decreased with increasing altitude. Whereas the average number of recorded nymphs converted per 60 minutes of flagging reached 15.2–25.7 nymphs in 700–799 m a. s. l. and 3.3–23.3 in 800–899 m, it was 4–9.6 nymphs in 900–999 m, 1.5–1.7 nymphs in 1000–1099 m and only 0.2 nymph in 1100–1299 m a. s. l. The observed shift of the tick altitudinal distribution limit at the same time results in extension of areas with potential risk of tick-borne diseases.

Key words: Ixodes ricinus, vertical distribution, Czech Republic, climate change, altitude

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INTRODUCTION

Altitudinal distribution limit of the tick *Ixodes ricinus* was formerly found to be situated in approximately 700–800 m above sea level (a. s. l.) in Central Europe according to many years field experiences (cf. 1, 2). This empirical distribution limit was confirmed by Daniel (1) and Daniel et al. (3) in long term field experiment in 1981–1983 just in the Krkonoše Mts. Survival potential of individual developmental stages of *I. ricinus* that were transferred into suitable habitats in altitudes from 630 to 1550 m was modelled in the experiment. The climatic and microclimatic conditions were monitored simultaneously. The results revealed that ticks in localities above 700 m a. s. l. could continue in their development for a certain period, however they never finished the whole life cycle and did not give rise to a local tick population. Low temperatures and unfavourable humidity did not allow finishing development of particular stages before energy exhaustion. This altitude was also considered to be the uppermost limit of the virus of tick-borne encephalitis (TBE) infection and also of other infections transmitted by ticks.

A shift in geographical and altitudinal distribution limits of the tick *I. ricinus* towards north and increasing tick density was observed in the last 20 years in Sweden (4, 5). Swedish researchers pointed out connection of this phenomenon with observed climatic changes. Most recently Daniel et al. (2) published data from the Šumava Mts. (Czech Republic) indicating a shift of tick altitudinal distribution limit over 1000 m a. s. l. In connection with tick spread, there is an increasing risk of dissemination of tick-borne diseases – Lyme borreliosis and TBE (2, 6, 7).

Changes in the incidence of tick-borne diseases (especially TBE in the last decade) were so apparent and alarming that investigation of its potential causes has been included in the WHO/EC project "Climate Change and Adaptation strategies for Human Health in Europe" (8). Research into the vertical changes in the distribution of their major vector – the tick *I. ricinus* – has become important part of resolved project tasks.

The aim of the present study was to monitor present status of the altitudinal distribution of the tick *I. ricinus* in the Krkonoše Mts. and to evaluate its potential changes.

MATERIAL AND METHODS

Study Area

The Krkonoše Mts. are situated in the north part of the Czech Republic on the Czech – Polish border. They are the highest range of the Sudetes with the highest peak of Sněžka (1602 m a. s. l.). Many of their summits surpass the timberline, which is situated approximately at 1250 m a. s. l. They have a temperate climate, marked by seasonal periodicity. Because of the relative proximity of the Atlantic Ocean and prevailing western winds, the highest ridges intercept wet, cold oceanic masses, as shown by high rainfall and snowfall and low air temperatures throughout the year. Mean annual temperature range between 6 °C and 0 °C (Žacléř [600 m a. s. l.] 6.1 °C; Špindlerův Mlýn [720 m a. s. l.] 4.7 °C; Szrenica [1362 m a. s. l.] 1.9 °C; Sněžka [1602 m a. s. l.] 0.2 °C). Precipitation increases with altitude, from about 800 mm in the foothills, to 1200 to 1400 mm on the summits. There is a permanent snow cover for seven months (from about mid-October to mid-May) at the highest elevations. The mean depth of snow cover ranges from 120 to 200 cm. Majority of the area of the Krkonoše Mts. is covered by forest ecosystems (83% of the area of the Krkonoše National Park). Most of the natural deciduous and mixed forests in the submontane belt have been cut down and replaced by single-dominant plantations of Norway spruce *(Picea abies)*. Both native and planted mountain spruce forests dominated in montane belt. At present all forest stands show some degree of damage due to air pollution. Knee-timber stands, virgin and secondary mat-grasslands and subarctic mires are concentrated in the subalpine belt around the summit plateau. The highest peaks reach up to the alpine belt. (9)

Mean annual temperature reached 5.3 ºC in 2002 and 5.0 ºC in 2003. Annual amount of precipitation was 1308 mm in 2002 and 756 mm in 2003. Mean temperature in the period from the 1st May to 30th September was 12.9 ºC in 2002 and 13.5 ºC in 2003. Amount of precipitation in this period reached 511 mm in 2002 and 312 mm in 2003 (data from the meteorological station near Rýchorská bouda in 1001 m a. s. l.; 50º 39´ 37″ N, 15º 50´ 57″ E).

Monitoring of Tick Presence on Dogs in 2001–2002

Monitoring of tick presence on dogs was used to obtain preliminary data about tick distribution in the studied area. Questionnaires prepared for this purpose were distributed to dog owners (mainly foresters and chalets permanent inhabitants) living or staying in the area of the Krkonoše Mts. Each questionnaire comprised data about a dog (race, size, sex, age, hair type – long/short, usage of antiparasitic preparations) and about recorded ticks (date of finding, in the case of recurrent findings a period from/until ticks were found, number of found tick individuals, their approximate size – to assess a developmental stage, localisation on a dog, locality (localities) of dogs motion with its brief characteristics, dogs motion – free/led). Respondents were asked to fill in positive as well as negative records and to check their dogs after every outdoor walk. They should monitor tick presence on their dogs from the beginning of May to the end of October and they were asked to preserve collected ticks in supplied tubes with 70% ethyl alcohol. They were also asked to note their possible experiences with tick occurrence on their dogs in the previous years. Fifty questionnaires were distributed in 2001, more than 100 in 2002. Altogether 32 fill in ones were returned back in 2001, 56 in 2002. As regards evaluation of obtained data, when a dog moved at more localities with different altitudes between two following tick checking, locality with the lowest altitude was considered as a locality of tick infestation.

Monitoring of Host–Seeking Ticks by Flagging Method

Two altitudinal transects were chosen for tick monitoring by flagging with respect to the results of tick monitoring on dogs in 2001 and to predictive map of tick incidence (10). The first transect (No. 1) in the central part of the Krkonoše Mts. reached from 620 to 1270 m a. s. l. It included six localities (C1–C6) in altitudinal distance of approximately 100 meters. Studied localities with their brief characteristics (name; geographical coordinates; altitude; habitat specification):

- locality C1–V končinách; 50º 38´ 43″ N, 15º 37´ 45″ E; 620 m a. s. l.; an edge of a submontane meadow and a Norway spruce *(P. abies)* forest with abundant deciduous trees in the forest margin *(Acer pseudoplatanus, Alnus glutinosa)*;
- locality C2–Vápenice; 50º 39´ 06″ N, 15º 37´ 42″ E; 700–765 m a. s. l.; an edge of a submontane meadow and Norway spruce forest with abundant deciduous trees in the forest margin *(A. pseudoplatanus, Fraxinus excelsior, Sorbus aucuparia)*;
- locality C3 – Hříběcí Boudy; 50º 40´ 40″ N, 15º 37´ 30″ E; 800–850 m a. s. l.; an edge of a montane meadow and a Norway spruce forest with abundant deciduous trees in the forest margin *(A. pseudoplatanus, S. aucuparia, A. glutinosa)*;
- locality C4 – Husí Boudy; 50º 40´ 54″ N, 15º 39´ 00″ E; 900–920 m a. s. l.; an edge of a montane meadow and a Norway spruce forest with a few deciduous trees in the forest margin *(S. aucuparia, Betula pendula)*;
- locality C5 – Přední Rennerovky; 50º 41´ 18″ N, 15º 39´ 10″ E; 1030–1100 m a. s. l.; an edge of a clear-cut and a Norway spruce forest;
- locality C6 – Klínové Boudy; 50º 42´ 38″ N, 15º 39´ 20″ E; 1160–1270 m a. s. l.; an edge of a montane meadow and a Norway spruce forest.

The second transect (transect No. 2) in the eastern part of the Krkonoše Mts. reached from 600 to 1020 m a. s. l. and it comprised five localities (E1–E5) in altitudinal distance of approximately 100 meters. Studied localities with their brief characteristics (name, geographical coordinates, altitude, habitat specification):

- locality E1 – Bystřice; 50º 37´ 25″ N, 15º 51´ 22″ E; 600–620 m a. s. l.; an edge of a submontane meadow and a mixed forest *(P. abies, Fagus sylvatica, A. pseudoplatanus, F. excelsior, B. pendula, Quercus robur, Corylus avellana, S. aucuparia)*;
- locality E2 – Sklenářovice; 50º 38´ 10″ N, 15º 51´ 18″ E; 710–750 m a. s. l.; an edge of a submontane meadow and a mixed beech (F. sylvatica) and Norway spruce forest with abundant deciduous trees in the forest margin *(Populus tremula, Salix caprea, A. pseudoplatanus, B. pendula, F. excelsior)*;
- locality E3 – Rýchory, Ochranná kaple; 50º 38´ 10″ N, 15º 50´ 37″ E; 780–820 m a. s. l.; an edge of a submontane meadow and a Norway spruce forest with abundant deciduous trees in the forest margin *(B. pendula, S. caprea, A. pseudoplatanus, A. glutinosa, S. aucuparia)*;
- locality E4 – Rýchory, Pod Sokolkou; 50º 38´ 45″ N, 15º 51´ 18″ E; 870–930 m a. s. l.; an edge of a montane meadow and a Norway spruce forest;
- locality E5 – Rýchory, the top of a mountain ridge; 50º 39´ 00″ N, 15º 51´ 40″ E; 960–1020 m a. s. l.; edges of a montane meadows and a mixed forest *(P. abies, F. sylvatica)* with a few deciduous trees in the forest margin *(S. caprea, S. aucuparia, Betula spp.)*.

Flagging of host-seeking ticks on both transects was conducted three times a year in late spring (from the end of May to half of June), in summer (during the first two decades of July) and in early autumn (during the first two decades of September) in 2002 and 2003. (Localities C5 and C6 were not monitored in autumn 2002. Monitoring at locality E3 started in summer 2002, at locality E4 only in autumn 2002.) Localities below 1000 m a. s. l. were monitored at least for one hour at each time, sites above 1000 m at least for two hours. A standard white flannel flag 50 x 70 cm was used. A flag was carefully checked for ticks after every 15 flag motions. All recorded tick specimens belonged to the species *Ixodes ricinus* (L.).

Moreover ticks were monitored by flagging at another 13 localities situated above 800 m a. s. l. in 2003. All these localities comprised edges of meadows and Norway spruce or mixed beech and spruce forests. They were visited only once and monitored for one to two hours.

Table 1. *Results of monitoring of the tick Ixodes ricinus presence on dogs in different altitudinal zones in 2001 – 2002.*

Altitude (m a. s. l.)	Number of checking		Number of recorded ticks
	positive	negative	
400–499	4	4	18
500–599	13	2	104
600–699	25	0	352
700–799	28	6	284
800–899	21	3	122
900–999	7	2	91
1000–1090	9	3	67
1100–1199	4	0	19
1200–1299	4	1	34

Table 2. *Number of individual stages of the tick Ixodes ricinus recorded by flagging on the transect No. 1 in 2002–2003*

Locality*	Altitude (m a. s. l.)	Total flagging time (minutes)	Larvae	Nymphs	Females	Males	Sum of all stages
C1	620	420	26	171	0	2	199
C2	700–765	380	0	96	1	1	98
C3	800–850	375	20	36	0	0	56
C4	900–920	370	0	59	0	0	59
C5	1030–1100	560	0	15	0	0	15
C6	1160–1270	665	0	2	0	0	2

** For the locality abbreviations see the chapter Material and Methods*

Table 3. *Number of individual stages of the tick Ixodes ricinus recorded by flagging on the transect No. 2 in 2002–2003*

Locality*	Altitude (m a. s. l.)	Total flagging time (minutes)	Larvae	Nymphs	Females	Males	Sum of all stages
E1	600–620	385	102	358	2	2	474
E2	710–750	380	5	163	0	1	169
E3	780–820	335	72	130	0	2	211
E4	870–930	245	0	33	0	0	33
E5	960–1020	1060	0	27	0	0	27

** For the locality abbreviations see the chapter Material and Methods*

RESULTS

Table 1 reports results of the monitoring of tick presence on dogs in 2001–2002. The majority of positive records and also the highest number of recorded tick individuals were reported from 600 to 900 m a. s. l. However the dog infestation by ticks was frequently found out at localities above 900 m a. s. l. and ticks were reported from places up to 1299 m a. s. l. Several respondents placed the first records of ticks on their dogs in higher altitudes (800–1300 m a. s. l.) to the end of 90-ies of the last century and they observed increasing trend in their abundance from that time. Two employees of the Krkonoše National park Authority permanently living in the locality Hříběcí Boudy (50º 40′ 40″ N, 15º 37′ 40″ E; 750–850 m a. s. l.) independently dated the first tick records on their cats and dogs in this locality back to the turn of 80-ies and 90-ies of the last century. From that time they have recorded ticks regularly every year. Two respondents permanently living in Harrachov (690 m a. s. l.) and Dolní Dvůr (600 m a. s. l.) observed tick occurrence on their pets already in the end of March in 2001 and 2002, although there was still patchily snow cover.

The results of tick monitoring by flagging on the transect No. 1 shows Table 2 and on the transect No. 2 Table 3. The majority (81.2%) of all recorded individuals were nymphs of *I. ricinus*, which were recorded at all localities of both transects. The highest situated records came from locality C6, where one nymph was

Table 4. *Number of individual stages of the tick Ixodes ricinus recorded by flagging on once visited localities in 2003*

Altitude (m a. s. l.)	Number of localities	Total flagging time (minutes)	Larvae	Nymphs	Females	Males	Sum of all stages
800–899	2	90	0	5	0	0	5
900–999	1	60	3	4	0	0	7
1000–1099	7	570	0	16	0	0	16
1100–1199	1	90	0	0	0	0	0
1200–1299	2	225	0	1	0	0	1

recorded in 1260 m a. s. l. on 30. 5. 2003, another one in 1180 m a. s. l. on 10. 7. 2002. Adults (merely 0.8% of all recorded individuals) were recorded only at localities up to 820 m a. s. l. on both transects, larvae up to 850 m a. s. l. The majority of nymphs recorded in altitude between 870–1000 m a. s. l. on both transects (localities C4, E4 and a part of E5) were found just on the edge of meadow and forest under branches of outer Norway spruce trees, which reached nearly up to ground. Whereas only a few nymphs were recorded on open places on a meadow-forest edge as well as in an inner forest edge. Also almost all recorded nymphs from localities above 1000 m a. s. l. (localities C5, C6 and a part of E5) were found under spruce branches reaching up to the ground as in the previous case.

Table 4 provides results from 13 once visited localities. At 8 from 10 localities situated between 800–1099 m a. s. l. ticks nymphs were recorded. One nymph was also recorded at one from three monitored localities located above 1100 m a. s. l. The locality with positive tick record (Liščí louka; 50º 41´ 08″ N, 15º 41´ 55″ E) included edge of mountain meadow and Norway spruce forest and the nymph was found there in 1230 m a. s. l. on 13. 6. 2003. As in the case of regularly monitored localities almost all the nymphs were found under spruce branches reaching up to the ground.

Fig. 1 compares average numbers of recorded nymphs converted per 60 minutes of flagging at localities of both regularly monitored transects in 2002–2003 and at once visited localities. Average number of recorded nymphs generally decreased with increasing altitude. There are quite high differences between numbers of nymphs recorded at localities of both transect situated in 700–900 m a. s. l. On the other hand numbers of nymphs recorded per 60 minutes at localities above 900 m a. s. l. are very similar.

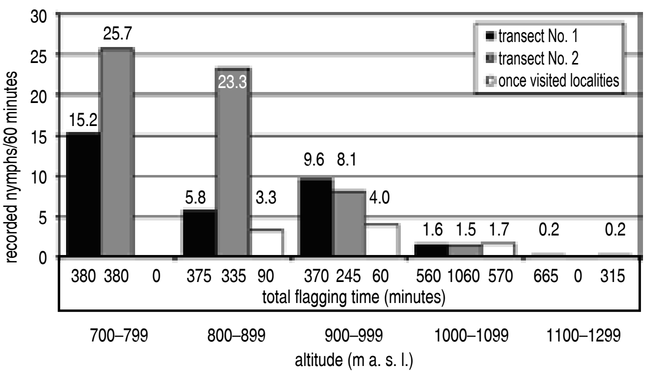

Fig. 1. *Average number of recorded nymphs of the tick Ixodes ricinus converted per 60 minutes of flagging in altitudes above 700 m a. s. l. at studied localities of transect No. 1, 2 and at once visited localities.*

Thus 4–9.6 nymphs per 60 minutes of flagging were recorded at localities situated between 900–990 m a. s. l., at localities between 1000–1099 m a. s. l. it was 1.5–1.7 nymphs per 60 minutes and at localities above 1100 m a. s. l. it was 0.2 nymph per 60 minutes.

Five fully verified cases of human infestation by nymphs of the tick *I. ricinus* from altitudes above 800 m a. s. l. were recorded in 2002–2003 among the Krkonoše National Park Authority staff (altitude, date of infestation): 880–940 m a. s. l, 28. 6. 2002; 1000–1050 m a. s. l., 12. 7. 2002; 1100–1200 m a. s. l., 4. 9. 2002; 1250 m a. s. l., 21. 7. 2003; 1356–1554 m a. s. l., 17. 6. 2003.

DISCUSSION

Both monitoring methods revealed an obvious shift in altitudinal distribution limit of the tick *I. ricinus* in the Krkonoše Mts. Naturally the results of monitoring of the tick presence on dogs should be evaluated very carefully with respect to impossibility of accurate location of dog infestation. However these results exactly correspond with data obtained by flagging of host-seeking ticks. The tick *I. ricinus* recently penetrates even up to a timberline (approximately 1250 m a. s. l.) in the Krkonoše Mts. In comparison with experimental results of Daniel (1) concerning ticks surviving in different altitudes in 1981–1983 in the same area, the altitudinal distribution limit of the tick shifted in last 20 years for approximately 500 altitudinal meters. This finding corresponds with results of similar study of Daniel et al. (2) from another mountain area in the Czech republic – from the Šumava mountain range. Comparison of these findings with historical data of Rosický (11) and Černý (12) which summarise tick distribution in the whole Czech Republic indicates that an altitudinal distribution limit of the tick *I. ricinus* has rapidly shifted most probably in the whole territory of Central Europe during the last two decades.

Naturally the number of recorded ticks rapidly decreased with increasing altitude. This general trend was already demonstrated for example by Černý et al. (13). High differences in numbers of recorded nymphs per 60 minutes of flagging between the lowest situated localities of both transects (localities C1 and C2 versus E1 and E2) most likely resulted from different types of habitats at these localities. Whereas both localities of transect No. 1 were primary meadow – spruce forest ecotones, the other ones of transect No. 2 were meadow – mixed forest ecotones. Deciduous and mixed forests usually provide more suitable habitat for ticks than coniferous ones and they show higher tick abundance. On the other hand higher situated localities of both transects as well as once visited localities including predominantly meadow

– spruce or in several cases meadow – mixed beech and Norway spruce forest ecotones, show very similar nymph numbers. Obtained abundance data from higher altitudes thus could be used for tick number estimations and for prediction of possible human infestation by tick in higher altitudes.

The fact that all ticks recorded above 850 m a. s. l. were nymphs is not surprising because nymphs are the most frequent developmental stages recorded by flagging. Relatively abundant records of host-seeking nymphs in high altitudes during both vegetation seasons of the biannual monitoring period indicate that the tick *I. ricinus* most probably has to complete its life cycle even near the timberline at the present. Also records of engorged females on dogs from the highest altitudes in the present study and a record of one host-seeking larva by flagging in 1080 m a. s. l. at locality C5 on 8. 6. 2004, two larvae in 960–1000 m a. s. l. at locality E5 on 9. 6. 2004 and another three larvae at the same locality on 19. 7. 2004 (Materna, unpublished) support this hypothesis. According to experimental results of Daniel (1), there was a possibility of tick introduction (especially engorged females) into higher altitudes by their hosts even in the past. However according to his results they could survive there for a long time and even oviposit, but they could not complete the developmental cycle and give rise to a new local population. Records of host-seeking nymphs in high altitudes suggest that during last two decades ticks introduced by birds or mammals could originate here new local populations. Especially red deer overwintering in lower foothills of the Krkonoše Mts. and in a spring migrating back up to subalpine belt, may serve as important sources of tick introduction into higher altitudes.

Observed preference of habitats under branches of outer Norway spruce trees just on the edge of meadow and forest by host-seeking nymphs in higher altitudes may have two reasons. First, these places are highly preferred by small mammals and serve also as the shelters for roe-deer – both potential tick hosts. Secondly, they can probably provide favourable microclimate conditions during the whole year.

What are the probable causes of so considerable shift of tick altitudinal distribution limit? There are two main factors affecting ticks distribution: sufficient number of hosts for all developmental stages and suitable microclimatic conditions. Because the blood source is not the limiting factor of tick altitudinal distribution in suitable habitats in Central Europe (cf. 1), the shift in its altitudinal distribution was most likely evoked by climatic changes during last decades. Lindgren et al. (5) found that increasing density and a shift in the geographical and altitudinal distribution limits of the tick *I. ricinus* towards north and towards higher altitudes observed between the early 1980s and mid-1990s in Sweden seems to be related to climatic changes. Mild winters and extended spring and autumn seasons of the 1990s in Sweden are according to their results the primary reason of observed changes in tick density and distribution.

The influence of climate modifications on the tick population and consequently TBE morbidity showed Daniel et. al. (14). Similarly Zeman and Beneš (15) who analysed the geographic-temporal pattern of TBE human cases in the Czech Republic confirmed that the impact of climate warming on the vertical distribution of this disease in Central Europe is evident. The increase of *I. ricinus* distribution limit implies a substantial extension of the areas with its occurrence and with risk of tick-borne diseases. Although not all habitats in higher altitudes are suitable for the tick *I. ricinus*, the risk of infestation by this vector is amplified by the fact that many of these areas are exploited for recreation and out-door activities. That this conclusion is not just a theoretical consideration was shown by verified tick human infestations reported on the territory of the Krkonoše Mts. in the present study as well as by TBE cases registered in the mountains parts of the Šumava Mts. (2) and records of ticks infected with borreliae causing Lyme disease from higher altitudes of the same mountain area (16).

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