Soil reaction and tick abundance *Ixodes ricinus*

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Abstract

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Tick *Ixodes ricinus* is a carrier of several diseases. At present, its activity in Slovakia was discovered in the winter months. It also extends to higher altitudes. Therefore it is necessary to know the ecology and optimal conditions suitable for its existence. Although considerable attention is devoted to the health research of tick, little is known about its claim on the soil environment, because as a representative of a temporary edaphone initial ontogenetic stages (eggs and larvae) survives in the soil. For this reason, the research focused on the soil pH as an important environmental factor that can influence the development and abundance of tick. The research was carried out on the plots with tick high abundance in the Veľká Fatra Mts at an altitude of - 760 meters asl. We found that mull humus form with a thin film of last year's litter fall (the Oon necrotic subhorizon) with a pH narrow range of 5.9–6.0 (rounded) was convenient for developmental stages of this tick. Below this horizon the soil type of cambisol with pH values in the upper range of 5.45–5.79 was produced. The soil reaction was therefore a slight acid. In this environment, a relatively high density of tick was in May, with the secondary mild autumn peak.

Key words

Cambisol, ecology, humus form, *Ixodes ricinus*, soil properties, Veľká Fatra Mts

Introduction

Ixodes ricinus – tick ordinary is epidemiologically most important tick of the families Ixodidae, one of 20 tick species occurring in Slovakia. Its danger lies in the ability of its body to transmit pathogens that cause Lyme disease, anaplasmosis, tick-borne encephalitis, and babesiosis.

Geographic and vertical expansion of this tick is dependent on a number of biotic and abiotic factors. The *Ixodes ricinus* tick is mesophilic, spring activity begins when the temperature rises to 4 to 5 °C (Hubálek et. al., 2003). The temperature required for the metamorphosis of the larva to the eggs is between 8 to 11 °C (Daniel, 1993). Activities *of Ixodes ricinus* correlate positively with a moisture content exceeding 80%, with the exception of parasitic hosting (Eisen and Lane, 2002).

Under the vertical occurrence, certain changes are detected, which originate from climate socio-economic factors. While Mačička (1955) indicates the occurrence of this tick at an altitude of 600–900 m asl in Slovakia, Bullová et al. (2008) monitored the incidence of *Ixodes ricinus* in Choč Mountain at a height of 1,230 m asl. Hrkľová (2010) describes the occurrence of all stages of the development of *Ixodes ricinus* on the area of Malinô Brdo, Veľká Fatra Mts at 1,044 m asl. Majláthová et al. (2015) reported the incidence of the tick above 1,400 m asl in the Veľká Fatra Mts and a relatively high density of *Ixodes ricinus* on sites up to an altitude of 1,000 m (60–120 ticks/an hour collection).

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The presence and density of tick´s hosts, and vegetation structure are important biotic factors for the incidence of ticks. The essential factor is primarily the structure of vegetation mainly within the habitat. Gray et al. (1998) describe the importance of the litter fall (fallen leaves) that protects ticks against cold and dry, and then creates optimal climatic conditions with high humidity. Some nymphs are lain down to the stage of rest generally in the upper layer of soil. Eisen and Lane (2002) have studied the ontogenesis of ticks in the laboratory and found spawning in a number of 1,000 eggs at *I. ricinus*.

Within the morphology frame of an ordinary tick a significant sexual dimorphism was observed in adults, the female body surface is covered with a scutum, which reaches from 1/3 to 1/2 the length of the body and allows the increased size of the body. Chelicerae responsible for penetration through the skin of the host are placed on the head at the terminal position. Paired cheliceras and pedipalps serve as sensory organs and centrally located *hypostom*, with backward pointing little teeth fixing the tick body in the skin of the host. On the first pair of limbs there is Haller organ which detects CO_2, heat and a host motion (Černý and Rosický, 1971).

Ixodes ricinus belongs to outer ticks. All developmental stages (larvae, nymphs and adults) are active blood-sucking forms. The female lays eggs in the topsoil or in fallen leaves where the ongoing development and metamorphosis of the individual stages run (Filippova, 1977). Tick belongs to temporary zooedaphone as geophiles i.e. edaphophils (Stašiov, 2006). Therefore ticks form part of zooedaphone, whom currently an increased attention is generally paid to (Stašiov, 2009; Stašiov and Svitok, 2014).This research of *Ixodes ricinus* was carried out in the soil type of Cambisol. Literary sources have not so far stated details relating to the soil pH and the occurrence and abundance of *I*xod*es ricinus.*

The main aim of the study is to determine what soil reaction (pH) occurs at locations with high abundance and density of incidence of the ordinary tick *I. ricinus*, thus on the locations with its optimal occurrence. Characterization of humus form and some properties of environment on localities with high tick abundance is the next goal.

Materials and methods

The research was carried out on the plot of Škutovky, northwest of the village of Liptovská osada, in the Veľká Fatra Mts located at an altitude of 760 m asl. The research area has eastern exposure with an inclination of 5°. Dimensions of investigated meadows were 80 × 15 m and the edge of the forest parallel with the meadow was wide about six meters.

On weathered granite massif there were mostly sandy-loamy soils broaden. On the rocks crystalline core and also on our research areas Cambisol was developed containing three horizons. The land is inhabited by a number of organisms, while in general, the greater the number and diversity, the greater the stability of the soil as an ecosystem (Stašiov, 2006).

The factors that affect the life cycle of ticks include rock, soil, climate, geographical factors, plants and animals. Studied sites belong to temperate and cold climate areas. The territory has a mountainous precipitation regime with a total annual rainfall of 1,000 to 1,400 mm (Miklós, 2002), which ranks among the wettest areas of Slovakia. The average annual temperature was 7 °C. An average temperature in July was 16 °C and in January –5 °C.

Geological substrate, landforms, soil and climatic conditions of Veľká Fatra Mts, together with other factors have contributed to the development of diverse habitats. Different plant and animal species (Halaša and Šľachta, 1968) have developed on them. Mainly particular forest communities maintain its natural character which demonstrates the development of forest communities of the Carpathian type with the occurrence of numerous rare and endangered species. The forests were mixed *Acer pseudoplatanus*, *Acer platanoides*, *Fraxinus excelsior*, *Sorbus aucuparia*, *Tilia cordata* and *Tilia platyphyllos*. Beech forests occupied the largest forest land. Plant communities of rocky habitats with rare taxa were of a relict nature. Mountain orientation and limestone bedrock caused that more thermophilic species were spread in the Veľká Fatra Mts compared to other high mountains.

Fieldwork

The research was focused on ecotone, i.e. a temporary band mixed, hardwood-conifer forest with rich undergrowth of scrub (a forest edge habitat) and a parallel meadow ecosystem (a meadow habitat). From April to November 2015, host seeking nymphal and adult ticks were sampled once a month depending on local weather conditions by the methods of flag dragging through vegetation using a flannel flag (1 × 0.7 m) in the morning considering the temporal and spatial appetence behaviour of ticks, i.e. during the highest questing activities (timed dragging). The number of ticks collected using the flag during one hour collection represented the apparent density of ticks. Timed dragging was conducted by teams of 4 or 5 persons, with each person dragging for a respective time, making together 1 hour. According to Peťko et al. (1996) an hour collection represents an area of 600 m^2 (in the pastures and woods with shrubs) to 800 m^2 (the meadows and parks).

Soil sampling was focused on surface soil whose characteristics most influence the abundance and a life cycle of ticks. For the research, one transect was chosen on the meadow and forest edge ecotones. On each transect three trench soil pits were dug up. Soil samples were taken from each horizon of the soil pit – surface humus horizon (the Oo horizon), followed by a layer of 5–10 cm (the A horizon) and a depth of 20–25 cm (the Bv horizon), making together 18 soil samples. Soil samples were dried at a laboratory temperature and processed according to the pH appropriate method.

Laboratory work

The soil pH was measured in a soil suspension. The common ratio of soil to water was 1:2.5 (10 g of soil to 25 ml of distilled water). At surface horizon organic matter, this ratio was 2:25 (2 g of soil sample was added to a 100 ml beaker and mixed with 25 ml of distilled water). After stirring, the soil sample suspension was left standing for 24 hours motionlessly. After this time, the soil pH was measured using multifunction equipment 340i WTW on 2 decimal places.

The data were analysed using the Statistical Package of SPSS 19.0. A data normal distribution was treated by the Kolmogorov-Smirnov test. When processing the pH data, basic statistical characteristics were calculated – an arithmetic mean, a standard deviation, coefficient of variation, variation margin, and a median. Since the tick data did not follow a normal distribution, variable was analysed using a nonparametric test. The difference in the tick abundance between the forest edge and meadow was tested for significance using the non-parametric Friedman test. A p-value of 0.05 was considered as statistically significant.

Results

The results of fieldwork

Meadow

The meadow had 100% coverage of grassland vegetation (grasses, herbs, mosses – *Entodon schreberi*). Humus form was turf mull. The Oon litter fall sub-horizon was practically absent, residues of dead plant organs occurred rarely. On this habitat, 3 trench soil pits were dug up with the following characteristics:

- Meadow 1 – the humus form was Mull, a thickness of Au was 11 cm.
- Meadow 2 – the humus form was Mull, a thickness of Au was 15 cm.
- Meadow 3 – the humus form was Mull, a thickness of Au was 17 cm.

Unlike the soil in ecotone (the forest edge), the Au horizon was weakly humic, gray-brown, sharply demarcated from the substrate horizon – this meadow was in the past probably plowed to a depth of 25 cm, a content of skeleton was about 20%.

The meadow average relative abundance of *Ixodes ricinus* was 2.1 ± 3.7 individuals per one dragging hour. Composition of ticks collected was 71.4% and 28.5% for nymphs and adults, respectively.

The edge of the forest

An ecotone coat of stand was overgrown with shrubs and hazel (*Corylus avellana)*, Goat willow (*Salix caprea)*, Sloe (*Prunus spinosa*) and Midland hawthorn (*Crataegus laevigata)*. In the undergrowth, there were forest and meadow grasses and herbs – St. John's Wort (*Hypericum perforatum)*, European sanicle – (*Sanicula europaea*) etc. The herbaceous layer covers 100% of the surface of the site.

On this stand compared to other habitats, the relatively high average abundance of 11.7 ± 9.6 was found, in the range of 9–104 ticks per one dragging hour. Composition of ticks collected was 85.4% and 14.5% for nymphs and adults, respectively. Results showed that this study area had convenient conditions for the tick life cycle. The research of seasonal dynamics has pointed to two peaks of the tick frequency over the studied period (Fig. 1). It is certainly linked with the occurrence of hosts in this habitat.

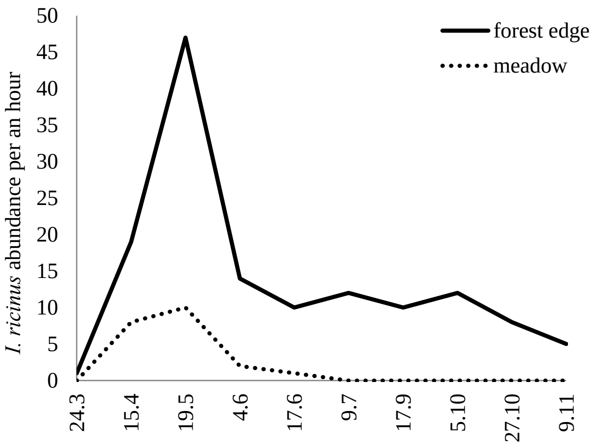

Fig. 1. Seasonal dynamics of *Ixodes ricinus* (timed dragging).

The difference in tick abundance between the forest edge and meadow habitats was statistically evaluated. Due to the fact that the result data did not follow a normal distribution (the Kolmogorov-Smirnov test), this difference was tested using the non-parametric Friedman test.

On forest edge habitat, 3 trench soil pits were dug up with the following characteristics:

- Edge of forest 1 – the Oon horizon was predominantly formed nearly with decomposed litter of grasses and shrubs. The humus form was a mull with a thickness of 1 cm. The Au horizon was 12 cm thick.
- Edge of forest 2 – the humus form was also mull with a total thickness up to 1 cm. In undergrowth synusia, in addition to herbs and grasses, there was also moss (*Entodon schreberi*) presented which might indicate a certain acidification. The Au horizon was 11 cm thick.
- Edge of forest 3 – the humus form was a mull with a thickness up to 1 cm. The Au horizon was 11 cm thick. The composition of the surface vegetation was analogous to the plots 1 and 2.

In the meadow ecosystem, there was Cambisol with surface diagnostic horizons Oo (sub-horizon Oon), Umbrian surface diagnostic horizons Au and subsurface diagnostic horizons Bv. The humus form was turf mull.

In ecotone of the forest edge, there was also Cambisol with surface diagnostic horizons Oo (sub-horizon Oon), Umbrian surface diagnostic horizons Au and subsurface diagnostic horizons Bv. The humus form was mull.

The results of laboratory work

The basic statistical characteristics of soil pH (Table 1) comprised mean data, standard deviations and coefficients of variation of different soil profiles present. Each value represents the arithmetic mean calculated from sub-horizon Oon and two mineral horizons (A, B). Thus the arithmetic mean was given by properties of organic Oo and organo-minerals A and mineral B horizons. Despite the different structure and composition of biota, results' variability was low. The coefficient of variation was less than 8%, the standard deviation "s", did not exceed a value given by the product of 0.43× pH and the span of pH arithmetic means for the Cambisol fluctuated in an interval of 5.4–5.8 (rounded).

Table 1. Statistical values of pH of trench probe, including Oo surface horizons

Locality	Area	$\overline{x}$	s	v_k
Škutovky	Meadow 1	5.777	0.0902	1.56%
Škutovky	Meadow 2	5.780	0.2425	4.19%
Škutovky	Meadow 3	5.790	0.3959	6.83%
Škutovky	Forest edge 1	5.700	0.3905	6.85%
Škutovky	Forest edge 2	5.453	0.3066	5.62%
Škutovky	Forest edge 3	5.643	0.4206	7.45%

$\bar{x}$, arithmetic mean; s, standard deviation; v_k, coefficient of variation.

The arithmetic mean, median, standard deviations, coefficients of variation and outliers for the Oon surface humus sub-horizon on individual research areas were compared (Table 2). This horizon was found on all areas and localities, therefore its values were compared with each other. In addition, it was a horizon whose properties were very important to the developmental cycle of the tick. It was formed of the litter which was already subjected to some changes and therefore this horizon differed terminologically and was designated as a horizon or a layer of litter. It was directly influenced by a composition of the litter (necromass) of biocenose (plants, herbs, grasses etc.). Analysis results rounded up to one decimal place were of very low variability (5.9 to 6.0 pH) with a coefficient of variation below 5%.

Table 2. Statistical pH values of Ool horizons of meadow habitats and forest edges

Area	$\overline{x}$	$\tilde{x}$	s	v_k (%)	Min-max
Meadow	5.78	5.77	0.24	4.99	5.64–6.20
Forest edge	6.00	6.05	0.19	3.10	5.79–6.15
Together	5.93	5.91	0.21	4.04	5.64–6.20

$\bar{x}$, arithmetic mean; s, standard deviation, v_k, coefficient of variation.

The pH statistical characteristics of the trench soil pits were compared (Table 1), no statistically significant higher pH values were found on the forest edge (pH 6.00). Neither statistically significant lower pH values were found on the meadow, for more acid environment with a pH of 5.78 (Table 2).

Significance test of the arithmetic mean difference of the pH by using the analysis of variance is given in Table 3. The analysis confirmed that the difference of arithmetic mean of the pH on the research areas was not statistically significant and therefore, properties of areas are very similar. No significant differences were achieved with the 95% probability.

Table 3. Hypothesis testing the differences of arithmetic means of pH values at individual stations

	Meadow 1	Meadow 2	Meadow 3	Forest edge 1	Forest edge 2	Forest edge 3
Meadow 1		0.99503	0.98013	0.88612	0.54662	0.80334
Meadow 2	0.99503		0.98509	0.8812	0.54251	0.79854
Meadow 3	0.98013	0.98509		0.86649	0.53028	0.78416
Forest edge 1	0.88612	0.8812	0.86649		0.6453	0.91569
Forest edge 2	0.54662	0.54251	0.53028	0.6453		0.72279
Forest edge 3	0.80334	0.79854	0.78416	0.91569	0.72279	

Discussion

There are cumulative factors which influence the relation of the ticks and the host as possible vector of range spectra pathogens. Globally these factors influence the incidence of tick-transmitted diseases (Lindgren, 1998; Sarah and Jonathan, 2010). The non-parasitic stage of ticks requires the special condition of environment. The abiotic factors as temperature, precipitation influence plant communities, the host animals as well as the occurrence of people in landscapes. The metamorphosis of the ticks takes place in the surface layers of soil or forest litter (Daniel et al., 2015). Certainly the micro-environment influences the development of the stage of ticks and consequently their abundance. Despite of many works about influence of environmental condition on the ticks abundance there is missing a systematic study of the soil environment.

Tick metamorphosis begins in the Oon organic surface sub-horizon. From soil and genetic point of view, it is called the necrotic sub-horizon of slightly altered to strongly decomposed residues of forest and a meadow litter fall which represents stages of its decomposition and transformation in the direction from top to bottom (Societas pedologiga slovaca, 2014). The thickness of the Oo litter fall horizon is more than 3 mm, more than 20% of combustible organic matter and at least one of sub-horizons (Oon necrotic – Oof fermentation (detritus) – Ooh humic sub-horizon) is presented. Only Oon litter sub-horizon was found on our research areas, it is basically a layer of the last year's litter fall.

The humus form of mull that was found on our research plot was produced in mild to warm climate on soils adequately supplied with nutrients and sufficiently moist. Cambisol on our study areas was formed from non-carbonate parent rocks. Proper soil-forming substrate was rich in skeleton and therefore a sufficient mass of soil material was quite susceptible to weathering, thus constantly releasing nutrients. As for the grain size, the soil on the research plots was loamy according to a field trial.

Mountains orientation of a north-south direction, bedrock, soil and climatic conditions provided good conditions for thermophilic plant and animal species in the Veľká Fatra Mts. Favourable effect of temperature and soil moisture also explained why the ticks throve so well in this environment. Moreover, our research plots border with shrub undergrowth of hazel, hawthorn and blackthorn and just such an environment was according to current knowledge appropriate for the survival of ticks, their hosts and their developmental stages.

The pH is one of the most important properties of the soil as it affects not only the solubility of the substances in the soil and their utility for biota, but also the sorption of cations and the structure of the soil, and thus soil physical properties and biochemical reactions. Soil pH substantially affects living conditions for soil organisms, including ticks. Therefore one of the goals of our research was to determine what a soil reaction (pH) occurs at locations with a high abundance of ticks. It appeared that the pH of the Oon necrotic sub-horizon suited well to ticks in a confidence interval of pH 5.6–6.2 with a 95% probability that was in the slightly acidic range. Mentioned range may be considered convenient for the incidence of ticks.

In addition, we found that the pH of the Oon necrotic sub-horizon was in a very narrow range of arithmetic means (5.87 to 6.00). The coefficient of variation fluctuated around 4% and the standard deviation was equalled to 0.21× pH at a variation range 0.56× pH (Table 2). Extreme readings were in the range pH from 5.64 to 6.20. Below the Oo litter fall horizon, Cambisol was produced with a pH from 5.45 to 5.79 in a mineral soil in a depth of up to 25 cm, confirming again that a maximum of tick-abundance was in a slightly acidic pH. Since our investigations were the first study on the analysis of the tick distribution associated with a soil reaction, our genuine pH results couldn't be compared with those of literature sources.

The occurrence of the tick *Ixodes ricinus* was characterised by seasonally curve (Fig. 1) with two peaks in May and in October (the incidence of ticks was lower compared to that in May). This bimodal seasonal activity dynamics of host-seeking *I. ricinus* was also found in the study of Schwarz et al. (2009) who studied the tick distribution based on plant communities, with peaks in June and October. This shift of tick abundance peak in June may be given by local climatic conditions. *I. ricinus* starts its host-seeking activity at 2.5 °C and 45% relative humidity (Hubálek et al., 2003; Lindgren et al., 2000), that was why the tick lowest occurrence was recorded on 24 March. The abundance peaks were next associated with soil relative moisture, which was reflected in the statistically significant large difference in the average tick occurrence between the forest edge habitat (11.7 ± 9.6 ticks per hour) and the meadow habitat (2.1 ± 3.7 ticks per hour). The seasonal questing activity of *I. ricinus* may be associated with major variations in biotic (host species, host abundance and behavior, and vegetation structure) as well as abiotic factors (climate) (Gern et al., 2008). From this point of view, ticks were sampled by flagging at temperature up to 24 °C (Perret et al., 2000). The high air temperature above 24 °C leads to decrease in numbers of questing ticks due to the low desiccation resistance of ticks (Martin et al., 1990). Ticks descend more often from the vegetation into the soil to rehydrate. If ticks frequently have to leave their questing places due to rehydration, they will rapidly exhaust their energy reserves before they find a host and die (Gern et al., 2008). The relative humidity directly above the soil is given by the soil water content. As the ticks have to rehydrate in the soil, the number of collected ticks correlate to the soil water content (Guerra et al., 2002).

From July till the end of sampling period, no *I. ricinus* ticks were found on the meadow habitat which can be advocated by a dry period, while in the forest edge habitat with the higher relative soil moisture the numbers of collected ticks were above 10, except for November. This result was in coincidence with the finding of Maetzel et al. (2005) stating that no ticks were found in open habitats like agricultural fields. Similar seasonal dynamics were also found in other species zooedaphone (Macko, 2008; Rendoš et al., 2012).

Favoured habitats of this tick are coniferous and mixed deciduous woodlands (Lindstrom and Jaenson, 2003). The tree composition of a forest is determined by a moisture gradient involving soil aeration, soil nutrient supply, and microclimatic features (Curtis, 1959). The factors interacting at the microclimatic level within the topsoil and leaf litter sub-horizon appeared to have an important influence on tick survival. Soil texture, in addition to the topography, determines the extent of drainage, and the level of moisture of the ground layer (Guerra et al., 2002). Excessive moisture in the soil may be deleterious to tick survival since they overwinter in the topsoil and leaf litter sub-horizon. Leaf litter is a requisite component for the survival of immature stages of ticks (Schulze et al., 1995; Gray et al., 1998).

Ticks are found everywhere where environmental factors are suitable to them. With climate warming, ticks are even getting to higher positions above 1,000 m asl. In the course of research, a rare occurrence of ticks was confirmed even in the NNR Skalná Alpa at an altitude of 1,450 m asl.

It was discovered that edges of forests satisfied ticks in a better way, thus forest ecotone is more convenient for them than meadow ecosystems (Maetzel et al., 2005). Ticks live mainly on the edges of deciduous and coniferous forest in the shrub and herbaceous undergrowth. Especially nymphs use an Oon sub-horizon (a layer of litter) for its development. (Schulze et al., 1995).

Conclusion

By assessment of selected abiotic factors, soil pH, composition of forest ecotones and meadows in the mountain area of Veľká Fatra Mts, we have come to a finding that they provide favourable conditions for the occurrence of ticks and a real danger for the development of infections of humans and animals (grazing) – Lyme disease, tick-borne encephalitis, anaplasmosis and other tick-borne infectious and parasitic diseases of humans. Forest ecotone satisfies all the requirements needed to maintain the life cycle of the tick. Its conditions are very suitable for the survival of hosts needed for the livelihood of ticks. As these were the first and original results of ecopedological conditions for the occurrence of the tick *Ixodes ricinus*, further research is needed to clarify our findings.

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