Ticks (Acari: Ixodidae) Parasitizing Red Foxes (Vulpes vulpes) in Slovakia and New Data About Subgenus Pholeoixodes Occurrence

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Abstract

Background Distribution and biology of Pholeoixodes ticks is not very well understood. The goal of the study was to collect new data on the Pholeoixodes tick occurrence in Slovakia.

Methods Tick infestation of red foxes in the regions of Košice, Prešov, Bratislava and Žilina was studied during the period 2017–2018. Ticks were collected from the fur of animals using tweezers and identified using appropriate keys. In total, 146 red foxes (Vulpes vulpes) were investigated.

Results In total, 39 (26.7%) of animals were found to be infected with ticks from five species. Pholeoixodes ticks were found on 13 (3.4%) of the foxes: Ixodes hexagonus (Leach, 1815) on 5 specimens (3.4%) in the Košice, Prešov and Žilina regions; I. crenulatus (Koch, 1844) on 8 specimens (5.5%) in the Prešov and Bratislava regions; Ixodes ricinus (Linnaeus, 1758) collected from 25 (17.2%) foxes in every locality; Dermacentor reticulatus (Fabricius, 1794) from 5 foxes (3.4%) in the Košice, Prešov and Žilina regions; Haemaphysalis concinna (Koch, 1844), from 4 foxes (2.8%) from the Košice region.

Conclusions Ixodes hexagonus has been previously recorded in Slovakia. However, this is the first finding of I. crenulatus in the country. The morphological features of the I. crenulatus specimens found in Slovakia were identical to those of ticks described in Poland and descriptions given in identification keys.

Keywords Ixodes crenulatus · Ixodes hexagonus · Vulpes vulpes

Introduction

The subgenus Pholeoixodes (Schulze, 1942), includes tick species occurring in the Palearctic, Nearctic and Neotropical Zones. They are distinguished from other ticks of the Ixodes genus by many morphological features, such as a short capitulum and thickset legs. They are also characterised by the presence of relatively short internal spurs on the coxae of the first legs, or in some cases, their complete absence. In addition, the tarsi of the first leg are stepped below Haller’s organ and the genital aperture is located between the coxae 3. While three pairs of setae can be seen on the anal plate of nymphs, five are present in adult females [14, 30, 37]. All Pholeoixodes ticks engage in a nest-dwelling mode of feeding, residing in the nests and burrows of their hosts. Hence, Pholeoixodes specimens are only occasionally caught by flags and drags, and most of the data on their occurrence has been based on the specimens collected from hosts [30, 37].

Of the Pholeoixodes ticks, five species affecting carnivores in Europe have been described: Ixodes canisuga Johnston, 1849; Ixodes (Pholeoixodes) crenulatus Koch 1844; Ixodes (Pholeoixodes) hexagonus Leach, 1815; Ixodes (Pholeoixodes) rugicollis Schulze et Schlottke, 1929; Ixodes (Pholeoixodes) kaiserii Arthur, 1957 [13, 30, 37].

Ixodes canisuga (Johnston 1849)

Ixodes canisuga is associated with mammals which inhabit burrows. The most commonly infested species are medium-sized Mustelidae and Canidae, such as the red fox (Vulpes vulpes) and badger (Meles meles), among others [10, 35, 43]. This tick species is also a common parasite of domestic dogs and has been found on cats [25]. The known distribution of this tick ranges from Great Britain, Ireland and France in the
West, to Austria and Germany in the East [10, 31, 39, 43], and Portugal to the south [35]. In addition, *Ixodes canisuga* has been observed in the countries of southern Europe, i.e., Hungary, Romania, Croatia, Serbia, Bosnia and Herzegovina, although these have only been recorded as single sightings [9, 13, 16, 27].

**Ixodes (Pholeoixodes) crenulatus** (Koch 1844)

*Ixodes crenulatus* is the second tick species to be associated with carnivores; however, infestations have also been observed in large insectivores and large rodents [30, 37]. Its documented range includes Poland, Ukraine and Romania [13, 17, 18, 26, 30, 42]. Its southernmost border runs along northern Iran, Afghanistan and Kazakhstan [14, 30, 37].

**Ixodes (Pholeoixodes) hexagonus** (Leach, 1815)

*Ixodes hexagonus* is associated with hedgehogs, *Erinaceus europaeus* and *E. roumanicus*, as main hosts and the broad spectrum of medium-sized burrow-inhabiting mammals. Apart from hedgehogs, it is commonly found on red foxes and mustelids, including the badger (*Meles meles*), European pine marten (*Martes martes*), stoat (*Mustela erminea*), European polecat (*Mustela putorius*) and occasionally the otter (*Lutra lutra*) [4, 7, 8]; it has also been found on large rodents, including beavers (*Castor fiber*) [30, 37, 39]. The distribution of *I. hexagonus* covers almost the whole of Europe, ranging from the British Islands and Atlantic coast on the west [31], across all the countries in the central Europe to the eastern border of Romania. The tick is common in Austria, the Czech Republic [6, 7, 39], Poland [18, 19, 24, 45], Slovakia [5, 23, 32] and Germany [10, 33, 43]. Filippova [14] did not demonstrate its occurrence to the east of the River Bug. The range extends to southern Scandinavia in the north and the Mediterranean coast in the south, where it is regarded as a rare tick species [12, 14, 37]. Isolated populations have been found in northern Africa and the Middle East [41, 42].

![Fig. 1 Map of Slovakia with marked counties, where the samples were collected](Image)

**Ixodes (Pholeoixodes) kaiseri** (Arthur, 1957)

*Ixodes kaiseri* has a poorly known range. Its described occurrence covers south-eastern Europe from Romania and Moldavia, extends across southern Ukraine to Kazakhstan to the east, and towards Israel and Egypt to the south [13, 14]. Wodecka et al. [44] report the presence of *Pholeoixodes* females, nymphs and larvae from raccoon dogs and badgers, and these were morphologically distinguished from *I. crenulatus* and *I. hexagonus*. An analysis of thirty-four ITS2 sequences revealed high similarity to *I. kaiseri*.

**Ixodes (Pholeoixodes) rugicollis** (Schulze et Schlottke, 1929)

*Ixodes rugicollis* is rarely observed in Europe, with only isolated observations being recorded in localities in France, eastern Germany, Switzerland, Poland and Romania [8, 13, 33, 37, 42]. Ticks (nymphs and adult females) were collected from dogs in the Podkarpackie voivodeship and from badgers in the Dolnośląskie voivodeship in Poland. However, no detail has been provided regarding their exact coordinates and developmental stage [38].

**Materials and Methods**

The present study was conducted in Slovakia during the period 2017–2018, in the regions of Košice, Prešov, Bratislava and Žilina (Fig. 1). Red foxes were legally shot during the monitoring of the effect of oral antirabies vaccination by the State Veterinary and Food Administration of the Slovak Republic. The research was conducted in compliance with the internationally required guidelines under the special permit of the Ministry of Environment of the Slovak Republic. Ectoparasites were collected from animal skin and fur by combing and using tweezers, and preserved in 70% ethanol until further study. The ticks were determined using appropriate keys to
distinguish the central and east-European species by Filippova [14], Nosek and Sixl [29] and Siuda [37].

The structure of tick groups were characterised by the prevalence and intensity of infestation, the factors commonly used in the description of parasitocenoses. However, because there is often no correlation between the intensity of infestation and the incidence of parasites, the index of infection \((Z)\) is additionally used. \(Z\) is calculated according to the formula
\[ Z = \frac{A \times B}{C^2}, \]
where: \(A\) = the number of parasites of the given species, \(B\) = the number of foxes infested with this same parasite species, \(C\) = the number of foxes examined in the sample. The index is able to prove the dominant parasite species, following the influent and accessory species in the grouping [3, 11, 20], independent of the number of hosts and parasite population size.

**Results**

In total, 146 red foxes (Vulpes vulpes) were investigated; 112 from the Prešov region, 9 from the eastern part of the Košice region, 17 from the Bratislava region in western Slovakia, and 8 from the Žilina region in the central northern part of Slovakia. Ticks were found on 39 of the animals tested from the regions: 8 from Žilina, 9 from Košice, 10 from Bratislava, and 12 from Prešov. Five species of ticks were found on the foxes: Ixodes ricinus, Dermacentor reticulatus, Haemaphysalis concinna, Ixodes hexagonus and Ixodes crenulatus (Table 1).

Among them, Pholeoixodes ticks were found on 13 foxes— I. crenulatus on two foxes from Bratislava, one from Košice region and five from Prešov, I. hexagonus on one specimen from Košice region, one from Prešov and three from Žilina. There were some mixed infestations: I. ricinus + I. crenulatus on single specimens from Prešov and from Košice; I. ricinus + I. hexagonus on single specimens from Košice and from Žilina; D. reticulatus + I. hexagonus on one animal from Košice. There were no mixed infestations with I. crenulatus and I. hexagonus.

The analysis of prevalence and index of infection \(Z\) show that the dominant species in all localities was Ixodes ricinus—the prevalence of infestation was 5.4–66.6%, mean 17.2%; the index of infection \(Z\) 0.44–1.40, mean 0.76. The influent species were I. hexagonus and I. crenulatus, the prevalence was \(I. \) hexagonus) 0.9–37.5%, mean 3.4%, \(Z = 0.02–0.14\), mean 0.03, and \(I. \) crenulatus) 4.5–11.1%, mean 5.5%, \(Z = 0.01–0.73\), mean 0.14, respectively. Accessory species were H. concinna and D. reticulatus. H. concinna was present in two localities—Košice and Bratislava, the prevalence was 11.1 and 17.6%, mean 2.8%, \(Z = 0.02\) and 0.84 respectively. D. reticulatus occurred in three localities—Košice, Prešov, Bratislava, the prevalence was 0.9–17.6%, mean 3.4%, \(Z = 0.01–0.05\), mean 0.02.

| Fox sampling areas | Ixodes ricinus | Ixodes crenulatus | Ixodes hexagonus | Haemaphysalis concinna | Dermacentor reticulatus |
|-------------------|----------------|-------------------|------------------|------------------------|-------------------------|
| Košice n=9 (9) 100.0% | 1 F 1 N 0.9% 0.14 | 1 F 1 N 0.9% 0.14 | 1 F 1 N 0.9% 0.14 | 1 | 1 |
| Bratislava n=17 (10) 58.8% | 5 F 2 M 1 N 11.1% 0.11 | 5 F 2 M 1 N 11.1% 0.11 | 5 F 2 M 1 N 11.1% 0.11 | 1 | 1 |
| Prešov n=12 (12) 100.0% | 1 F 1 N 0.9% 0.14 | 1 F 1 N 0.9% 0.14 | 1 F 1 N 0.9% 0.14 | 1 | 1 |
| Žilina n=8 (8) 100.0% | 6 F 2 M 1 N 75.0% 1.22 | 6 F 2 M 1 N 75.0% 1.22 | 6 F 2 M 1 N 75.0% 1.22 | 1 | 1 |
| In total n=146 (39) 26.7% | 25 F 15 M 3 N 26.7% 1.22 | 25 F 15 M 3 N 26.7% 1.22 | 25 F 15 M 3 N 26.7% 1.22 | 5 | 5 |

The number of foxes investigated is provided (n), the number of foxes infested with ticks (1), the number of ticks (2), the prevalence of infestations (3), the index of infection (Z).
Two species of *Pholeoixodes* ticks were identified. These displayed the morphological features typical of the *Pholeoixodes* subgenus and enabled the species identification, i.e., *I. hexagonus* and *I. cenulatus*. All of these features distinguished the investigated ticks in accordance with all used determination keys, i.e., Filippova [14], Nosek and Sixl [29] and Siuda [37]. The most important features are compiled in Table 2. These are the tarsi of the first leg stepped below Haller’s organ, the genital aperture located between coxae 3 and the relatively short capitulum. The *auriculae* size, the length of the *cornua*, and the length of coxae 1 internal spurs distinguished the males and females of these species, while the *cornua* length, the length of coxae 1 internal spurs, and the presence/absence of coxae 2–3 external spurs distinguished the nymphs (Fig. 2). The morphological nomenclature was used according to Estrada-Peña [12].
| Table 2 | The most important morphological features of *I. hexagonus* and *I. crenulatus*, able the species identification (after: 4, 12–14, 16, 19, 37) |
|---------|---------------------------------------------------------------------------------------------------------------------------------|
| **Ixodes hexagonus** | **Ixodes crenulatus** |
| **Female** | **Male** | **Nymph** | **Larvae** | **Female** | **Male** | **Nymph** | **Larvae** |
| **Hypostome** | Narrowed at the apex with a clear indentation at the top | About 2 times shorter than the basis capituli; the widest in the front 1/3 of the length |
| **Basis capituli** | Rectangular; postero-lateral angles slightly salient; ending anterily as cone-like protuberance | With latero-ventral protuberances | Subrectangular dorsally; postero-lateral angles slightly salient; anterofac ending not cone-like |
| **Porose areas** | Presence of double longitudinal ridges between the porose areas |
| **Cornua** | Present | Present | Absent | Absent |
| **Scutum** | Nearly as broad as long | Present | Absent | Absent |
| **Cervical grooves** | Faint, wavy, reaching posterior border | Faint, strongly divergent | Shallow, wavy, reaching the posterior border | Chiefly visible as elongate divergent depressions |
| **Lateral grooves** | Slight ridge on the antero-lateral borders | Short |
| **Coxae I** | Postero-internal spur distinct and sharp-ended | Postero-internal spur well defined | Small and short postero-internal spur | Postero-internal spur relatively short and bluntly angled | Postero-internal spurs at most slightly pointed | Postero-internal spur absent |
| **Coxae II** | Slight protuberances at the postero-lateral angles | Unarmed |
| **Coxae III** | Slight protuberances at the postero-lateral angles | Unarmed |
| **Coxae IV** | Slight protuberances at the postero-lateral angles | Unarmed |
| **Median ventral plate** | About as broad as long | | | | | |
Discussion

There is the first evidence of *Ixodes crenulatus* in Slovakia. This species had not been recorded before the present study; however, its occurrence has previously been noted in Poland [17, 18], Czech [28] and Ukraine [1, 14]. *Ixodes hexagonus* has been identified in various parts of the country, including the Ondavská Highlands and Tribeč Mountain [5, 23, 28], as well as the south-eastern area [22].

The structure of tick groupings on foxes is typical for the European population of this host. The prevalence of infestation is typical and similar to values noted in the same geographic latitude. The available data in Central Europe show the infestation of foxes with *I. ricinus* from 17.9 to 82.2% [21, 36], with *D. reticulatus* from 24.5 to 27% [18, 40], *I. canisuga* from 4.8 to 35.3% [25, 40], *I. crenulatus* 2.9% [18], *I. hexagonus* 1.8 to 37.5% [18, 34].

Index of dominance *Z* demonstrates, that the dominant species is *I. ricinus* usually, subdominants *Pholeoixodes* ticks. Other tick species have the accessory character. However, the status of influent and accessory species was locally differentiated, i.e., index *Z* shows that in Prešov, *I. crenulatus* (*Z* = 0.73) dominates and *I. ricinus* is an influent species. Near Bratislava the influential species is *H. concinna* (*Z* = 0.10), and this same species is an accessory in the Košice locality (*Z* = 0.02); however, the southern locality of Bratislava is the occurrence area of this species. Other investigated localities lie on the border of *H. concinna* occurrence [29, 37]. On all localities, *D. reticulatus* is the accessory species—however, its occurrence in Slovakia has changed during last decades; previously, it was classified as a rare species [2].

A review of available literature indicates a difference of opinion regarding whether two tick species are present, i.e., *Ixodes crenulatus* Koch, 1844 and *Ixodes canisuga* Johnston, 1849, or whether they constitute a single species *Ixodes crenulatus*, and concur with the synonym *Ixodes canisuga*. New publications of Estrada-Peña [13] and Guglielmone [15] have postulated the presence of two species, based on a comprehensive review of descriptions, while authors from eastern and central Europe, notably Filippova [14], Siuda [37] and Nowak-Chmura [30], postulate that *I. canisuga* and *I. crenulatus* are synonymous, i.e., a single species. As mentioned above, *I. crenulatus* has mainly been recorded in Poland and Ukraine, while *I. canisuga* has typically been observed in other European countries.

Two other *Pholeoixodes* species that affect carnivores, *I. kaiseri* and *I. rugicollis*, were not found during the study, and have not been recorded in Slovakia previously. However, according to the literature cited above, their described occurrence range covers Slovakia, and their discovery is only a question of time.

| Table 2 (continued) |
|---------------------|
| *Ixodes hexagonus*  |
| Female | Male | Nymph | Larvae |
| Anal grooves | Pointed in front of anus | Rounded in front of anus |
| *Ixodes crenulatus*  |
| Female | Male | Nymph | Larvae |
| Anal grooves | Pointed in front of anus | Rounded in front of anus |
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Authors contributions GK and MS designed the study and prepared the manuscript draft. GK made the ticks species determination. BV organised material collection and postmortem of foxes; MM and ZH participates in the material collection. All authors reviewed the manuscript and participated in the final version.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest of any sort with anyone.

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