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Short communication

Ixodid ticks on ruminants, with on-host initiated moulting (apolysis) of *Ixodes*, *Haemaphysalis* and *Dermacentor* larvae

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ABSTRACT

To screen the host-dependent abundance of hard tick (Acari: Ixodidae) developmental stages on ruminants in South Hungary, red and roe deer, as well as goats and sheep were examined in a season, when larvae and nymphs are active. Altogether 2271 ticks were collected. In the relevant period the prevalence of tick-infestation was significantly higher among goats, than among sheep kept in the very same area, most likely in association with the browsing habit of the former. Roe deer and goats were found to be important hosts for *Ixodes ricinus* and *Haemaphysalis concinna* larvae, in contrast to the view that this stage does not usually feed on medium-sized mammals. Interestingly, one third of *I. ricinus* larvae and one larva of *H. concinna* and of *Dermacentor reticulatus* collected from goats in the same herd in August have started the moulting process (showed apolysis) on their host, despite being three-host ticks. This is the first survey involving four species of domestic and wild ruminants in Europe to compare the host-preference of ixodid ticks in the same region.

1. Introduction

Hard ticks (Acari: Ixodidae) feed on the blood of vertebrates and are regarded as the most important transmitters of disease agents in the temperate zone (Jongejan and Uilenberg, 2004; Kalluri et al., 2007). In Europe few one- or two-host ticks occur in the Mediterranean region (Estrada-Pena et al., 2004), north of which only three-host ticks are indigenous (Babos, 1964). The latter means that larvae, nymphs and adults will feed on three consecutive hosts, usually on small, then on medium, finally on large-size animals (Mejlon and Jaenson, 1997); and moulting always takes place in the environment. Although not host-specific, each hard tick species and/or developmental stage may have different host preferences (Babos, 1964).

In Europe the comparative importance of ruminants as hosts for ixodid ticks, including their developmental stages, is hardly known. The present survey was undertaken to compensate for this lack of information. With emphasis on subadult ticks, the study was carried out in a period, when larvae and nymphs are active (Hornok and Farkas, 2009). Domestic small ruminants were chosen as target hosts because of their extensive keeping, and on account of their different feeding preference of vegetation (i.e. grazing vs. browsing in case of sheep and goats); utilizing the opportunity that sheep and goats are frequently kept together (Animut and Goetsch, 2008), e.g. female goats to nurture lambs. In addition, red and roe deer represent different size ranges that are important factors influencing tick burdens (Mejlon and Jaenson, 1997). The aim was to collect, analyse and present data that reflect the actual host-preference of tick species and their developmental stages.

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2. Materials and methods

2.1. Sample collection

Ticks were collected during the summer months of 2011, from fresh carcasses of 32 red deer (*Cervus elaphus*) and 35 roe deer (*Capreolus capreolus*) shot in Southern Hungary according to hunting regulations. For comparison, 110 goats and 375 sheep (that were kept together by the same owners, on four places in the relevant region) were also sampled alive at regular (monthly) intervals. The whole skin surface of each animal was carefully examined. All specimens of ticks were removed by forceps and immediately put into 70% ethanol. Identification of species was done according to standard keys (Babos, 1964).

2.2. Data analyses

Exact confidence intervals (CI) for abundance rates (i.e. individuals of one species as a percentage of all ticks from the same host) were calculated according to Sterne's method at the level of 95% (Reiczigel, 2003). Monthly intraspecific proportions of developmental stages and prevalence rates (extensities) of tick-infestation were compared by Fisher's exact test. Differences were regarded significant when $P \leq 0.05$.

3. Results

3.1. Prevalence of tick-infestation

All red and roe deer of the study were found tick-infested. During the consecutive three months (June, July and August) the prevalence of infestation among sheep was 1.6% (6 of 375), 0.3% (1 of 375) and 0.5% (2 of 375), respectively, whereas for goats it was 100%, 23.6% (26 of 110) and 30% (33 of 110), respectively. This means that goats were significantly more frequently tick-infested than sheep grazing the very same area ($P < 0.0001$).

3.2. Abundance and predilection sites of tick species

Altogether 2271 ticks were collected in the study period. On roe deer *Haemaphysalis concinna* predominated (90.9% of all ticks, CI: 89.3–92.3%), whereas on goats *Ixodes ricinus* (90.4% of all ticks, CI: 86.5–93.5%) (Table 1). The proportion of these two species was more equilibrated on red deer (38.7% vs. 61.3%, respectively). Two females of *Dermacentor reticulatus* were also found on sheep in June, and one larva of this species on a goat in August.

I. ricinus subadults were present exclusively on the upper part of the front legs, or in the axillary and inguinal regions. The majority of *H. concinna* individuals were removed from the head of roe deer. As a unique predilection site for this species, nymphs and larvae were attached deeply in the external ear of several animals.

3.3. Host-association of adult ticks

Adults of both *I. ricinus* and *H. concinna* were found during all three months (Table 1). Discounting one nymph, on red deer only adults of *I. ricinus* occurred. Throughout the study period females of *I. ricinus* outnumbered males on all evaluated host species. However, for *H. concinna*, on red deer female ticks, while on roe deer male ticks were consistently more prevalent, which was a significant difference between the two host species ($P < 0.0001$).

3.4. Host-association of nymphs

Nymphs of both *I. ricinus* and *H. concinna* were found during all three months (Table 1). The number of *I. ricinus* nymphs, although relatively low, reached and surpassed the number of adults on both roe deer and goats by August. On these two hosts nymphs of *H. concinna* became more prevalent, than adults already in July, as contrasted to red deer (only in August). In July and August the nymph-to-adult ratio of *H. concinna* was significantly greater on roe deer, than on red deer ($P < 0.0001$).

3.5. Host-association of larvae

Larvae of *I. ricinus* appeared only in July and exclusively on roe deer and goats (Table 1). On the latter host the larva-to-nymph ratio became significantly higher ($P < 0.0001$) by the end of the summer. From July large populations of *H. concinna* larvae were also present on roe deer (Table 1), but their proportion to nymphs did not differ significantly between these two hosts. Interestingly, one third of *I. ricinus* larvae (22 specimens), plus one *H. concinna* and one *D. reticulatus* larva collected from goats in the same herd in August have started the moulting process (showed apolysis) on their host (Fig. 1).

4. Discussion

This is the first survey involving four species of domestic and wild ruminants in Europe to compare the host-preference of ixodid ticks, including their developmental stages, in the same region.

Monthly distribution of engorged ticks in the present study corresponded to previous observations on questing individuals of relevant species (Hornok, 2009). Attachment sites for *H. concinna* on roe deer were consistent with those already reported for *Ixodes* ticks (Kiffner et al., 2011), except for the location of subadults in the external ear as a novel finding. Predilection sites of *I. ricinus* nymphs and larvae on goats indicated that immature stages most likely remain in association with the legs.

The predominance of female *I. ricinus* on all hosts, as well as male *H. concinna* on roe deer also reflected the pattern among questing individuals of these species (Hornok and Farkas, 2009). However, on red deer females of *H. concinna* were more frequently found, than males. This inconsistency of engorged tick sex ratio between hosts is similar to the one described for Haemaphysalinae ticks in a previous study (Stich et al., 2008), and – together with different abundance rates of ixodid species on red and roe deer – may depend on their habitat (Prokešová et al., 2006).

With relevance to factors that may influence the role of ruminants as hosts for ixodid ticks within the same habitat, sheep and goats were compared, since they are frequently

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Table 1
Monthly distribution of *Ixodes ricinus* (Ir) and *Haemaphysalis concinna* (Hc) larvae (L), nymphs (N) and adults according to the four hosts. Data are presented to show the overall importance of ruminant species as hosts, excluding individual intensities of tick-infestation.

		June					July					August				
		Total	L	N	♂	♀	Total	L	N	♂	♀	Total	L	N	♂	♀
Red deer	Ir	232	–	1	35	196	5	–	–	–	5	72	–	–	17	55
	Hc	156	1	7	42	106	29	–	3	5	21	10	–	8	2	–
Roe deer	Ir	93	–	5	20	68	24	3	5	7	9	15	–	8	3	4
	Hc	27	–	–	18	9	818	84	636	66	32	467	224	235	5	3
Goats	Ir	146	–	9	23	114	26	10	4	–	12	100	65	19	–	16
	Hc	2	–	–	1	1	7	1	6	–	–	20	9	11	–	–
Sheep	Ir	12	–	–	–	12	1	–	1	–	–	2	–	–	–	2
	Hc	4	–	–	1	3	–	–	–	–	–	–	–	–	–	–

kept together (Animut and Goetsch, 2008). Throughout the study period significantly more goats harboured ticks, than sheep in the same pasture. This was probably due to differences between their feeding preferences (i.e. contact with vegetation type): sheep like to graze the middle of pastures with short grass, whereas goats tend to browse along the margins, going into dense shrubs and eating leaves from higher. Therefore the latter have more opportunities to get infested with ixodid species – as exemplified by questing forest ticks, *I. ricinus* and *H. concinna* (Uspensky, 2002) – that are associated with lower vegetation around bushes and trees.

Occurrence of developmental stages on the host are also determined by contacts with the vegetation, because larvae, nymphs and adults of hard ticks are known to quest at different levels above the ground (Mejlon and Jaenson, 1997). This may explain why *I. ricinus* adults were highly prevalent on red deer, and nymphs of *H. concinna* on roe deer during this survey. On the other hand, due to questing of larvae at low level, only small mammals are regarded as important hosts for this stage according to the so-called "host size-dependent questing strategy hypothesis" (Tälleklint and Jaenson, 1994). Contrarily, based on the above data, roe deer and goats as medium-sized mammals appear to be important hosts for *H. concinna* and *I. ricinus* larvae, respectively. Thus, for these two tick species, the theory should be carefully interpreted. Although it is true that climbing of adults (nymphs) higher on the vegetation confines their occurrence to medium or large mammals, but – since legs of any size animals can pick up ticks even from the ground level – host size-restriction is less evident for larvae.

Regarding further development of ixodid larvae, for three-host ticks (including *I. ricinus*, *H. concinna* and *D. reticulatus*) it is essential that they moult to nymphs after an incubation period following detachment (Fourie et al., 2001), i.e. only in the environment. For this reason it was unexpected to find in the present study larvae of the above three species that initiated moulting (showed apolysis) on goats, although this occurred only in one herd and only in August. Since the facultative two- or three-host life cycle is known to be exclusive for the genus *Hyalomma* (Alahmed and Kheir, 2003), another explanation appears to be more plausible for this unusual phenomenon. In particular, relevant animals were kept in an area with meadows rich in *Serratula* spp. These plants have poor forage quality and are eaten only by goats (Corio-Costet et al., 1996), but contain high amounts of phytoecdysteroids (Adler and Grebenok, 1995; Báthori et al., 1990). Such ecdysteroids were shown to promote apolysis and moulting in vitro (Rees, 2004), but not yet in vivo – when ticks ingest blood meal from animals feeding on the plants. However, this assumption seems likely, because ecdysteroid concentrations in *Serratula* spp. peak during blossoming, i.e. in August (Báthori et al., 2000), when on-host apolysis was noted in the present study. Nevertheless, the role of plant-derived hormones in the on-host initiated moulting (apolysis) of three-host ticks needs to be confirmed with experimentation.

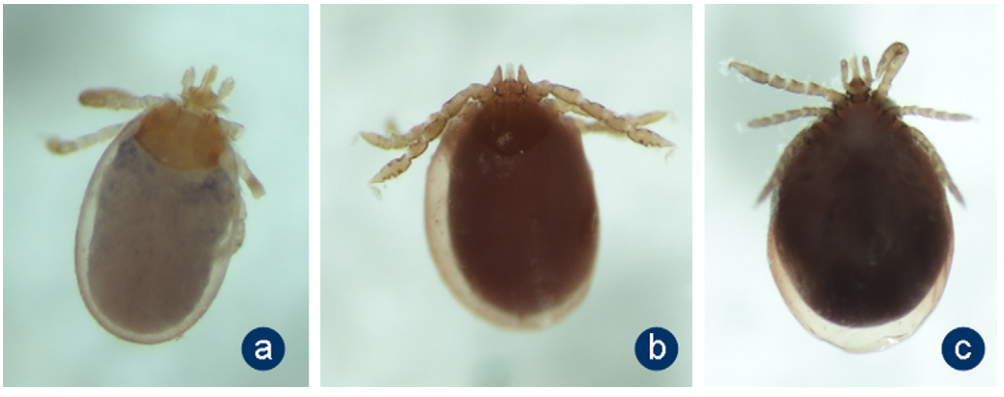

Fig. 1. Larva of (a) *Dermacentor reticulatus*, (b) *Haemaphysalis concinna* and (c) *Ixodes ricinus* which initiated moulting (with apolysis) on goats.

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In conclusion, this is the first report to indicate that the moulting process of three-host ticks can be initiated naturally on the host. Medium-sized ruminants – both sylvatic and domestic – were demonstrated to be important hosts for subadult ixodid ticks, depending on their habitat and/or foraging behaviour. Therefore these results have epidemiological significance from the point of view of tick-borne diseases, i.e. the known stage-dependent (transstadial–transovarial) transmission of pathogens.

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