Ixodes persulcatus [Schulze 1930] (Acari: Ixodidae) in eastern Finland

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Data concerning the occurrence and abundance of the taiga tick *Ixodes persulcatus* in eastern Finland (North Karelia and Kainuu regions) are presented. Sampling was carried out in May 2008 and June 2009, around the University of Joensuu Mekrijärvi Research Station and the City of Kuhmo. In 2008 and 2009, the abundance of adult taiga ticks in the first study area was 0.17 and 0.13 ticks per flag-km, respectively. Only a single *I. persulcatus* specimen was found in the second study area (abundance was 0.02 specimens per flag-km). All ticks (635 specimens) collected from cats and dogs in south-eastern Finland were identified as *Ixodes ricinus*.

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1. Introduction

Changes in the range of natural focus infections, including infections transmitted by ixodid ticks, is a "hot" topic in recent discussions on emerging infectious diseases (Lindgren *et al.* 2000, Randolph 2000, Broker & Gniel 2003, Zeman & Benes 2003, Sumilo *et al.* 2006, Eisen 2008, Gray *et al.* 2009). The main reason for this interest is the growing abundance and distribution of *Ixodes* ticks – the main vectors of tick-borne encephalitis (TBE) and Lyme borreliosis.

In the 1950's, the northwestern limit of the range of *Ixodes persulcatus* ran across Russian

Karelia connecting the points N 63°15 E 33°15 and N 61°15 E 31°55 (Lutta & Shulman 1954). Later (1960's–70's), the species was also noted on the NW shore of Lake Ladoga near the city of Sortavala (Korenberg 1985). More recently there have been several records of the ticks from the Kokkola Archipelago in western Finland (Jääskeläinen *et al.* 2006), which is still further north from the long-standing NW limit of its distribution. Furthermore, the abundance of the taiga tick within its historical range in Russian Karelia has increased markedly over the past few decades (Bespyatova *et al.* 2006). In this study we investigate the species composition and abun-

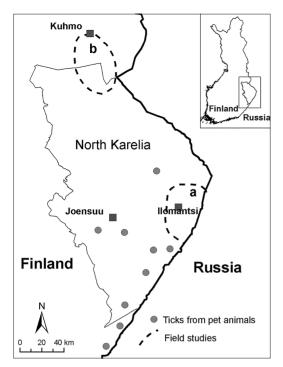


Fig. 1. Field study areas. Mekrijärvi Research Station and its surroundings. – a. 20–30 May 2008 and 3–8 June 2009; Kuhmo area (Friendship Park). – b. 9–13 June 2009. Separate dots denote sites where ticks were collected from dogs and cats.

dance of ticks in eastern Finland near the Russian border to obtain base-line data on the abundance of *Ixodes persulcatus* and *I. ricinus* in this area.

2. Material and methods

Tick sampling took place in East Finland (Northern Karelia and Kainuu regions), around the University of Joensuu Mekrijärvi Research Station and the City of Kuhmo (Fig. 1). The surveys were carried out in May 2008 and in June 2009.

Tick abundance was estimated using standard methods. Adults and nymphs were collected by flagging (a square of white waffle cotton cloth $(0.7 \times 1.1 \text{ m})$ is pulled along the ground and over vegetation), and the abundance was converted to "ticks per 1 flag-kilometer". Around Mekrijärvi, 160 and 75 flag-km were covered in 2008 and 2009 respectively. About 100 transects of varying length were covered. Within Kuhmo (2009 only), 44 flag-km were sampled in 28 transects. Alto-

gether, 20 male ticks 19 females and 4 nymphs were collected in the study period. The ticks were kept in moist bandage wrapped in foil or plastic bags and stored at $+4^{\circ}$ C before identification.

Additional samples of adult ticks were collected from cats and dogs during June–September 2008 with the help of pet owners in Liperi, Parikkala, Pyhäselkä, Uukuniemi, Kesälahti, Tohmajärvi, Värtsilä, Kitee and various parts of Joensuu town. Ticks collected from these animals were fixed in 70% ethanol (with a collecting information label). A total of 635 ticks (192 male, 443 female) were collected from pets.

The abundance of tick larvae and nymphs was estimated from the infestation rate on small mammals captured in traps. In total, 32 transects with 25-50 traps each were arranged and exposed during 2-5 nights, amounting in total to 3,525 trapnights (2,925 in Mekrijärvi and 600 in Kuhmo). We captured 45 small mammals (43 of them (from Mekrijärvi) belonging to three species: common shrew Sorex araneus Linnaeus, 1758 (16 specimens), bank vole Myodes glareolus (Schreber, 1780) (24 specimens) and field vole Microtus agrestis (Linnaeus, 1761) (1 specimen). These yielded 13 nymphs and 16 larvae of Ixodes. The tick infestation rate of small mammals was calculated as prevalence (Pr) – the percentage of infected hosts, and as abundance index (Ab) mean abundance of Ixodes per small mammal (Margolis et al. 1982). The larvae and nymphs were fixed in 70% ethanol. For further identification, temporary mounts with Fora-Berlese medium were prepared. The tick species were identified using morphology, following Filippova (1977).

Taxonomic identity of the ticks in 2 pools of 3 and 4 ticks (collected by flagging) and 5 individual ticks (collected from pets) was confirmed by sequencing part of ITS2 rRNA. For this purpose the ticks were pre-treated by rinsing with 96% ethanol and twice with physiological NaCl solution. The ticks were homogenized in 200 μ l of medium 199 on Earle's solution (PIPVE, Russia). Nucleic acids (both RNA and DNA) were isolated using a standard kit protocol ("RNA/DNA extraction kit for blood serum or plasma samples" Lytech, Russia). ITS2 rRNA was amplified by PCR with primers 3SA and JB9A (Barker 1998). This method is originally to determine rhipi-

| Site | No. of collectors (pet owners) of ticks | No. of ticks | | |
|------------|--|--------------|-------|--|
| | | Females | Males | |
| Patvinsuo | 1 | 1 | 0 | |
| Joensuu | 7 | 103 | 26 | |
| Liperi | 1 | 2 | 2 | |
| Pyhäselkä | 1 | 6 | 2 | |
| Tohmajärvi | 7 | 159 | 70 | |
| Värtsilä | 2 | 14 | 23 | |
| Kitee | 18 | 119 | 49 | |
| Kesälahti | 4 | 17 | 4 | |
| Uukuniemi | 1 | 22 | 7 | |
| Parikkala | 1 | 0 | 9 | |
| Total no. | | 443 | 192 | |

Table 1. *Ixodes ricinus* collected from cats and dogs from various Finnish municipalities along the western side of the Finnish-Russian border in 2008.

cephaline ticks. However, it has also been used to estimate the phyletic relationships among *Ixodes* spp. (Fukunaga *et al.* 2000).

To determine the infection rates of *Borrelia burgdorferi* (*sensu lato*) and tick-borne encephalitis virus in the ticks RNA was reversely transcribed using M-MLV reverse transcriptase kit (Promega, USA) and random hexanucleotides (Syntol, Russia), according to the manufacturer's protocol. The 16S rRNA of the *Borrelia burgdorferi* (*sensu lato*) complex was identified using PCR with the LD primer set of Marconi & Garon (1992). The presence of TBE virus was demonstrated using primers specific to NS5 regions of the TBEV genome: TBEL1 (5'-TCT-GAG-GGA-GAC-ACA-CTT-GG-3') and Kgg57 (5'-TGC-TGA-ACA-CAT-CCA-TTC-C-3').

The ticks in 5 pools of 2–4 ticks (13 male, 2 female) and 9 individual ticks (3 male, 6 female) collected by flagging in Mekrijärvi were tested by this method.

3. Results

All tick larvae and nymphs collected from small mammals from the studied areas belonged to two species *I. persulcatus* Schulze, 1930 (10 larvae, 2 nymphs) and *I. trianguliceps* Birula, 1895 (6 larvae, 11 nymphs). These were found only at the

Table 2. Numbers, prevalence and abundance of *Ixodes persulcatus* larvae and nymphs on small mammals in Mekrijärvi area.

| | No. | Pr% | Ab | | | |
|-------------------------------------|-------------|-------------|------|--|--|--|
| Common shrew S | Sorex arane | us (n = 16) | | | | |
| Larvae | 8 | 6.2 | 0.5 | | | |
| Bank vole Myodes glareolus (n = 26) | | | | | | |
| Larvae | 2 | 7.7 | 0.1 | | | |
| Nymphs | 2 | 7.7 | 0.1 | | | |
| Total | | | | | | |
| Larvae | 10 | 7.1 | 0.2 | | | |
| Nymphs | 2 | 4.8 | 0.05 | | | |
| | | | | | | |

No. = Number of ticks

n = Number of small mammals

Pr% = Prevalence: percentage of infected hosts

Ab = Abundance index: mean abundance of *I. persulcatus* per small mammal

Mekrijärvi site. These equated to a prevalence (and abundance) of *I. persulcatus* of 7.1% (0.2 larvae per rodent) and 4.8% (0.05 nymphs per rodent) for larvae and nymphs respectively (Table 2). At the other site (Kuhmo), only two small mammals were captured (a bank and a field vole), and no ticks were found on them.

All adult ticks (39 specimens) collected by flagging were identified as *I. persulcatus*. For 2 tick pools (of 3 and 4 ticks) identification by morphological criteria was confirmed by sequencing part ITS2 rRNA (GenBank HM055905, HM072039). The obtained sequence was clear enough to rule out the possibility of having several species in those two pools. The only specimen of *I. ricinus* (Linnaeus, 1758) identified was a nymph found in the south of the study area (N62°18.3; E30°48.9).

In 2008 and 2009, the abundance of adult taiga ticks in the first study polygon (Fig. 1a) was 0.17 and 0.13 ticks per flag-km, respectively, and *I. persulcatus* was found in 11 of 96 transects surveyed (Table 3). All of the finds were located in the immediate vicinity of settlements (or abandoned hamlets), and this represents the most typical habitat of this species. Mean abundance of adult taiga ticks in these transects was 2.6 and 0.8 ticks per flag-km in 2008 and 2009, respectively.

The only *I. persulcatus* specimen (female) in the second polygon (Fig. 1b) was found in one

| Site id. | Coordinate | 2008 | | 2009 | |
|----------|----------------------|--|---|-------------------------------------|--|
| | | No. of ticks (males/females/ nymphs) | No. of ticks per flag-km (males/females/ nymphs) | No. of ticks (males/ females) | No. of ticks per flag-km (males/ females) |
| Mekrijär | rvi (a in Fig. 1) | | | | |
| 1 | N62°42' E31°21.8' | 3/2/0 | 1.1/0.8/0 | 1/0 | 0.7/0 |
| 2 | N62°40.2' E31°19.9' | 3/4/0 | 4.7/6.2/0 | 0 | 0 |
| 3 | N62°50.2' E31°16.7' | 0/0/2 | 0/0/0.6 | _ | _ |
| 4 | N62°51.9' E31°29.4' | 2/3/0 | 0.6/1.0/0 | 1/1 | 0.6/0.6 |
| 5 | N62°46' E30°59.3' | 2/0/1 | 0.2/0/0.1 | 1/1 | 0.1/0.1 |
| 6 | N62°50.5' E30°46.0' | 3/1/0 | 1.4/0.5/0 | 1/1 | 0.6/0.6 |
| 7 | N62°49.8' E30°41.3' | 0/3/0 | 0/1.4/0 | 0/1 | 0/0.6 |
| 8 | N62°39' E31°01.2' | 1/0/0 | 0.2/0/0 | - | - |
| 9 | N62°42.9' E30°35.8' | _ | _ | 1/0 | 0.7/0 |
| 10 | N63°11' E31°01.6' | _ | _ | 0/1 | 0/0.5 |
| 11 | N63°16.9' E30°36.4' | - | - | 1/0 | 0.7/0 |
| Kuhmo | (b in Fig. 1) | | | | |
| 12 | N63°57.1'; E29°43.2' | - | - | 0/1 | 0/0.7 |

Table 3. Abundance of *Ixodes persulcatus* in twelve study locations in south-eastern Finland. Dashes = not studied.

 $(N63^{\circ}57.1; E29^{\circ}43.2)$ of the 28 transects surveyed in 2009. This equated to an abundance of 0.02 ticks per flag-km.

The ticks collected from cats and dogs from various parts of North Karelia in 2008 (Table 1) were all identified as *I. ricinus* by morphological criteria. However, it was not possible to identify to species 5 specimens by morphological criteria. Partial sequencing of ITS2 rRNA identified these ticks as *I. ricinus* (GenBank HM055901-HM055904, HM208308).

No *Borrelia burgdorferi* (sensu lato) complex spirochaetes or TBE virus-infected ticks were detected. We were somewhat surprised with the result that no *Borrelia* was found in ticks examined by us, because it is usually found in the area (Bugmyrin *et al.* 2008). But we do not have bases to doubt the correctness of the technique, as the material was processed not as a separate sample but in parallel with ticks from other regions (the Moscow region, Karelia) for which positive results were received. TBE virus, on the other hand, is usually found only in low frequency (Jääskeläinen *et al.* 2010). Accordingly, our negative result for it in total of 29 ticks was not unexpected.

4. Discussion

The first conclusion from this study is that viable *I. persulcatus* populations exist in eastern Finland. The evidence for this is the number of positive transects, the repeated recording of ticks in the transects, and the presence of all development stages. The specific identity of ticks was determined not only by morphological criteria, but was confirmed by genotyping.

Most taiga ticks were found in the area between N 63° in the north and N 62° 30 in the south. The northernmost finding of *I. persulcatus* was from a site 60 km south of Kuhmo (Table 3), suggesting that more sampling is necessary to define the northern border of *I. persulcatus* distribution in eastern Finland.

The estimated abundances of taiga ticks in the studied area are very low for all stages – much lower than in central and eastern Karelia (Russia). For example, in some parts of Russian Karelia the abundance of adult ticks *I. persulcatus* in 2008 was greater than 20 ticks per flag-km (Bugmyrin *et al.* 2008), while in the Mekrijärvi area the abundance was two orders of magnitude lower – 0.17 in 2008 and 0.13 in 2009. The lower abundance in 2009, estimated using monitoring sur-

veys (Table 2), could have been the result of a slightly later survey (2 weeks later in the season) or of unfavourable weather conditions with rain.

The distribution of *I. persulcatus* was confined to areas close to the Finnish-Russian border and the species was not found in samples provided by pet owners from villages and towns. The areas where people live are dry, bushy and overgrown, and less favourable for *I. persulcatus*. On the other hand, these territories with a high anthropogenic influence are typical habitat of *I. ricinus* in Russian Karelia (Lutta *et al.* 1959).

We suspect that human exploitation and landuse structure restrict the distribution and abundance of I. persulcatus in the eastern Finnish landscape. Forests are intensively managed in Finland and their structure is quite different in Russian and Finnish Karelia. Mires and moist forests have practically all been drained in Finland. Both intensive management resulting into sparse stands, and well-maintained drainage decrease favourable habitats for I. persulcatus. So far it has been found at very low abundance and little can be said about its population dynamics, although the population in the Ilomantsi area seems to be well established. Further studies are needed concerning the life-cycle and hosts, and the abundance of I. persulcatus in different biotopes.

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