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Original article

# Comparative analysis of the roles of *Ixodes persulcatus* and *I. trianguliceps* ticks in natural foci of ixodid tick-borne borrelioses in the Middle Urals, Russia

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ABSTRACT

Long-term studies on natural foci of ixodid tick-borne borrelioses (ITBB) have been performed in Chusovskoi district of Perm region, the Middle Urals, where the vectors of these infections are represented by two ixodid tick species: the taiga tick *Ixodes persulcatus* and many times less abundant vole tick *I. trianguliceps*. Over 10 years, more than 6000 half-engorged ticks were collected from small forest mammals using the standard procedure, and 1027 *I. persulcatus* and 1142 *I. trianguliceps* ticks, individually or in pools, were used to inoculate BSK-2 medium. As a result, 199 *Borrelia* isolates were obtained. Among them, 177 isolates were identified, and the *rrf*(5S)–*rrl*(23S) intergenic spacer sequence was determined in 57 isolates. The prevalence of *Borrelia* infection in *I. persulcatus* larvae and nymphs averaged 31.0 and 53.3%, while that in *I. trianguliceps* larvae, nymphs, and adult ticks was five to ten times lower: 2.6, 10.2, and 8.1%, respectively. Each of the two tick species was found to carry both ITBB agents circulating in the Middle Ural foci (*Borrelia garinii* and *B. afzelii*), but the set of genogroups and genovariants of these spirochetes in *I. trianguliceps* proved to be far less diverse. According to the available data, this tick, compared to *I. persulcatus*, is generally less susceptible to *Borrelia* infection (especially by *B. afzelii*). Taking into account of its relatively low abundance, it appears that *I. trianguliceps* cannot seriously influence the course of epizootic process in ITBB foci of the study region, whereas highly abundant *I. persulcatus* with the high level of *Borrelia* infection is obviously a key component of these parasitic systems. A similar situation may well be typical for the entire geographic range shared by the two tick species.



## Introduction

The taiga tick *Ixodes persulcatus* and the vole tick *Ixodes trianguliceps* are the most widespread ixodid tick species in the forest zone of Eurasia. The former is the main vector for the majority of tick-borne infections occurring in the Eurasian temperate belt (Korenberg et al., 2013). The latter is a three-host parasite that feeds mainly on small mammals at the larval, nymph, and adult stages (Filippova, 1977, 2010). This tick does not attack humans but is involved in the circulation of pathogens in the natural foci of no less than seven infections (Kovalevskii and Nefedova, 2013). The range of *I. persulcatus* spreads from the western to eastern border of Russia, in places extending to the territories of neighboring countries (Korenberg et al., 1969; Korenberg, 1979), and that of *I. trianguliceps* covers the area from the British Isles to Lake Baikal (Korenberg and Lebedeva, 1969). Thus, the ranges of these ticks almost completely lie within the part of Eurasia known to be endemic for ixodid tick-borne borrelioses (ITBB). Many natural ITBB foci in European Russia and Siberia are inhabited by both *I. persulcatus* and *I. trianguliceps*. The parasitic systems of these foci are usually formed by *Borrelia garinii* and *B. afzelii*, which are transmitted to humans mainly by adult *I. persulcatus* ticks (Korenberg et al., 2013). The fact of their spontaneous infection by *Borrelia* was first revealed when analyzing unfed adult ticks collected in the northwestern and eastern part of the species range in 1986–1987. The circulation of *B. garinii* and *B. afzelii* in populations of this vector was subsequently confirmed by hundreds of isolates from almost throughout its range (Korenberg et al., 2002a). Pathogenic *Borrelia* from *I. trianguliceps* were first isolated in 1994. In particular, several *B. garinii* isolates were obtained from nymphs collected on small mammals in the east of Perm region, in forests of the Middle Urals (Gorelova et al., 1996), where *Borrelia* had already been detected by that time in adult *I. persulcatus* ticks (Korenberg et al., 1993). In subsequent years, we continued large-scale bacteriological

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examination of *I. persulcatus* and *I. trianguliceps* from this region, along with studies on the basic aspects of their ecology that determine the significance of these tick species for maintaining *Borrelia* circulation among small mammals, the main reservoir hosts of ITBB agents.

The results have shown that human-pathogenic *B. garinii* and *B. afzelii* simultaneously circulate in the region, each of them being represented by two genetic subgroups described previously (Postic et al., 1994; Masuzawa et al., 1996): NT29, 20047 (*B. garinii*) and VS461, NT28 (*B. afzelii*). Infection by these spirochetes has been detected in eight species of small rodents and insectivores, with its overall prevalence exceeding 15%. Neither any significant differences in host specificity between these *Borrelia* genospecies (Korenberg et al., 2002a) nor temporal variation in their relative abundance have been revealed.

The prevalence of *Borrelia* infection in unfed adult *I. persulcatus* ticks varies between years from about 30 to 60%, and the density (absolute numbers) of infected ticks is usually several hundred or, in some years, over a thousand individuals per hectare. Among ixodid ticks, only *I. trianguliceps* occurs in the region together with the taiga tick. *Ixodes trianguliceps* in forests of the Middle Urals is approximately five times less abundant than *I. persulcatus*, with its ecology being similar in some aspects to that of preimaginal life stages of the latter species (Kovalevskii et al., 2013). Although the seasonal activity pattern in *I. trianguliceps* is bimodal, unlike in *I. persulcatus*, its major peak in early summer coincides with the single seasonal peak of abundance of *I. persulcatus* larvae and nymphs. The two tick species inhabit all basic types of forest biotopes and parasitize all 19 small mammal species inhabiting the study region. The range of main hosts is similar for both species, especially in case of larvae. In *I. persulcatus*, it includes the most abundant representatives of small forest mammals, the bank vole (*Myodes glareolus*) and common shrew (*Sorex araneus*). The same species, together with the northern red-backed vole (*M. rutilus*), are food hosts for the majority of *I. trianguliceps* ticks. Both tick species prefer mature male hosts. They readily feed together on the same individuals, and no apparent signs of competition between them have been revealed. At the common peak of tick abundance in early summer, more than one-third of small mammals examined (35.2%) proved to be infested by *I. persulcatus* and *I. trianguliceps* ticks simultaneously, and most of these animals belonged to the aforementioned main host species with a proven status of *Borrelia* carriers in the study region (Gorelova et al., 1995; Korenberg et al., 2002a, 2011a). All this is evidence that ecological prerequisites for the involvement of ticks in the process of *Borrelia* circulation in natural ITBB foci of the Middle Urals are equally favorable for *I. persulcatus* and *I. trianguliceps.* The main purpose of this study was to analyze to what extent these prerequisites are implemented and to evaluate the actual roles of the two tick species in the functioning of natural ITBB foci. This is the first attempt to analyze the results of our long-term research from such an aspect, and no comparative data of this kind have been published previously.

## Materials and methods

### *Study area*

Our long-term studies (1994–2003) have been performed in the surroundings of the village of Mys (Chusovskoi district, Perm region, Russia; 58°33′ N, 57°28′ E). The study region, located in the low mountains of the Middle Urals, is characterized by the prevalence of southern taiga ecosystems with almost ubiquitous distribution of *I. persulcatus* tick and active epidemic manifestations of ITBB foci (Alypova et al., 2002). The etiological and biocenotic structure of these foci and specific features in the dynamics of the epizootic process in them have been described in detail previously (Korenberg et al., 2002a, 2002b, 2011a, 2011b; Kovalevskii et al., 2004).

### *Collection of ticks*

Ticks were collected in May to August from small forest mammals trapped with Sherman's live traps and anesthetized with ether. The traps were set as described (Kovalevskii et al., 2013). Feeding ticks were carefully removed with forceps, examined to determine the species and life stage of each individual, and, when necessary, stored live at 4 °C for no more than 1–2 days prior to isolating *Borrelia.*

### *Spirochete isolation*

Ticks were washed in two portions of 70% ethyl alcohol and placed on a glass slide in a drop of sterile saline for dissection. In larvae, the gnathosoma and legs were removed, and the body was placed in a plastic tube with 2 mL of BSK-2 medium (Sigma, United States). In nymphs and adult ticks, the complex of internal organs was isolated and placed in the same volume of the medium. The tubes were sealed and incubated at 32 °C for 1–2 months, with weekly screening under a dark field microscope. Positive cultures were transferred to new tubes with fresh BSK-2 medium (8 mL) and grown at 32 °C for up to several weeks (Gorelova et al., 1996; Postic et al., 1997).

### *PCR–RFLP analysis*

The isolates were identified by PCR–RFLP analysis of the *rrf* (5S)–*rrl* (23S) rRNA intergenic spacer, as described (Postic et al., 1994, 1997). The results were compared with previous data on identification of *Borrelia* isolates from the internal organs and skin biopsies of small forest mammals trapped in the same area (Korenberg et al., 2006). All identified isolates are stored in the *Borrelia* culture collection at the Vector Laboratory of the Gamaleya Research Institute of Epidemiology and Microbiology (Korenberg et al., 2006).

### *DNA sequencing*

The *rrf–rrl* spacer in 57 isolates from *I. persulcatus* and *I. trianguliceps* ticks was PCR-amplified with primers previously designed for this purpose (Derdakova et al., 2003), and the resulting fragments (245–271 bp) were sequenced (Nefedova et al., 2005). Each genetic subgroup of *B. garinii* or *B. afzelii* in the study region was represented by several genovariants slightly differing in the *rrf–rrl* spacer sequence (Korenberg et al., 2011b). The spacer sequences obtained for 16 isolates were deposited in GenBank under accession nos. AM748060, AY772048–AY772051, AY772054, and AY772055 (isolates from *I. persulcatus*) and AM748054, AM748058, AM748059, AY772197–AY772201, and AY772206 (from *I. trianguliceps*).

### *Statistical analysis*

In statistical analysis, a 0.95 level of significance was used. For parameters expressed in percent values ($P$), standard error ($m_p$) and confidence interval ($2\ m_p$) were calculated. Differences between test parameters were evaluated using Student's $t$-test ($t$). The strength of correlation was measured with Pearson's $r$ coefficient.