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Original article

Ixodes ricinus ticks (Acari, Ixodidae) as a vector of *Borrelia burgdorferi* sensu lato and *Borrelia miyamotoi* in Lower Silesia, Poland – Preliminary study

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ABSTRACT

Ixodes ricinus is the primary vector of *Borrelia* spirochetes in Europe, including both the Lyme borreliosis (LB) group and the relapsing fever (RF) group. The aim of the study was to estimate the prevalence of different genospecies from the *B. burgdorferi* s.l. complex and *B. miyamotoi* in questing *I. ricinus* collected in chosen areas in Lower Silesia, SW Poland. A total of 599 *I. ricinus* ticks were investigated using the PCR-RFLP method. The calculated overall minimum infection rate of ticks with *Borrelia* spirochetes in Lower Silesia was 15.5%. Five different restriction patterns, characteristic of *B. afzelii*, *B. garinii*, *B. burgdoreri* s.s., *B. valaisiana*, and *B. miyamotoi*, were obtained and confirmed by DNA sequencing. At least 14% of ticks were infected with *B. burgdorferi* s.l. while *B.afzelii* was the dominant genospecies (68.5%). The MIR for *B. miyamotoi* was calculated at 2%. Four co-infections in single adult ticks were found: *B. miyamotoi*/*B. afzelii*, *B. miyamotoi*/*B. burdorferi* s.s., *B. miyamotoi*/*B. garinii*, and *B. afzelii*/*B. burgdorferi* s.s. The results of this study confirm the risk of LB and RF occuring in both urban and protected areas.

Introduction

Ixodes ricinus, the most important tick species in Europe from a medical and veterinary point of view, is a primary vector of different pathogenic bacteria (including spirochetes *Borrelia* spp., rickettsiae *Anaplasma phagocytophilum*, *Rickettsia* spp. of the spotted fever group [SFG].), viral agents (tick-borne encephalitis viruses—TBEV), and piroplasms (*Babesia* spp., *Theileria* spp.). Of these, *Borrelia* spirochetes, mainly *B. burgdorferi* sensu lato (s.l.), are the most commonly identified pathogens in *I. ricinus*. However, tick-borne *Borrelia* spp. are clustered into three major phylogenetic groups, i.e., the Lyme borreliosis (LB) group, the relapsing fever (RF) group, and the reptile-associated borreliae (Franke et al., 2013). Spirochetes belonging to the first group are transmitted by hard ticks of the genus *Ixodes*, and mainly by *I. ricinus* in Europe, which cause the most common tick-borne disease in the Northern Hemisphere. Lyme borreliosis is a widely distributed zoonosis recorded in the United States and nearly all European countries including Poland. In Poland, over nine thousand (23.6/100,000) human cases of LB were noted in 2010 (www.pzh.gov.pl). The majority were reported in northeastern Poland but the whole country can be considered an endemic area. During 2010, about 560 cases (19.5/100,000) were recorded in Lower Silesia. At present, borreliae from the LB group constitute at least 19 genospecies included in the *B. burgdorferi* sensu lato complex (Rizzoli et al., 2011). Of them, in Europe, *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto (s.s.), *B. bavariensis* (formerly *B. garinii* OspA serotype 4), and *B. spielmanii* are known to cause disease in humans while the pathogenicity of others, such as *B. lusitaniae*, *B. valaisiana*, and *B. bissetii* remains uncertain, although they have also recently been reported to cause diseases (Gern et al., 2010; Rizzoli et al., 2011). Six species of *B. burgdorferi* s.l. complex have so far been identified in Poland in *I. ricinus* ticks: *B. afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. lusitaniae*, *B. valaisiana*, and *B. bavariensis* (Wodecka, 2011). Their distribution, however, is known only fragmentarily.

Spirochetes of the second group belonging to the genus *Borrelia*, responsible for RF infections, are transmitted by lice bites (louse-borne RF), like *B. recurrentis* which is spread from person to person *via* the human body louse (*Pediculus humanus corporis*) or ticks (tick-borne RF). Tick-borne RF infections, characterized

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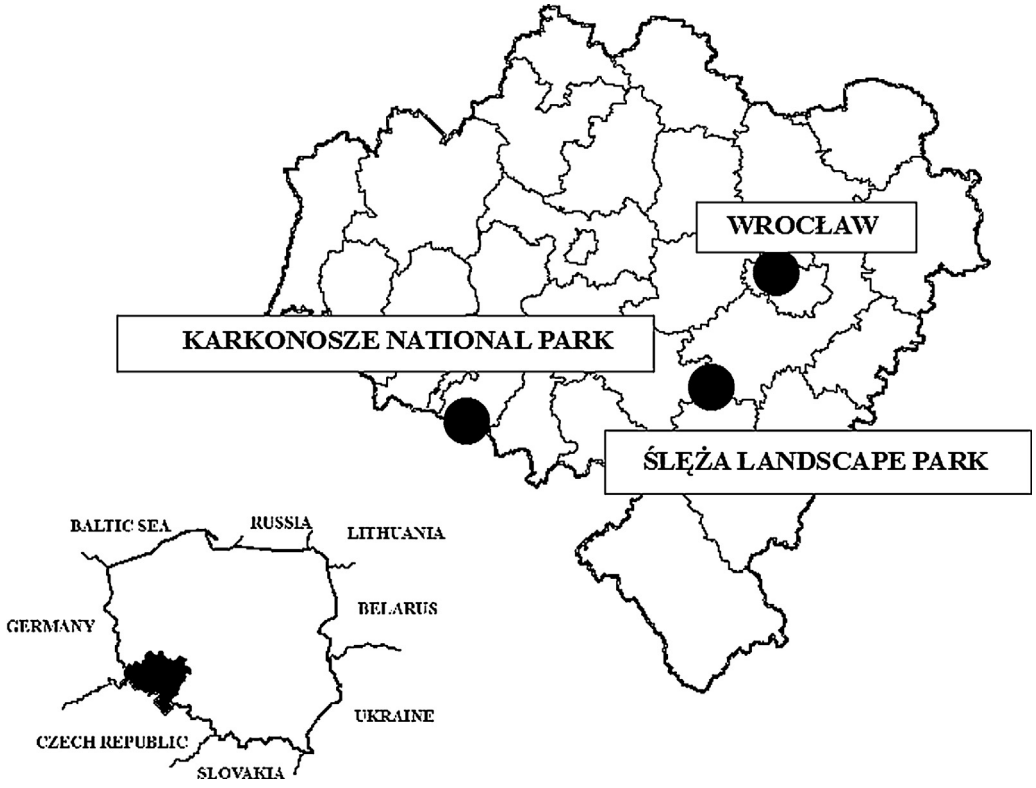

Fig. 1. Tick collecting habitats in Lower Silesia in 2009.

by an influenza-like illness with periodic febrile episodes as the main symptom, are mainly transmitted by soft ticks (of the family Argasidae) of the genus *Ornithodoros* (Fraenkel et al., 2002). The RF group includes several species, such as *B. hermsii*, *B. coriaceae*, *B. turicatae*, *B. parkeri* in North America, *B. crocidurae*, *B. duttonii*, *B. graingeri* in Africa, and *B. caucasica*, *B. latyschewii*, *B. microti*, *B. persica* in Asia (Wodecka, 2012). However, RF-associated *Borrelia* species were found also in hard ticks: *B. miyamotoi* was first isolated from *I. persulcatus* in Japan in 1995 (Fukunaga et al., 1995), and *B. lonestari* from *Amblyomma americanum* in the USA in 1996 (Barbour et al., 1996; Armstrong et al., 1996). In the USA, *I. scapularis* infected with *B. miyamotoi* was first reported by Scoles et al. (2001). Then report of *B. miyamotoi*-like borrelia in *I. ricinus* was confirmed and in Europe in Sweden (Fraenkel et al., 2002).The aim of the study was to present preliminary data on the prevalence of genospecies from *B. burgdorferi* s.l. complex and *B. miyamotoi* in questing *I. ricinus* collected in chosen areas of Lower Silesia, southwestern Poland.

Materials and methods

Study area and tick collection

Studies were conducted from March to May 2009 (during tick spring activity) in three chosen habitats in Lower Silesia: in the city of Wrocław (the Osobowicki Forest), in the Ślęża Landscape Park, and in the Karkonosze National Park (Fig. 1). The Osobowicki Forest (140 ha), constituting a part of the ecological corridor of the Odra river, is a suburban forest used for recreation; the area is regularly visited by deers (Gottfried, 2006). The Ślęża Landscape Park (ŚLP), established in 1988, constitutes an important recreational ground for the inhabitants of Wrocław and its environs. The Ślęża Massif, located roughly 35 km southwest of Wrocław, is the highest elevation of the Sudetes Foothills, and surpasses the surrounding plains by about 500 m (Kondracki, 1994). The predominating habitats of the Massif are the mixed deciduous and coniferous forests of the mountainous and upland varieties. The Karkonosze National Park (KNP), located in the Karkonosze Mountains in southwestern Poland, is the highest area of the Sudetes mountain system (the highest peak, Śnieżka, is 1602 m above sea level). It was created in 1959 to cover an area of 5575 ha. The aboriginal hardwood and mixed forests have been largely replaced with spruce monocultures. KNP is a popular skiing and hiking destination for tourists.

Host-seeking ticks were mostly collected on forest paths and trails (ŚLP and KPN) and at the edge of the woods using a standard flagging method. The collected ticks were placed separately (adults) or in pools of five specimens (nymphs) in vials with 70% ethanol for further investigation.

DNA extraction

DNA was extracted from *I. ricinus* through the lysis of crushed ticks in ammonium hydroxide (NH_4OH) (Rijpkema et al., 1996). Adult specimens were processed individually while nymphs were pooled as described above. The lysates were then stored at −20 °C.

Detection and identification of Borrelia DNA using PCR-RFLP techniques

To detect *Borrelia* DNA, a nested PCR targeting the *fla* gene was used. The first reaction was based on outer primers 132f and 905r which amplify a 774 bp fragment of gene while the second reaction involved inner primer pair: 220f and 824r yielding an amplification product of 605 bp (Wodecka et al., 2009). Each PCR reaction was performed in a reaction volume of 20 μL containing 0.5 μL RUN Taq polymerase (1 U/1 μL) (A&A Biotechnology, Gdynia, Poland), 2 μL 10× PCR buffer (A&A Biotechnology, Gdynia, Poland), 2 μL dNTPs mixture (10 mM) (Fermentas, Lithuania), 0.4 μL of appropriate primers, 12.7 μL double-distilled water (13.7 μL for nested PCR), and 2 μL of the processed tick sample or 1 μL of the obtained PCR product for nested PCR. *B. burgdorferi* s.l.-positive tick lysates