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Borrelia miyamotoi infection in *Apodemus* spp. mice populating an urban habitat (Warsaw, Poland)

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ABSTRACT

The two of three species of city-inhabiting (Warsaw, Poland) *Apodemus* spp. mice were showed to be infected with *Borrelia miyamotoi,* spirochete bacterium considered to be a tick-borne relapsing fever (TBRF) disease causative agent. The pathogen infection was ascertained based on bacterium DNA presence in a blood (obtained from the lateral tail vein) of the mice, using the nested polymerase chain reaction (PCR) technique. The *B. miyamotoi* carriers populated not only green spaces situated in the suburban areas but also recreational parks and lawns in the city centre proper. Moreover, we found no significant differences in the infection rate depending on mouse species. The total prevalence of infection at the level of 5.6% suggests the potential role of urban-settled rodents in the spreading of the tick-borne zoonosis, which pose high risk to public health.

1. Introduction

Borrelia miyamotoi is a spirochete bacterium considered to be a tick-borne relapsing fever (TBRF) disease causative agent. The first reports of human *B. miyamotoi* infection were published in Russia in 2011 (Platonov et al., 2011); since then cases of the disease have been described in Europe, the United States and Asia (Hovius et al., 2013; Krause et al., 2013; Sato et al., 2014). The pathogen has been found in several of the hard-bodied *Ixodes* tick species also known to be vectors of the *Borrelia burgdorferi* s.l. complex (the agents of Lyme borreliosis) and a wide range of other tick-borne pathogens. The *B. miyamotoi* zoonotic reservoir remains unidentified, but it is known that small rodents as well as passerine birds could serve as the main pathogen hosts (Burri et al., 2014, Wagemakers et al., 2016). So far, studies have focused only on wildlife living in natural ecosystems (Barbour et al., 2009; Taylor et al., 2013; Wagemakers et al., 2016; Salkeld et al., 2018). Given that some species of these vertebrate groups also inhabit urban areas, the principal goal of our study was to determine the *B. miyamotoi* infection prevalence in rodents inhabiting this specific type of environment. Three *Apodemus* mice species were selected for this study (the striped field mouse *A. agrarius*, yellow-necked mouse *A. flavicollis* and wood mouse *A. sylvaticus*). Our previous research showed that in the Warsaw (Poland) habitat, these species comprise in total 85% of the whole small mammal community, as opposed to voles, which decisively avoid the urban environment and are absent within the most human-changed areas (Gortat et al., 2014). In ticks inhabiting green, recreational areas within the city of Warsaw, *B. miyamotoi* prevalence was detected at the level of 0.31% (Kowalec et al., 2017). This finding prompted us to study urban-inhabiting mice which potentially could serve as the *B. miyamotoi* reservoir and participate in the pathogen circulating scheme, therefore having a public health-threatening impact.

2. Materials and methods

Our investigations were performed in the city of Warsaw, Poland (52°12′N, 21°02′E; approx. 2 million residents). The rodent-trapping locations were within green spaces situated strictly in the city centre proper (e.g. municipal parks, lawns, squares) as well as in surrounding suburban areas (e.g. suburban forests, set-aside areas). The trapping sessions were carried out in September 2011, and the animal collection details have been described elsewhere (Gryczyńska et al., 2018).

B. miyamotoi infection was ascertained based on bacterium DNA presence in a small volume (approx. 50 μl) of blood obtained from the lateral tail vein of the mice. Genomic DNA was extracted using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, USA) in accordance with the manufacturer's protocol. All the samples were analysed using

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Table 1
Number of *Apodemus* mice captured and infected with *Borrelia miyamotoi*, by trapping area, at sites in the city of Warsaw, Poland.

Species	Northern suburbs	City centre	Southern suburbs	Total
	No. infected (%)/No. captured			
striped field mouse	3 (6.1)/49	4 (5.4)/74	4 (11.8)/34	11 (7.0)/157
yellow-necked mouse	1 (2.6)/38	0 (0)/19	2 (7.4)/27	3 (3.6)/84
wood mouse	0 (0)/5	0 (0)/2	0 (0)/4	0 (0)/11

the nested PCR technique with the use of *B. miyamotoi* flagellin gene marker (*flaB*), with published primers and unmodified reaction conditions (Wodecka et al., 2009; Kowalec et al., 2017). Amplicons were sequenced (Genomed, Poland) and nucleotide sequences were compared with data stored in GenBank databases using the BLAST-NCBI programs (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results

We found that *B. miyamotoi* infected *Apodemus* spp. mice within the Warsaw agglomeration, with a total prevalence of infection of 5.6% (n = 252 individuals tested). Among the mice species captured, the highest prevalence of infection (7%, n = 157 individuals tested) was detected for the striped field mouse. The yellow-necked mouse was infected with lower prevalence (3.6%, n = 84 individuals tested), whereas no wood mouse was found to be infected (n = 11 individuals tested) (Table 1). No statistically significant differences were found in the infection rate depending on mouse species ($\chi^2 = 1.91$; DF = 2; P = 0.38). Within green areas located inside the city centre, only four infected individuals were ascertained, all of them representing striped field mouse species. The trapping locations where these mice were found were situated relatively close to one other (Fig. 1). A majority of the *B. miyamotoi* carriers populated suburban locations, where the anthropogenic disturbances are relatively small. Even so, neither in the striped field mice nor in the yellow-necked mice did we find any statistically significant differences in the infection prevalence depending on trapping area ($\chi^2 = 1.87$; DF = 1; P = 0.17 and $\chi^2 = 0.2$; DF = 1; P = 0.65).

4. Discussion

Our most important finding – the first such report from Poland – is the confirmation of *B. miyamotoi* infection in city-inhabiting rodents, consequently highlighting their potential role in the spread of tick-borne, medically significant zoonosis, which pose high risk to public health. To date, *B. miyamotoi* infection has been reported mainly in questing ticks. Studies have dealt with individuals populating different kinds of habitats: natural (e.g. Netherlands 2.5%–3.84% and Belgium 1.14%; Cochez et al., 2015; Wagemakers et al., 2016) as well as urban (e.g. Switzerland 2.5% and Poland 3.5%; Oechslin et al., 2017; Kubiak et al., 2019).

Data in the literature indicate that infection prevalence in vertebrates potentially responsible for maintenance of the pathogen is spread out across the world, but still very rare. In the United States, *B. miyamotoi* has been identified in *Peromyscus* spp. mice (*P. boylii*, *P. californicus*, *P. leucopus*) and dusky-footed woodrats (*Neotoma fuscipes*), with infection rates ranging 6%–33% in different host species (Barbour et al., 2009; Salkeld et al., 2018). In addition, blood samples derived from rodents (both *Apodemus* spp. mice and voles) captured in Japan were pathogen positive at a rate of 6.9% (0%–9.4%) (Taylor et al., 2013).

In Europe *B. miyamotoi* infection has been confirmed in wild rodents inhabiting forested areas, and the yellow-necked mouse was found to be the most frequently infected host, with infection rates ranging 0.4%–9.3% in various countries (Hungary, Szekeres et al., 2015; Romania, Kalmár et al., 2019; Slovenia, Cerar et al., 2015; Slovakia, Hamšiková et al., 2017). The yellow-necked mouse has also been reported to transmit *B. miyamotoi* to 23.8% of xenodiagnostic ticks in a study conducted in Switzerland (Burri et al., 2014). These reports seem to confirm the yellow-necked mouse's capacity to act as a *B. miyamotoi* competent reservoir. In comparison to our study, the infection rate found in the wood mouse captured within the Netherlands was relatively high (14%), but contrarily, that study did not capture a single yellow-necked mouse that was infected (presumably because of the small sample size) (Wagemakers et al., 2016). The wood mouse, being typical for natural environments, basically avoids urban habitats, so we captured too few individuals to allow conclusions about this species' participation as a *B. miyamotoi* reservoir in the human altered environment. On the

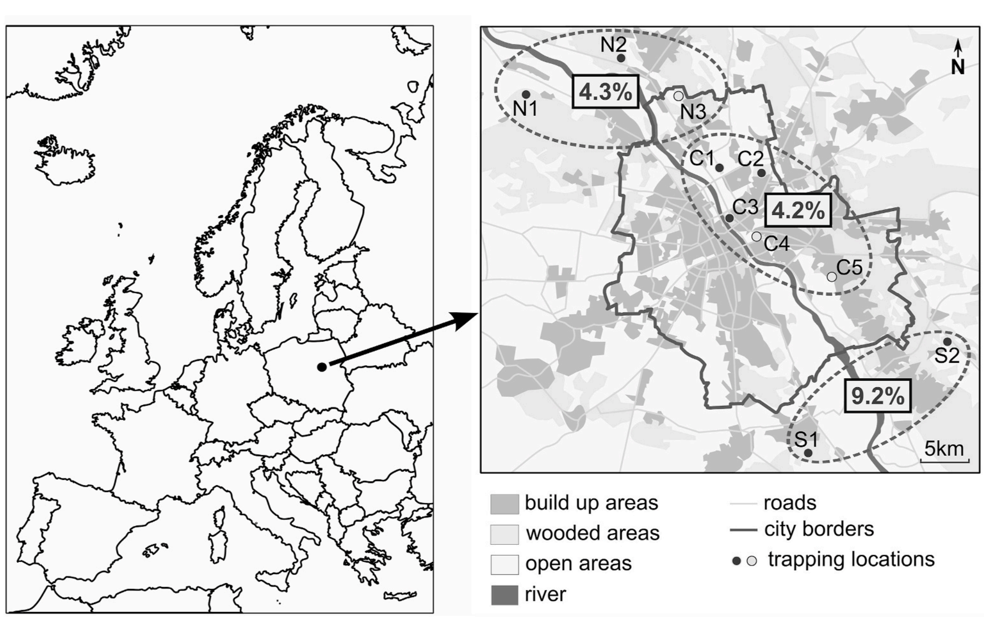

Fig. 1. Scheme of the study area (city of Warsaw, Poland) showing the arrangement of mice-trapping locations. Black points – locations where *B. miyamotoi* infected mice were present; White points – locations where none of the captured mice were *B. miyamotoi* infected; N1–N3 – locations within *n*orthern suburbs; C1–C5 - locations within city centre; S1–S2 – locations within southern suburbs; numbers in boxes – *B. miyamotoi* prevalence in mice inhabiting respective areas.

contrary, the striped field mouse readily migrates into urban agglomerations, indeed being the most popular rodent species in many cities. Our investigation found the largest number of striped field mouse individuals inhabiting the study area, and its infection prevalence was recorded as the highest. In previous studies conducted within natural habitats, however, none of the captured striped field mouse individuals was *B. miyamotoi* infected (Szekeres et al., 2015; Kalmár et al., 2019).

In our investigation carried out in the city of Warsaw, sites stretching from suburban areas towards the urban core exhibited a certain diversity in the host infection pattern. Mice inhabiting locations situated strictly in the city centre are efficient at transferring infected ticks only in areas where their mobility is not restricted due to the specific urban infrastructure (spatial isolation due to street network, open areas, densely built-up areas). By contrast, in the larger suburban forests and set-aside areas surrounding Warsaw, also used intensively for recreational activity by Warsaw residents and their companion animals, infected rodents were found in each trapping location.

Our results are relevant not only to the issue of determining the natural *B. miyamotoi* reservoir, but also to understanding the dynamics of host-pathogen interactions in the specific environment of an urban habitat. Our data are in accordance with assumptions about the increasing importance of tick-borne pathogens and the attendant hazard for humans, especially in urban landscapes.

Declaration of competing interest

All authors declare no conflicts of interest relevant to this paper.

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