*Epidemiology and Infection*

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# Urban rodent reservoirs of *Borrelia* spp. in Warsaw, Poland

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## Original Paper

**Cite this article:** Gryczyńska A, Gortat T, Kowalec M (2018). Urban rodent reservoirs of *Borrelia* spp. in Warsaw, Poland. *Epidemiology and Infection* **146**, 589–593. https://doi.org/10.1017/S095026881800033X

Received: 19 October 2017
Revised: 21 December 2017
Accepted: 29 January 2018
First published online: 5 March 2018

**Key words:**
*Apodemus* spp; *Borrelia burgdorferi sensu lato* complex; Lyme borreliosis; urban habitat

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**Abstract**

The anticipated worldwide surge in urban environments is generating ever-greater interest in the study of host–pathogen interactions in this specific type of habitat. We investigated the potential of city-inhabiting rodents to serve as the main Lyme borreliosis agents (*Borrelia* spp.) reservoir. We also tried to verify if anthropogenic disturbances changing the vertebrate species community composition may also alter the scheme of *Borrelia* spp. circulation. A total of 252 *Apodemus* mice (*A. agrarius*, *A. flavicollis*, *A. sylvaticus*) were captured in Warsaw (Poland), at sites classified into different zones of anthropogenic disturbance, ranging from suburban forests to municipal parks strictly in the city centre. *Borrelia* spp. infection, ascertained based on bacterium DNA presence in the rodents' blood, was found only in *A. agrarius* and *A. flavicollis* (7.6 and 6%, respectively). Only one species from the *Borrelia* genus – the mammal-associated species *B. afzelii* – was found in the mice studied. We found no statistical evidence of a correlation between infection in *Apodemus* mice and the zone of anthropogenic disturbance where the mice were caught. Non-homogeneous concentrations of *Borelia* spp. infected specimens within the strict city centre area suggest a lack of contact between members of particular mice subpopulations, and their responsibility for relatively high, but local *Borrelia* spp. infection.

## Introduction

Lyme borreliosis (also known as Lyme disease) is a tick-borne zoonosis caused by spirochetes bacteria belonging to the *Borrelia burgdorferi sensu lato* complex. Among a dozen or so validly named species comprising this *Borrelia* complex that have been identified to date, ten infect humans (*B. afzelii*, *B. bavariensis*, *B. bissettii*, *B. burgdorferi*, *B. garinii*, *B. kurtenbachii*, *B. lusitaniae*, *B. mayoni*, *B. spielmanii* and *B. valaisiana*), causing a wide spectrum of clinical manifestations [1–3]. In terms of epidemiology, Lyme borreliosis is one of the most common and important diseases spread by ticks. The causative agents of Lyme borreliosis circulate between *Ixodes* ticks (in Europe mainly *I. ricinus*) and a large number of vertebrate hosts in an enzootic cycle. A tick must engorge on the blood of an infected vertebrate to acquire spirochetes, then become infected and be able to transmit them by feeding on another vertebrate to complete the cycle. Several dozen animal species can serve as *Borrelia* spp. potential reservoirs with small mammals, particularly rodents and insectivores, constituting the main group of vertebrates susceptible to maintaining the pathogen in nature [4]. Evidence has also been reported of ground-dwelling birds and lizards serving as competent reservoirs for this pathogen, although the role of birds and reptiles is relatively low as compared with that of mammals [5–8].

The various reservoir groups are associated with different *Borrelia* species, due to the fact that they differ with respect to serum complement sensitivity [9, 10]. The species *B. afzelii* was, until recently, regarded as a strictly rodent-connected group, appearing only in ticks feeding on mammals, mainly on small rodents, but not infective for birds [11]. Recent findings have altered this hypothesis, however, by showing that birds are also becoming carriers of ticks infected with this species, and in future presumably may establish a competent reservoir for it [12–14]. Currently, birds are considered as a competent reservoir hosts for *B. burgdorferi*, *B. garinii*, *B. valaisiana* and *B. turdi* [6, 7, 15–17]. At the same time, the *B. lusitaniae* species is recognised to be associated with lizards [8, 18].

In this study, we sought to investigate the potential of small rodents to serve as the main Lyme borreliosis bacteria reservoir when living under the specific conditions typical of an urban habitat. In addition, we sought to elucidate whether anthropogenic disturbances which alter the composition of the vertebrate species community and limit their interactions are, thereby, capable of modifying the scheme of circulation and maintenance in the environment of the respective species of *Borrelia* spp.

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## Methods

### Study area, mice collection

This study was performed in the city of Warsaw, Poland (52°12′N, 21°02′E; approx. 2 million residents) and surrounding non-urban areas. Small mammals were trapped at 10 locations situated in areas exhibiting different degrees of anthropogenic disturbance, at various distances from the city centre. Based on our previous research, we know that the city spatial structure (roads, buildings, etc.), urban infrastructure level and the percentage of the area covered by vegetation could influence the small mammal community composition [19]. Rodent-trapping locations situated in the larger suburban forests and set-aside areas surrounding Warsaw, with relatively small anthropogenic disturbance, were classified into the Suburb Zone (S1–S5), whereas locations situated in municipal parks, squares and lawns in the city centre proper were classified into the Centre Zone (C1–C5) (Fig. 1). Nonetheless, all these areas are used intensively for recreational activity by Warsaw residents and their companion animals.

The trapping sessions were carried out for seven subsequent days in each location during September 2011 (at time of high rodent densities). At each location, a transect running about 600 m was delineated, along which live traps (two per point) for small mammals were set up every 20 m. Wheat grains and fruits were used as bait. The standard CMR method was used to trap the rodents. All captured mice (genus *Apodemus*) were individually marked and their species and sex were determined. A small volume (approx. 50 µl) of the rodents' blood was obtained from the lateral tail vein and stored in vials containing EDTA buffer (our study was related to a broader project investigating the genetic structure of Warsaw-inhabiting rodents; as such, we gathered no data concerning tick densities in the habitat or mice infestation). After all these procedures the mice were released into the same habitats where they had just been caught.

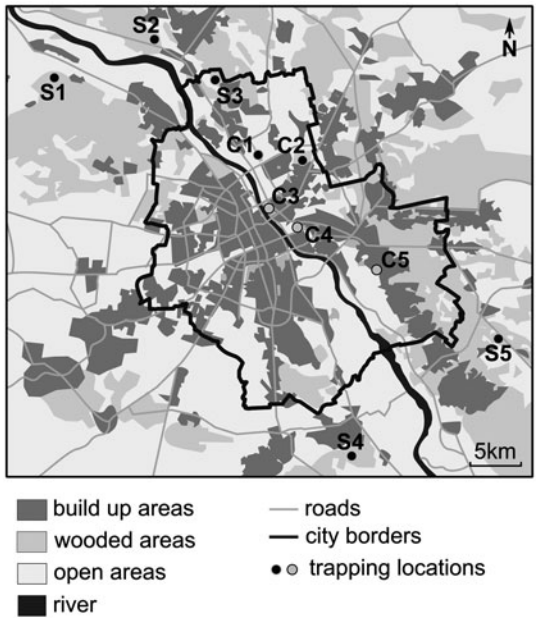


**Fig. 1.** Scheme of the study area (city of Warsaw, Poland) with the arrangement of mice-trapping locations. Black points – locations where *Borrelia* spp. infected mice were present; White points – locations where *Borrelia* spp. infected mice were not caught.

Individuals recaptured in subsequent days were released and were not rerecorded in the data analysis.

### Laboratory procedures

*Borrelia* spp. infection was ascertained based on bacterium DNA presence in rodents' blood [20].

Genomic DNA was extracted using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, USA) in accordance with the manufacturer's instructions. For further analyses the samples were stored at −20 °C.

All the samples were tested for the presence of *Borrelia* spp. DNA using the nested polymerase chain reaction (PCR) technique with Dream Taq polymerase and DreamTaq Green Buffer (Thermo Fisher Scientific Inc., USA), based on two-stage amplification of the fragment of the *fla* gene, coding for a bacterium flagellar protein (Thermal Cycler C1000; BioRad Laboratories, USA). The first-stage product was 774 base pairs in size, and the second-stage product was a 605 base-pair fragment of the *fla* gene. The thermal profiles and PCR primer sequences used in this study were those published [21], albeit slightly modified: initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 52 °C (I PCR) or at 55 °C (II PCR) for 30 s and then extending at 72 °C for 60 s for a total of 34 cycles in both of the stages, and also final elongation for 7 min. (I PCR 132f: 5′-TGGTATGGGAGTTTCTGG-3′, 905r: 5′-TCTGTCATT GTAGCATCTTT-3′; II PCR 220f: 5′-CAGACAACAGAGGGA AAT-3′; 824r: 5′-TCAAGTCTATTTTGGAAAGCACC-3′). DNA isolated from a *Borrelia fla* gene positive *I. ricinus* tick from Poland, sequenced and determined as *B. afzelii* was used as a positive control and a sterile water as a negative control. The second-stage products were separated by electrophoresis in 1.5% agarose gel in TAE buffer and visualised with Midori Green stain (Nippon Genetics, Germany) in UV light, wavelength 300 nm (BioRad Laboratories, USA).

Amplicons were purified using the Axygen Clean-up purification kit (Axygen, USA) and sequenced (Genomed, Poland) in one direction using the internal primer 824r. In order to compare nucleotide sequences with data stored in GenBank databases (http://www.ncbi.nih.gov/Genbank/index.html), BLAST-NCBI programs were used (http://www.ncbi.nlm.nih.gov/BLAST/).

## Results

A total of 252 rodents were caught: 157 striped field mice *A. agrarius*, 84 yellow-necked mice *A. flavicollis* and 11 wood mice *A. sylvaticus*.

We found *Borrelia* spp. infection in the striped field mouse and yellow-necked mouse with the prevalence of 7.6% (12/157) and 6% (5/84), respectively (together representing a total of 6.8% of all the mice captured; 17/252) (Table 1). Given that no wood mouse was found to be infected, and also in view of the small number of wood mouse specimens trapped, we decided to exclude this species from further analyses.

No statistically significant differences were found in the infection rate depending on mouse species ($\chi^2 = 0.24$; DF = 1; $P = 0.63$). Moreover, neither in the striped field mice nor in yellow-necked mice did we find any statistically significant differences in *Borrelia* spp. infection depending on mouse sex ($\chi^2 = 1.97$; DF = 1; $P = 0.16$ and $\chi^2 = 0.87$; DF = 1; $P = 0.35$).

Examining the prevalence of *Borrelia* spp. infection in *Apodemus* mice populating particular green areas within the

https://doi.org/10.1017/S095026881800033X Published online by Cambridge University Press

**Table 1.** *Borrelia* spp. infection in *Apodemus* mice, by zones of anthropogenic disturbance, Warsaw, Poland

| Zone of anthropogenic disturbance (habitat) | No. of rodent-trapping locations/locations with *Borrelia*-infected mice | No. of striped field mouse (*Apodemus agrarius*) infected (%)/captured | No. of yellow-necked mouse (*Apodemus flavicollis*) infected (%)/captured | No. of wood mouse (*Apodemus sylvaticus*) infected (%)/captured | Total infected (%)/captured |
|---|---|---|---|---|---|
| Suburb Zone (natural forests and set-aside areas surrounding city) | 5/5 | 9 (10.8)/83 | 3 (4.6)/65 | 0 (0)/9 | 12 (7.6)/157 |
| Centre Zone (municipal parks, squares and lawns) | 5/2 | 3 (4.1)/74 | 2 (10.5)/19 | 0 (0)/2 | 5 (5.3)/95 |
| Total | 10/7 | 12 (7.6)/157 | 5 (6)/84 | 0 (0)/11 | 17 (6.8)/252 |

city of Warsaw, we found no statistical evidence of a correlation between this parameter and zone of anthropogenic disturbance where the mice were caught ($\chi^2 = 0.53$; DF = 1; $P = 0.47$). Seven out of 10 trapping locations were populated by the infected mice (Fig. 1). All of the sites situated in the Suburb Zone were inhabited by these specimens. In contrary, mice living only at two (C1 and C2) out of five locations with the greatest anthropogenic disturbance, strictly in the city centre (Centre Zone), were *Borrelia* spp. infected (Table 1).

All the mice found to be infected in our study carried only a single species from the *Borrelia* genus – the rodent-associated *B. afzelii*. Using BLAST and phylogenetic analyses (MEGA 7.0) of a 563 bp fragment of the *fla* gene, among 17 *B. afzelii* sequences obtained in the study, we detected seven variants of nucleotide sequences (Table 2). The nucleotide identity/similarity of the *fla* gene fragments of the obtained *B. afzelii* sequences was very high (99.6–100%). One most frequent variant (A1) present in either *A. agrarius* or *A. flavicolis* was identical with five reference *B. afzelii* sequences in GenBank from *A. agrarius* (KF894064, KF894070), *Myodes glareolus* (KF894068) and *Ixodes ricinus* (KX646195, DQ016619) from Poland. Other variants (B1–B6) were highly similar, although single amino-acid substitution occurred in three sequences. The obtained sequences were deposited in GenBank (Table 2).

## Discussion

The anticipated worldwide surge in areas of urbanisation and intensive growth in the population of humans residing in large agglomerations could lead to alterations in wildlife–pathogen interactions in the near future [22–24]. As a habitat becomes altered by urbanisation, its composition of species and their biology usually change. To verify the potential impact of anthropogenic disturbances on pathogen maintenance, we resolved to carry out our study in an urban environment, seeking to ascertain whether the scheme of *Borrelia* spp. (*B. burgdorferi sensu lato* complex) circulation in these specific circumstances differs from that known in natural habitats.

Given that other research carried out in the city of Warsaw, at sites from suburban areas towards the urban core, documented a

**Table 2.** Variants of *fla* gene sequences obtained in this study

| Species | Variant (563 bp of *fla* marker) | Host (no. of sequences) | GenBank Accesion | Highest similarity in GenBank | | | Amino-acid substitution (site) | Reference in GenBank |
|---|---|---|---|---|---|---|---|---|
| | | | | Similarity %, (nucleotide identity) | Accession | Host | | |
| *B. afzelii* | A1 | *A. agrarius* (7) | KY626318 | 100 (562/562) | KX646195 | *Ixodes ricinus* | no | *B. afzelii* K78: CP009058 |
| | | *A. flavicolis* (4) | KY626319 | 100 (561/561) | DQ016619 | *Ixodes ricinus* | no | |
| | | | | 100 (553/553) | KF894064 | *Apodemus agrarius* | no | |
| | | | | 100 (556/556) | KF894070 | *Apodemus agrarius* | no | |
| | | | | 100 (553/553) | KF894068 | *Myodes glareolus* | no | |
| | B1 | *A. agrarius* (1) | KY626320 | 99.8 (561/562) | KX646195 | *Ixodes ricinus* | no | |
| | B2 | *A. agrarius* (1) | KY626321 | 99.6 (560/562) | | | V:A (211) | |
| | B3 | *A. agrarius* (1) | KY626322 | 99.8 (561/562) | | | N:D (122) | |
| | B4 | *A. agrarius* (1) | KY626323 | 99.8 (561/562) | | | no | |
| | B5 | *A. agrarius* (1) | KY626324 | 99.8 (561/562) | | | T:C (130) | |
| | B6 | *A. flavicolis* (1) | KY626325 | 99.8 (561/562) | | | no | |

https://doi.org/10.1017/S095026881800033X Published online by Cambridge University Press

relatively high proportion of host-seeking *Borrelia* spp. infected *I. ricinus* ticks (6.1–23.5% [25–27]), the occurrence of infected vertebrates considered to be tick hosts and a competent reservoir for this bacterium was expected in our study in the Warsaw habitat. These animals not only participate in pathogen perpetuation, but also infect consecutive tick generations.

Previous studies showed that rodents belonging to the genus *Apodemus* may comprise a competent reservoir for *Borrelia* spp. bacteria in natural habitats uninfluenced by human activity. Not only was pathogen DNA found to be present in the investigated mice tissues (with the prevalence of 4.3–11%), but they were also found to have the ability to infect tick larvae feeding on them [28–31]. Our results confirmed that *Apodemus* mice can be infected in similar level and potentially serve as a reservoir of *Borrelia* spp. bacteria also in a large urban agglomeration. Our results also suggest no species-dependent differences in mouse involvement in pathogen circulation in the Warsaw environment.

Previous research and our current results have shown diversity in the habitation pattern and non-homogeneous concentrations of particular *Apodemus* species within the Warsaw area [19]. In the larger suburban forests and set-aside areas surrounding Warsaw (Suburb Zone), all of the investigated mouse species are present, but the strict city centre (Centre Zone) is almost exclusively populated by the striped field mouse, and other mouse species were occasionally present. Furthermore, the three mouse species selected for our investigation are known to comprise in total 85% of the whole small mammal community dwelling in Warsaw [19]. At the same time, voles (genus *Microtus* and *Myodes*) are occasionally encountered in suburban forests and set-aside areas, and they are absent in most urban green areas within the strict city centre. Consequently, the striped field mouse could potentially serve as the basic rodent member of *Borrelia* spp. competent reservoir in the most urbanised parts of Warsaw.

On the other hand, it has been clearly demonstrated that in natural habitats, the involvement of voles in *Borrelia* spp. maintenance is significantly greater than that of mice. Studies carried in Lithuania and Norway have found the yellow-necked mouse to be less efficient in transmitting *Borrelia* spp. to ticks than the bank vole (*M. glareolus*) and field vole (*Microtus arvalis*) [29, 30]. Research in central Italy found the infection rates of *Apodemus* spp. mice and bank voles to be 6.93 and 11.9%, respectively [31]. The authors suggest species-specific differences in susceptibility to *Borrelia* spp. infections and a higher propensity of voles *vs.* mice to transmit the infection to feeding larvae as potential reasons for this variation.

Because voles decisively avoid the urban environment, this kind of habitat, therefore, lacks the most common vertebrate participants establishing a competent *Borrelia* spp. reservoir. As such, it would seem that this role may be acquired by other small rodent species – e.g. mice from the genus *Apodemus*, constituting both an *I. ricinus* tick host group and a competent reservoir for pathogens carried by them. Nevertheless, based on our results, the lack of voles in the study area did not entail any significant increase in the prevalence of *Borrelia* spp. infection in *Apodemus* mice as compared with infection levels ascertained for these species inhabiting natural woodlands [30, 31].

It is known from the literature that urban environments may promote pathogen transmission through increased host contact rates, and additionally across gradients of urbanisation the incidence of some zoonotic pathogens has been found to be highest in urban cores [23, 24]. Our present results partly run counter to these hypotheses, because infected mice were found in all of the suburban locations, and only in two (close to each other) out of five sites placed in the city centre. But the infection prevalence in mice subpopulations living in these two locations was much higher than in suburban ones. So, we can expect that mice living there were in close liaison with each other and did not have contact with members of remaining subpopulations. Our previous studies showed that small mammals inhabiting cities are constricted in their mobility by the specific urban structure (street network, open areas, densely built-up areas). In the case of small rodents inhabiting Warsaw, the great spatial isolation and consequently the specific genetic structure has been shown to cause a lack of contact between members of particular subpopulations of the mammals populating the strict city centre [32, 33]. This is the potential reason why even though striped field mice are present in relatively great densities in green patches located in the city centre proper, they are responsible only for local *Borrelia* spp. maintenance and circulation, and probably do not currently comprise the main pathogen reservoir in the Warsaw habitat as a whole.

We may, therefore, conjecture that other groups of vertebrates may be participating in this process, with greater involvement as compared with natural circumstances. In large agglomerations, the other wildlife vertebrates (ungulates, carnivores) able to fulfil the above roles are still relatively rare within the strict centre. Also, the densities of these species' populations in suburbs are small compared to those encountered in natural habitats. Presumably, red squirrels (*Sciurus vulgaris*) and European hedgehogs (*Erinaceuseuropaeus*) commonly occurring in the city centre proper could serve as potential *Borrelia* spp. reservoirs. Furthermore, in urban habitats, as compared with natural ones, birds could become more important as competent *Borrelia* spp. reservoirs. Many species of passerine birds have adapted to the urban environment with great flexibility and are persistently present even in city centres. Considering their intensive and easy mobility, birds can act as transport vehicles for ticks among different green areas within the city. They are also potential disseminators of *Borrelia* spp. spirochetes in the urban habitat, either as carriers of infected ticks or as reservoir hosts of the pathogen [5, 6, 17, 34, 35].

Based on the example of Warsaw, we can state that the distinctive wildlife community composition typical of urban ecosystems can alter host–pathogen interactions. A lack of certain important mammal species, or their occurrence at low abundances, may affect diversity in the circulation of particular *Borrelia* species as compared to the natural habitat. Mammal interactions and consequently pathogen transmission are also hindered by the specific city centre structure. Therefore, potential relative growth can be expected in the relevance of birds, in relation to other groups of vertebrates, in establishing a reservoir for Lyme borreliosis agents in the urban habitat. This, in turn, could lead to an increasing proportion of *Borrelia* spp. species associated with avian reservoir groups *vs.* species occurring in rodents. Medical reports have shown that the respective *Borrelia* species are known to be linked to different Lyme borreliosis processes and distinctive clinical manifestations [2].

To complete the data concerning the contribution of city-inhabiting vertebrates in the *Borrelia* spp. circulation scheme, studies should investigate other species of mammals and birds populating urban areas. This is very important given that green areas within the city are used as places of recreation and leisure by humans and their companion animals. Thus, the continual

https://doi.org/10.1017/S095026881800033X Published online by Cambridge University Press

renewal of our understanding of the dynamics of host–pathogen interactions in the urban environment allows us to better understand the channels by which the disease spreads and consequently to better manage and limit the risk of *Borrelia* spp. exposure to humans, companion animals and wildlife.

**Acknowledgements.** Approval for all the procedures used in this study was obtained from the First Warsaw Local Ethics Committee for Animal Experimentation, no. 21/2010. We are grateful to Daniel J. Sax for language editing, to Dr Miłosława Sokół for her help with applying statistical methods and to Dr Robert Meronka for the work he put into preparing the scheme of the study area. This study was financed in part by the National Science Centre (Grant No. N N304 169539).

**Declaration of interest.** None.

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https://doi.org/10.1017/S095026881800033X Published online by Cambridge University Press