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Zakażenie kleszczy *Ixodes ricinus* *Bartonella* spp. w makroregionie lubelskim

Infection of *Ixodes ricinus* ticks with *Bartonella* spp. in the Lublin macroregion

Streszczenie

Wstęp. Choroby przenoszone przez kleszcze *Ixodes ricinus* charakteryzują się naturalną ogniskowością, celowe jest zatem badanie środowiskowych uwarunkowań transmisji patogenów wektorowanych przez te stawonogi.

Cel. Celem pracy było określenie stopnia zakażenia kleszczy bakteriami z rodzaju *Bartonella* spp., czynnika etiologicznego choroby kociego pazura, choroby Carriona i gorączki okopowej.

Materiały i metody. Przebadano 1182 kleszczy *Ixodes ricinus* zebranych na pięciu wybranych stanowiskach makroregionu lubelskiego (Zwierzyniec, Żyrzyn, Parczew, Włodawa, Dąbrowa). W celu wykrycia DNA *Bartonella* spp. zastosowano metodę łańcuchowej reakcji polimerazy (PCR), a jako markera genetycznego użyto genu *gltA* kodującego syntezę cytrynianową.

Wyniki. Ogólny odsetek zakażeń *Bartonella* spp. w makroregionie lubelskim wyniósł 1,1%. Najwyższy odsetek zakażeń stwierdzono u kleszczy zebranych w Dąbrowie (3,4%) a znacznie niższy w Parczewie (0,9%). Na trzech pozostałych terenach badań (Zwierzyniec, Żyrzyn, Włodawa) nie stwierdzono obecności genu *gltA* w zebranych kleszczach. Podobny odsetek zakażeń stwierdzono u samców i samic (2,2% i 2,0%), natomiast nie wykryto genu *Bartonella* spp. wśród nimf.

Wnioski. Mimo niskiego odsetka zakażeń *Bartonella* spp. w województwie lubelskim (1,1%), nie można wykluczyć kleszczy *Ixodes ricinus* jako potencjalnego wektora tego patogenu, co może mieć znaczenie dla prawidłowej diagnostyki i profilaktyki chorób odkleszczowych.

Summary

Introduction. Diseases transmitted by *Ixodes ricinus* ticks are naturally focal, which justifies the study on environmental conditions of the transmission of pathogens vectored by these arthropods.

Aim. The aim of the study was to determine the level of infection of ticks with *Bartonella* spp., which is the etiological factor of cat scratch disease, Carrion's disease and trench fever.

Materials and methods. 1182 *Ixodes ricinus* ticks collected from five selected sites in the Lublin macroregion (Zwierzyniec, Żyrzyn, Parczew, Włodawa, Dąbrowa). To detect *Bartonella* spp., the polymerase chain reaction method (PCR) was applied; the genetic marker was the *gltA* gene encoding the citrate synthase.

Results. The total percentage of infections with *Bartonella* spp. in the Lublin macroregion was 1.1%. The highest proportion of infections was found in ticks harvested in Dąbrowa (3.4%), while that detected in Parczew was much lower (0.9%). In three remaining study areas (Zwierzyniec, Żyrzyn, Włodawa) the *gltA* gene was not found in the collected ticks. The levels of infection in males and in females were similar (2.2% and 2.0%), while in nymphs *Bartonella* spp. gene was not detected.

Conclusions. Despite the low percentage of infections with *Bartonella* spp. in the Lublin province (1.1%), *Ixodes ricinus* ticks cannot be excluded as a potential vector of this pathogen, which may be important for the appropriate diagnostics and prophylactics of tick-borne diseases.

Słowa kluczowe: kleszcze, *Ixodes ricinus*, *Bartonella* spp.

Key words: ticks, *Ixodes ricinus*, *Bartonella* spp.

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INTRODUCTION

Bartonella spp. are Gram-negative alphaproteobacteria. At the beginning of 1990s only two diseases caused by those bacteria were known: Carrion's disease (etiological factor: *B. bacilliformis*) and trench fever produced by *B. quintana* [1]. Now, 24 species in this genus have been identified [2], almost half of them being pathogenic for humans (among others, *B. henselae*, *B. elizabethae* [3], *B. alsatica* [4], *B. koehlerae* [5], *B. vinsonii* subsp. *arupensis* [6]). The clinical picture and the course of *Bartonella* infection may vary, from mild, asymptomatic forms to life-threatening ones [7].

Carrion's disease had two phases: the first, called Oroy fever, is characterised by raised temperature, anaemia, jaundice; the fatality rate in untreated patients is 44-88%. In the chronic phase, patients develop a cutaneous rash transforming into lesions called "Peruvian wart" (verruca peruana) [8].

The homeless, people living in poor sanitary conditions and alcoholics most frequently suffer from trench fever, which is related to their exposure to body lice – the main vector of the bacteria. The symptoms include fever for 1-3 days combined with headaches and vertigo, and pain in the shins. The symptoms relapse every 4-6 days, but were less intense with every relapse. *B. quintana* can also produce endocarditis, bacterial angiomatosis and lymphadenopathy [9]. The most widespread bartonellosis (usually caused by *B. henselae*) is the cat scratch disease (CSD) whose symptoms are swelling and inflammation of lymph nodes near the site of cat scratch or bite [10].

In 2000-2006, bartonellosis were classified as belonging to 15 „emerging” diseases in Europe. This is an important public health problem. Cases of those diseases were reported in France and Portugal [11]. Apart from such species as *B. bacilliformis* that occur mainly in Peru, infections with *Bartonella* spp. are found – in an increasing number – all over the world. The groups particularly exposed to the bacteria are the homeless, drug addicts and veterinary surgeons. The percentage of *Bartonella*-seropositive persons in the United States ranges from 2% in blood donors, to 7.1% in veterinarians, to 46% in drug addicts. In France, the homeless patients with skin symptoms who showed the prevalence of anti-*B. quintana* antibodies were 54% of the examined population. The study on healthy adult people in Germany and Greece found that the proportion of persons with anti-*B. henselae* antibodies is 30% and 19.8%, respectively. In Italy 61.6% of the examined children were seropositive. 77.5% adults and children from the areas endemic for Carrion's disease in Peru had anti-*B. bacilliformis* antibodies [1]. 8.7% of the healthy population in Spain is seropositive for *B. henselae*, and no statistically significant differences according to sex, place of residence, or contact with animals were found. Only in the group of 30-44 years old patients the antibodies were found more frequently [12]. Immunoglobulin for *B. quintana* was detected in 56% of the homeless and 51% of blood donors in Japan [13]. A similar study conducted in Sweden shows that in 62.5% of the homeless there are anti-*Bartonella* spp. antibodies (those for *B. elizabethae* are most frequently found), compared to 18% of seropositive blood donors [14].

Mammals are a reservoir for *Bartonella* spp., most species showing preference for one type of host. Cats may

be infected with *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, while dogs – by *B. vinsonii* subsp. *berkhoffii*, *B. henselae*, *B. clarridgeiae*, *B. washoensis* and *B. quintana* [15]. A reservoir for bacteria of the *Bartonella* genus may also be wild rodents and ruminants, such as: rats (*B. elizabethae* and *B. grahamii*), bank voles and wood mice (*B. grahamii*) [16], as well as roe deer (*B. schoenbuchensis*), and domestic animals: sheep and cattle (*B. bovis*) [17-19].

Transmitters of *Bartonella* spp. are blood-sucking arthropods. A vector of *B. bacilliformis* is the sand fly of the *Lutzomyia* genus [20], of *B. quintana* – the body louse (*Pediculus humanus humanus*) and probably the head louse (*Pediculus humanus capitis*) [21]. *B. henselae*, the etiological factor of cat scratch disease (CSD) is transmitted by the cat flea (*Ctenocephalides felis*) [22].

Also other arthropods, including ticks, may participate in spreading bacteria of the *Bartonella* genus.

AIM

The aim of the study was to identify the level of infection of *Ixodes ricinus* ticks (at each of their developmental stages) with bacteria of the *Bartonella* spp. genus in various forest biotopes in the Lublin macroregion.

MATERIALS AND METHODS

Collection of ticks. Unfed ticks (550 nymphs, 325 males and 307 females) were collected during two vegetation seasons in spring and summer in 2005 and 2006 from the forest area of 5 districts in the Lublin macroregion: Zwierzyniec, Żyrzyn, Parczew, Włodawa, Dąbrowa. Ticks were collected by dragging a woollen flag over lower vegetation at the peripheral and inner parts of deciduous and mixed forests, including suburban localities and recreational areas. Collected ticks were placed in glass tubes in 70% ethanol for further investigation.

DNA isolation. A total 1182 *Ixodes ricinus* ticks were examined. Protozoan DNA was isolated from ticks after removal from alcohol by boiling in 0.7 M ammonium hydroxide, according to Rijpkema [23], and stored at -70°C. Adults ticks were prepared separately while nymphs in pools of 5 specimens.

Detection of *Bartonella* spp. DNA by PCR. Ticks lysates were examined for the presence of *Bartonella* spp. DNA using amplification by polymerase chain reaction (PCR). The set of following primers was applied: pair of primers: BhCS.781p (5'-GGGGACCAGCTCATGGTGG-3') and BhCS.1137n (5'-AATGCAAAAAGAACAGTAACA-3'). These primers are specific for the *gltA* gene encoding the citrate synthase described by Norman *et al.* [24]. In each PCR reaction were applied: matrix DNA (2.5 µl), 50 pmol of each primer (Eurogentec, Seraing, Belgium), 0.25 U thermostable polymerase DNA (DyNAzyme™ II DNA F-501S, Finnzymes Oy, Espoo, Finland), 10x reaction buffer mixture of dNTPs nucleotide (2.5 mM), nuclease-free water (Applied Biosystems). The size of the amplified DNA fragment was 380 base pairs (bp). The nuclease-free water was used as a negative control. The positive control was obtained from slides of the *Bartonella* IFA IgG Kit (FOCUS Technologies, Cypress, USA).

The amplification reactions were carried out in PTC-150 thermal cycler (MJ Research Inc., Waltham, MA, USA)

according to Norman *et al.* [24]: samples were incubated for 1 min at 95°C and then thermally cycled 35 times, at 20 sec. in 95°C, 51°C for 30 sec. and 72°C for 2 min.; final extension lasted 7 min. Products of amplification were identified in 2% agarose gel after electrophoresis in standard conditions and staining with ethidium bromide solution (2 µg/ml). Amplified fragments were visualized and analysed under UV light in Gel Documentation and Analysis System (In Genius, Syngene Biotech, UK).

Statistical analyses. The data were analysed by χ^2 test and t-Student with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

The total percentage of *Ixodes ricinus* ticks infection with *Bartonella* spp. in the Lublin macroregion was 1.1%. The highest proportion of infected specimens was found in Dąbrowa (3.4%), while that in Parczew was much lower (0.9%). At three examined sites (Zwierzyniec, Żyrzyn, Włodawa) *Bartonella* DNA was not found in the examined ticks. Males were the most infected tick developmental stage (2.2%). Similar results were obtained for females (2.0%). Infection with *Bartonella* spp. was not found in nymphs (Table 1). The correlation of the level of infection with *Bartonella* spp. with the developmental stage was found statistically significant (for males: $p < 0.01$). A high statistical significance was also detected in the case of the correlation between the frequency of infection with the pathogen and the site of tick harvesting ($p < 0.001$), where positive results were found in specimens collected in deciduous and mixed forests. No infection was found in ticks harvested in coniferous forests.

DISCUSSION

Up to date, reports on tick infection with *Bartonella* spp. pathogen have been scarce. It is believed that the main vectors of these bacteria are mosquitoes, the human louse, the cat flea and the mite. However, as early as 1990s, the role of ticks as the pathogen transmitter began to be considered, when in 30% of patients with the cat scratch disease caused by *B. henselae* no contact with cats was reported [25].

The proportion of infections of *I. ricinus* ticks with *Bartonella* spp. found in the Lublin region is one of the lowest in comparison with the results from other parts of Poland.

Bartonella spp. DNA was found in 3.7% of *Ixodes ricinus* ticks collected from vegetation and animals in southern, central and eastern Poland.

Bacterial strains most frequently identified in ticks collected from animals were *B. henselae* and *B. quintana*. A significantly lower level of infection (0.5%) was detected in *Dermacentor reticulatus* [26]. Studies on ticks collected from deer show that ticks do not infect with *Bartonella* during the process of feeding. The level of infection with *B. capreoli* in ticks harvested from roe deer in the vicinity of Szczecin was 7.7% [27, 28].

In France the percentage of infections with *B. schoenbuschensis* in *I. ricinus* ticks was 9.8% [29]. Proportions similar to those detected in our own study (1.1%) were found in Czech Republic (1.2%) [30] and in Italy (1.4%) [31]. Studies conducted in Sweden, Great Britain, Hungary and central Slovakia did not identify any *Bartonella* species in the examined ticks [32, 33].

In the United States much higher levels of infections in ticks: *Ixodes scapularis* (34.5%) [34] and *Ixodes pacificus* (19.2%) [35]. However, in *Amblyomma americanum* ticks the percentage of infections with *Bartonella* (0.45%) was comparable to that in *Dermacentor reticulatus* ticks (0.5%) [36]. The level of infections in *Rhipicephalus sanguineus* ticks from California (3.2%) is similar to the percentage of infections found in *Ixodes ricinus* in Poland [37]. In Iowa (USA) *Bartonella* DNA was also identified in soft ticks *Carios kelleyi* (3.2%) where the analysis of 16S-23S ITS gene sequence showed 100% homology with *B. henselae* sequence [38].

Studies of tick populations in Korea confirmed the presence of *Bartonella* in 5.2% of the specimens, where DNA of the pathogen was identified, among others, in *Ixodes* spp. (0.8%), *I. persulcatus* (0.1%), *Heamaphysalis longicornis* (4.0%) [39]. Other studies in Korea show that the percentage of *Bartonella* in unfed *Heamaphysalis longicornis* ticks collected from small mammals is 35.2% [40]. In China high *Bartonella* levels were found in *Heamaphysalis longicornis*: 30.0% – 41.3% and in *I. sinensis*: 3.3% – 42.3% [41].

As opposed to the results obtained in Poland, in Russia the percentage of *Bartonella* infections in *Dermacentor reticulatus* ticks was 21.4%, *B. henselae* and *B. quintana* as prevailing species. In *I. persulcatus*, however, the identified level of infections was much higher (37.6%) than in the same tick species in Korea [39, 42].

TABLE 1. Prevalence of *Bartonella* spp. in *Ixodes ricinus* ticks in Lublin region, presented by stage/sex and districts.

Districts	Stage/sex of <i>Ixodes ricinus</i> ticks							
	Nymphs		Males **		Females		Total ***	
	Pos/N	%	Pos/N	%	Pos/N	%	Pos/N	%
Zwierzyniec	0/170	-	0/32	-	0/24	-	0/226	-
Żyrzyn	0/130	-	0/67	-	0/48	-	0/245	-
Parczew	0/90	-	0/68	-	2/77	2.6	2/235	0.9
Włodawa	0/40	-	0/57	-	0/56	-	0/153	-
Dąbrowa *	0/120	-	7/101	6.9	4/102	3.9	11/323	3.4
Total**	0/550	-	7/325	2.2	6/307	2.0	13/1182	1.1

N (number of examined ticks); Pos (number of infected ticks); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (χ^2 test).

In Australia, in ticks from Ixodidae family (species: *I. tasmani* and *I. trichosuri*), collected from the koala, *Bartonella* spp. infections were found in 21.0% of the specimens [43].

A study conducted by Cotté *et al.* on *I. ricinus* ticks fed with blood infected with *B. henselae*, prove that ticks are competent vectors of *B. henselae*. The transmission of *B. henselae* from ticks to the blood via the tick mouthparts was confirmed. It was also shown that *B. henselae* is also transmitted transstadially. *B. henselae* was detected in eggs laid by females fed with infected blood, while in nymphs hatched from the eggs the pathogen DNA was not found. In addition, an infectious character of *Bartonella* was confirmed with experiments on cats: the cats infected with salivary glands of ticks fed with infected blood developed bacteremia [44].

In Poland, studies on patients with clinical symptoms of bartonellosis identified *B. henselae* in 21% (1998), 30% (1999), 55% (2000) and 40% (2001) of the examined population. Most seropositive patients came from the Mazowieckie province (30.1%) and the Lower Silesian province. Infections most frequently occurred at the age of 8-16 and 40-50 years. In 2001 in Poland, the incidence of the cat scratch disease was 0.10 for 100,000 inhabitants. This result can be compared to the incidence ratio of 9.30 for 100,000 people in USA, 12.5 in the Netherlands, and 0.69 in Belgium. The morbidity in Poland in 2007 was 0.25 for 100,000 people. The fact that bartonellosis is seldom diagnosed in Poland is probably due to a low awareness of first contact physicians and to the absence of regularly performed diagnostic tests for this disease [45, 46]. Studies on the groups exposed to the disease showed the prevalence of specific antibodies in 48.3% of homeless alcoholics, 45% of veterinary surgeons, 53.3% of cat owners, in comparison to 24% of seropositive blood donors [47].

Examination of ticks for infections with *Bartonella* spp. makes it possible to predict such infections in humans, which is very important for the appropriate diagnostics and prophylactics of tick-borne diseases.

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