

Occurrence of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in *Ixodes ricinus* ticks collected from selected areas of Opolskie Province in south-west Poland

Marek Asman^{1,A-D,F}, Joanna Witecka^{1,C,E}, Krzysztof Solarz^{1,C,E-F}, Alicja Zwonik^{1,C}, Piotr Szilman^{1,B}

¹ Department of Parasitology, Medical University of Silesia, Katowice, Poland

A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

Asman M, Witecka J, Solarz K, Zwonik A, Szilman P. Occurrence of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in *Ixodes ricinus* ticks collected from selected areas of Opolskie Province in south-west Poland. Ann Agric Environ Med. doi: 10.26444/aaem/110214

Abstract

Introduction. Ticks (Acari: Ixodida) are vectors and/or reservoirs of many pathogens, i.e. *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti*. These pathogens are ethiological agents of such diseases as Lyme borreliosis, human granulocytic anaplasmosis and human babesiosis.

Objective. The aim of the study was to evaluate the role of the *Ixodes ricinus* in the transmission of *Borrelia burgdorferi* sensu lato, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia burgdorferi* sensu stricto, *Anaplasma phagocytophilum* and *Babesia microti* in Opolskie Province in Poland.

Materials and method. DNA from 222 ticks was isolated by the ammonia method. The pair of primers specific to the flagelline gene was used to detect of *B. burgdorferi* s. l. To detect of genospecies of this spirochete, three pairs of internal primers were used. In turn, two pairs of primers specific to the 16S rDNA gene and the 18S rRNA were used, respectively, for the detection of *A. phagocytophilum* and *B. microti*. *Borrelia burgdorferi* s. l., *A. phagocytophilum*, and *B. microti* were detected in 4.5%, 2.7% and 5.4% of examined ticks, respectively.

Results and conclusions. Of the ten ticks infected with *B. burgdorferi* s. l., *B. afzelii* was found in seven, undefined genospecies in two, and mixed infection with *B. afzelii* and *B. burgdorferi* s. s. in one. The study demonstrated the potential risk of exposure of humans and animals to infections of *B. burgdorferi* s. l., *A. phagocytophilum* and *B. microti* in the examined area of Poland.

Key words

Ixodes ricinus, Opolskie Province, Poland, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi* sensu lato genospecies

INTRODUCTION

It is widely known that *Ixodes ricinus* is the most common tick species in Europe and in Poland. In these areas, this ectoparasite is also the main vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* [1]. These pathogens are etiological agents of Lyme borreliosis, human granulocytic anaplasmosis and babesiosis [2, 3, 4]. The presence of *B. burgdorferi* s. l. in ticks from different localities in Poland may vary to a considerable extent [5]. In some regions, more than 60% of ticks may be infected with this spirochete [6]. The first case of borreliosis in Poland was reported by Januszkiewicz and Kieda in 1987 in the province of Western Pomerania [7]. Since then, the number of cases of this disease in Poland has continued to grow, and in 2003 a record number of infected people were noted (more than 3,500 subjects) [8]. This multisystem and multicentre disease has a seasonal character. It has been observed that

the prevalence rates of borreliosis increase in the summer and autumn seasons [9]. At present, about 19–20 genospecies of this bacteria have been identified. In Europe, the following five are confirmed pathogens in humans responsible for causing Lyme borreliosis: *Borrelia afzelii*, *Borrelia garinii*, *Borrelia burgdorferi* sensu stricto, *Borrelia bavariensis* and *Borrelia spielmani* [10]. Particular genospecies are responsible for different clinical symptoms, i.e. *B. burgdorferi* s. s. causes mainly chronic arthritis, *B. garinii* neuroborreliosis, and *B. afzelii* – acrodermatitis chronica atrophicans (ACA) [11]. Most probably, *B. afzelii* is the dominant genospecies in Poland [12].

The prevalence of *A. phagocytophilum* in *I. ricinus* in some regions of Poland may reach even more than 75% [13]. In turn, the first case of human granulocytic ehrlichiosis (granulocytic anaplasmosis) in Poland was reported by Tylewska-Wierzbanowska et al. [14]. The symptoms of infection with *A. phagocytophilum* in humans may resemble flu, and in extreme cases it may lead to many adverse complications, including haemolytic and immunological abnormalities [14]. At present, in some regions of Poland, seroactivity against this rickettsia in human reaches even 20% [15].

Address for correspondence: Marek Asman, Medical University of Silesia in Katowice, Department of Parasitology, Poland
E-mail: masman@sum.edu.pl

Received: 22.11.2018; accepted: 08.06.2019; first published: 17.07.2019

The presence of *B. microti* in ticks also varied between different localities of Poland and in some regions may reach more than 40% [16]. The first case of human *babesiosis* in Poland was reported in a sailor who returned from Brasil [17]. The first symptoms of this infection are flu-like and, in extreme cases, may affect the kidneys, lungs, myocardium, spleen and liver, causing symptoms such as hepatosplenomegaly [4]. To-date in Europe, only 29 cases of human *babesiosis* have been noted, but it is probable that the scale of the problem may be significantly larger. The reason may be rare diagnosis of this disease caused mainly by the non-specific symptoms in humans [18].

Ticks and tick-borne pathogens are a significant threat to human health. Despite numerous studies conducted in Poland on the occurrence of tick borne pathogens in *I. ricinus* ticks, there are still areas where this knowledge is not widely known, and the Opolskie Province located in south-western Poland is one such place. There are many recreational areas in this province and about 36 nature reserves, among others.

OBJECTIVE

The aim of the presented study was to assess the occurrence of *B. burgdorferi* s. l., and its three genospecies (*B. afzelii*, *B. burgdorferi* s. s. and *B. garinii*), *A. phagocytophilum* and *B. microti* in *I. ricinus* ticks, and at the same time, to assess the risk of exposure of humans and animals to infection by these pathogens in selected areas of the Opolskie Province in south-west Poland.

MATERIALS AND METHOD

Ticks were collected by flagging in selected areas of the Opolskie Province: Bryksy (50°17'22"N, 17°58'31"E), Jarnołtówek (50°17'05"N, 17°25'29"E), Grodzisko (50°18'14"N, 17°55'45"E) in April and May in 2016 – 2017, and in May 2018 (Fig. 1). The DNA from single individuals was isolated by the ammonia method [19]. The isolated DNA was measured spectrophotometrically with a 260/280 nanometer wave



Figure 1. The sites sampled for ticks on the territory of Opolskie Province (Poland). A – sampling site in Bryksy (50°17'22"N, 17°58'31"E); B – sampling site in Grodzisko (50°18'14"N, 17°55'45"E)

length. The PCR and nested PCR methods were used to detect pathogens in the ticks. A pair of primers Fla1/Fla2 specific to the flagelline gene was used to detect *B. burgdorferi* s. l. [20]. In turn, to detect genospecies of this spirochete, three specific pairs of primers were used: BA1/BA2 for *B. afzelii*, BG1/BG3 for *B. garinii*, and BB1/BB2 for *B. burgdorferi* s. s. [20]. Whereas to detect *A. phagocytophilum*, two pairs of primers, ge3a/ge10r and ge9f/ge2r, specific to the 16S rDNA gene were used [21]. *B. microti* was detected with the use of two pairs of primers, Bab1/Bab4 and Bab2/Bab3, specific to the 18S rRNA coding gene for the small ribosome subunit [22]. The PCR and nested PCR products were separated electrophoretically in 2% ethidium bromide stained agarose gels, and visualized and photographed in an Omega 10 analyser (UltraLum, USA). Statistical analysis was performed using CSS-Statistica for Windows 10. Statistical significance was declared at the *p* value of less than 0.05. Results were analysed using an χ^2 test.

RESULTS

A total of 222 *I. ricinus* ticks were collected and analyzed for the presence of *B. burgdorferi* s. l., *B. afzelii*, *B. garinii*, *B. burgdorferi* s. s., *A. phagocytophilum*, and *B. microti*. The percentage of infected ticks was the highest in Grodzisko (22.8%), distinctly lower in Jarnołtówek (12.5%), and was the lowest in Bryksy (3.8%). It should be noted that the differences in percentages of infected ticks between Bryksy, Grodzisko and Jarnołtówek were statistically significant (Yates corrected $\chi^2 = 13.87$ and 4.11 , $p = 0.0002$ and 0.043 , respectively), whereas the difference between Grodzisko and Jarnołtówek was insignificant (Yates corrected $\chi^2 = 2.74$, $p = 0.098$). Generally, the molecular studies showed that the collected ticks were most frequently infected with *B. microti*, with the exception of Bryksy (Tab. 1). The remaining detected pathogens were present in a smaller percentage of the collected ticks (Tab. 1), but these differences were statistically insignificant (Yates corrected χ^2 , $p > 0.7$). The spirochetes *B. burgdorferi* s. l., and the rickettsiae *A. phagocytophilum* occurred mainly in ticks collected in Grodzisko (Tab. 1; Fig. 2). These pathogens were present in 7.6%, and 5.4% of ticks collected from this locality, respectively (Tab. 1). In the other two studied areas, these pathogens were present in smaller numbers of collected ticks (Tab. 1). In turn, the highest percentage of ticks infected with *B. microti* was reported in ticks collected in Jarnołtówek (12.5%). But this percentage was only slightly higher than that reported in Grodzisko (9.8%) (Tab. 1); the difference was statistically insignificant (Yates corrected $\chi^2 = 0.20$, $p = 0.67$). It should be stressed that *B. burgdorferi* s. l. and *A. phagocytophilum* were absent in ticks collected in Jarnołtówek, whereas *B. microti*

Table 1. Number and percentage of *Ixodes ricinus* ticks infected with *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in the studied areas of Opolskie Province

Studied area	Number of studied ticks	<i>Borrelia burgdorferi</i> sensu lato	<i>Anaplasma phagocytophilum</i>	<i>Babesia microti</i>
Bryksy	106	3 (2.8%)	1 (0.9%)	0 (0.0%)
Jarnołtówek	24	0 (0.0%)	0 (0.0%)	3 (12.5%)
Grodzisko	92	7 (7.6%)	5 (5.4%)	9 (9.8%)
Total	222	10 (4.5%)	6 (2.7%)	12 (5.4%)

Table 2. Number and percentage of particular developmental stages of *Ixodes ricinus* infected with *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in the studied areas of Opolskie Province

Developmental stage	Number of studied ticks	<i>Borrelia burgdorferi</i> sensu lato	<i>Anaplasma phagocytophilum</i>	<i>Babesia microti</i>
Female	33	1 (3.0%)	0 (0.0%)	4 (12.1%)
Male	45	3 (6.7%)	2 (4.4%)	5 (11.1%)
Nymph	144	6 (4.2%)	4 (2.8%)	3 (2.1%)
Total	222	10 (4.5%)	6 (2.7%)	12 (5.4%)

was not detected in ticks from Bryksy (Tab. 1). The piroplasm *B. microti* were found mainly in the adult stages of both *I. ricinus* while *B. burgdorferi* s. l., and *A. phagocytophilum* were present mainly in males of this species (Tab. 2). Females were statistically more frequently infected with *B. microti* than with *B. burgdorferi* s. l. (Yates corrected $\chi^2 = 4.61$, $p = 0.0317$). Males were also more frequently infected with *B. microti* (11.1%) than with *B. burgdorferi* and *A. phagocytophilum* (6.7% and 4.4%, respectively); these differences, however, were statistically insignificant (Yates corrected χ^2 , $p \geq 0.1$).

A further nested PCR analysis showed nine mono-infections and one co-infection of a particular genospecies of *B. burgdorferi* s. l. In the case of mono-infections, *B. afzelii* was the dominant genospecies in Grodzisko, whereas the co-existence of *B. afzelii* and *B. burgdorferi* s. s. was found only in Bryksy (Tab. 3).

Table 3. Occurrence of particular genospecies of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks collected in the studied areas of Opolskie Province

Studied area	Number of <i>Borrelia burgdorferi</i> sensu lato positive ticks	<i>Borrelia afzelii</i>	Unidentified genospecies	<i>Borrelia afzelii</i> + <i>Borrelia burgdorferi</i> sensu stricto
Bryksy	2	0	2	1
Jarnołtówek	0	0	0	0
Grodzisko	7	7	0	0
Total	10	7	2	1

DISCUSSION

The studies conducted in Poland on the occurrence of *B. burgdorferi* s. l. in *I. ricinus* showed that the percentage of ticks infected with this pathogen varies in different regions of the country [5]. In southern Poland, the number of *I. ricinus* infected ticks ranges from 0% – 79.3% [23]. In the areas investigated in the current study, a significantly lower percentage of ticks infected with *B. burgdorferi* s. l. was shown. This may be caused by the geographical differences in local environmental conditions between the sampling areas, including the level of urbanization, percentage of forest areas, or the number and variety of hosts. The studies conducted in 1996–2007 in south-eastern Poland showed that the biotope may serve as an indicator of the level of infection of ticks with various pathogens, *B. burgdorferi* s.l., among others. Especially, the biotopes with conditions suitable for small rodents and medium mammals which are reservoirs for these pathogens, may have an influence on the

number of ticks infected with *B. burgdorferi* s.l. [24]. The low number of ticks infected with this spirochete in the studied areas of Bryksy and Grodzisko may be caused by the type of biotope. These areas were surrounded by fields, and as shown in the study by Hubelaek et al. [25] and Bartosik et al. [24], the highest risk of infection with the *B. burgdorferi* s. l., exists in deciduous and mixed forests, whereas the lack of this pathogen in ticks from Jarnołtówek where mixed forest areas dominated, may be caused mainly by the low number of studied ticks. To-date, a total of nine *B. burgdorferi* s. l. genospecies have been detected in *I. ricinus* ticks in Poland [26]. In general, the dominant genospecies both in Poland and in Europe is *B. afzelii* [12]. The results obtained in the current study may confirm this general fact.

In turn, studies conducted in eastern Poland by Cisak et al. [27] showed the dominance of *B. burgdorferi* s. s. *B. afzelii* was also stated in these areas, but less frequently. Whereas the lack of *B. garinii* in the studied areas may be corroborated, the study conducted by Asman et al. in the areas of Beskid Żywiecki may suggest that this genospecies is rarely stated in ticks in southern Poland [28]. These differences may indicate that the dominance of each genospecies of this spirochete may vary in different parts of Poland.

In Europe, *A. phagocytophilum* occurred in 0.4–66.7% of examined ticks [29]. In Poland, the percentage of ticks infected with *A. phagocytophilum* differs, but in the southern part of the country this prevalence ranges from 2.6% in the south-western part, to even 76.7% in the south-eastern part [13, 30]. The prevalence of *A. phagocytophilum* in the areas investigated in the current study was not much higher than the prevalence in the south-western part of Poland. This may have been caused by similar environmental conditions in the studied areas. Moreover, in some regions of Poland, this rickettsia was reported mainly in adult *I. ricinus* ticks [15, 31, 32]. In turn, the studies conducted by Chmielewska-Badora et al. [33] in eastern Poland showed that females of *I. ricinus* were more frequently infected with this rickettsia than males and nymphs. The presented study may partially confirm this rule because this pathogen was reported more frequently in males than in nymphs, while the analyzed females were negative for this pathogen.

The prevalence to *B. microti* in ticks in Poland is also variable and ranges from 0.6% of ticks in northern Poland to 5.4% of ticks in eastern Poland, or even 50% in some areas of southern Poland [31, 34, 35]. The results presented in the presented study are almost twice as high as the results obtained by Kiewra et al. [30] in south-western Poland. These results may indicate that the prevalence of this protozoan in the southern part of Poland increases as one moves eastward. This trend may be caused by the difference in local environment, especially by the level of urbanization and the number of rodents. Nevertheless, the highest percentage of ticks infected with *B. microti* was stated in Jarnołtówek, located in the Opawskie Mountains, dominated mainly by mixed forest areas. This result may suggest that in this type of biotope, besides the high risk of tick-borne infections with *B. burgdorferi* s. l. and tick-borne encephalitis viruses, there exists also a high risk of infection with protozoans *B. microti*.

CONCLUSIONS

The presence of *B. burgdorferi* s. l., *A. phagocytophilum* and *B. microti* in *I. ricinus* was shown in ticks collected in the examined areas of Grodzisko. In the other two studied areas, only *B. burgdorferi* s. l. and *A. phagocytophilum* or only *B. microti* were stated in the collected ticks. The presented results confirm the role of this ectoparasite in spreading these pathogens in Poland, and they are also the first reports on the prevalence of these pathogens in ticks in the examined areas of the Opolskie Province. Moreover, the obtained results confirmed that *B. afzelii* is one of the dominant genospecies in Poland. The possibility of co-existence of more than one genospecies in one tick was also confirmed.

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