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The occurrence of three tick-borne pathogens in *Ixodes ricinus* ticks collected from the area of the Kraków-Częstochowa Upland (Southern Poland)

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ABSTRACT

The common tick *Ixodes ricinus* is the main vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in Europe. The aim of this study was to estimate the potential risk of exposure to common tick-borne diseases in the recreational areas of the Kraków-Częstochowa Upland, Poland with particular emphasis on the city of Częstochowa. The DNA from 459 *I. ricinus* ticks was used to detect *B. burgdorferi* s. l., *A. phagocytophilum* and *B. microti* by PCR and nested PCR methods. Generally, infectious agents were found in 26.3% of all the examined ticks: *B. microti* was found in 23.3%, and *A. phagocytophilum* in 2.4% of the ticks. The protozoan was found mainly in females and nymphs, while *A. phagocytophilum* was detected mainly in adults. The co-existence of *B. microti* and *A. phagocytophilum* was found in 2.1% of the examined ticks. The presence of *B. burgdorferi* s. l. was not revealed in the examined material. The conducted studies demonstrate the high potential risk of exposure of tourists and pilgrims to *B. microti*, and a low risk of exposure to *A. phagocytophilum* and *B. burgdorferi* s.l. in the examined areas of the Kraków-Częstochowa Upland. Furthermore, the possible co-existence of *A. phagocytophilum* and *B. microti* in 3 individual ticks means that infection by multiple infectious agents is possible. The obtained results highlight the need to conduct further research on tick-borne pathogens in this region of Poland.

Keywords *Borrelia burgdorferi* sensu lato; *Babesia microti*; *Anaplasma phagocytophilum*; tourism; exposure risk

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Introduction

Ticks (Acari: Ixodida) are commonly known parasites of animals and humans. In Europe, the main vector of *Borrelia burgdorferi* sensu lato, *Babesia microti* and *Anaplasma phagocytophilum* is the common tick *Ixodes ricinus* (Balmelli and Pifaretti 1995; Blanco and Oteo 2002; Kiewra and Lonc 2004; Nowak-Chmura 2013; Yabsley and Shock 2013). These pathogens are etiological agents of borreliosis, human babesiosis and human granulocytic anaplasmosis, respectively (Spielman 1976; Burgdorfer *et al.* 1982; Maeda *et al.* 1987). Lyme disease counts among the most dangerous tick-borne diseases. There are three main clinical symptoms of this disease: chronic arthritis, neuroborreliosis and acrodermatitis chronica atrophicans (ACA) (Balmelli and Pifaretti 1995). In Poland, the first case of this polymorphous disease in

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humans was described in West Pomerania (Januszkiewicz and Kieda 1987). The rickettsiae *A. phagocytophilum* causes human granulocytic anaplasmosis (Oehme *et al.* 2002; Grzeszczuk *et al.* 2004). The first case of this disease in Europe was recorded in Slovenia (Petrovec *et al.* 1997). In Poland, the first case of human anaplasmosis was reported in 2001 (Tylewska-Wierzbanowska *et al.* 2001). The symptoms of this disease are flu-like: fever, myalgias, arthalgias and headache (Blanco and Oteo 2002). Babesiosis in Europe was described for the first time in Yugoslavia (Boustani and Gelfand 1996). The initial symptoms of this disease are often under-recognised and confused with a cold or flu. Up to 9 weeks after the infection, we can observe non-specific symptoms such as headaches, muscle aches, fever, chills or general fatigue (Rożej-Bielicka *et al.* 2015). Humans can be infected by these pathogens by a tick bite if they encounter the common vector *Ixodes ricinus* in meadows, forests, parks and gardens (Nowak-Chmura 2013).

The Kraków-Częstochowa Upland is one of the most attractive tourist regions in Poland. This region is situated between two towns – Kraków and Częstochowa (Figure 1), popular recreational places with a lot of tourist attractions. This is particularly true for the city of Częstochowa, which is a well-known European pilgrimage city. In this city, there are a lot of parks where citizens and tourists spend their free time. The most popular, especially among pilgrims, is Jasna Góra Park located under the Jasna Góra Monastery. Work and recreation in the area of the Kraków-Częstochowa Upland can be associated with an increased risk of human exposure (forestry workers, tourists, citizens) to ticks. However, we still know little about the potential exposure risk for the transmission of tick-borne pathogens within these densely inhabited regions. So far, studies on tick fauna have only been conducted in some parts of this region. During these studies, only *I. ricinus* was found, suggesting that *I. ricinus* is the most common tick species in Poland. In addition, these faunistic studies confirmed that the tick density in the studied areas depends on the humidity conditions and air temperature (Siuda *et al.* 1992; Nowak *et al.* 2009; Solarz *et al.* 2007, 2010; Nowak-Chmura 2013).

Based on this previous work, the objective of the present study was to determine the potential risk of exposure of tourists and pilgrims to tick-borne infections. We therefore screened *I. ricinus* ticks from the selected recreational areas of the Kraków-Częstochowa Upland, with a particular emphasis on the recreational areas of Częstochowa, for the presence of *B. burgdorferi* s.l., *A. phagocytophilum* and *B. microti*.

Materials and methods

To the study on the occurrence of tick-borne pathogens, *I. ricinus* ticks from the collection of the Department of Parasitology, Medical University of Silesia in Katowice, Poland were used. The ticks were previously collected by flagging (Pet'ko *et al.* 1997) during the spring and autumn peaks in their activity in 2013-2014. Ticks came from the selected recreational areas of Klucze, Jaroszowiec, Pazurek, Rabsztyn, Ogrodzieniec, Mirów, Jasna Góra Park in Czestochowa (the common name for two closely situated little parks – the Staszic Park, and the 3rd of May Park, located close to the Jasna Góra Monastery), Aniołowski Forest in Częstochowa, Lisiniec Park in Częstochowa (Figures 1 and 2).

Evaluation of the environmental impact severity

The DNA was isolated from whole ticks by the ammonia method (Guy and Stanek 1991). The ticks were removed from alcohol and dried on filter paper. They were then placed in a sterile plastic tube with 100µl 0.7 M NH_4OH. Next, ticks were crushed mechanically. The samples were boiled at 100°C for 15 minutes in a TB-941U thermobloc (JWElectronic, Poland). Then, the caps were opened and samples were boiled at 100° C for 10 minutes to remove the ammonia. Next, the samples were centrifuged at 12 000 rotations per minute [rpm] for 5 minutes in a microcentrifuge (Hettig, Germany). After centrifugation, the supernatant was transferred to a new plastic tube. The concentration of DNA was measured spectrophotometrically in the

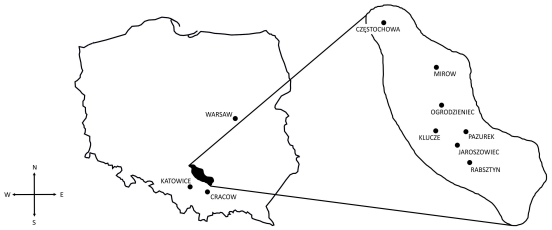


Figure 1 Locations of the Kraków-Częstochowa Upland in Poland and the location of the studied areas.

PEARL nanosphectrophotometer (Implen, Germany). DNA was stored at -20°C for further analysis.

Borrelia burgdorferi s. l. in ticks was detected using the Fla1/Fla2 primer pair, specific for the flagelline gene (Wodecka and Skotarczak 2000). The amplification mixture was as follows: 200 ng of the DNA, 2 µL of the reaction buffer diluted 10x, 0.5 µL of 2 mM dNTPs, 0.8 µL of each primer and 0.1 µL of *Taq* polymerase (ThermoScientific, USA). The sample was topped up with molecular grade water to the total volume of 20 µL. The PCR conditions were as follows: the initial denaturation (3 min at 95 °C), the proper denaturation (30 sec at 94 °C), primer annealing (45 sec at 54 °C), elongation (45 sec at 72 °C), and the final elongation (7 min at 72 °C). A total of 35 cycles was performed. The positive control of the DNA of *Borrelia afzelii* was isolated from culture and was obtained by courtesy of dr Angelina Wójcik-Fatla, PhD from The Institute of Agricultural Medicine in Lublin. To detect *B. microti* in ticks, two primer pairs – Bab1/Bab4 and Bab2/Bab3 – specific to the 18S rRNA gene of the small ribosome subunit were used (Pershing *et al.* 1992). The first PCR conditions were



Figure 2 Locations of the study areas in Częstochowa City.

as follows: the initial denaturation (1 min at 94 °C), the proper denaturation (1 min at 94 °C), primer annealing (1 min at 60 °C), elongation (2 min at 72 °C) and the final elongation (7 min at 72 °C); 35 cycles were performed. The amplification mixture of the first PCR was as follows: 200 ng of the DNA, 2.5 µL of the reaction buffer diluted 10x, 1 µL of 2 mM dNTPs, 1 µL of each primer and 0.1 µL of *Taq* polymerase (ThermoScientific, USA). The sample was topped up with molecular grade water to the total volume of 25 µL. The conditions of the second PCR were the same as the first, except that 30 cycles were performed. The amplification mixture of the second PCR was as follows: 1µL of the first amplification product, 2.5 µL of the reaction buffer diluted 10x, 1 µL of 2 mM dNTPs, 1 µl of each primer and 0.1 µL of *Taq* polymerase (ThermoScientific, USA). The sample was again topped up with molecular grade water to the total volume of 25 µL. The positive control of *B. microti* was obtained courtesy of dr Angelina Wójcik-Fatla, PhD from The Institute of Agricultural Medicine in Lublin.

To detect *A. phagocytophilum* in ticks two primer pairs – ge3a/ge10r and ge9f/ge2r – specific to the 16S rDNA gene were used (Massung *et al.* 1998). The conditions of the first PCR were as follows: the initial denaturation (2 min. at 95 °C), the proper denaturation (30 sec at 94 °C), primer annealing (30 sec at 55 °C), elongation (1 min at 72 °C) and the final elongation (5 min at 72 °C). A total of 40 cycles was performed. The amplification mixture of the first PCR was as follows: 200 ng of the DNA, 2.5 µL of the reaction buffer diluted 10x, 1 µL of 2 mM dNTPs, 1 µL of each primer and 0.1 µL of *Taq* polymerase (EURx, Poland). The sample was topped up with molecular grade water to the total volume of 25 µL. The conditions of the second PCR were the same as described for the first, except that 30 cycles were performed. The amplification mixture of the second PCR was as follows: 1 µL of the first amplification product, 2.5 µL of the reaction buffer diluted 10x, 1 µL of 2 mM dNTPs, 1 µL of each primer and 0.1 µL of *Taq* polymerase (EURx, Poland). The sample was topped up with molecular grade water to the total volume of 25 µL. The positive control of *A. phagocytophilum* was obtained courtesy of dr Angelina Wójcik-Fatla, PhD from The Institute of Agricultural Medicine in Lublin.

Amplifications and reamplifications were carried out in a MJ Mini thermal cycler (BioRad, USA). The reaction products were separated electrophoretically in 2% ethidium bromide stained agarose gels at 80 V for 2 hrs, and then were visualized and photographed in the Omega 10 analyser (UltraLum, USA). The expected product sizes were as follows: 482 base pairs [bp] for *B. burgdorferi* s.l., 238 bp and 154 bp for *B. microti*, 932 bp and 546 bp for *A. phagocytophilum*.

The statistical analysis was performed using CSS-Statistica for Windows version 12, with a significance threshold of p-value < 0.05. Results were analyzed using the χ^2 test. Differences in the prevalence of pathogens in ticks were compared using the Yates corrected χ^2 test (2 x 2).

Results

A total of 459 *I. ricinus* ticks was collected, including 178 females, 138 males and 143 nymphs; all were screened for detection of *B. burgdorferi* s. l., *A. phagocytophilum* and *B. microti*. Pathogens were found in 26.3% of the examined ticks. *B. microti* was found in 23.3%, and *A. phagocytophilum* was found in 2.4%. The protozoan was reported mainly in females and nymphs, whereas the rickettsiae was found mainly in males. The co-existence of both infectious agents was shown only in nymphs and males (Table 1). The presence of *B. burgdorferi* s. l. was not shown in the examined material.

The highest percentage of ticks infected with *B. microti* was reported in Rabsztyn (41.4%), Jaroszowiec (32.4%), Częstochowa (26.4%), Mirów (24.6%) and Ogrodzieniec (20.5%). It should be stressed that the difference in the percentage of infected ticks was statistically significant between Rabsztyn and Częstochowa (χ^2= 5.05; p = 0.025), whereas the difference was insignificant between Rabsztyn and Jaroszowiec (χ^2= 1.75; p = 0.19). Moreover, differences in the percentage of ticks infected with *B. microti* were also statistically significant between

Table 1 Number and percentage of developmental stages of ticks infected with *Borrelia burgdorferi* sensu lato, *Babesia microti* and *Anaplasma phagocytophilum* in the studied areas of the Kraków-Częstochowa Upland.

Developmental stage	Number of studied specimens	1 pathogen			2 pathogens
		Borrelia burgdorferi sensu lato	*Babesia microti*	*Anaplasma phagocytophilum*	*Anaplasma phagocytophilum* + *Babesia microti*
Female	178	0 (0.0%)	49 (27.5%)	4 (2.2%)	0 (0.0%)
Male	138	0 (0.0%)	22 (15.9%)	5 (3.6%)	1 (0.7%)
Nymph	143	0 (0.0%)	36 (25.1%)	2 (1.4%)	2 (1.4%)
Total	459	0 (0.0%)	107 (23.3%)	11 (2.4%)	3 (0.6%)

Rabsztyn and Mirów or Rabsztyn and Ogrodzieniec (χ^2= 5.09, p = 0.0241 and χ^2= 8.44, p = 0.0037, respectively). The remaining differences, between Mirów and Jaroszowiec or Częstochowa, between Ogrodzieniec and Jaroszowiec or Częstochowa, and between Mirów and Ogrodzieniec were statistically insignificant (χ^2; p > 0.1 in all cases).

Anaplasma phagocytophilum was shown mainly in ticks collected in the areas of Rabsztyn (4.8%) and Czestochowa (4.2%). The co-existence of *B. microti* and *A. phagocytophilum* was reported only in 2.1% of the ticks collected in Częstochowa. (Table 2).

Within Częstochowa, ticks were infected mainly with *B. microti* (Jasna Góra Park and Lisiniec Park). However, *A. phagocytophilum* was found in Jasna Góra Park and Aniołowski Forest. The co-existence of *B. microti* and *A. phagocytophilum* was found only in ticks from the Aniołowski Forest area (Table 2). The difference in the percentage of ticks infected with *B. microti* was statistically significant (χ^2= 9.27; p = 0.0023) between the Jasna Góra Park and Aniołowski Forest, but not between other localities in Częstochowa (χ^2; p > 0.1).

In total, in Czestochowa, pathogens were found in 32.86 % of the examined ticks. *B. microti* was found mainly in adults (31.8% of females and 25% of males), whereas *A. phagocytophilum* was mainly found in males and nymphs (5.7% and 4.5%, respectively) (Table 3). None of these values were significantly different (χ^2; p > 0.05).

Discussion

So far, studies of tick fauna have only been conducted in the areas of the Kraków-Częstochowa Upland in Poland. These previous faunistic surveys showed a high risk of exposure of humans

Table 2 Number and percentage of ticks infected with *Babesia microti* and *Anaplasma phagocytophilum* in the studied areas of the Kraków-Częstochowa Upland.

Study locations		The number of studied specimens	1 pathogen		2 pathogens
			Babesia microti	*Anaplasma phagocytophilum*	*Anaplasma* + *Babesia microti*
Klucze		65	6 (9.2%)	0 (0.0%)	0 (0.0%)
Jaroszowiec		37	12 (32.4%)	1 (2.7%)	0 (0.0%)
Pazurek		64	9 (14.0%)	1 (1.5%)	0 (0.0%)
Rabsztyn		41	17 (41.4%)	2 (4.8%)	0 (0.0%)
Ogrodzieniec		39	8 (20.5%)	0 (0.0%)	0 (0.0%)
Mirów		73	18 (24.6%)	1 (1.3%)	0 (0.0%)
Częstochowa	Jasna Góra Park	19	8 (42.1%)	1 (5.2%)	0 (0.0%)
	Aniołowski Forest	90	19 (21.1%)	5 (5.5%)	3 (3.3%)
	Lisiniec Park	31	10 (32.2%)	0 (0.0%)	0 (0.0%)
		140	37 (26.4%)	6 (4.2%)	3 (2.1%)
Total		459	107 (23.3%)	11 (2.4%)	3 (0.6%)

Table 3 Number and percentage of development stages of ticks infected with *Babesia microti* and *Anaplasma phagocytophilum* in the studied areas of Częstochowa.

Developmental stage	Number of studied specimens	1 pathogen		2 pathogens
		Babesia microti	*Anaplasma phagocytophilum*	*Anaplasma phagocytophilum* + *Babesia microti*
Female	44	14 (31.8%)	1 (2.2%)	0 (0.0%)
Male	52	13 (25.0%)	3 (5.7%)	1 (1.9%)
Nymph	44	10 (22.7%)	2 (4.5%)	2 (4.5%)
Total	140	37 (26.4%)	6 (4.2%)	3 (2.1%)

and animals to ticks in this region (Siuda *et al.* 1992; Nowak *et al.* 2009; Solarz *et al.* 2007, 2010; Nowak-Chmura 2013). Research on pathogens transmitted by these ticks was not conducted. However, it is commonly known that *I. ricinus* is the main vector and/or reservoir of *B. burgdorferi* s.l., *B. microti* and *A. phagocytophilum* in other regions of Poland (Nowak-Chmura 2013), highlighting the need for a more indepth study of their presence in the Kraków-Częstochowa Upland area .

From previous work, the highest percentage of ticks infected with *B. burgdorferi* s. l. was found in the Upper and Lower Silesia province, Mazowieckie, Lubelskie and Western Pomerania province (Kiewra 2014). The studies conducted in areas of southern Poland also showed that the infection prevalence ranged from 4% in the areas of Upper Silesia to up to 62% in areas of Beskid Żywiecki (Pet'ko *et al.* 2002; Asman *et al.* 2012). The presence of *B. burgdorferi* s. l. has not been shown in the present study. The lack of spirochaetes in the studied areas may be caused by the low number of the studied ticks. However, this suggests that the risk of human exposure to these bacteria may be low compared to other regions of Poland. It could also be that infected ticks were missed by our PCR method; indeed, only a single PCR procedure was used rather than a more powerful nested procedure. As *Borrelia* intensities in questing ticks may vary, it is also possible that ticks infected at low intensities were missed. However, as previous work in Poland used the same protocol (Wójcik-Fatla et al. 2009), our results may suggest that infection risk is still lower in the Kraków-Częstochowa Upland region compared to other regions.

The studies conducted in northern and north-eastern Poland showed the presence of *B. microti* in 0.6%-2.3% of ticks (Stańczak *et al.* 2004; Siński *et al.* 2006). Comparable results were obtained in central-eastern, eastern and south-eastern Poland. In these areas, the percentage of infected ticks ranged from 3.1% to 3.5% (Wójcik Fatla *et al.* 2009; Sytykieicz *et al.* 2012; Kiewra *et al.* 2014), whereas studies conducted in the areas of southern Poland showed varying levels of ticks infected with *B. microti,* ranging from 0%-3.9% in ticks collected from people, 35% in ticks collected from the Żywiec Landscape Park to 50.87% in ticks collected from areas of the Tarnogórski district (Asman *et al.* 2012; Albertyńska *et al.* 2016; Asman *et al.* 2014; Asman *et al.* 2015). The results of the present study are higher than those obtained from northern, eastern, central and south-western Poland and comparable to those of southern Poland. These results may indicate a high risk of exposure of humans to this pathogen in the Kraków-Częstochowa Upland region.

In Europe, the prevalence of ticks infected with *A. phagocytophilum* ranges from 0.4 to 66.7% (Blanco and Oteo 2002; Smrdel *et al.* 2010). The studies conducted in southern Poland showed similar prevalence ranges from 2.6% in Lower Silesia, 38.9% in Upper Silesia to even 76.7% in the territory of the Niepołomnice Forest (Cisak *et al.* 2005; Cuber *et al.* 2011; Asman *et al.* 2013; Asman *et al.* 2014;Kiewra *et al.* 2014). The studies conducted in eastern Poland by Wójcik-Fatla *et al.* (2009) showed only 4.9% of ticks infected with *A. phagocytophilum*. However, studies conducted by Stańczak *et al.* (2004) in northern Poland, Skotarczak *et*

al. (2008) in north-western Poland and Tomasiewicz *et al.* (2004) in mid-eastern Poland, Sytykiewicz *et al.* (2012) in central eastern Poland showed this rickettsia in 14%, 5.5%, 13.1% and 8.5% of examined ticks, respectively. Studies conducted in northern, mid-eastern and south-eastern Poland showed the pathogen mainly in adult ticks (Stańczak *et al.* 2004; Tomasiewicz *et al.* 2004; Cisak *et al.* 2005). Generally, our findings confirmed that *A. phagocytophilum* is reported mainly in adult ticks. The exceptions are ticks collected from the recreational areas of Częstochowa, where a higher percentage of nymphs were infected with this pathogen than adult ticks. Overall, these results are within the European average and match results obtained in western, mid-eastern, south-eastern and south-western Poland. However, they are lower than results obtained in recreational areas of southern Poland. Based on the presented results, a low risk of exposure to *A. phagocytophilum* for tourists and pilgrims in the Kraków-Częstochowa Upland is suggested.

There are known cases of co-existence of two or more pathogens in one tick. However, such a phenomenon usually only occurs in a small percentage of studied ticks (Stańczak *et al.* 2004; Wójcik Fatla *et al.* 2009; Asman *et al.* 2013; Asman *et al.* 2015). The study conducted by Asman *et al.* (2012, 2014) in southern Poland showed the co-existence of *A. phagocytophilum* and *B. microti* in 0% to 5% of ticks. The studies conducted in other regions of Poland showed the co-existence of these two pathogens ranging from 1.1% in eastern Poland, to 1.8% in central eastern Poland, and up to 10.6% in northern Poland (Stańczak *et al.* 2004; Wójcik Fatla *et al.* 2009; Sytykiewicz *et al.* 2012). Our results are therefore similar to those obtained in southern, eastern and central-eastern Poland, and suggest low levels of co-infections with *A. phagocytophilum* and *B. microti* ticks in the areas of the Kraków-Częstochowa Upland.

Conclusions

The conducted study suggests a high potential risk of exposure of humans to *B. microti.* and a low potential risk for *A. phagocytophilum* and *B. burgdorferi* s.l. in the Kraków-Częstochowa Upland area. The co-existence of the studied infectious agents was low, but may lead to difficulties in disease diagnostics. However, the presence of *B. microti* and *A. phagocytophilum* in both adults and nymphs confirms that both developmental stages of *I. ricinus* may be reservoirs and/or vectors for these pathogens. As the apparent lack of *B. burgdorferi* s.l. in the studied ticks may suggest low prevalence and/or infection intensities of this pathogen in the studied area, further studies using more powerful detection techniques are called for. The obtained results indicate that tourists, pilgrims and other people who visit, or spend their free time in these areas of the Kraków-Częstochowa Upland should use official preventive measures to avoid tick bites.

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References

Albertyńska M., Rozwadowska B., Mendera-Bożek U., Jasik K.P., Okła H., Słodki A., Słodki J., Swinarew A.S., Cieślik-Tarkota R. 2016. The presence of *Borrelia burgdorferi* sensu lato, *Babesia microti*, *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*/*Ehrlichia muris* and tick borne encephalitis virus (TBEV) in ticks from the Silesian Province. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Interrelationships in the host-ectoparasite-pathogen system. Lublin: Koliber. p. 67-74 [in Polish].

Asman M., Gąsior T., Pająk C., Cuber P., Szilman P., Szilman E., Solarz K. 2012. Occupational risk of infections with *Borrelia burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *B. garinii* and *B.*

afzelii in agricultural workers on the territory of Beskid Zywiecki. In: Buczek A., Błaszak Cz. (Eds). Arthropods. The medical and economic importance. Lublin: Akapit. p. 163-170.
Asman, M., Gąsior T., Jacek E., Cuber P., Solarz K. 2012. Isolation of rickettsiae *Anaplasma phagocytophilum* and protozoans *Babesia* sp. in castor bean ticks *(Ixodes ricinus*) collected from patients of public health centres in the territory of Upper Silesia. In: Buczek A. Błaszak Cz. (Eds). Arthropods. The medical and economic importance. Lublin: Akapit. p. 171-180.
Asman M., Nowak M., Cuber P., Strzelczyk J., Szilman E., Szilman P., Trapp G., Siuda K., Solarz K., Wiczkowski A. 2013. The risk of exposure to *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Babesia* sp. and co-infections in *Ixodes ricinus* ticks in the territory of Niepołomice Forest (southern Poland). Ann Parasitol., 59: 13-19.
Asman M., Pindel Ł., Solarz K. 2014. The risks of occupational exposure to spirochaetes *Borrelia burgdorferi* sensu lato, rickettsiae *Anaplasma phagocytophilum* and protozoans *Babesia microti* in the area of Zywiecki Landscape Park. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Threat to human and Animals health. Lublin: Koliber. p. 153-140 [in Polish].
Asman M., Solarz K., Cuber P., Gąsior T., Szilman P., Szilman E., Tondaś E., Matzullok A., Kusion N., Florek K. 2015. Detection of protozoans *Babesia microti* and *Toxoplasma gondii* and their co-existence in ticks (Acari: Ixodida) collected in Tarnogórski district (Upper Silesia, Poland). Ann Agric Environ Med., 22: 80-83. doi:10.5604/12321966.1141373
Balmelli T., Pifaretti J.-C. 1995. Association between different clinical manifestation of Lyme disease and different species of *Borrelia burgdorferi* sensu lato. Res Microbiol., 146: 329-340.doi:10.1016/0923-2508(96)81056-4
Blanco J.-R., Oteo J.-A. 2002. Human granulocytic ehrlichiosis in Europe. Clin Microbiol Infect., 8: 763-772. doi:10.1046/j.1469-0691.2002.00557.x
Boustani M.-R., Gelfand J.-A. 1996. Babesiosis. Clin Infect Dis., 22: 611-614. doi:10.1093/clinids/22.4.611
Burgdorfer W., Barbour A.-G., Hayes S.-F., Benach J.-L., Grunwaldt E., Davis J.-P. 1982. Lyme disease-a-tick-borne spirochetosis. Science, 216: 1317-1319. doi:10.1126/science.7043737
Cisak E., Chmielewska-Badora J., Zwoliński J., Wójcik-Fatla A., Polak J., Dutkiewicz J. 2005. Risk of tick borne bacterial diseases among workers of Roztocze National Park (South-Eastern Poland). Ann Agric Environ Med., 12: 127-132.
Cuber P., Asman M., Solarz K., Szilman E., Szilman P. 2011. The prevalence of *Anaplasma phagocytophilium* and *Babesia* sp. in ticks (Acari: Ixodida) collected from recreational areas of Silesian Province. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Human and animal parasites. Lublin: Akapit. p. 105-114.
Grzeszczuk A., Stańczak J., Kubica-Biernat B., Racewicz M., Kruminis-Łozowska W., Prokopowicz D. 2004. Human anaplasmosis in north - eastern Poland: seroprevalence in humans and prevalence in *Ixodes ricinus* ticks. Ann Agric Environ Med., 11: 99-103.
Guy E., Stanek G. 1991. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. J Clin Pathol., 29: 610-611. doi:10.1136/jcp.44.7.610
Januszkiewicz J., Kieda A. 1987. Cases of borreliosis (Lyme disease) in Western Pomerania. Epidemiol Rev., 41: 324-329 [in Polish].
Kiewra D., Lonc E. 2004. Biology of *Ixodes ricinus* (L.) and its pathogens in Wrocław area —Wiad Parazytol., 50: 259-264 [in Polish].
Kiewra D. 2014. Ocena wektorowej roli kleszczy *Ixodes ricinus* L. 1758 (Acari: Ixodidae) w transmisji kretków Borrelia burgdorferi s. l. na terenie Polski, ze szczególnym uwzględnieniem Dolnego Śląska. Wrocław: I-Bis. [in Polish].
Kiewra D., Zaleśny G., Czułowska A. 2014. The risk of infection with *Anaplasma phagocytophilum* and *Babesia microti* in Lower Silesia, SW Poland. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Threat to human and animal health. Lublin: Koliber. p. 103-110.
Maeda K., Markowitz N., Hawley R.-C., Ristic M., Cox D., McDade J.-E. 1987. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. New Eng J Med., 316: 853-856. doi:10.1056/NEJM198704023161406
Massung R.-F., Slater K., Owens J.-H., Nicholson W.-L., Mather T.-N., Solberg V.-B. and Olson J.-G. 1998. Nested PCR Assay for detection of granulocytic ehrlichiae. J Clin Microbiol., 36: 1090-1095.
Nowak M., Siuda K., Solarz K., Góra A., Cuber P. 2009. A risk of infection with ticks of *Ixodes ricinus* (Linnaeus, 1758) species (Acari: Ixodidae) in south-eastern Poland depending on the daily and seasonal rhythm. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Invasion and their control. Lublin: Akapit. p. 31-43.
Nowak-Chmura M. 2013. Fauna kleszczy (Ixodida) Europy Środkowej. Kraków: Wydawnictwo Naukowe Uniwersytetu Pedagogicznego. [in Polish].
Oehme R., Hartelt K., Backe H., Brockmann S., Kimmig P. 2002. Foci of tick borne diseases in southwest Germany. Int J Med Microbiol., 291: 22-29. doi:10.1016/S1438-4221(02)80005-4
Pet'ko B. 2002. Lyme borreliosis in carpathian region of central Europe – ecological aspect of diagnostics. In: Buczek A., Błaszak Cz. (Eds). Arthropods. Arthropods in medicine. Lublin: Koliber. p. 93-104.
Pet'ko B., Siuda K., Stanko M., Tresova G., Karbowiak G., Fricova J. 1997. *Borrelia burgdorferi* sensu lato In the *Ixodes ricinus* ticks In southern Poland. Ann Agric Environ Med., 4: 263-269.
Persing D.-H., Mathiesen D., Marshall W.-F., Telford S.-R., Spielman A., Thomford J.-W., Conrad P.-A. 1992. Detection of *Babesia microti* by polymerase chain reaction. J Clin Microbiol., 30: 2097–2103.
Petrovec M., Lotric-Furlan S., Zupanc T.-A., Strle F., Brouqui Ph., Roux V., Dumler J.-S. 1997. Human disease in Europe caused by a granulocytic *Ehrlichia* species. J Clin Microbiol., 35: 1556-1559.
Rożej-Bielicka W., Stypułkowska-Misiurewicz H., Gołab E. 2015. Human Babesiosis. Epiemiol Rev., 69: 489-494.

Siński E., Pawełczyk A., Bajer A., Behenke J.-M. 2006. Abundance of wild rodents, ticks and environmental risk of Lyme borreliosis: a longitudinal study in an area of Mazury Lakes District of Poland. Ann Agric Environ Med., 13: 295-300.

Skotarczak B., Adamska M., Sawczak M., Maciejewska A., Wodecka B., Rymaszewska A. 2008. Coexistence of tick borne pathogens in game animals and ticks in western Poland. Vet Med., 53: 668-675. doi:10.17221/1975-VETMED

Siuda K., Solarz K., Deryło A., Buczek A., Sadowski T., Kwiatkowski S. 1992. Studies on ticks (Acari: Ixodida) from the Krakow-Czestochowa Upland with particular consideration of the occurrence and quantity of the population of *Ixodes ricinus* (L.) (Ixodidae) on the territories affected at various stages of antropopressure. Prądnik, 5: 235-245 [in Polish].

Smrdel K.-S., Serdt M., Duh D., Knap N., Županc T.-A. 2010. *Anaplasma phagocytophilum* in ticks in Slovenia. Parasites & Vectors, 3: 102. doi:10.1186/1756-3305-3-102

Spielman A. 1976. Human babesiosis on Nantucket Island: transmission by nymphal Ixodes ticks. Am J Trop Med Hyg., 25: 784-787. doi:10.4269/ajtmh.1976.25.784

Solarz K., Piec M., Batoryna O., Kwiecień W., Szilman P. 2007. Number of *Ixodes ricinus* L. (Acari: Ixodida: Ixodidae) in the territory of Ojców National Park. Prądnik, 17: 113-120 [in Polish].

Solarz K., Asman M., Cuber P., Gomółka P., Komosińska B., Nazarkiewicz M. 2010. The abundance of *Ixodes ricinus* L. tick (Acari: Ixodida: Ixodidae) in the Zachwyt Valley (Ojców National Park) during the autumn peak of tick activity. Prądnik, 20: 323-332 [in Polish].

Stańczak J., Mohammed Gabre R., Kruminis-Łozowska W., Racewicz M., Kubica-Biernat B. 2004. Ixodes riciuns as a vector of B*orrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and **Babesia microti** in urban and suburban forests. Ann Agric Environ Med., 11: 109-114.

Sytykiewicz H., Karbowiak G., Hapunik J., Szpechciński A., Supergan-Marwicz M., Goławska S., Sprawka I., Czerniewicz P. 2012. Molecular evidence of *Anaplasma phagocytophilum* and *Babesia microti* co-infections in Ixodes ricinus ticks in central-eastern region of Poland. Ann Agric Environ Med., 19: 45-49.

Tomasiewicz K., Modrzewska R., Buczek A., Stańczak J., Maciukajć J. 2004. The risk of exposure to *Anaplasma phagocytophilum* infection in mid-eastern Poland. Ann Agric Environ Med., 11: 261-264.

Tylewska-Wierzbanowska S., Kruszewska D., Chmielewski T., Żukowski K., Żabicka J. 1996. Ticks as a reservoir of Borrelia burgdorferi and Coxiella burnetii in Poland. Epidemiol Rev., 50, 245-51 [in Polish].

Wodecka B., Skotarczak B. 2000. Genetic diversity of Borrelia burgdorferi s. l. in *Ixodes ricinus* ticks collected in north-west Poland. Wiad Parazytol., 46: 475-478 [in Polish].

Wójcik-Fatla A., Szymańska J., Wdowiak L., Buczek A., Dutkiewicz J. 2009. Coincidence of three pathogens (*Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti*) in *Ixodes ricinus* ticks in the Lublin macroregion. Ann Agric Environ Med., 16: 151-158.

Yabsley M.-J, Shock B.-C.2013. Natural history of Zoonotic Babesia:Role of wildlife reservoirs. Int J Parasitol Parasites Wildl, 2: 18-31. doi:/10.1016/j.ijppaw.2012.11.003