

## PREVALENCE OF *BABESIA MICROTI* IN *IXODES RICINUS* TICKS FROM LUBLIN REGION (EASTERN POLAND)

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**Abstract:** A total of 1,367 *Ixodes ricinus* ticks collected from 5 districts of the Lublin region (eastern Poland) were examined for the presence of *Babesia microti* DNA by PCR and nested-PCR. As many as 74 ticks (5.4%) were found to be infected with *Babesia microti*. The infection rate varied significantly with stage/sex of ticks ( $\chi^2=16.48543$ ,  $df=2$ ,  $p<0.000264$ ). The infection rates in females and males amounted to 6.4% and 8.8% respectively and were significantly higher ( $p=0.006$  and  $p=0.0001$  respectively) compared to minimum infection rate in nymphs that was equal to 2.8%. The prevalence of infection showed also a significant variability depending on geographic location within the Lublin region ( $\chi^2=18.62812$ ,  $df=4$ ,  $p<0.000932$ ). The highest rates of infection with *Babesia microti* were noted in ticks collected from the areas of Puławy district situated in the northern part of region and the suburban Lublin district situated in the central part of the region (8.0% and 7.3% respectively). Mediocre infection rates (respectively 3.4% and 3.3%) were found in ticks from the Parczew and Włodawa districts situated in eastern part of the region and covered with humid lakeland forests. The lowest infection rate (0.5%) was noted in ticks from the Zamość district situated in southern part of the region. In conclusion, the infection rate of *Ixodes ricinus* ticks with *Babesia microti* found in this study is higher compared to the majority of data reported by Polish and other European authors, and indicates a potential risk of human infection during occupational or recreational exposure to tick bite.

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### INTRODUCTION

The protozoans *Babesia microti* and *Babesia divergens*, the etiologic agents of human babesiosis are obligate intracellular pathogens that invade, infect and kill the red blood cells [10, 12, 21, 24]. They are parasites of small mammals and cattle, transmitted to humans by ixodid ticks which acquire the infection by feeding on infected hosts [8, 15, 23, 34, 35].

Human infections with *Babesia* spp. have been reported from many countries mainly from the northern part of the

USA, [3, 24] and from Europe [20, 22, 29, 26]. The first documented case of human babesiosis in Europe was described in Yugoslavia in 1957 [7] and in Poland in 1997, as a disease presumably imported from Brazil, caused by *B. microti* [19]. While in the USA, *Babesia microti* is the commonest cause of human babesiosis [3, 18, 24], in Europe this disease is evoked predominantly by *Babesia divergens*, mostly in splenectomised patients [6, 12, 27, 29]. Nevertheless, the cases of human babesiosis caused by *B. microti* and the presence of antibodies against this parasite in people exposed to tick

bite were described also in Europe [5, 9, 19, 22, 26, 29, 44, 45], and this species is considered worldwide as an emerging human pathogen [11, 12, 20, 21, 23, 24]. Moro *et al.* [28] and Swanson *et al.* [43] presume that coinfection with *Babesia microti* and *Borrelia burgdorferi* may increase severity of the disease.

The prevalence of *Babesia* protozoans in ixodid ticks, usually with use of polymerase chain reaction (PCR) method was studied by the scientists on various continents [1, 2, 6, 7, 14, 33, 41, 46]. In Poland, such studies have been also carried out, mostly in the northern and western parts of the country, less in southern and eastern regions [25, 31, 35, 36, 37, 38, 39, 40, 42].

The objective of the present study was to determine the prevalence of *Babesia microti* in *Ixodes ricinus* ticks from different localities of the Lublin region, which is the mid-eastern part of Poland, adjacent to the Ukraine.

## MATERIALS AND METHODS

**Collection of ticks.** Unfed *Ixodes ricinus* ticks (adults and nymphs) were collected during spring/summer seasons in 2005 and 2006 from the forested areas of 5 districts of the Lublin region (eastern Poland). Of these, 2 districts (Parczew, Włodawa) harboured wet lakeland forests, while the other 3 districts (Zamość, Puławy, Lublin) harboured dry upland forests. 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 of 1,367 *I. ricinus* ticks (377 females, 375 males, and 615 nymphs) were examined. Protozoan DNA was isolated from ticks after removal from alcohol by boiling in 0.7 M ammonium hydroxide, according to Rijpkema [32], and stored at -70°C. Adult ticks were prepared separately while nymphs in pools of 5 specimens.

**Detection of *Babesia microti* DNA by PCR and nested-PCR.** Ticks lysates were examined for the presence of *Babesia microti* DNA using amplification by polymerase chain reaction (PCR) and confirmatory reamplification by nested-PCR. The set of following primers was applied: • pair of outer primers Bab1 (5'-CTT AGT ATA AGC TTT TAT ACA GC-3') and Bab4 (5'-ATA GGT CAG AAA CTT GAA TGA TAC A-3'); • and pair of inner primers Bab2 (5'-GTT ATA GTT TAT TTG ATG TTC GTT T-3') and Bab3 (5'-AAG CCA TGC GAT TCG CTA AT-3'). These primers are specific for a gene encoding the nuclear small subunit ribosomal RNA (SS-rDNA) [42], described by Persing *et al.* [30].

In each PCR reaction were applied: • matrix DNA, • primers Bab1, Bab4 (Eurogentec, Seraing, Belgium), • DNA of *Babesia microti* merozoites (obtained from the Department of Parasitology, University of Warsaw) as a

positive control, • redistilled water as a negative control, • mixture of dNTP nucleotides (DNA, Gdańsk, Poland), • thermostable polymerase (DyNAzyme™ II DNA, Finnzymes Oy, Espoo, Finland). The size of the amplified DNA fragment was 238 base pairs (bp).

In each nested-PCR reaction were applied: • DNA of first amplification, • primers Bab2, Bab3 (Eurogentec, Seraing, Belgium), • DNA of *Babesia microti* merozoites (obtained from the Department of Parasitology, University of Warsaw) as a positive control, • redistilled water as a negative control, • mixture of dNTP nucleotides (DNA, Gdańsk, Poland), • thermostable polymerase (DyNAzyme™ II DNA, Finnzymes Oy, Espoo, Finland). The size of the amplified DNA fragment was 154 bp.

The amplification and reamplification were carried out in PTC-150 thermal cycler (MJ Research Inc., Waltham, MA, USA) according to Stańczak *et al.* [42]. Products of amplification and reamplification were identified in 2% agarose gel after electrophoresis in standard conditions and staining with ethidium bromide solution (2 µg/ml). Amplified fragments were visualised in transilluminator under UV light (UV-953, JW Electronic, Warsaw, Poland).

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

## RESULTS

A total of 74 out of 1,367 examined *Ixodes ricinus* ticks (5.4%) were found to be infected with *Babesia microti*. The infection rate varied significantly with stage/sex of ticks ( $\chi^2=16.48543$ ,  $df=2$ ,  $p<0.000264$ ). The infection rates in females and males amounted to 6.4% and 8.8% respectively and were significantly higher ( $p=0.006$  and  $p=0.0001$  respectively) compared to minimum infection rate in nymphs that was equal to 2.8% (Tab. 1). The prevalence of infection also showed a significant variability depending on geographic location within the Lublin region ( $\chi^2=18.62812$ ,  $df=4$ ,  $p<0.000932$ ). The highest rates of infection with *Babesia microti* were noted in ticks collected from the areas of Puławy district situated in the northern part of region and the suburban Lublin district situated in the central part of region (8.0% and 7.3% respectively) (Tab. 2). Mediocre infection rates

**Table 1.** Prevalence of *Babesia microti* in ticks collected from Lublin region, presented by stage/sex.

Stage/sex	Number of ticks infected/examined	Percent of infected ticks
Females	24/377	6.4%
Males	33/375	8.8%
Nymphs	17/615	2.8%*
Total	74/1367	5.4%

\* minimum infection rate

**Table 2.** Prevalence of *Babesia microti* in ticks collected from Lublin region, presented by districts.

District	Number of ticks infected/examined	Percent of infected ticks
Zamość	1/182	0.5%
Puławy	35/440	8.0%
Parczew	9/265	3.4%
Włodawa	5/153	3.3%
Lublin	24/327	7.3%
Total	74/1367	5.4%

(respectively 3.4% and 3.3%) were found in ticks from the Parczew and Włodawa districts situated in the eastern part of the region and covered with humid lakeland forests. The lowest infection rate (0.5%) was noted in ticks from Zamość district situated in the southern part of the region.

## DISCUSSION

Compared to the data hitherto reported by Polish authors who examined *Ixodes ricinus* ticks for the presence of *Babesia microti* DNA in various parts of the country, the mean infection rate obtained in the present study (5.4%) is higher than the percentages reported by Stańczak *et al.* (2.3%) for ticks from suburban and urban forests in cities of northern Poland [42] and by Siński *et al.* (0.6%) for ticks from the Mazury Lakes District in northeastern Poland [35]; it is within a broad range of 1.9-16.3% reported by different authors as an infection rate of ticks from northwestern Poland with *Babesia* spp. DNA [25, 31, 36, 37, 38, 39, 40] and higher compared to results obtained by Skotarczak *et al.* [39] who have not detected the *Babesia* spp. DNA in 515 *I. ricinus* ticks collected from birds and vegetation in west-central Poland.

The mean infection rate of ticks with *Babesia* found in the present study is higher compared to the majority of those reported by different authors from the countries of Central and Western Europe. Thus, Hartel *et al.* [14] detected *Babesia* spp. in 1.0% of *I. ricinus* ticks from southern Germany, Rudolf *et al.* [33] found a prevalence rate of *Babesia microti* equal to 1.5% in *I. ricinus* nymphs collected from south Moravia in the Czech Republic, Sreter *et al.* [41] reported a minimum prevalence of *Babesia microti* in Hungarian *I. ricinus* ticks which amounted to 0.9%, Hilpertshauer *et al.* [16] detected *Babesia* spp. in 3.7% of ticks collected from ruminants in southern Switzerland, and Casati *et al.* [6] found in *I. ricinus* ticks from 4 regions of Switzerland the infection rate with *Babesia* spp. (*Babesia divergens*, *Babesia microti*, *Babesia* sp. EU1) equal to 0.8%. The only higher value is that reported by Duh *et al.* [7] who found *Babesia* spp. DNA in 9.6% of *I. ricinus* ticks collected in Slovenia.

Compared to the data reported infection of other tick species with *Babesia microti*, our result is higher compared to the infection rate of *Ixodes scapularis* in Maine, USA, which amounted to 1.9% [17], to the minimum infection rate of 3.2% determined for *Ixodes spinipalpis* in Colorado, USA [4], and to that of *Ixodes persulcatus* in Russia which was equal to 0.9% [2], but lower compared to the infection rate of 7.1% reported for *Ixodes ovatus* in Japan [46] and to that of 8.4% determined for *Ixodes scapularis* in New Jersey, USA [1].

In conclusion, the infection rate of *Ixodes ricinus* ticks with *Babesia microti* found in this study is higher compared to the majority of data reported by Polish and other European authors, and indicates a potential risk of human infection during occupational or recreational exposure to tick bite.

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