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ISOLATION AND AMPLIFICATION BY POLYMERASE CHAIN REACTION DNA OF *BABESIA MICROTI* AND *BABESIA DIVERGENS* IN TICKS IN POLAND

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Abstract: *Babesia microti* and *B. divergens*, the etiological agents of human babesiosis, are transmitted by the bite of *Ixodes ricinus*. The purpose of this study was differentiation of those two species in ticks collected in urban woods in the city Szczecin (north-western Poland). The prevalence of the DNA of *Babesia* were investigated by PCR amplification with primers to the fragment from a gene encoding the nuclear small-subunit ribosomal RNA (SS-rDNA). We examined a total of 533 specimens of *Ixodes ricinus.* The mean infection rate was 16.3%. Our results indicate that a *B. microti* and *B. divergens* - specific PCR test may provide a sensitive tool also for the laboratory diagnosis of human babesiosis.

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INTRODUCTION

The babesias are intraerythrocytic parasites classified within the phylum Apicomplexa, class Aconidasida, order Piroplasmida. Babesiosis is a world-wide distributed protozoal zoonosis, and *Babesia* spp. is one of the most ubiquitous blood parasites of mammals. These tick-transmitted protozoans infect various vertebrate reservoir hosts. The first demonstrated case of human babesiosis in the world was reported in Europe, in 1957 [16]. Since then, about 30 further babesial cases in man have been reported in Europe. Most of them (76%) were caused by *B. divergens*, a cattle parasite [4]. Hundreds of human infections transmitted by ticks carrying the rodent parasite *B. microti* have been reported in the USA [4, 5, 7–9, 17, 18]. In North America, human babesiosis, like Lyme disease and ehrlichiosis, are transmitted by *Ixodes scapularis*, whereas in Europe - by *Ixodes ricinus* [6].

In 1999, we demonstrated a thorough analysis of the quantitative and rate per cent occurrence of *B. microti* by detecting its DNA in all developmental stages of *I. ricinus* [13, 14]. In this study, we tested *I. ricinus* for the presence of DNA of both *B. microti* and *B. divergens.* The same specimens of *I. ricinus* were tested for the presence of *Ehrlichia* spp. and *Borrelia burgdorferi* sensu lato [11, 12, 15, 18, 19].

MATERIAL AND METHODS

Collection of ticks. The ticks were collected in natural habitats (urban woods in the Szczecin city) by dragging 1 m^2 piece of flannel cloth over the vegetation. Sex and developmental stage of each tick were determined. Out of the total 533 specimens collected 27.8% were females, 24.8% males, 43.9% nymphs and 3.5% larvae. Each tick was studied for the presence of DNA of *B. divergens* and *B. microti.*

Preparation of tick samples for PCR. *I. ricinus* ticks were processed for PCR by lysis in ammonium hydroxide method [5].

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Table 1. Prevalence (%) of infection *Ixodes ricinus* by *B. microti* and *B. divergens*.

Species of *Babesia*	Females			Males			Nymphs			Larvae			Total		
	N	PCR positive		N	PCR positive		N	PCR positive		N	PCR positive		N	PCR positive	
		N	%		N	%		N	%		N	%		N	%
B. microti	148	22	14.9	132	19	14.4	234	26	11.1	19	4	2.1	533	71	13.3
B. divergens	148	10	6.7	132	6	4.5	234	0	0.0	19	0	0.0	533	16	3.0
Total	148	32	21.6	132	25	18.9	234	26	11.1	19	4	2.1	533	87	16.3

PCR testing. We used as a target a fragment from a gene encoding the nuclear small-subunit ribosomal RNA (SS-rDNA). Primers Bab1 - Bab4 (expected PCR product has a size of 238 bp) described by Persing [9] and Persing *et al.* [10] and primers A(23) - C (589 bp) described by Conrad *et al.* [2] were used. As positive amplification controls, we used 1 ng of *B. microti* merozoite DNA from the University of Warsaw, Poland, and 10 ng of *B. divergens* merozoite DNA from the Precigout Laboratoire de Biologie Cellulaire et Moleculaire, Montpellier, France, one reaction each. Negative amplification control consisting of 5 µl of distilled water added to the PCR buffer was also included in each PCR test. The reactions for detection of *B. microti* consisted of an initial denaturation (1 min at 94ºC) followed by 35 rounds of temperature cycling (94ºC for 1 min, 55ºC for 1 min and 72ºC for 2 min). For detection of *Babesia divergens*, the PCR reaction consisted of an initial denaturation (1 min at 94ºC) followed by 40 rounds of temperature cycling (94ºC for 1 min, 70ºC for 45 s and 72ºC for 1 min). The products of PCR were analysed on 2% agarose gels in 1 × TRIS-buffer.

RESULTS

As Table 1 shows, the overall infection rate for both babesias was 16.3% and was higher for *B. microti* (13.3%) than for *B. divergens* (3.0%). Coinfection by *B. microti* and *B. divergens* was not found, there occurred only a single-species tick infection. The infection rate for both babesias was 20.3% in adult ticks, 11.1% in nymphs and 2.1% in larvae. The highest infection rates, both with *B. microti* and *B. divergens*, were found in females and males of *I. ricinus*, while the larvae and nymphs had only DNA of *B. microti*.

DISCUSSION

The maintenance of *Babesia* spp. is dependent on two classes of hosts; the specific tick vector must feed on a vertebrate reservoir. Our results show the competence of ticks *I. ricinus* as a vector and reservoir of *B. microti* and *B. divergens* in north-western Poland. The infection rate was higher for *B. microti* than *B. divergens*. The females and males of *I. ricinus* had DNA of both babesias, while nymphs and larvae of *B. microti* only. Our earlier investigation showed a difference between the prevalence of DNA of *B. microti* in spring-summer and summer-autumn seasons of ticks collection [13].

Fujisaki [3], Healy and Ristic [6], Kuttler [8], Telford *et al.* [17], suggest that human babesiosis is probably caused most often by *Babesia microti* in North America and *B. divergens* in Europe. There have been only two reported cases of human babesiosis in Europe which were caused by *B. microti* [7]. In our present investigations we determined by PCR the presence of both babesias in *I. ricinus* ticks collected in north-western Poland. We used the sequences of the primers prepared in the USA by Persing *et al.* [10].

According to Homer *et al.* [7], the actual frequency of babesial infections in humans is probably much greater than the number of reported cases because babesiosis is usually undiagnosed. The laboratory diagnosis of babesiosis consists in demonstration of either a specific antibody [1] or intraerythrocytic inclusions on Giemsa-stained peripheral blood smears. However, the relatively common absence of parasites during early disease and in many stages of disease in normosplenic hosts makes the test relatively insensitive. Our results indicate that a *B. microti* and *B. divergens* - specific PCR (with species-informative region) test may provide a sensitive tool also for the laboratory diagnosis of human babesiosis.

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