PCR detection of Babesia microti and Babesia divergens in ticks

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PCR detection of *Babesia microti* and *Babesia divergens* in ticks

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ABSTRACT

We investigated the presence of *Babesia microti* and *B. divergens* in *Ixodes ricinus* ticks collected in a Lyme borreliosis endemic area of Northern Poland. We used as the PCR target the fragment from a gene encoding the nuclear small-subunit ribosomal RNA (SS-rDNA). We have made a thorough analysis of the quantitative and rate per cent occurrence *Ixodes ricinus* from two areas of forest in province of Szczecin, known as highly recreative and frequented of many people resort. A total of 533 specimens collected in 13.3% was detected DNA of *B. microti* and in 3.0% of *B. divergens.*

Introduction

Babisial protozoan parasites, are referred to as piroplasmas, because of their pear-shaped intraerythrocytic stage (Fujisaki 1997). The genus *Babesia* comprises more than 100 species of tick-transmitted pathogens infect a wild and domestic vertebrate hosts (Tilford *et al.* 1993). Within the last few years, hundreds of cases of human babesial infections have been reported in USA and Europe (Healy and Ristic 1988; Kuttler 1988; Teleford et al 1993; Fujisaki 1997). Thus human babesiosis, like lyme disease and ehrlichosis, is emerging as tick-borne zoonosis. In North America human babesiosis are transmitted by *Ixodes scapularis*, in Europe by *Ixodes ricinus* (Healy and Ristic 1988). Human babesiosis, which is caused by infection with *Babesia microti* has recently been diagnosed with increasing frequency in USA (Pershing et al 1992). Healy and Ristic (1988), Kuttler (1988), Telford et al (1993), and Fujisaki (1997) suggest that human babesiosis most often is probably caused by *Babesia microti* in North America and *B. divergens* in Europe. In our investigations we determined by PCR the presence both babesias in *Ixodes ricinus* collected in North Poland.

We have made a thorough analysis of the quantitative and rate per cent occurrence of stages *Ixodes ricinus* from areas of forest in province of Szczecin, known as highly recreative and

frequented of many people resort. Before that the same places we tested on *Borrelia burgdorferi* presence in *Ixodes ricinus* (Skotarczak 1997, Skotarczak and Wodecka 1998, Skotarczk et al. (1998, 1998a, 1998b, 2000).

MATERIAL AND METHODS

Collection of ticks. Investigations were performed in 1999 year, in two areas of forest in province of Szczecin; Dabie Forest Park and Forest of Goleniów. Obtained specimens were regard of sex and growing stage during each collection. Each tick body was tested for PCR to detect *B. divergens* and/or *B. microti*.

Preparation of tick samples for PCR. *I. ricinus* ticks were processed for PCR by three methods, but lysis in ammonium hydroxide (Guy and Stanek 1991) was and crushed with a pipette tip. The suspensions in sealed vial were boiled for 20 min at 96 ^{0}C, the most effective. The tick whole body was inmmersed in 70% ethanol and air-dried on filter paper. It was immersed in 100 µl of 0.7 M ammonium hydroxide and crushed with a pipette tip. The suspension in a sealed vial was treateded for 20 min at 96 ^{0}C. After heat treatment, the cap was opened for 10 min to remove ammonia. The negative controls – 0.7 M ammonium hydroxide without tick lysate were included in each PCR. The lysates were stored at -20 ^{0}C.

PCR testing. We used the fragment from a gene encoding the nuclear small-subunit ribosomal RNA (SS-rDNA) as a PCR target by Pershing (1993). As a positive amplification controls, we used DNA of *B. microti* merozoite. The merozoites in mouse blood were obtained from University of Warsow, Poland. The DNA of *B. divergens* merozoite was obtained from Precigout Laboratoire de Biologic Cellulaire et Moleculaire Montpellier. In the place of parasite DNA, 5 µl of distilled water were added to PCR buffer was also included in each a PCR as negative amplification control. The reaction steps for detection of *B. microti* consisted of an initial denaturation (1 min at 94 ^{0}C) followed by 35 rounds of temperature cycling (94 ^{0}C for 1 min, 55 ^{0}C for 1 min and 72 ^{0}C for 2 min). For detection of *Babesia divergens*, PCR program consisted of an initial denaturation (1 min at 94 ^{0}C) followed by 40 rounds of temperature cycling (94 ^{0}C for 1 min, 70 ^{0}C for 45 s and 72 ^{0}C for 1 min). Five microliters of each reaction was electrophoresed in 2% agarose gels in 1 x TRIS-buffer.

RESULTS

The results of the extensivity (%) of infection *Ixodes ricinus* by *B. microti* and *B. divergens* in Dobie Forest Park and Forest of Goleniów (Szczecin) shows table 1. The overall

prevelance of *Babesia* parasites, in teh ticks population studied, was 1.3% for DNA of *B. microti* and 3.0% for *B.divergens*.

TABLE 1 *B. microti* and *B. divergens* in *Ixodes ricinus* collected in Szczecin urban forests

Place of collection	*B. microti*			*B. divergens*		
	No. Of ticks	Positive in PCR	Positive %	No. Of ticks	Positive in PCR	Positive %
Dabie Forest Park	305	26	8.5	305	16	5.2
Forest of Goleniów	228	45	19.7	228	0	0.0
Total	533	71	13.3	533	16	3.0

DISCUSSION

Since 1960, *Babesia microti* has been considered to be a medically important parasite (Karbowiak et al 1999). Clinical cases of human babesiosis were reported in the USA (Grunwaldt 1977, Scharfinan and Taft 1977, Steketee *et al.* 1985; Pruthi *et al.* 1995; White *et al.* 1998). In Europe, babesiosis caused by B. *microti* infection was described by Sebek *et al.* (1977), Krampitz *et al.* (1986) and Brausser and Gorenflot (1996). In Poland, a case of human babesiosis was reported in 1997 in Szczecin (Humiczewska and Kuzna 1997).

B. microti has, as a host and reservoir numerous wild and domestic mammals, especially small rodents (Karbowiak *et al.* 1999). The studies on cohabitating populations of small mammals at three locations in Poland (Karbowiak and Sinski 1996; and Karbowiak *et al.* 1999) showed presence of *B. microti* in seven species of rodents and 4 species of shrews.

Our studies showed the presence DNA of *B. microti* and *B. divergens* in tick-vector by PCR for the first time in Poland.

Healy and Ristic (1988), Kuttler (1988), Telford et al (1993), Fujisaki (1997) suggest that human babesiosis most often is probably caused by *B. microti* in North America and *B. divergens* in Europe. In our investigations we determined the presence both *Babesia* species in *Ixodes ricinus* collected in North Poland by PCR. We used the primers designed in USA by Persing(1992) and Persing *et al.* (1993). The laboratory diagnosis of babesiosis rests on

demonstration of either a *B. microti*-specific antibody (Chisolm et al. 1978) or intererythrocytic inclusions on Giemsa-stained peripherial blood smears. However, the relatively absent of parasites during early phase of the infection and in many stages of disease in nonsplectomized hosts makes the test relatively insensitive. Our results have indicated that a *B. microti* and *B. divergens*-specific PCR tests may provide sensitive methods for the laboratory diagnosis of human babesiosis.

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