Polish Journal of Microbiology
2010, Vol. 59, No 2, 95–97

ORIGINAL PAPER

The Molecular Evidence of *Babesia microti* in Hard Ticks Removed from Dogs in Warsaw (central Poland)

WOJCIECH ZYGNER[1*], PIOTR BĄSKA[1], MARCIN WIŚNIEWSKI[1] and HALINA WĘDRYCHOWICZ[1, 2]

[1] Division of Parasitology and Parasitic Diseases, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland
[2] Laboratory of Molecular Parasitology, W. Stefański Institute of Parasitology, Polish Academy of Sciences, Warsaw, Poland

Received 12 December 2009, revised 7 February 2010, accepted 15 February 2010

Abstract

The purpose of this study was to specify the occurrence and prevalence of *Babesia microti* in hard ticks removed from dogs in Warsaw (central Poland). Among 590 collected ticks, 209 were identified as *Ixodes ricinus*, and 381 as *Dermacentor reticulatus*. *B. microti* DNA was detected in 11 out of 590 (1.86%) samples of ticks. The DNA of the parasite was detected only in lysates from female *I. ricinus* ticks (11 out of 193; 5.7%). The result of this study is the first evidence of *B. microti* in *I. ricinus* ticks in Warsaw.

K e y w o r d s: *Babesia microti*, *Ixodes ricinus*, human babesiosis, Poland

Introduction

Among hard ticks, *Ixodes ricinus* is the most common tick occurring in Poland. The parasite plays an important role as a vector and reservoir of many viral, bacterial and protozoan pathogens (Siuda, 1993). One of these pathogens is *Babesia microti*, a protozoan parasite responsible for human babesiosis. Although *I. ricinus* is considered the final host of *B. microti*, one of the most recent studies from Poland showed that the tick *Dermacentor reticulatus* can also be infected with this parasite (Welc-Falęciak *et al.*, 2008). The disease caused by *B. microti* occurs mainly in North America where the parasite is transmitted by *I. scapularis* (formerly *I. dammini*). Although *B. microti* was detected in ticks and rodents in Europe, *B. divergens* and *B. venatorum* are considered as the main cause of human babesiosis on this continent (Brasseur and Gorenflot, 1996; Herwaldt *et al.*, 2003; Homer *et al.*, 2000; Šebek *et al.*, 1977). In Poland human babesiosis caused by *B. microti* was described only in one case, but the infection was imported from Brazil (Humiczewska and Kuźna-Grygiel, 1997). These literature data seem to suggest that in Europe human babesiosis is caused only by *B. divergens* and *B. venatorum*. However, Hildebrandt *et al.* (2007) confirmed the first autochthonous case of human *B. microti* infection in Europe. The result of that study suggests that there is a risk of *B. microti* infection in humans in Europe. Thus, *B. microti* detected in rodents and ticks in Poland can be also infective for humans. Many studies from our country showed that *B. microti* occurs (in ticks and rodents) in northern, south-western and eastern Poland (Karbowiak *et al.*, 1999; Pawełczyk *et al.*, 2004; Siński *et al.*, 2006; Skotarczak *et al.*, 2002; Skotarczak and Cichocka, 2001; Skotarczak and Sawczuk, 2003; Stańczak *et al.*, 2004; Welc-Falęciak *et al.*, 2008; Wójcik-Fatla *et al.*, 2009). However, to the best knowledge of the authors of this study, there is no any study on the occurrence of *B. microti* in central Poland.

The aim of this study is to continue the previous surveys (Zygner and Wędrychowicz, 2006; Zygner *et al.*, 2008). In those works two species of ticks in dogs from Warsaw were detected. Among all collected ticks 35.4% were identified as *I. ricinus* and 64.6% as *D. reticulatus*. PCR revealed that 11% of *D. reticulatus* ticks harboured DNA of *Babesia canis*, 6.2% of

* Corresponding author: W. Zygner, Division of Parasitology and Parasitic Diseases, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8, 02-786 Warsaw, Poland; phone: (+48) 22 593 60 45; fax: (+48) 22 593 60 48; **e-mail: wojciechzygner@yahoo.pl**

I. ricinus harboured *Borrelia afzelii*, and DNA of *Anaplasma phagocytophilum* was detected in 2.9% of *I. ricinus* ticks. In the presented study the authors estimated the prevalence of *B. microti* infection in ticks collected form dogs in Warsaw.

Experimental

Materials and Methods

Tick sampling. During 2 years, from March 2003 to February 2005, ticks were collected in Warsaw veterinary clinics from dogs presented for veterinary care. Among 590 collected ticks, 381 were identified as *D. reticulatus*, and among them, 137 as male and 244 as female specimens. The other 209 out of 590 ticks were identified as *I. ricinus*, and among them, 16 specimens were males and 193 were females. Larval or nymphal stages of ticks were not found in these dogs (Zygner and Wędrychowicz, 2006). All collected ticks were attached to the skin of the examined dogs except 16 collected *I. ricinus* male specimens. All *D. reticulatus* ticks and most of *I. ricinus* female ticks (171) were engorged or partly engorged. Only 22 collected *I. ricinus* female and all *I. ricinus* male ticks were no engorged.

DNA extraction. All collected ticks were kept at – 70°C (Jouan® VX 530 Series 2) until the isolation of DNA was performed. Before DNA extraction ticks were washed in 70% ethanol and sterile water, they were then homogenized in 100 ml PBS with sterile pestle. DNA was extracted from individual ticks using the Genomic Mini kit (A&A Biotechnology) according to the manufacturer's instructions, preceded by 6 h digestion with Proteinase K. The efficiency of DNA isolation was confirmed by electrophoresis in a 1.5% agarose gel. Isolated DNA was stored at – 70°C.

Amplification of *B. microti* DNA. Lysates from ticks were used to detect DNA of *B. microti*. PCR was performed according to Persing *et al.* (1992) with the primers Bab-1 (5' CTT AGT ATA AGC TTT TAT ACA GC 3') and Bab-4 (5' ATA GGT CAG AAA CTT GAA TGA TAC A 3') used to amplify the 18S rDNA gene fragment of *B. microti*. The expected product was about 238 bp in size. As a positive control the authors used DNA lysate from the *I. ricinus* tick infected with *B. microti*. The infection in this specimen was confirmed by PCR and sequencing of the PCR product, which revealed to be 100% identical with a fragment of the *B. microti* 18S ribosomal DNA gene under accession no. AY693840 in the GenBank® database. All PCRs were carried out in MJ Research PTC-200 thermal cycler. The size of the PCR product was analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

Sequencing of PCR products. The PCR products with the expected amplicon size were isolated from the agarose gel using the Gel-Out kit (A&A Biotechnology). Next, all PCR products were sequenced to verify the presence of *B. microti*. The sequencing reaction was carried out on the AbiPrism® Genetic Analyser using computer program GeneScan® Analysis Software. The obtained sequences were compared to sequence data available in the GenBank® using the BLASTN 2.2.17 program (http://www.ncbi.nlm.nih.gov/BLAST/). New sequence was submitted to the GenBank® database.

Results

B. microti DNA was detected in 11 out of 590 (1.86%) samples of ticks. The DNA of the parasite was detected only in lysates from female *I. ricinus* ticks (11 out of 193; 5.7%). Three out of 11 infected ticks were collected as partly engorged ticks and 8 out of them were collected as no engorged specimens. The 11 products were sequenced. The sequences showed 100% similarity with 18S rDNA partial sequence of *B. microti* (accession no AY693840, Gray strain isolated from human). The sequence obtained in this study was submitted to the GenBank® database under accession no. EU882727.

Discussion

The result of this study is the first proof for the presence of *B. microti* in *I. ricinus* ticks in Warsaw. The prevalence of infected female *I. ricinus* ticks is similar to the results of previous studies from the Mazury Lakes District and Lublin macroregion (Siński *et al.*, 2006; Wójcik-Fatla *et al.*, 2009). In Mazury Lakes District *B. microti* DNA was detected in 4 out of 92 (4.35%) collected female *I. ricinus* ticks. In the Lublin macroregion 25 out of 409 (6.11%) female *I. ricinus* ticks harboured *B. microti* DNA. The highest prevalence of *B. microti* infection in female *I. ricinus* ticks was detected in north-western Poland, where 14.9–15.3% of female specimens were infected with this pathogen (Skotarczak and Cichocka, 2001; Skotarczak *et al.*, 2002). However, in opposition to the mentioned works, in this study DNA of *B. microti* was detected only in female ticks. This result probably is the consequence of very small number of collected male ticks. The authors of this study did not detect *B. microti* DNA in *D. reticulatus* ticks. This result differs from the results of the previous study form north-eastern Poland (Welc-Falęciak *et al.*, 2008) in which 2 out of 17 larvae and 16 out of 398 nymphs of *D. reticulatus* ticks were infected with *B. microti*.

Detection of *B. microti* DNA in ticks collected from dogs shows that these ticks were probably infected as developmental stages like larva or nymph. This statement is based on the fact that the dog is not intermediate host of *B. microti* (Uilenberg, 2006) and 8 of infected ticks were collected as no engorged ticks. However, 3 out of infected specimens were collected as partly engorged and all these specimens were attached to the skin of dogs. It seems probable that the blood of dogs in the collected ticks was not the origin of *B. microti* DNA but this cannot be excluded.

Detection of *B. microti* DNA in Warsaw seems to suggest that there can be a risk of infection for humans. The question, does *B. microti* in Europe have any zoonotic potential seems to be still open. Although detection of *B. microti* DNA in ticks and rodents in many European countries, there was only one confirmed case of autochthonous human babesiosis in Europe. It seems important to mention that this first case of human *B. microti* infection in Europe was caused by a variant which showed 100% similarity with that zoonotic Gray strain (accession no AY693840). This fact shows that detected in Warsaw *B. microti* can have zoonotic potential as well.

Literature

Brasseur P. and A. Gorenflot. 1996. Human babesial infections in Europe. *Rocz. Akad. Med. Bialymst.* 41: 117–122.

Herwaldt B.L., S. Cacciò, F. Gherlinzoni, H. Aspöck, S.B. Slemenda, P. Piccaluga, G. Martinelli, R. Edelhofer, U. Hollenstein, G. Poletti and others. 2003. Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerg. Infect. Dis.* 9: 942–948.

Hildebrandt A., K.P. Hunfeld, M. Baier, A. Krumbholz, S. Sachse, T. Lorenzen, M. Kiehntopf, H.J. Fricke and E. Straube. 2007. First confirmed autochthonous case of human *Babesia microti* infection in Europe. *Eur. J. Clin. Microbiol. Infect. Dis.* 26: 595–601.

Homer M.J., I. Aguilar-Delfin, S.R. Telford III, P.J. Krause and D.H. Persing. 2000. Babesiosis. *Clin. Microbiol. Rev.* 13: 451–469.

Humiczewska M. and W. Kuźna-Grygiel. 1997. A case of imported human babesiosis in Poland (in Polish). *Wiad. Parazytol.* 43: 227–229.

Karbowiak G., M. Stanko, L. Rychlik, W. Nowakowski and K. Siuda. 1999. The new data about zoonotic reservoir of *Babesia microti* in small mammals in Poland. *Acta Parasitol.* 44: 142–144.

Pawełczyk A., A. Bajer, J.M. Behnke, F.S. Gilbert and E. Siński. 2004. Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland. *Parasitol. Res.* 92: 270–284.

Persing D.H., D. Mathiesen, W.F. Marshall, S.R. Telford, A. Spielman, J.W. Thomford and P.A. Conrad. 1992. Detection of *Babesia microti* by polymerase chain reaction. *J. Clin. Microbiol.* 30: 2097–2103.

Siński E., A. Bajer, R. Welc, A. Pawełczyk, M. Ogrzewalska and J.M. Behnke. 2006. *Babesia microti*: Prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of north-eastern Poland. *Int. J. Med. Microbiol.* 296 (Suppl 1): 137–143.

Siuda K. 1993. *Ticks of Poland (Acari: Ixodida), Part II, Taxonomy and Distribution* (in Polish). Polskie Towarzystwo Parazytologiczne, Warsaw.

Skotarczak B. and A. Cichocka. 2001. Isolation and amplification by polymerase chain reaction DNA of *Babesia microti* and *Babesia divergens* in ticks in Poland. *Ann. Agric. Environ. Med.* 8: 187–189.

Skotarczak B. and M. Sawczuk. 2003. Occurrence of *Babesia microti* in ticks *Ixodes ricinus* on selected areas of western Pomerania (in Polish). *Wiad. Parazytol.* 49: 273–280.

Skotarczak B., B. Wodecka and A. Cichocka. 2002. Coexistence DNA of *Borrelia burgdorferi* sensu lato and *Babesia microti* in *Ixodes ricinus* ticks from north-western Poland. *Ann. Agric. Environ. Med.* 9: 25–28.

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

Šebek Z., B. Rosický and W. Sixl. 1977. The occurrence of Babesiasis affecting small terrestrial mammals and the importance of this zoonosis in Europe. *Folia Parasitol.* 24: 221–228.

Uilenberg G. 2006. *Babesia* – A historical overview. *Vet. Parasitol.* 138: 3–10.

Welc-Falęciak R., A. Bajer, J.M. Behnke and E. Siński. 2008. Effects of host diversity and the community composition of hard ticks (Ixodidae) on *Babesia microti* infection. *Int. J. Med. Microbiol.* 298 (Suppl 1): 235–242.

Wójcik-Fatla A., J. Szymańska, L. Wdowiak, A. Buczek and J. Dutkiewicz. 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.

Zygner W., S. Jaros and H. Wędrychowicz. 2008. Prevalence of *Babesia canis*, *Borrelia afzelii*, and *Anaplasma phagocytophilum* infection in hard ticks removed from dogs in Warsaw (central Poland). *Vet. Parasitol.* 153: 139–142.

Zygner W. and H. Wędrychowicz. 2006. Occurrence of hard ticks in dogs from Warsaw area. *Ann. Agric. Environ. Med.* 13: 355–359.