Annals of Agricultural and Environmental Medicine 2015, Vol 22, No 1, 80–83
www.aaem.pl

ORIGINAL ARTICLE

Detection of protozoans *Babesia microti* and *Toxoplasma gondii* and their co-existence in ticks (Acari: Ixodida) collected in Tarnogórski district (Upper Silesia, Poland)

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Asman M, Solarz K, Cuber P, Gąsior T, Szilman P, Szilman E, Tondaś E, Matzullok A, Kusion N, Florek K. 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. 2015; 22(1): 80–83. doi: 10.5604/12321966.1141373

Abstract

Introduction. Ticks (Acari: Ixodida) are vectors of many pathogens i. a: *Babesia microti* [Bm] and *Toxoplasma gondii* [Tg]. In Poland, *Ixodes ricinus* [Ir] is the main vector of both pathogens. This tick species and pathogens transmitted by them are a significant threat to human and animal health.

Objectives of the study. Detection of the protozoans Bm and Tg in ticks collected in the Tarnogórski district area.

Material and methods. The ticks were collected from vegetation and pets in the spring period of their activity on the territory of the Tarnogórski district. The parasites were preserved in 70% ethanol. DNA was isolated by ammonia method. Bm was detected by nested-PCR using specific primers for the 18S rRNA sequence. To detect the Tg a commercial kit was used. The PCR products were separated on 2% ethidium bromide stained agarose gels and visualised under UV light.

Results. It was showed that all collected ticks belonged to the species Ir. Bm was detected in 50.87% and Tg in 64.91% of all examined ticks. Co-existence of these both protozoans in 36.84% of total examined ticks was noted.

Conclusions. The study showed a high risk of exposure to Bm and Tg in the studied area. Ticks Ir play an important role in the transmission of Bm in this region. Demonstrating a high percentage of ticks collected from animals infected with Tg may indicate their important role in the transmission of this pathogen, but it requires a further studies.

Key words

Babesia microti, *Toxoplasma gondii, Ixodes ricinus*, ticks, Upper Silesia, co-existence

INTRODUCTION

Ticks (Acari: Ixodida) are important vectors of many pathogens (viruses, rickettsia, bacteria and protozoa) dangerous for the health of humans as well as wild and domestic animals. Some of these pathogens (e.g. *Toxoplasma gondii*) may also affect the health of owners in contact with their pets [1]. The incidence of tick-borne disease in Poland has been rising steadily through the past decade, along with the range expansions of tick species and tick-borne infectious agents, such as *Borrelia burgdorferi, Anaplasma phagocytophilum, Babesia microti* and *T. gondii* [2, 3, 4]. In Central Europe, including Poland, the main vector of those pathogens is *Ixodes ricinus* [6, 7]. It is believed that global warming and climate change have an impact on the increase in the number of ticks in Central Europe, thus on an increased risk of exposure to ticks and tick-borne pathogens [8, 9].

The genus *Babesia* comprises nearly 100 species of tick-transmitted protozoan pathogens that infect a wide variety of vertebrate hosts including humans [10]. Babesiosis is a common and clinically significant tick-borne haemoprotozoan disease with a worldwide distribution. The piroplasms of domestic animals have been the subject of intense research interest and molecular-based re-classification during the last 10 years. In animals, including humans, infection by these haemoparasites results in a wide range of clinical presentations, from subclinical disease to serious illness characterised by fever, pallor, jaundice, splenomegaly, weakness and collapse associated with intra- and extra-vascular haemolysis, hypoxic injury, systemic inflammation and thrombocytopenia [10, 11, 12].

Toxoplasmosis is a widespread zoonotic disease caused by *T. gondii* and can constitute a serious diagnostic and treatment problem [13]. Its congenital infection causes cerebral and ocular damage in newborns, and an acquired infection in immunocompromised individuals, such as AIDS patients, may result in toxoplasmic encephalitis and disseminated toxoplasmosis [14, 15]. Human infections are caused mainly by genotypes I and II of *T. gondii*. Type II has been isolated from patients with congenital toxoplasmosis and AIDS, whereas type I and II strains are often isolated from animals [16, 17].

The aim of the presented study was to assess the risk of exposure of humans and animals to the protozoans *B. microti* and *T. gondii* in the Tarnogórski district, and to estimate the degree of co-infection of these two pathogens in ticks.

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Received: 03 April 2014; Accepted: 02 September 2014

MATERIALS AND METHOD

DNA was isolated from 60 randomly selected castor bean ticks (*I. ricinus*) collected from vegetation in the spring period of their activity in two selected places in Tarnogórski district. At the same time, ticks from 28 dogs and 26 cats (each tick came from one individual) in one infirmary in Tarnowskie Góry were collected. The ticks from vegetation were collected by flagging and those from animals by a veterinarian using tweezers. The collected material was preserved in plastic sample-tubes containing 70% ethyl alcohol. Next, the ticks were determined to the species and developmental stages under a stereomicroscope Olympus SZ-40 (Olympus, Japan). DNA was isolated from 114 ticks by the ammonia method. A single tick was removed from alcohol and air dried on a filter paper, then immersed in 100µl 0.7 M NH_4OH and crushed mechanically. Next, the homogenate was boiled for 15 min at 100°C. The caps were then opened and probes boiled for 10 min at 100°C to remove ammonia. Next, the samples were centrifuged at 12,000 rpm for 5 min and the filtrates transferred to new sample tubes. The concentration of DNA was measured spectrophotometrically using a Pearl Nanospectrophotometer (Implen, Germany).

To detect *B. microti* in ticks, nested-PCR with two pairs of the specific primers for an 18S rRNA coding gene for a small ribosome subunit were used [4, 18]. For the amplification, Bab1 and Bab4 primers were used. This reaction consisted of the initial denaturation at 94°C for 1 min and 35 cycles. Each cycle consisted of proper denaturation at 94°C for 1 min, primers annealing at 60°C for 1 min and elongation at 72°C for 2 min. The final elongation proceeded at 72°C for 7 min. For the reamplification, Bab2 and Bab3 primers were used. Only the positive samples from amplification were reamplified. The PCR conditions of this reaction were identical with the amplification, only the number of cycles was reduced to 30.

Nested-PCR was also used to detect *T. gondii* in ticks. To detect this protozoan, diagnostic kit PK 40 (Blirt SA, Poland) was used. This kit used the specific primers for a gene fragment coding of 65 kDa antigenic protein of *T. gondii*. The PCR condition of amplification and reamplification are enclosed with the kit manual.

The PCR products were separated electroforetically using 2% ethidium bromide stained agarose gels, and examined under UV light. The expected PCR product sizes were: 238 base pairs [bp] (PCR I) and 154 bp (PCR II) for *B. microti* and 308 bp for *T. gondii* (PCR I and PCR II).

RESULTS

A total of 114 ticks were examined. It was shown that all of them belonged to the *I. ricinus* species. The occurrence of *B. microti* was detected in 58 (50.87%) and *T. gondii* in 74 (64.91%) of the total number of examined ticks (Fig. 2, 3). Co-existence of these both protozoans was shown in 42 (36.84%) of the total number of examined ticks.

The highest level of ticks infected with *B. microti* was shown in the Tarnowskie Góry area (90.0% of ticks examined in this area), and in ticks collected from dogs (Fig. 1; Tab. 1, 2). In the same area, ticks infected with *T. gondii* also dominated (Fig. 1; Tab. 1). All ticks collected from cats were infected with *T. gondii* and in all these ticks co-existence of *B. microti* and *T. gondii* was demonstrated (Tab. 2).

Among all tick specimens collected from the Tarnogórski district the highest infection level of both these pathogens in adult forms was demonstrated; respectively, 58.32% for *B. microti* and 76.04% for *T. gondii*. No co-infection of *B. microti* and *T. gondii* in nymph was noted. In adult forms – 43.75%. The highest percentage of infected ticks in the studied area were females and the lowest were nymphs (Tab. 3).

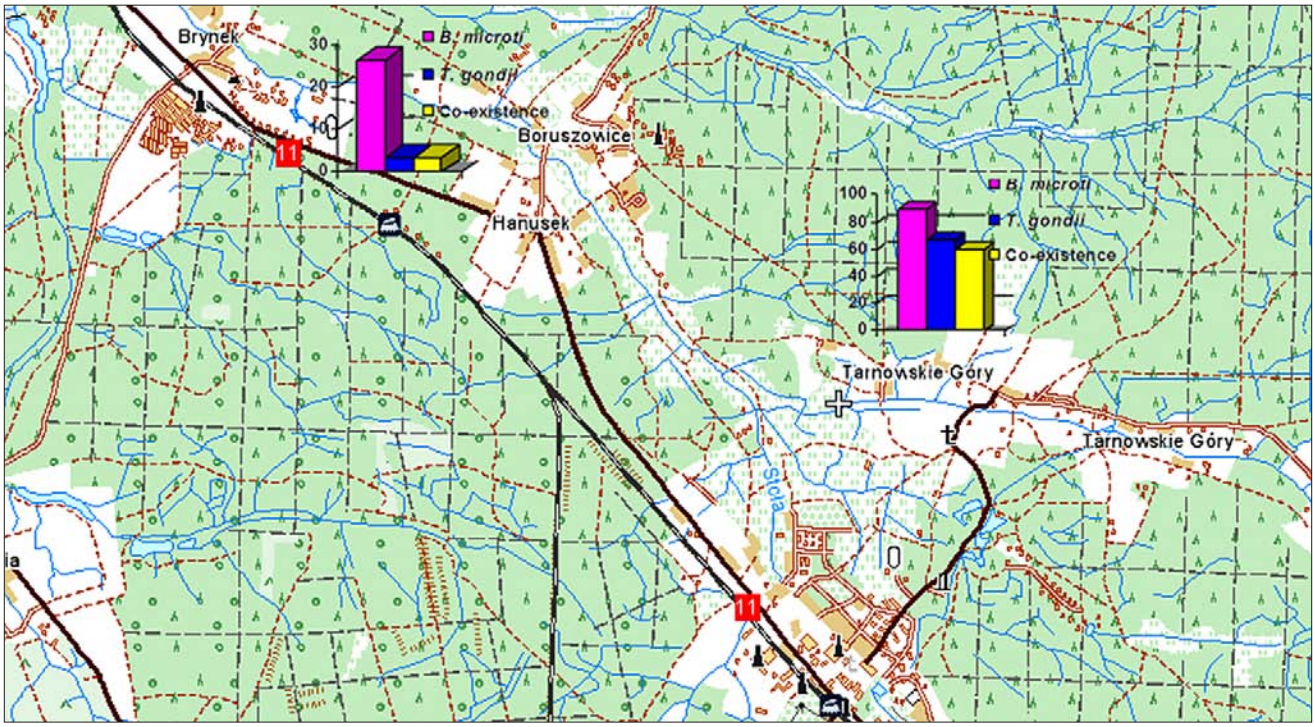

Figure 1. Occurrence of *B. microti* and *T. gondii* in ticks collected from vegetation in the studied area (in percent)

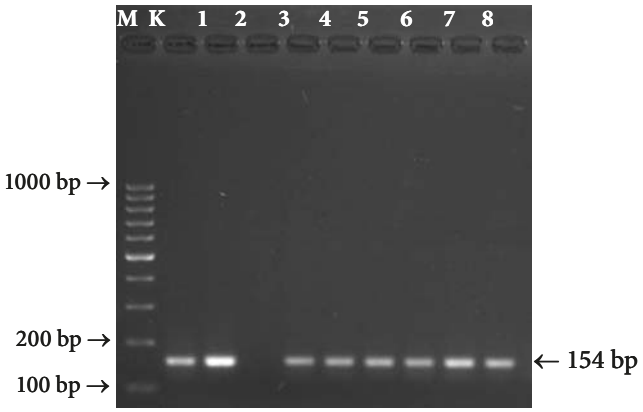

Figure 2. Nested-PCR amplification product of 18S rRNA coding gene for a small ribosome subunit for *Babesia microti*.
M – molecular height marker (EURx, Poland); K – positive control for *B. microti*; 1 and 3–8 – positive samples; 2 – negative sample

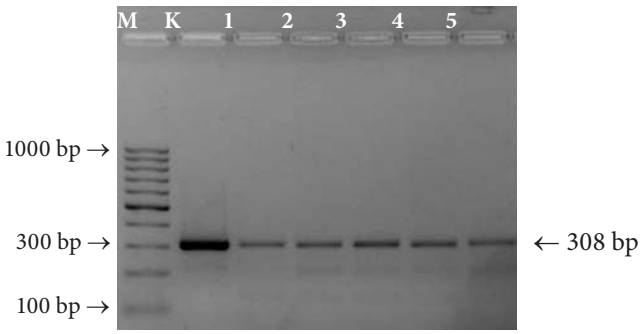

Figure 3. PCR amplification product of a gene fragment coding of 65 kDa antigenic protein of *Toxoplasma gondii*.
M – molecular height marker (Blirt SA, Poland); K – positive control of *T. gondii*; 1–5 – positive samples

Table 1. Occurrence of *Babesia microti* and *Toxoplasma gondii* in examined ticks collected by flagging from the selected sites in the Tarnogórski district area

Site	No. of examined ticks N=100%	*Babesia microti*	*Toxoplasma gondii*	Co-existence of *B. microti* and *T. gondii*
Tarnowskie Góry	30	27 (90.0%)	20 (67.0%)	18 (60.0%)
Boruszowice	30	8 (27.0%)	1 (3.32%)	1 (3.32%)
Total	60	35 (58.32%)	21 (35.0%)	19 (32.0%)

Table 2. Occurrence of *Babesia microti* and *Toxplasma gondii* in examined ticks collected from pets in Tarnogórski district area

Animal	No. of examined ticks (N=100%)	*Babesia microti*	*Toxoplasma gondii*	Co-existence of *B. microti* and *T. gondii*
Dog	28	19 (67.85%)	27 (96.42%)	19 (67.85%)
Cat	26	4 (15.40%)	26 (100%)	4 (15.40%)
Total	54	23 (42.60%)	53 (98.15%)	23 (42.60%)

Table 3. Occurrence of *Babesia microti* and *Toxplasma gondii* in studied developmental stages of all examined ticks in Tarnogórski district area

Developmental stage	No. of examined ticks (N=100%)	*Babesia microti*	*Toxoplasma gondii*	Co-existence of *B. microti* and *T. gondii*
Male	4	1 (25%)	3 (75%)	1 (25%)
Female	92	55 (59.80%)	70 (76.10%)	41 (44.56%)
Nymph	18	2 (11.10%)	1 (5.55%)	0 (0%)

DISCUSSION

The obtained results confirmed that *I. ricinus* ticks are the main vector of *B. microti* in Poland [2, 3, 4]. The research conducted in the Upper Silesian and Małopolska province (southern Poland) showed the occurrence of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia* sp. in *I. ricinus* ticks [9, 19, 20, 21, 22, 23]. These studies showed that the invasion extensiveness of *Babesia* sp. in ticks varied from 0 in individuals collected from humans to 60% in ticks collected form vegetation in the Niepołomnice Forest [19, 20, 23]. The studies conducted in northern Poland by Stańczak et al. showed 2.3% ticks infected with *B. microti* [3]. However, a study conducted in the Lublin macroregion (southeast Poland) showed 3.52% ticks infected with *B. microti* [4]. The obtained results demonstrated the highest extent of *B. microti* infection in examined ticks compared to the data from northern and eastern Poland. The percentage of infected ticks in the Tarnogórski district varied from 15.4% among ticks collected from cats, to even 90% among ticks collected from vegetation in Tarnowskie Góry.

The studies demonstrated that all examined developmental stages of ticks were infected with *B. microti*. The highest extent of infection with this protozoan was revealed in female ticks. A similarly high level of *Babesia* sp. infection in females collected from recreational areas of the Silesian province was obtained in 2011 by Cuber et al. [19]. However, the study conducted by Stańczak et al. and Wójcik-Fatla et al. showed a lower level of *B. microti* infection in all examined developmental stages of ticks [3, 4]. The high extent of *B. microti* infection in female ticks obtained in the presented study may be caused by the fact that some of the ticks were collected from animals and the samples were collected in the spring period of ticks activity.

The results of the presented study also showed that *I. ricinus* can probably be a vector for the other dangerous protozoan – *T. gondii*. The research carried out by Sroka et al. demonstrated that these ticks may be involved in the spread of this pathogen [5]. The conducted studies confirmed this thesis because the presence of *T. gondii* was found both in hungry and engorged ticks. In the studied area, an almost five times higher extent of *T. gondii* infection in ticks was demonstrated than in the studied areas of northwestern and eastern Poland [13, 24]. In their studies, Sroka et al. showed that the prevalence of this protozoan was the highest in females [24]. The conducted studies also found this regularity. Our results of the current study indicate that *T. gondii*, apart from the well known transmission by the alimentary route with oocysts shed by cats in faeces and with cysts present in the pork and beef meat, could also probably be transmitted by tick vectors.

There are commonly known co-infections of the three most popular of pathogens transmitted by ticks: *B. burgdorferi* s. l., *B. microti* and *A. phagocytophilum* [3, 4, 19, 23, 25]. In 2009, Sroka et al. showed that among ticks there may exist co-infection of *T. gondii* and *B. burgdorferi* sensu lato, but it occurs only in a small percentage of infected ticks [13]. In contrast, the presented study demonstrated that this protozoan may co-exist with a different protozoan transmitted by these ticks – *B. microti*. This co-existence is on a high level and requires further studies.

CONCLUSIONS

The presented study showed a high risk of exposure of humans and animals to infection with *B. microti* and *T. gondii*. Ticks *I. ricinus* and dogs played an important role in the transmission of *B. microti* in the study area. It was demonstrated that both adult stages and nymphs can be infected with *B. microti* and *T. gondii*. The presence of *T. gondii* in 35% of examined ticks collected from vegetation, and in 100% collected from cats, may indicate a high risk of exposure to infection by this pathogen in the study area (especially among cats owners). Demonstrating a high percentage of ticks collected from animals infected with *T. gondii* may indicate their important role in the transmission of this pathogen, but this presumption requires further studies.

Acknowledgements
The studies received support from Iceland, Lichtenstein and Norway through the EEA Financial Mechanism and the Norwegian Financial Mechanism (Grant No. PL0343). This work was also supported by the Medical University of Silesia in Katowice through Grant No. KNW-1- 136/K/3/0. The authors are grateful for the successful cooperation with the veterinarians from the 'Vetmedicor' Animal Clinic in Tarnowskie Góry, Poland, who kindly provided the ticks collected from pets, and performed the survey among pet owners.

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