Acta Sci. Pol.
Silv. Colendar. Ratio Ind. Lignar. 17(4) 2018, 317–323
FORESTRY AND WOOD TECHNOLOGY
www.forestry.actapol.net pISSN 1644-0722 eISSN 2450-7997 http://dx.doi.org/10.17306/J.AFW.2018.4.29

SHORT COMMUNICATION

Received: 5.08.2018
Accepted: 8.11.2018

THE POTENTIAL ROLE OF ROE DEER (*CAPREOLUS CAPREOLUS*) AND MEADOW TICK (*DERMACENTOR RETICULATUS*) AS A RESERVOIR OF *TOXOPLASMA GONDII*, *BORRELIA BURGDORFERI* SENSU LATO, *ANAPLASMA PHAGOCYTOPHILUM* AND *BABESIA MICROTI* IN WESTERN POLAND

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ABSTRACT

The research was carried out at the Trzciel Breeding Center in the Trzciel Forest District. Samples were collected at random from hunted roe deer during the 2016/17 hunting season. Blood samples of 19 killed animals were collected, labeled and stored at –18°C. The pathogens were detected with the use of PCR and nested PCR methods. To detect *T. gondii* in animal blood and ticks the two pairs of primers specific to the B1 gene were used. The presence of *B. burgdorferi* s.l. was detected with the use of a pair of primers specific to the flagelline gene, while two pairs of primers specific to the gene coding 16S rRNA were used to detect *A. phagocytophilum*. *B. microti*, on the other hand, was detected with the use of two pairs of primers specific for the 18S rRNA gene. The presence of the reaction products with the size of base pairs [bp] 531 bp for *T. gondii*, 482 for *B. burgdorferi* s.l., 932 bp and 546 bp for *A. phagocytophilum*, 238 bp and 154 and for *B. microti* were treated as positives. Neither *T. gondii* nor *B. burgdorferi* s.l. were detected in any samples, *A. phagocytophilum* was present in two samples (10.2%) and *B. microti* was present in seven (36.8%). *T. gondii* was present in 2% of *Dermacentor reticulatus* ticks.

Keywords: roe deer, *Dermacentor reticulates*, *Toxoplasma gondii*, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*

The study was financed by the Silesian University of Medicine in Katowice, statutory research project no. KNW-1-158/K/7/1.

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INTRODUCTION

The European roe deer is the smallest Cervidae in Poland, in the western part of the country it is quite common and often found in the vicinity of human settlements. This species is often infested by ticks (Michalik et al., 2009; Ruiz-Fons and Gilbert, 2010; Skotarczak et al., 2008), and thus can be a reservoir of many pathogens causing tick-borne diseases such as *Borrelia burgdorferi* spp., *Anaplasma phagocytophilum*, *Babesia* spp. or a virus responsible for tick-borne encephalitis (Adamska 2006; Crippa et al., 2002; de la Fuente et al., 2008; Overzier et al., 2013; Pantchev et al., 2017; Stuen et al., 2002; Weststrate et al., 2017; Vor et al., 2010). Diseases transmitted by ticks are becoming a serious problem, not only in Poland itself, but also throughout Europe (Nowak-Chmura, 2013). There are many factors affecting this situation, such as climate change. In addition, many countries in the world are struggling with aging of the human population, and the number of people with weakened immunity, more susceptible to infection and prone to more severe illnesses is increasing (Grzeszczuk, 2008). The risk groups of diseases transmitted by ticks include people who have the most frequent contact with forest biotopes, including employees of the State Forests and wood factories. We should never forget about people gathering mushrooms and forest fruits (Rizzoli et al., 2011), as well as tourists. In addition to diseases such as Lyme disease, human granulocytic anaplasmosis or babesiosis, it is also worth looking at toxoplasmosis caused by protozoan *Toxoplasma gondii*. People who eat undercooked meat or have direct contact with a sick animal, e.g. during eviscerating, may become an intermediate host for this protozoan. This is particularly important in environments and countries where game is a popular food (Dubey, 1994; Gamarra et al., 2008; Ross et al., 2001; Sacks et al., 1983). All species of birds and mammals, including the European roe deer, can be an intermediate host for *T. gondii*, while the final hosts for this parasite are only animals from the Feline family such as cats, wildcats, ocelots, etc. (Włodarczyk et al., 2013).

The main purpose of this paper is to determine the level of infection in European roe deer and ticks by *Toxoplasma gondii*, *Anaplasma phagocytophilum*, *Babesia microti* and *Borrelia burgdorferi* sensu lato from the Trzciel Breeding Centre. In addition, we examine ticks collected from vegetation from the area of the Trzciel Breeding Centre in 2014 to determine the level of infection by *Toxoplasma gondii*. Presented analyses are only a preliminary study.

MATERIAL AND METHODS

Blood samples from female roe deer were taken during the 2016/17 hunting season from the Trzciel Breeding Centre. The Research area was described in Opalińska et al. (2016). A total of 19 blood samples were the research material. Every blood sample was collected from the body cavity during processing the animal carcass after hunting. The 3 ml blood was collected into plastic tubes with sprayed EDTA and frozen in –20°C. The DNA was isolated from the 1.5 ml blood using the commercial Gene MATRIX Quick Blood DNA Purification Kit (EURx, Polska), according to the manufacturer's manual. The concentration of DNA was measured using a PEARL nanospectrophotometer (Implen, Germany). Then the samples were frozen at –80°C for further analysis.

To study the presence of *T. gondii* in *Dermacentor reticulatus* ticks from the same area, a total of 125 DNA isolates, 116 from males and 9 from females, frozen at –80°C were used. The ticks were collected in 2014 from forest low vegetation; the species determination process and their previous analysis regarding other pathogens is described in Opalińska et al. (2016).

The pathogens were detected in animal blood samples and ticks using PCR and nested PCR methods. The amplification and re-amplification products were applied as follows:

To detect *Toxoplasma gondii* in animal blood and ticks, two pairs of primers specific to the B1 gene were used (Sroka et al., 2009). The amplification conditions were as follows: initial denaturation at 94°C for 120 s, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 90 s. The final elongation proceeded at 72°C for 120 s. A total of 30 cycles were performed. The re-amplification conditions were the same as in the first PCR, but the number of cycles was reduced to 20.

Borrelia burgdorferi sensu lato was detected in blood with the use of a pair of primers Fla1/Fla2

Table 1. Number and percentage of roe deer blood samples infected with *Toxoplasma gondii*, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in Trzciel Breeding Centre (western Poland)
Tabela 1. Liczba i procent prób krwi sarny zainfekowanych przez *Toxoplasma gondii*, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* z terenu Ośrodka Hodowli Zwierzyny Trzciel (zachodnia Polska)

The studied material Materiał badawczy	Number of studied samples Numer próby *N* = 100%	Pathogen – Patogen			
		Toxoplasma gondii	*Borrelia burgdorferi* sensu lato	*Anaplasma phagocytophilum*	*Babesia microti*
Blood – Krew	19	0 (0.0%)	0 (0.0%)	2 (10.5%)	7 (36.8%)

specific to the flagelline gene fragment (Wodecka and Skotarczak, 2000; Wójcik-Fatla et al., 2009). The PCR conditions were as follows: preliminary denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 54°C for 45 s, elongation at 72°C for 45 s and final elongation at 72°C for 7 min. A total of 35 cycles were performed.

To detect *Anaplasma phagocytophilum* two pairs of primers Ge3a/Ge10r and Ge9f/Ge2r specific to the gene coding 16S rRNA were used (Massung et al., 1998). The amplification conditions were as follows: preliminary denaturation at 95°C for 2 min, after which 40 cycles were performed. Each cycle included denaturation at 94°C for 30 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min. The final elongation proceeded at 72°C for 5 min. The nested PCR reaction conditions were the same as in the first PCR, but the number of cycles was reduced to 30.

To detect *Babesia microti* we used two pairs of primers: Bab1/Bab4 and Bab2/Bab3, specific to the 18S rRNA gene (Persing et al., 1992; Wójcik-Fatla et al., 2009). Conditions of the PCR amplification were as follows: preliminary denaturation at 94°C for 1 min, then denaturation at 94°C for 1 min, annealing at 60°C for 1 min, elongation at 72°C for 2 min and final elongation at 72°C for 7 min 35 cycles were performed. The conditions of nested PCR reaction were the same as in the first PCR, but the number of cycles was reduced to 30.

The amplification and re-amplification products were separated electrophoretically in 2% ethidium bromide staining gels at 80V for about 2 hours. Then the gels were visualised under ultra violet light and photographed in the Omega 10 analyser (UltraLum, USA). The presence of reaction products of 482 base pairs [bp] for *B. burgdorferi* s.l., 932 bp and 546 bp for *A. phagocytophilum*, 238 bp and 154 for *B. microti* and 531 bp for *T. gondii* were treated as positives. The positive control was taken from the laboratory of the Institute of Agricultural Medicine, courtesy of Angelina Wójcik-Fatla.

RESULTS

In total, 19 blood samples were studied for the presence of *T. gondii*, *B. burgdorferi* s.l., *A. phagocytophilum* and *B. microti*. The pathogens were found in 47.4% blood samples. *Anaplasma phagocytophilum* was identified in 10.5% and *B. microti* in 36.8% of studied roe deer blood samples. However, the presence of *T. gondii*, *B. burgdorferi* s.l. in the roe deer blood was not detected (Table 1). The analysis for the presence of *T. gondii* in *D. reticulatus* showed this protozoan only in 1.6% (2 ind. – males) of studied ticks.

DISCUSSION

Results presented in this paper are a preliminary study on a relatively small sample. So far there have been few records of *T. gondii* in animals and ticks, as DNA detection has not been used until recently (Zając et al., 2017) – most of the previous studies used the MTA method (Dubey and Desmonts, 1987; Gamarra et al., 2008; Sobrino et al., 2007). This study together with a previous paper by Opalińska et al. (2016) is comprehensive, we examined ticks and roe deer from the same area.

In *Dermacentor reticulatus* ticks were found in 1.6% of positive samples (2 ind.) – a lower rate than reported by Wójcik-Fatla et al. (2015) 3.2% (range 0–16.7%) and Zając et al. (2017) – 2.1%. Thus we have confirmed the presence of *T. gondii* in *D. reticulates*

from Poland. In turn, Sroka et al. (2009) recorded a much higher rate, 13.2%, but they examined other tick species – *I. ricinus*.

We detected no DNA of *T. gondii* in roe deer blood. All the available data concerning the prevalence of *T. gondii* in wild ruminants are based on the antibody detection modified agglutination test (MAT), the dye test (DT), the indirect antibody test (IFAT) and the direct agglutination test (DAT), so a direct comparison between the results is not possible, although it indicates the scale of the problem. In Norway the prevalence of *T. gondii* in ruminants was quite high: 33.9% in roe deer, 12.6% in moose, 7.7% in red deer and 1% in reindeer (Vikøren et al., 2004). In Italy the prevalence of *T. gondii* in roe deer was 27% (Gaffuri et al., 2006), while in the Czech Republic it was 13% (Hejlíček et al., 1997). More data is available for Spain: in 2006 Gauss et al. found 21.8% of the roe deer had antibodies to *T. gondii* and Gamarra et al. in 2008 – 33.9%. In the analyzed countries, both far and close to Poland, the prevalence of *T. gondii* is much higher. Roe deer have a similar level of prevalence compared to other deer species, e.g. in Spain 15.6% of red deer and 24% of fallow deer had antibodies (Gauss et al., 2006). Gamarra et al. (2008) suggests that the level of prevalence depends on rainfall and humidity in the area – the higher the humidity, the more *T. gondii* in animals. In western Poland annual rainfall is low (550–600 mm), which can explain the lack of pathogens in roe deer from this area.

We also examined the roe deer blood samples for the presence of DNA of *B. burgdorferi* s.l., *A. phagocytophilum* and *B. microti*. DNA of A. *phagocytophilum* was found in two (10.5%) samples and DNA of *B. microti* in seven (36.8%) samples. We found no bacterial DNA, but we did find *B. microti* in five samples (4%) in *Dermacentor* ticks (Opalińska et al., 2016). Prevalence for *A. phagocytophilum* is low, lower than in other European studies, e.g. in Germany, where 96.1% of roe deer were infected (Kauffmann et al., 2017), in Poland Michalik et al. (2009) found 9.6%, Welc-Falęciak et al. (2013) reported 37.3% and Skotarczak et al. (2008) – 53.6% of roe deer infected by *A. phagocytophilum*.

So far, *Babesia* sp. protozoa found in Cervidae in European papers included *Babesia capreoli*, *B. divergens* and *divergens*-like, *B. EU1* and *B. venatorum* (Andersson et al., 2016; Bastian et al., 2012; Bonnet et al., 2007; Cancrini et al., 2008; Cezanne et al., 2017; Hoby et al., 2009; Kauffmann et al., 2017; Michel et al., 2014). *Babesia microti* was found only in Korea (Hong et al., 2017). The level of prevalence is average – we can find countries with lower and higher percentages of infected roe deer, e.g. Austria at 5.1% (Cezanne et al., 2017), Switzerland – 26% (Hoby et al., 2009) and 10.7% (Michel et al., 2014), Sweden – 52% (Andersson et al., 2016), France – 58–94% in anti-body detection (Bastian et al., 2012) and 59% when the DNA detection method was used (Bonnet et al., 2007), as well as Germany at 62.8% (Kauffmann et al., 2017). We decided to detect only *B. microti*, because this genospecies is most dangerous to humans. Additionally, the nested PCR method was also applied to detect only *B. microti*.

Roe deer can be a host of many different pathogens, e.g. in Western Poland it can be a source of *A. phagocytophilum* and *B. microti* together with the vector *D. reticulatus.*

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SARNA EUROPEJSKA (*CAPREOLUS CAPREOLUS*) I KLESZCZ ŁĄKOWY (*DERMANOCENTOR RETICULATUS*) JAKO POTENCJALNY REZERWUAR *TOXOPLASMA GONDII*, *BORRELIA BURGDORFERI* SENSU LATO, *ANAP ASMA PHAGOCYTOPHILUM* I *BABESIA MICROTI* NA TERENIE ZACHODNIEJ POLSKI

ABSTRAKT

Badania były prowadzone na terenie Ośrodka Hodowli Zwierzyny Nadleśnictwa Trzciel. Próbki krwi pobrano od 19 pozyskanych zwierząt podczas sezonu łowieckiego 2016/17, następnie przechowywano je w temperaturze –18°C w celu dalszych analiz molekularnych. Patogeny wykrywano za pomocą metody PCR i nested PCR. W celu wykrycia *T. gondii* we krwi zwierzęcej i kleszczach zastosowano dwie pary starterów swoistych dla genu B1. Obecność *B. burgdorferi* s.l. wykrywano, używając pary starterów specyficznych dla genu flageliny. Do wykrycia *A. phagocytophilum* zastosowano dwie pary starterów specyficznych dla genu kodującego 16S rRNA. Natomiast do wykrycia *B. microti* użyto dwóch par starterów określonych dla genu rRNA 18S. Obecność produktów reakcji o wielkości: par zasad [bp] 531 bp dla *T. gondii*, 482 dla *B. burgdorferi* s.1., 932 bp i 546 bp dla *A. phagocytophilum*, oraz 238 bp i 154 dla *B. microti* traktowano jako pozytywne. We krwi saren nie wykryto *T. gondii* ani *B. burgdorferi* s.l., natomiast *A. phagocytophilum* została wykazana w dwóch próbkach (10,2%) oraz *B. microti* – w siedmiu (36,8%). *T. gondii* wykryto w 2% kleszczy *Dermacentor reticulatus.*

Słowa kluczowe: sarna europejska, kleszcz łąkowy, *Toxoplasma gondii*, *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Babesia microti*