Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

Investigation of *Babesia* spp. in sympatric populations of *Dermacentor reticulatus* and *Ixodes ricinus* ticks in Lithuania and Latvia

Jana Radzijevskaja*, Dalytė Mardosaitė-Busaitienė, Asta Aleksandravičienė, Algimantas Paulauskas

Vytautas Magnus University, Vileikos 8, Kaunas, LT-44404, Lithuania

ARTICLE INFO

Keywords:
Babesia canis
Babesia venatorum
Babesia microti
Dermacentor reticulatus
Ixodes ricinus
Baltic countries

ABSTRACT

The objectives of the present study were to investigate the presence of the *Babesia* parasites in *Dermacentor reticulatus* ticks along its current distribution range in Lithuania and Latvia, and *Ixodes ricinus* in Lithuania, to characterize the detected *Babesia* spp. using partial sequencing of 18S rRNR gene, and to determine the prevalence of the *Babesia* pathogens in ticks from different locations of Lithuania and Latvia. From 2013 to 2015, four *D. reticulatus* nymphs and 2255 questing adults were collected from 40 locations in Lithuania, and 181 questing adult *D. reticulatus* specimens from 11 locations in Latvia. In Lithuania, 16 questing *I. ricinus* nymphs and 354 questing adults were collected from 12 locations with the sympatric existence of *D. reticulatus* and *I. ricinus*. In Lithuania, *Babesia* spp. were detected in 1.2% (26/2259) of *D. reticulatus* and in 9.5% (35/370) of *I. ricinus* ticks. The overall prevalence of *Babesia* in *D. reticulatus* ticks from Latvia was 2.8% (5/181). Sequence analysis of partial 18S rRNA gene of positive samples indicated the presence of *B. canis* and *B. venatorum* in *D. reticulatus* ticks, whereas *B. microti* and *B. venatorum* were detected in *I. ricinus* ticks. Our study is the first investigation on the prevalence and molecular characterization of zoonotic *B. canis, B. venatorum*, and *B. microti* in ixodid ticks in Lithuania, and of *B. canis* in *D. reticulatus* ticks in Latvia. It is also the first report of *B. venatorum* in *D. reticulatus* ticks.

1. Introduction

Babesiosis is recognized as an important tick-borne infectious disease in humans and animals caused by different intraerythrocytic protozoan *Babesia* parasites. The *Babesia* species have been reported circulating among the vertebrate hosts and vectors in many countries worldwide and are considered to be emerging pathogens. In Europe, *I. ricinus* tick is the main vector of the *Babesia* species (*B. divergens, B. venatorum*, and *B. microti*) causing human babesiosis (Hildebrandt et al., 2013), while the other tick – *D. reticulatus* – has been recognized as the most important vector of *B. canis*, the causative agent of canine babesiosis (Schaarschmidt et al., 2013). During the last decades, a spread of canine babesiosis due to *B. canis* to the previously non-endemic areas has been reported in Europe (Solano-Gallego and Baneth, 2011). The previous studies conducted in different European countries showed that the prevalence of *B. canis* in adult *D. reticulatus* varies from 0% to 14.8% (reviewed by Földvári et al., 2016). A recent study demonstrates that *D. reticulatus* has expanded its range in the Baltic countries and the presence of *D. reticulatus* has been confirmed in new areas in Lithuania and Latvia (Paulauskas et al., 2015). Although cases of autochthonous canine babesiosis in dogs due to *B. canis* were reported in Lithuania and Latvia (Berzina et al., 2013; Paulauskas et al., 2014), there is no information of *Babesia* infection in *D. reticulatus* ticks. *Babesia* spp. in *Ixodes* ticks have been reported in many European countries, including the Baltic countries (Radzijevskaja et al., 2008; Katargina et al., 2011; Caplagina et al., 2016) where *I. ricinus* is the most prevalent and widely distributed tick species. However, information on the prevalence of *Babesia* spp. in *I. ricinus* ticks in Lithuania is scarce.

The aims of the present study were to investigate the presence of the *Babesia* parasites in *D. reticulatus* ticks along its current distribution range in Lithuania and Latvia, and *I. ricinus* ticks in Lithuania.

2. Materials and methods

Ticks were collected from vegetation using the standard “flagging” method in different habitats: in open landscapes (meadows, abandoned

Abbreviations: ML, maximum-likelihood; BIC, Bayesian information criterion

* Corresponding author.
E-mail addresses: jana.radzijevskaja@vdu.lt (J. Radzijevskaja), dalyte.mardosaite-busaitiene@vdu.lt (D. Mardosaitė-Busaitienė), asta.aleksandraviciene@vdu.lt (A. Aleksandravičienė), a.paulauskas@gmf.vdu.lt (A. Paulauskas).

http://dx.doi.org/10.1016/j.ttbdis.2017.09.013
Received 8 June 2017; Received in revised form 24 August 2017; Accepted 10 September 2017

fields), in forest landscapes (among young tree stands, clearings of mixed deciduous woodland), in ecotones (grassy/woodland areas, grassy, woodland areas/lakes, rivers) during the monthsof March, April, and May from 2013 to 2015. March-May is the period of high activity of *D. reticulatus* tick in Lithuania, while May is the month of peak activity for both *I. ricinus* and *D. reticulatus*. Ticks were identified to the species level, stage and sex using morphological characteristics (Hillyard, 1996; Estrada-Peña et al., 2004).

The relative abundance of ticks was estimated by determining the average number of ticks per person per 30 min in each collection session (Nowak, 2011).

DNA extraction was carried out by lysis of ticks in ammonium hydroxide solution (2.5%) (Rijpkema et al., 1996). Nested PCR detection of the *Babesia* pathogens in *D. reticulatus* and in *I. ricinus* ticks was carried out according to the protocols described by Jefferies et al. (2007) and Rar et al., (2005, 2011) using primers BTF1/BTR1, BTF2/BTR2 and BS1/BS2, PiroA/PiroC, respectively. In each PCR run positive (DNA of *Babesia*-infected dogs and ticks, confirmed by sequencing) and negative controls (double-distilled water) were used. All positive PCR fragments were repeatedly used in PCR reactions (at least 3 times) to confirm positivity. PCR products of *Babesia*-positive samples were sequenced. The obtained partial 18S rRNA sequences were analyzed using Mega software package, version 6.05, and compared with the sequence data available from GenBank using the BLAST program. A phylogenetic tree was constructed using the maximum-likelihood (ML) method. The model of nucleotide substitution was determined according to the Bayesian information criterion (BIC) using the program jModelTest2 (Guindon and Gascuel, 2003; Darriba et al., 2012). Partial 18S rRNA sequences for representative samples were submitted to GenBank under the accession numbers: KY945498 KY945504 – *B. canis* sequences, KY945505, KY945506 and KY945507 – *B. venatorum*, and KY945508 – *B. microti*.

The prevalence of *Babesia* spp. infection in ticks was statistically analyzed by means of the Pearson's $\chi 2$ test using the statistical software package SPSS.

3. Results

A total of 2255 questing *D. reticulatus* adults and four nymphs were collected from 40 locations in Lithuania and 181 questing *D. reticulatus* adults from 11 locations in Latvia. In Lithuania, 16 questing *I. ricinus* nymphs and 354 questing adults were collected from 12 locations where both tick species co-occur (Table 1). The relative abundance of *D. reticulatus* ticks range in different sampling localities from 0 to 88, and *I. ricinus* from 0 to 17.5.

In Lithuania, *Babesia* spp. were detected in 26 of the 2255 (1.2%) questing adult *D. reticulatus* and in 35 of the 354 (9.9%) questing adult *I. ricinus* ticks (Table 1). None of the four *D. reticulatus* and 16 *I. ricinus* nymphs carried any *Babesia*. The prevalence of pathogens in *D. reticulatus* and *I. ricinus* ticks varied in different locations from 0% to 11% and from 0% to 32%, respectively. *Babesia*-infected *D. reticulatus* were found in 30% (12/40), while *Babesia*-infected *I. ricinus* in 80% (10/12) of sampling locations. *Babesia* pathogens in both tick species were detected in three of 12 sympatric populations of *D. reticulatus* and *I. ricinus* (Table 1). There was no significant difference either in the prevalence of *Babesia* spp. in *D. reticulatus* females and males ($\chi 2 = 1.135$; $p = 0.567$) or in the prevalence of *Babesia* spp. in *I. ricinus* females and males ($\chi 2 = 2.388$; $p = 0.303$) (Table 1).

In Latvia, *Babesia* pathogens in *D. reticulatus* were detected in three out of 11 sampling locations with the overall prevalence of 2.8% (5/181). The prevalence of the *Babesia* pathogens in different locations ranged from 1% (1/73) to 17% (3/18) and 25% (1/4). Only *D. reticulatus* males (7%; 5/76) were infected with *Babesia* pathogens. None of the 105 *D. reticulatus* females was found to be infected with *Babesia* spp. in Latvia (Table 1).

Altogether *Babesia* spp. DNA was detected in 31 *D. reticulatus* ticks. A total good quality 18S rRNA gene sequences were obtained and analyzed.

The sequence analysis of the 564-bp 18S rRNA gene showed that *Babesia* isolates derived from 17 *D. reticulatus* specimens were 100-99% identical to the corresponding *B. canis* sequences deposited in GenBank, while *Babesia* isolate from one *D. reticulatus* showed a 100% identity with *B. venatorum* (Fig. 1). A total of six single nucleotide polymorphisms in partial 18S rRNA gene of *B. canis* were identified by sequence analysis. Three genotypes of *B. canis* were distinguished on the basis of two observed nucleotide substitutions (GA/AG) in the positions 408 and 409 (610 and 611 in the whole-length ss rRNA gene). The first genotype (I) that displayed GA nucleotides in these positions was detected in eight of the 17 (50%) sequences. Four polymorphic variants were detected in this genotype of *B. canis*. The second genotype (II) was characterized by the presence of GA/AG nucleotides double peaks in these positions, and was observed in seven of the 17 (43.8%) sequences. The third genotype (III) with AG nucleotides combination was detected in one positive sample (6.2%) (Fig. 1).

Babesia spp. DNA was detected in 35 *I. ricinus* ticks. However, good quality 18S rRNA gene sequences were obtained for 15 samples. The sequence analysis of 300-bp 18S rRNA gene revealed that 60% (9/15) of *Babesia* isolates derived from *I. ricinus* were identical to each other and showed a 100% identity with the zoonotic *B. microti* 'Jena/Germany' type (EF413181; KP742796), while 40% (6/15) of isolates showed a 99–100% identity with *B. venatorum*. Two genotypes of 18S rRNA gene of *B. venatorum* with one nucleotide difference were detected. The phylogenetic analysis shows that our *B. venatorum* isolates from *D. reticulatus* and *I. ricinus* ticks were identical and similar to those obtained from ticks and humans in Europe (Fig. 1).

4. Discussion

In Lithuania, the highest prevalence of *B. canis* in *D. reticulatus* was detected in central (7%) and southern (11%) parts of the country; here populations of these ticks have been obtained since the last century and dogs are frequently diagnosed with canine babesiosis (Paulauskas et al., 2014). There was no significant difference in the prevalence of *Babesia* spp. in *D. reticulatus* in open areas, ecotones and forest landscapes ($\chi 2 = 3.08$; $p = 0.0794$; $\chi 2 = 3.34$; $p = 0.0674$; $\chi 2 = 0.9$; $p = 0.343$). The highest prevalence of *B. canis* in our study was detected among ticks collected in Latvia (17% in site 46 and 25% in site 42) − in the northern area of the zone of expansion of *D. reticulatus* ticks in Central Europe (Table 1). A similar high prevalence of *B. canis* in *D. reticulatus* has been reported in southern areas of the expansion zone in central Poland (14.8%), where the overall prevalence of infection in *D. reticulatus* was 4.18% (Mierzejewska et al., 2015).

Unfortunately, *Babesia* species identification and molecular characterization was not possible for all *Babesia*-positive samples. Good quality 18S rRNA gene sequences were obtained for 35 out of 66 samples. The reason for this was a low concentration of *Babesia* DNA in the tested samples.

Three 18S rRNA *B. canis* genotypes (from the four previously detected in *D. reticulatus* ticks and dogs in Europe (Beck et al., 2009; Schaarschmidt et al., 2013)) were found in *D. reticulatus* ticks in Lithuania. Earlier investigations of *B. canis* isolates from dogs revealed an association between the 18S rRNA genotype and virulence, and two genetically different groups A and B (designated in the present study as genotypes I and III, respectively) were identified (Adaszek et al., 2009; Carcy et al., 2015). It was found that the extent of thrombocytopenia was more severe in dogs infected with the 18S rRNA-B genotype. Our study demonstrates the prevalence in Lithuania of *B. canis* 18S rRNA-A genotype of lower virulence and 18S rRNA *B. canis* genotype, which displayed ambiguous nucleotides in 18S rRNA sequences (genotype II) (Fig.1). These two genotypes were previously detected in dogs from Lithuania (Paulauskas et al., 2014). Two 18S rRNA *B. canis* genotypes are present in Latvia: one genotype of higher virulence (genotype III)