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Prevalence and phylogenetic analysis of *Babesia* spp. in *Ixodes ricinus* and *Ixodes persulcatus* ticks in Latvia

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Abstract *Babesia* spp. are tick-borne protozoan parasites that have been reported in many European countries and are considered to be emerging pathogens. Several *Babesia* spp. have been identified in ticks in Latvia. Recently, canine babesiosis cases were diagnosed for the first time in Latvia; therefore, continued studies on the prevalence and occurrence of new species are warranted. In the present study, questing tick samples collected in 2005–2007 were screened for the presence of *Babesia* spp.; in total, 432 *Ixodes ricinus* and 693 *Ixodes persulcatus* ticks were analyzed. *Babesia* spp. were detected in 1.4 % of the *I. ricinus* ticks and in 1.9 % of *I. persulcatus* ticks. Sequencing revealed that ixodid ticks in Latvia contained *Babesia microti*, *Babesia capreoli*, and *Babesia venatorum. Babesia microti* was the most prevalent species, accounting for 58 % of all positive samples; moreover, two distinct *B. microti* genotypes were identified. Phylogenetic analysis of the full-length 18S rRNA gene of two *B. capreoli/B. divergens* isolates indicated a closer relationship to the *B. capreoli* clade than *B. divergens.* This is the first report of *B. venatorum* in *I. persulcatus* ticks in Latvia. Our results suggest that both *I. ricinus* and *I. persulcatus* ticks play important roles in the epidemiology of these zoonotic pathogens in Latvia.

Keywords *Ixodes ricinus* · *Ixodes persulcatus* · Questing ticks · *Babesia* spp. · Latvia

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Introduction

Babesiosis is recognized as an important tick-borne disease in humans and several animal species worldwide (Blaschitz et al. 2008). The disease affects a wide range of domestic and wild mammalian hosts. Depending on the causative species and immunological status of the host, babesiosis can manifest as severe, moderate or mild disease; most commonly, it is characterized by hemolysis, hemoglobinuria, fever and hypoxia (Kirtz et al. 2012; Michel et al. 2014). In Europe, three *Babesia* species are of particular interest in ruminants: *Babesia divergens*, *Babesia capreoli* and *Babesia venatorum* (Michel et al. 2014). Additionally, cattle are known to be a host for *Babesia bovis* and dogs for *Babesia canis* (Hunfeld et al. 2008). Most human infections worldwide are caused by *Babesia microti*; however, babesiosis caused by *B. divergens*, *Babesia duncani*, and *B. venatorum* has been reported in several countries (Vannier and Krause 2012; Gonzalez et al. 2014; Mørch et al. 2014).

Recently, the area where parasites have been detected in ticks and where the cases of babesiosis have been recognized in animals or men has expanded and new *Babesia* species have been found (Shock et al. 2014; Paulauskas et al. 2015). Therefore, local investigations are essential to assess the potential risk of human and animal disease (Halos et al. 2014). Such combined knowledge from different parts of the world helps both veterinarians and human health professionals understand the ecology of the disease agents and estimate global trends in the spread of the disease (Halos et al. 2014; Shock et al. 2014).

In Latvia (a northeast European country), two *Ixodes* tick species are commonly present: *Ixodes ricinus* is distributed in the central and western part and *Ixodes persulcatus* is prevalent in the central and eastern part of the country (Bormane et al. 2004; Karelis et al. 2012). Recent study showed several areas in Southern Latvia where *Dermacentor reticulatus* ticks that are the main vector of *B. canis canis* were found (Paulauskas et al. 2015). In addition, sporadically *Ixodes trianguliceps*, *Ixodes lividus* and *Ixodes apronophorus* have been found in this region (Salmane 2012).

Previously, *Babesia* spp. DNA was detected in pooled host-seeking *Ixodes* ticks and in *I. ricinus* ticks detached from migratory birds in Latvia (Bormane 2007; Capligina et al. 2014). Moreover, several cases of autochtonous canine babesiosis caused by *B. canis canis* were diagnosed in Latvia (Berzina et al. 2013).

The aims of this study were to (1) determine the prevalence of *Babesia* spp. in questing *Ixodid* ticks in Latvia, (2) identify the species/strains of *Babesia* spp., and (3) compare the occurrence of *Babesia* spp. in *I. ricinus* and *I. persulcatus*.

Materials and methods

Sample collection and DNA isolation

Ticks were collected in 2005–2007 using the flagging method during the tick peak activity (April–September) in 20 regions of Latvia (Fig. 1). Tick collection was performed in various habitats such as grassland, pasture, bushy areas as well as deciduous woodland. Ticks were preserved in 70 % ethanol and stored individually at −20 °C after species and stage identification prior to DNA extraction. DNA isolation from field-collected ticks was performed as described elsewhere (Capligina et al. 2014). To avoid possible seasonal differences, for the present study tick DNA samples were selected randomly and the

Fig. 1 Tick sampling sites and *Babesia* genospecies in Latvia. The name is provided only for regions included in the study. The sympatric area for *Ixodes persulcatus* and *I. ricinus* tick species according to Karelis et al. (2012) is highlighted in *grey*. The sympatric distribution area for both tick species is highlighted in *dark grey*; *I. persulcatus* predominated at more than 70 % according to this study. *Filled triangle B. microti*, *Filled* square *B. venatorum*, *Filled circle B. capreoli*

following selection criteria were applied: (a) samples from 20 regions of Latvia were included; (b) tick species and stage was clearly identified; (c) only *Ixodes* ticks samples were included; (d) adult tick samples (males and females) as well as nymphs were included and processed individually; and (e) DNA amount and quality was sufficient.

Detection and genotyping of *Babesia* spp.

For the amplification of the 18S rRNA gene fragment of *Babesia* spp., we used the outer primer pair 5-22F and 1661R for the primary PCR reaction and the inner primer pair 455-479F and 793-772R for the nested PCR reaction (Table 1; Birkenheuer et al. 2003).

Table 1 Primers used in this study

Pathogen/gene	Primer	Sequence (5′–3′)	Reference
Babesia spp./18S rRNA	5-22F	GTTGATCCTGCCAGTAGT	Birkenheuer et al. (2003)
	1661R	AACCTTGTTACGACTTCTC	
	455-479F	GTCTTGTAATTGGAATGATGGTGAC	
	793-772R	ATGCCCCCAACCGTTCCTATTA	
B. capreoli/18S rRNA	BcaprF	TGACTAAGGTTGAGCGTGAGAC	This study
	BcaprR	GCCATGGCAACGACAAATACG	

The PCR was performed in a final volume of 25 µl. The master mix (Thermo Scientific, USA) contained (per reaction) 1 × Taq Buffer with $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 200 µM of each dNTPs, 0.5 µM of each primer, 1.5 U of Taq DNA polymerase (recombinant) and 2 µl of DNA template (2 µl of the PCR products from the primary reactions was used as a template for the nested PCR reactions). The PCR assays were performed under the following conditions: an initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min; and a final elongation step at 72 °C for 5 min. Nested PCR assays were performed under the same conditions, but a reduced time (30 s) in the denaturation, annealing and elongation steps was used. To distinguish between the *B. divergens* and *B. capreoli* species, a full-length 18S rRNA gene was amplified and sequenced for these samples. For this task, primers BcaprF and BcaprR were designed. BcaprF is located 36-57 bases upstream of the gene, whereas BcaprR is located 102–122 bases downstream of the gene. The PCR was performed in a final volume of 20 µl. The master mix (Thermo Scientific) contained (per reaction) 1× Phusion Buffer with $MgCl_2$, 200 µM of each dNTPs, 0.3 µM of each primer, 0.4 U of Phusion Hot Start II DNA Polymerase and 1 µl of DNA template (5 ng/µl). The PCR assays were performed under the following conditions: an initial denaturation at 98 °C for 30 s; 40 cycles of denaturation at 98 °C for 10 s, primer annealing at 65 °C for 30 s, and elongation at 72 °C for 30 s; and a final elongation step at 72 °C for 5 min.

The PCR products were separated using electrophoresis on a 2 % agarose gel (TopVision Agarose, Thermo Scientific) in Tris–Acetate-EDTA buffer containing 0.2 µg of ethidium bromide/ml and were visualized with transillumination under UV light. All positive PCR products (ca. 400 bp for *Babesia* spp., ca. 2000 bp for *B. divergens*/*B. capreoli*) were purified from the agarose gel using the Gene JET Gel Extraction Kit (Thermo Scientific) and were subjected to sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sense strand was sequenced with the primers BcaprF, 5-22F and 455-479F and the antisense strand was sequenced with the primers BcaprR, 1661R and 793-772R to obtain the full 18S rRNA gene sequence. The DNA sequences generated in the present study were aligned and compared with DNA sequences deposited in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank) using the BLAST program (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST; Altschul et al. 1990).

Sequence and phylogenetic analysis

The 18S ribosomal RNA gene sequences of *B. microti*, *B. venatorum* (formerly *Babesia* sp. EU1), *B. divergens* and *B. capreoli* from the NCBI GenBank database were used for phylogenetic comparisons. The primer sequences were excluded from all amplicons so that they were not part of the original DNA template. A multiple sequence alignment was performed using the program CLUSTAL W Alignment (Thompson et al. 1994). The phylogenetic relationships between isolates were examined using the Neighbor-Joining method (Saitou and Nei 1987). The robustness of the generated tree was evaluated by bootstrap analysis of 500 replicates (Felsenstein 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezaki et al. 2004). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969) with the units in the number of base substitutions per site. The phylogenetic analyses were conducted in the MEGA software version 4.0 package (Tamura et al. 2007).

Statistical analysis

All ticks were processed individually, and the prevalence was expressed as a percentage. Statistical analysis was performed to compare the pathogen prevalence in *I. ricinus* and *I. persulcatus* ticks. A *P* value was calculated using the two-sided Fisher's exact test (GraphPad Software, La Jolla, CA, USA).

Results

Babesia spp. in ticks

In total, 1125 questing ticks from 20 regions located in various parts of Latvia were analyzed for the presence of *Babesia* spp. DNA (Fig. 1). Of these, 432 were identified as *I. ricinus* (127 males, 124 females and 181 nymphs) and 693 as *I. persulcatus* (257 males, 281 females and 155 nymphs). As expected, *I. ricinus* was solely found in the western and central part of the country, whereas *I. persulcatus* dominated in eastern Latvia in defined regions where it comprised more than 70 % of the collected ticks (Fig. 1). Both tick species were present in the northern part of the country, as well as some of the central and southern regions. *Babesia* spp. were identified in 1.4 % (6/432) of *I. ricinus* and 1.9 % (13/693) of *I. persulcatus* ticks (Table 2); this difference was not significant (Fisher's exact test: $P = 0.64$). *Babesia* spp. prevalence in males and females did not differ significantly for each tick species analyzed: 1.6 % for males and 3.2 % for females of *I. ricinus*; 1.2 % for males and 1.8 % for females of *I. persulcatus*; a similar result was obtained when both species were pooled: 1.3 % (5/384) for males and 2.2 % (9/405) for females (Table 2). Only in nymphs a difference in infection rate between species was found: *Babesia* spp. DNA was detected in 3.2 % of *I. persulcatus* nymphs and in none of the *I. ricinus* nymphs (Fisher's exact test: $P = 0.022$). In total, 1.8 % (14 of 789) adult ticks and 1.5 % (5 of 336) nymphs were *Babesia* spp. positive in this study; again, this difference was not significant (Fisher's exact test: $P = 1.0$).

Table 2 Prevalence of *Babesia* spp. in field-collected *Ixodes ricinus* and *Ixodes persulcatus* males, females and nymphs in Latvia

Ticks	No. of ticks	No. (%) of ticks infected with *Babesia* spp.			
		B. venatorum	*B. microti*	*B. capreoli*	Total
I. ricinus	432	3 (0.7)	3 (0.7)	0	6 (1.4)
Male	127	1 (0.8)	1 (0.8)	0	2 (1.6)
Female	124	2 (1.6)	2 (1.6)	0	4 (3.2)
Nymph	181	0	0	0	0
I. persulcatus	693	3 (0.4)	8 (1.2)	2 (0.3)	13 (1.9)
Male	257	1 (0.4)	2 (0.8)	0	3 (1.2)
Female	281	2 (0.7)	2 (0.7)	1 (0.4)	5 (1.8)
Nymph	155	0	4 (2.6)	1 (0.6)	5 (3.2)
Total	1125	6 (0.5)	11 (1.0)	2 (0.2)	19 (1.7)

Genotyping of *Babesia* spp. and phylogenetic analysis

Sequencing of the amplified 18S rRNA gene fragment and the subsequent phylogenetic analysis revealed that ixodid ticks in Latvia carried *B. venatorum*, *B. microti*, and *B. capreoli/B. divergens*, with *B. microti* accounting for 58 % (11/19) of all positive samples (Table 2; Fig. 2).

In total, three *I. ricinus* ticks (0.7 %) carried *B. venatorum* and three carried *B. microti*. *B. venatorum*, *B. microti* and *B. capreoli/B. divergens* were detected in 3 (0.4 %), 8 (1.2 %) and 2 (0.3 %) *I. persulcatus* ticks, respectively.

All six *B. venatorum* sequences obtained in this study were genetically similar (99–100 %) to those sequenced from ticks in Europe, Russia and China with 100 % identity to the *Babesia* spp. EU1 clone BAB20 reference sequence [GenBank: AY046575]; no separate clusters were observed during the phylogenetic analysis. *Babesia venatorum*-positive *I. ricinus* ticks were collected in Valka and Alūksne regions, whereas *I. persulcatus* ticks were collected in Madona and Alūksne; all these regions are located in northeast Latvia (Fig. 1). In contrast, two separate clades of *B. microti* were observed. Thus, a separate phylogenetic analysis of partial 18S rRNA gene sequences was conducted for the *B. microti* species (Fig. 3). The phylogenetic analysis of *B. microti* samples showed that 3 out of 11 *B. microti* sequences were identical to the Jena/Germany isolate [GenBank: EF413181] and clustered with the 'Jena/Germany' type. All three samples were detected in *I. ricinus* ticks from regions Rīga, Valka and Aizkraukle (Fig. 1). The remaining eight *B. microti* isolates that were obtained from *I. persulcatus* ticks formed a separate clade within the 'USA & Japan' lineage and showed a clear similarity (100 % homology) to the single isolate from Estonia [GenBank: HQ629933] reported by Kartagina et al. (2011) (Fig. 3). All eight samples were obtained in east Latvia, where *I. persulcatus* predominated at more than 70 % (Alūksne, Balvi, Gulbene, Madona and Rēzekne; Fig. 1). Importantly, a striking difference in the nucleotide positions 640 (A/G) and 725 (T/C) (nucleotide positions of the 18S ribosomal RNA gene were based on the reference sequence *B. microti* strain RI, GenBank: XR_001160977.1) was observed for the 18S rRNA genes of these *B. microti* sequences. Thus, the name 'Baltic' type was proposed for these *B. microti* samples for clarity.

Two of our *Babesia* spp. sequences (GenBank: KP742785 and KP742786) showed high similarity with the *B. divergens/B. capreoli* cluster (Fig. 2). *Babesia capreoli/B. divergens*-positive *I. persulcatus* ticks were collected in Gulbene and Madona regions (Fig. 1). Because the close relatedness for these species was noted previously, we sequenced the full-length 18S rRNA genes for these samples (Zintl et al. 2011). The analysis of full length sequences revealed that both sequences contained the *B. capreoli*-specific nucleotides G, T and T at positions 631, 663 and 1637, respectively (Malandrin et al. 2010). This finding explained the exact clustering of our *B. capreoli/B. divergens* sequences with *B. capreoli* and a slight segregation from *B. divergens* sequences on the phylogenetic tree (Fig. 2).

The obtained sequences were submitted to GenBank under the following accession numbers: *B. venatorum* sequences KP742787, KP742788, KP742789, KP742790, KP742791, and KP742792; *B. microti* sequences: KP742793, KP742794, KP742795, KP742796, KP742797, KP742798, KP742799, KP742800, KP742801, KP742802 and KP742803; and *B. capreoli* sequences: KP742785 and KP742786.

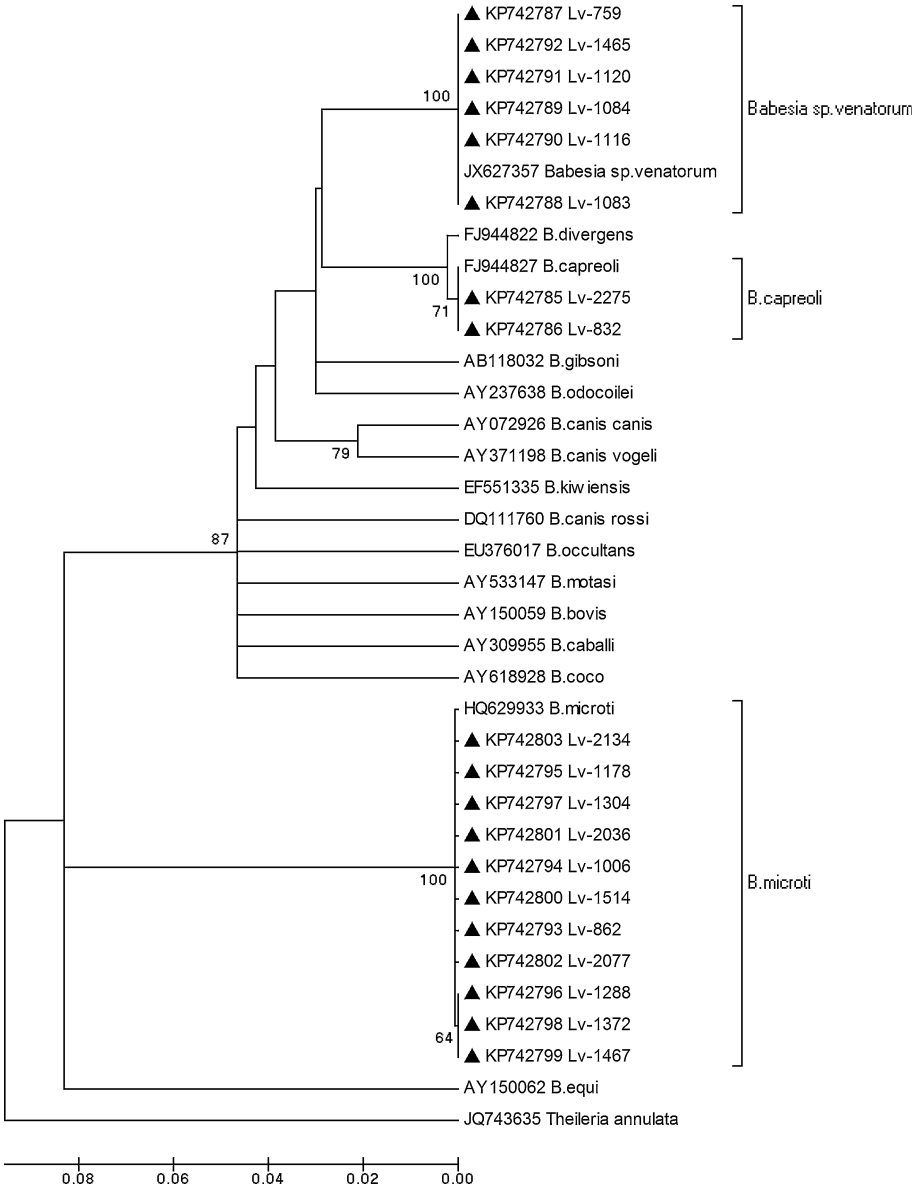

Fig. 2 Phylogenetic analysis of *Babesia* spp. Neighbour-joining tree showing the phylogenetic relationship of *Babesia* species based on the 18S ribosomal RNA gene sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (only values above 50 are displayed). The evolutionary distances are in the units of the number of base substitutions per site. *Theileria annulata* was used as an outgroup. *Filled triangle* our sample sequences identified in the present study

Fig. 3 Phylogenetic analysis of *Babesia microti*. Neighbour-joining tree showing the phylogenetic relationship of *B. microti* from ticks based on the 18S ribosomal RNA gene sequences. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (only values above 50 are displayed). *Theileria annulata* was used as an outgroup. The analyzed *B. microti* sequence names consist of the GenBank accession number, country of origin and tick species name. Designation: *I. canisuga—Ixodes canisuga*, *I. hexagonus—Ixodes hexagonus*, *I. persulcatus—Ixodes persulcatus*, *I. ricinus—Ixodes ricinus*; *Filled triangle B. microti* sequences newly identified in the present study ▶

Discussion

The present study revealed the presence of *Babesia* spp. in *Ixodes* ticks in Latvia. Our data showed that 1.4 % of *I. ricinus* and 1.9 % of *I. persulcatus* ticks were *Babesia* spp.-positive. Previous studies investigating pooled field-collected ticks in Latvia found *Babesia* spp. in 1.8 and 1.9 % of *I. ricinus* and *I. persulcatus*, respectively (Bormane et al. 2004; Bormane 2007). The prevalence of *Babesia* spp. on bird-feeding *I. ricinus* ticks from migratory birds in Latvia was reported to be 4 % (Capligina et al. 2014). The overall similarity of the data obtained suggest that the prevalence rate of infected ticks in Latvia has stayed low over the years. Similar results were obtained for *I. ricinus* and *I. persulcatus* ticks in the close neighbors of Latvia—the St. Petersburg region of Russia, Estonia, Lithuania and Belarus (0.5–3 %) (Alekseev et al. 2003; Radzijevskaja et al. 2008; Kartagina et al. 2011; Reye et al. 2013).

No significant differences in the *Babesia* spp. prevalence number were observed for either tick species or gender in this study. Interestingly, none of the nymphal *I. ricinus* ticks were positive, whereas 3.2 % (5/155) of *I. persulcatus* nymphs were infected with *Babesia* spp. Several studies in Europe have shown the presence of *Babesia* spp. in nymphal *I. ricinus* ticks; thus, one possible explanation for this result could be that a relatively small number of nymphs was investigated (181 and 155 for *I. ricinus* and *I. persulcatus*, respectively). Additionally, the prevalence rates could fluctuate among sampling sites (Gigandet et al. 2011).

Based on previous studies using field-collected ticks, the most common *Babesia* species in Latvia was *B. microti*, whereas *B. bovis* and *B. divergens* were rarely noted (Bormane 2007). The results of this study confirmed our previous observations: *B. microti* accounted for 58 % (11/19) of all *Babesia* spp.-positive samples. Again, the same outcome was obtained for the neighboring countries of Estonia, Belarus and the St. Petersburg region of Russia; the exception was Lithuania, where *B. divergens*-specific primers were used for the studies (Alekseev et al. 2003; Radzijevskaja et al. 2008; Kartagina et al. 2011; Reye et al. 2013). Two strains of *B. microti* were detected in Latvia based on the phylogenetic analysis of the partial 18S rRNA genes. The first strain was identical to the 'USA' type, which is distributed worldwide and has been reported to be pathogenic for humans (Gray et al. 2010). The second strain (named the 'Baltic' in this study) exhibited two nucleotide differences in the sequence region studied and formed a separate clade on the phylogenetic tree (Fig. 3). Importantly, the *B. microti* 'Baltic' strain was detected solely in *I. persulcatus* ticks. Thus, a possibility for a vector-pathogen specificity could be suggested. However, additional studies are required to address this phenomenon and to determine whether this strain of *Babesia* spp. poses any threat to human or animal health.

Babesia venatorum and *B. capreoli* were also identified in this study and accounted for 31.6 % (6/19) and 10.5 % (2/19) of *Babesia*-positive ticks, respectively. *Babesia*


AY648882 Switzerland I.ricinus
JX627356 Germany I.ricinus
KJ649305-KJ649312 Slovakia I.ricinus
▲ KP742796 Latvia I.ricinus
AY648883 Switzerland I.ricinus
KM610231 Ukraine I.ricinus
GQ856652 Belgium I.ricinus
KC007120 Germany I.ricinus
HQ629935-HQ629940 Estonia I.ricinus
HQ629934 Estonia I.persulcatus
AF494286 Switzerland I.ricinus
JQ886033 Germany I.ricinus
▲ KP742798 Latvia I.ricinus
AF373332 Slovenia I.ricinus
KC470048 Poland I.ricinus
EF413181 Jena/Germany type
KC007119 Germany I.ricinus
JX679169 Germany I.ricinus
GQ856653 Belgium I.ricinus
KJ508858 Slovakia I.ricinus
JX679171 Germany I.hexagonus
JQ886035-JQ886056 Germany I.ricinus
KJ863330-KJ863333 Italy I.ricinus
▲ KP742799 Latvia I.ricinus
JX627359 Germany I.ricinus
KC297160 Latvia I.ricinus
GU826703-GU826715 Luxembourg I.ricinus
JX679170 Germany I.canisuga
KJ508857 Slovakia I.ricinus
JQ711225-JQ711227 Belarus I.ricinus
GQ856649 Belgium I.ricinus
AY144692 Switzerland I.ricinus
Jena/Germany type
GU057383 Russia I.persulcatus
AF028344 USA I.dammini
KJ715163 China H.longicornis
AY144696 USA I.dammini
JQ993420 China I.persulcatus
AF028343 USA I.dammini
AY144697 USA I.dammini
JX679172 Germany I.ricinus
AF231348 USA type
AY144694 USA I.dammini
KJ486556 Russia I.persulcatus
GU057384 Russia I.persulcatus
83
USA type
AB050732 Hobetsu/Japan type
▲ KP742795 Latvia I.persulcatus
▲ KP742802 Latvia I.persulcatus
▲ KP742803 Latvia I.persulcatus
▲ KP742800 Latvia I.persulcatus
▲ KP742794 Latvia I.persulcatus
HQ629933 Estonia I.persulcatus
▲ KP742793 Latvia I.persulcatus
▲ KP742797 Latvia I.persulcatus
▲ KP742801 Latvia I.persulcatus
73
Baltic type
AB032434 Kobe/Japan type
KF170831 China Ha.longicornis
KF170830 China Ha.longicornis
91
73
Kobe/Japan type
KC470049 Poland I.ricinus
AY789075 Poland I.ricinus
KJ649287 Slovakia I.ricinus
AB071177 Munich/Germany type
80
Munich/Germany type
JQ743635 Theileria annulata
0.15
0.10
0.05
0.00

venatorum (formerly *Babesia* EU1) was recognized as a human pathogen; to date, the only known vector of *B. venatorum* was *I. ricinus* (Yabsley and Shock 2012). Moreover, this species was detected in *I. ricinus* ticks removed from migratory birds in Latvia, Norway and northwestern Russia (Hasle et al. 2011; Movila et al. 2011; Capligina et al. 2014). However, the results of this study showed, for the first time, that three *I. persulcatus* ticks were infected with *B. venatorum*. Interestingly, the phylogenetic analysis of partial 18S rRNA gene sequences did not show any differences between samples obtained from the two tick species. Thus, a role for *I. persulcatus* as a possible vector of clinically important *B. venatorum* could be proposed.

Several recent publications revealed that *B. divergens* sequences showed less than 100 % identity with the original *B. divergens* sequence based on changes in taxonomy and sequencing techniques; thus, these sequences have been renamed *B. capreoli* or *B. divergens*-like (Zintl et al. 2011; Michel et al. 2014). Additionally, *B. capreoli*, unlike *B. divergens*, lacks infectivity for gerbils and splenectomized cattle and has been isolated from asymptomatic roe deer (*Capreolus capreolus*) (Malandrin et al. 2010). Both *B. divergens*-like sequences in this study showed a remarkable similarity to both *B. divergens* and *B. capreoli*. In the current literature, two trends can be observed to distinguish between the two species, viz. the use of other domains such as rDNA ITS2 or a requirement for complete similarity between 18S RNA sequences in order to call an isolate *B. divergens*; otherwise, the isolate should be called *B. cf. divergens* or *B. capreoli* (Zintl et al. 2011; Michel et al. 2014). Thus, we sequenced the full-length 18S rRNA gene. The results of this study showed that both samples had identical sequences and should be named *B. capreoli.* Both Latvian *B. capreoli* isolates were detected in *I. persulcatus* ticks. To date, it has been accepted that *B. capreoli* is a separate species and does not pose a risk to humans or cattle because it cannot replicate in human or bovine erythrocytes (Malandrin et al. 2010). To the best of our knowledge, human babesiosis cases have not been reported in Latvia. In Europe, most clinical babesiosis cases in humans have been attributed to *B. divergens* and *B. venatorum* (formerly *Babesia* sp. EU1). *Babesia microti* infection of humans occurs mainly in the USA, although a case of autochthonous *B. microti* infection and serological evidence of infection have been reported in Europe (Øines et al. 2012; Silaghi et al. 2012; Yabsley and Shock 2012; Michel et al. 2014; Lempereur et al. 2015; Welc-Falęciak et al. 2015).

This study confirmed the presence of three *Babesia* species in *Ixodes* ticks in Latvia; two species (*B. microti* and *B. venatorum*) could be harmful for humans, especially those who are immunosuppressed or splenectomized. Because the results of the present study show a small but not negligible risk of human babesiosis in Latvia, babesiosis should remain on the differential list for Latvian human patients with adequate clinical findings and anamnesis.

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