Spectrum of Ixodidae Ticks Attacking Humans in Russian Siberia and Their Association with Tick-Borne Bacterial Agents

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Article

Spectrum of Ixodidae Ticks Attacking Humans in Russian Siberia and Their Association With Tick-Borne Bacterial Agents

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Abstract: Spectrum of ixodid ticks that bite people in Western Siberia significantly changed over the past two decades. In this study, we determined tick species attacking people in surroundings of Novosibirsk and the range of bacterial agents they infected with. This study included 301 ticks taken from people and 46% were *Ixodes pavlovsky*, followed by *Ixodes persulcatus* (19.6%), *I. persulcatus* / *I. pavlovskyi* interspecies hybrids (19.6%), and *Dermacentor reticulatus* (12.8%). Human DNA was determined in ticks, first demonstrating that all these tick species, including hybrids, were able effectively feed on humans. DNA of *Borrelia* spp., *Rickettsia* spp. and Anaplasmatacreae bacteria was detected in different tick species. *Borrelia garinii* prevailed in *Ixodes* species, being found in 8.8% ticks, whereas *B. afzelii* and *B. bavariensis* were found in single ticks. *Borrelia miyamotoi* was revealed in 3.7% ticks. Among *Rickettsia* spp., "*Candidatus* Rickettsia tarasevichiae" and *R. raoultii* were identified mainly in *I. persulcatus* and *D reticulatus* (44.8% and 26.3%, respectively), whereas *Rickettsia helvetica* was found only in 2.2% *I. pavlovsky.* The prevalence of *Anaplasma phagocytophilum*, *Ehrlichia muris* and *Neoehrlichia mikurensis* did not exceed 2%. The obtained results indicate a high risk for humans to be infected with agents of Lyme borreliosis, primarily *B. garinii*.

Keywords: *Ixodes pavlovsky*; *Ixodes persulcatus*; *Dermacentor reticulatus*; engorged ticks; human blood; *Borrelia burgdorferi* sensu lato (s.l.), *B. miyamotoi*; *Rickettsia* spp.; Anaplasmataceae

1. Introduction

Several species of ixodid ticks inhabit Novosibirsk province that is located in the southern part of Russian Western Siberia. Among ticks that bite humans, *Ixodes persulcatus*, *Ixodes pavlovskyi* and *Dermacentor reticulatus* are the most common, whereas the number of *Dermacentor marginatus* and *Dermacentor silvarum* is low (Livanova et al., 2011; Yakimenko et al., 2019).

Ixodes persulcatus has a wide distribution area in the forest part of Russia, from the North-Western to Far Eastern regions. Until the end of the last century, *I. persulcatus* was the predominant tick species in all examined locations of the Siberian forest zone. In contrast to *I. persulcatus*, *I. pavlovskyi* has a discontinuous distribution and inhabits the Far Eastern and Western Siberian regions (Bolotin et al., 1977; Pomerantsev, 1948; Zamoto-Niikura et al., 2023; Kovalevskiy, et al., 1975). In the last century, *I. pavlovskyi* was found mainly in mountain regions (Altai and Kuznetsk Alatau Mountains and Salair Ridge) of Siberia (Belyantseva et al., 1974; Sapegina et al., 1969; Kovalevskiy, et al., 1975). In the 21st century, interest in the study of *I. pavlovskyi* has increased sharply due to the rapid and significant spread of this tick. Nowadays, *I. pavlovskyi* inhabits not only foothills but also lowland biotopes; in particular, it dominates in areas around large cities, Novosibirsk and Tomsk (Livanova et al., 2011; Rar et al., 2017; Romanenko et al., 2015). Moreover, it was shown the presence of natural interspecies hybrids of *I. persulcatus* / *I. pavlovskyi* (hereinafter referred to as hybrids) in all examined locations of their simultaneous inhabitance, namely in the Republic of Altai and

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Novosibirsk and Tomsk provinces in Siberia as well as in Russky Island in the Far East (Kovalev et al., 2015; Rar 2019, Igolkina 2023a). Both these *Ixodes* species and their hybrids are infected with the same vector-borne agents, including highly pathogenic tick-borne encephalitis virus, agents of Lyme borreliosis (LB) and *Borrelia miyamotoi* disease (BMD) as well as several *Rickettsia* species and bacteria from the Anaplasmataceae family (Cleveland et al., 2022; Eisen 2020; Korenberg et al., 2010; Rar et al., 2017, 2019; Rollins et al., 2023). Notably, the prevalence of some agents varied depending on the tick species. Thus, *I. persulcatus* was significantly more frequently infected with *Borrelia bavariensis* and "*Candidatus* Rickettsia tarasevichiae" and less frequently with *Borrelia garinii* compared to *I. pavlovskyi* (Rar et al., 2017)

Unlike *Ixodes* spp., *Dermacentor* spp. ticks carry mainly rickettsial pathogens, most often *Rickettsia raoultii* and less often the highly pathogenic causative agent of Siberian tick typhus (STT), *Rickettsia sibirica* (Földvári et al., 2016; Kartashov et al., 2019; Rakov et al., 2023; Yakubovskij et al., 2023; Parola et al., 2013). Compared to *Ixodes* spp., *Dermacentor reticulatus* and *Dermacentor marginatus* inhabit drier and warmer areas located in forest-steppe and steppe zones, whereas *D. silvarum* inhabits forest areas; in lowland locations of Western Siberia the number of *D. silvarum* is low (Yakimenko et al., 2019)..

Despite the distribution of different ixodidae ticks in Western Siberia was examined in a number of studies (Livanova et al., 2011, 2015; Romanenko et al., 2015, 2017), the epidemiological significance of ticks recently invaded this region has not been sufficiently studied. To clarify this issue, in this study we examined the spectrum of ticks attacking humans in Novosibirsk province (Western Siberia), and their association with various bacterial agents.

2. Materials and Methods

2.1. Sampling

The study included ixodid ticks that were attached to people, as well as those removed from the body and clothing. The ticks were collected in diagnostic laboratories in Novosibirsk (Figure 1). In most cases, any information concerning tick species, developmental stage, degree of engorgement, and the location of sites, where ticks attacked humans, was absent.

Total DNA was extracted from ticks using "Real Best Extraction 100" ("Vector-Best", Novosibirsk, Russian Federation), according to the protocol, and 50 µl from 400 µl of extracted DNA from each tick were used for this study.

The study was approved by the Local Medical Ethical Committee of the Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia (Protocol No. 8, 03.08.2020).

Figure 1. The map shows the location of tick collection.

2.2. Tick Species Determination

Tick species determination was conducted based on results of species-specific PCR of the mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene and sequencing of the nuclear multicopy

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internal transcribed spacer (ITS2) fragment. For all samples, *cox*1 gene fragments were amplified using primers specific to *I. persulcatus*, *I. pavlovskyi* and *D. reticulatus* (Table 1). For subsequent sequencing, ITS2 fragment was amplified in each sample from *Ixodes* spp. ticks and a number of *Dermacentor* spp. ticks using primers indicated in Table 1.

Table 1. Primers used for identidication of tick species and bacterial agents.

Locus	Organism	Reaction	Primer name	Primer sequences 5′-3′	T# (°C)	References
ITS2	Ixodidae	conventional	F-ITS2	cacactgagcacttactctttg	57	[23]
			R1-ITS2	actggatggctccagtattc		
*cox*1	*I. persulcatus*	conventional	Ixodes-F	acctgatatagctttccctcg	55	[10]
			Ipers-R	ttgattcctgttggaacagc		
	I. pavlovskyi	conventional	Ixodes-F	acctgatatagctttccctcg	55	[10]
			Ipav-R	taatccccgtggggacg		
	Ixodidae	conventional	C1	accacaaagacattggaactatatat	50	[23]
			C2	aatccaggaagaataagaatatatac		
	D. reticulatus	conventional	Dret-F	ctaagacaacccggaacattaattg	60	This study
			Dret-R	aaaccctaaaagaccaattgcggc		
IGS	*B. burgdorferi* s.l.	Primary	NC1	cctgttatcattccgaacacag	50	[10]
			NC2	tactccattcggtaatcttggg		
		Nested	NC3	tactgcgagttcgcgggag	50	
			NC4	cctaggcattcaccatagac		
p66	*B. miyamotoi*	Primary	M3	ttctatatttggacacatgtc	50	[25]
			M4	cagattgtttagttctaatccg		
		Nested	M1	ctaaattattaaatccaaaatcg	50	
			M2	ggaaatgagtacctacatatg		
clpA	*B. burgdorferi* s.l.	Primary	clpAF1237	aaagatagatttcttccagac	50	[26]
			clpAR2218	gaatttcatctattaaaagctttc		
		Nested	clpAF1255	gacaaagcttttgatattttag	50	
			clpAR2104	caaaaaaaacatcaaattttctatctc		
p83/100	*B. burgdorferi* s.l.	Primary	F7	ttcaaagggatactgttagagag	50	[10]
			F10	aagaaggcttatctaatggtgatg		
		Nested	F5	acctggtgatgtaagttctcc	54	
			F12	ctaacctcattgttgttagactt		
gltA	*Rickettsia* spp.	Primary	glt1	gattgctttacttacgaccc	52	[10]

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			glt2	tgcatttctttccattgtgc		
		Nested	glt3	tatagacggtgataaaggaatc	53	
			glt4	cagaactaccgatttctttaagc		
	Ca. R. tarasevichiae	Nested	RT1	tactaaaaaagtcgctgttcattc	56	[10]
			RT2	tgttgcaaacatcatgcgtaa		
	SFGR	Nested	RH1	gtcagtctactatcacctatatag	54	[10]
			RH3	taaaatattcatctttaagagcga		
ompB	*Rickettsia* spp.	Primary	B1	atatgcaggtatcggtact	56	[27]
			B2	ccatataccgtaagctacat		
		Nested	B3	gcaggtatcggtactataaac	56	
			B4	aatttacgaaacgattacttccgg		
16S rRNA	Anaplasmataceae	Primary	Ehr1	gaacgaacgctggcggcaagc	57	[10]
			Ehr2	agtaycgraccagatagccgc		
		Nested	Ehr3	tgcataggaatctacctagtag	60	
			Ehr4	ctaggaattccgctatcctct		

T* - annealing temperature.

2.3. Identification of Engorged Ticks

To identify the engorged ticks and estimate the degree of engorgement, all ticks were examined for the presence of human DNA by RT-PCR with TaqMan probe targeted to the human unique TROSP gene as described previously (**Horsman et al., 2006**). To standardize this assay, DNA isolated from 100 µl of human blood and serially diluted 10-fold was used as positive control. The results were considered positive if cycle threshold (Ct) was < 40.

2.4. Detection and Genetic Characterization of Bacterial Agents

Identification of bacterial agents in tick specimens was carried out by genus-specific and species-specific PCR using primers specified in Table 1 and/or subsequent sequencing, as previously described (Rar et al., 2017).

Borrelia burgdorferi sensu lato (s.l.) and *B. miyamotoi* DNA was detected using multiplex PCR targeted to the 5S-23S rRNA intergenic spacer (IGS) of *B. burgdorferi* s.l. and the *p66* gene of *B. miyamotoi*. For positive *B. burgdorferi* s.l. specimens, additional PCR assays with primers specific to *clp*A and *p83/100* genes were carried out and the obtained PCR fragments were sequenced (Table 1). To determine *B. burgdorferi* genospecies, *clp*A gene sequences were analyzed using PubMLST (https://pubmlst.org/organisms/borrelia-spp) and Blastn (https://blast.ncbi.nlm.nih.gov). To determine the genospecies of samples, which could not be amplified by the *clp*A gene, the obtained p83/100 gene or IGS sequences were compared with available sequences using Blastn search.

Rickettsia spp. was identified in t*ick samples* using nested *PCR* with primers targeting the *glt*A and *omp*B genes, as described previously (Rar et al., 2017). To determine possible mixed infection, all positive samples were independently amplified using primers RT1 and RT2 specific to "*Candidatus* R. tarasevichiae", and primers RH1 and RH3 specific to spotted fever group rickettsiae (SFGR) (Table 1). The species of all SFGR were determined by sequencing of *glt*A or *omp*B gene fragments. For *R. helvetica*-positive samples, the sequences of a long fragment of the *omp*B gene with a total length of 3117 bp were determined, as previously described (Igolkina et al., 2023b).

Anaplasmataceae bacteria DNA was revealed by nested PCR using primers targeted to 16S rRNA gene (Table 1). For species determination, the obtained PCR fragments were sequenced.

2.5. Sequencing and Phylogenetic Analysis

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The obtained amplicons were gel purified in 0.6% SeaKem® GTG-agarose (Lonza, Haifa, Israel). *Sanger sequencing was* carried out *using* BigDye Terminator V. 3.1 Cycling Sequencing Kit (Applied Biosystems, CA, USA). Sanger reaction products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems Inc.). Obtained sequences were aligned, analyzed and compared using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The maximum likelihood (ML) method was used to construct phylogenetic trees using MEGA 7.0 software with 1000 bootstrap replicates (Kumar et al. 2016), based on obtained sequences and known sequences from the GenBank database available on 20 November 2024.

2.6. Statistical Analysis

The *Pearson's chi-square* test (http://www.socscistatistics.com/tests/chisquare/) was used for statistical analysis. $P < 0.05$ was considered as significant.

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2.7. Nucleotide Sequence Accession Numbers

Nucleotide sequences determined in the study are available in the GenBank database under accession numbers: PQ685972-PQ685977 and PQ682399-PQ682400 for *Dermacento*r; PQ682653-PQ682655 and PQ724397-PQ724411 for *Borrelia* spp.; PQ682631-PQ682652 for *Rickettsia* spp.

3. Results

3.1. Tick Species

A total of 301 specimens of ticks attacking humans in surroundings of Novosibirsk were examined. Tick species were determined based on analysis of the mitochondrial *cox*1 gene and nuclear ITS2. We failed to determine tick species in five specimens; these specimens were excluded and 296 specimens were investigated. Because of probable presence of *I. persulcatus* / *I. pavlovskyi* interspecies hybrids among examined ticks, all *Ixodes* spp. were genetically characterized by both mitochondrial and nuclear loci, as previously described (Rar 2019). A total of 137 *I. pavlovsky*, 58 *I. persulcatus* and 58 hybrids were identified.

Dermacentor reticulatus DNA was found in 38 specimens based on PCR using species-specific primers by the *cox*1 gene. For 13 specimens, ITS2 or *cox*1 fragments of *D. reticulatus* were sequenced to confirm the correctness of *D. reticulatus* identification using primers designed in this study. All determined ITS2 fragments were identical and had four polymorphic sites in positions, which differentiate *D. reticulatus* haplotypes previously identified in Eurasia (Figure 2) (Bilbija et al., 2023). The determined *cox*1 sequences of *D. reticulatus* were identical to those previously identified in Novosibirsk province (M867332) or differed from this sequence by one unique nucleotide substitution.

Based on sequences of ITS2 fragments, two *D. marginatus* and three *D. nuttalli* / *D. silvarum* were identified among the collected ticks. Two determined sequences of *D. marginatus* had five and six polymorphic sites; the location of these sites differed in these sequences. The probable haplotypes of *D. marginatus* corresponded to those of *D. marginatus* from Turkey (PP456826, etc) (Figure 2). Notably, this is the first determination of ITS2 sequences of *D. marginatus* collected in Russia.

The sequences of three *Dermacento*r spp. were closely related to available sequences of *D. nuttalli* and *D. silvarum* from Russian Siberia and China. Due to the high genetic similarity between *D. nuttalli* and *D. silvarum*, these tick species can be distinguished by morphology and distribution area rather than genetically (Figure 2). Two *D. nuttalli* / *D. silvarum* sequences (1069 bp fragments) were identical and showed the most similarity with *D. nuttalli* from Baikal region (KF241872), differing from it by the presence of four polymorphic sites. However, since the locations where these ticks were taken are known and correspond to the distribution area of *D. silvarum*, but not *D. nuttalli* (forest biotopes in the Republic of Altai), these ticks are probably *D. silvarum*. The sequence of the third tick was the most similar to those of *D. nuttalli* from Baikal region (KF241869) and China (OQ955291, OQ955293, etc), differing from them by three polymorphic sites (Figure 2). The determined in this study *D. nuttalli* / *D. silvarum* sequences differed between themselves by eight mismatches (seven substitutions and one indel) and seven polymorphic sites.

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Figure 2. Phylogenetic trees constructed by the ML method based on nucleotide sequences of 1078 bp fragment of ITS2 of *Dermacentor* spp. The scale bar indicates an evolutionary distance of 0.001 nucleotide per position in the sequence. Significant bootstrapping values (>70%) are shown on the nodes. Legend: ● – *D. nuttalli*; ♦ – *D. silvarum*; ■ – *D. marginatus*; ▲ – *D. reticulatus*.

3.2. Determination of Human DNA in Ticks

In order to determine the portion of engorged ticks and estimate the degree of their engorgement, all specimens were tested for the presence of human DNA by Taq-man real-time PCR targeted to the unique human TROSP gene. The quantity of human DNA corresponded to the volume of human blood. Among 296 tested specimens, human DNA was found in 72 (24.3%) ticks. Notably, most of them (55 samples) contained a small amount of human DNA, equivalent to 0.4-3.5 µl of human blood and only 17 ticks contained human DNA in amounts, corresponding to 5.5-106 µl of blood. None of the ticks contained human DNA in quantities equivalent to 3.5–5.5 µl of blood. Ticks may have picked up trace amounts of human material while moving across the skin or during their attachment. Thus, to exclude false-positive results, only ticks with the amount of human DNA equivalent to > 5.5 µl of blood were considered as engorged (Table 2).

The portion of ticks containing a small amount of human DNA (<3.5 µl of blood) varied from 13.8% to 25.9% between different tick species, being the lowest for hybrids and the highest for *I. persulcatus* (Table 2); the difference was not significant between any of the tick species ($p > 0.5$). The portion of engorged ticks (> 5.5 µl of blood) also varied depending on tick species and constituted 2.3%, 3.4%, 5.8%, and 10.3% for *D. reticulatus*, hybrids, *I. pavlovsky*, and *I. persulcatus*, respectively. The difference between different tick species was not significant ($p > 0.5$).

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Table 2. Human DNA in different tick species determined by RT-PCR.

Amount (µl) of human blood in a tick	The No (%) of ticks containing different amount of human blood				
	I. pavlovskyi (n=137)	*I. persulcatus* (n=58)	Hybrids (n=58)	*Dermacentor* spp. (n=43)	All species (n=296)
Nd (<0.4)	**106 (77.4)**	**37 (63.8)**	**48 (82.8)**	**33 (76.7)**	**224 (75.7)**
0.4-0.9	12	7	5	8	32
1.0-1.9	8	5	2	0	15
2.0-2.9	1	2	0	1	4
3.0-3.5	2	1	1	0	4
3.5-5.5	0	0	0	0	0
Subtotal 0.4-5.5	**23 (16.8)**	**15 (25.9)**	**8 (13.8)**	**9 (20.9)**	**55 (18.6)**
5.5-10	3	1	1	1	6
10-20	2	1	0	0	3
20-50	1	2	1	0	4
50-106	2	2	0	0	4
Subtotal 5.5-106	**8 (5.8)**	**6 (10.3)**	**2 (3.4)**	**1 (2.3)**	**17 (5.7)**

3.3. Detection of Borrelia spp. in Ticks

Spirochetes from *B. burgdorferi* s.l. species complex and *B. miyamotoi* were detected in *Ixodes* spp. but not *Dermacentor* spp. ticks. In total, *B. burgdorferi* s.l. was found in 30 (10.1%) ticks, including 19 (13.9%) *I. pavlovskyi*, 5 (8.6%) *I. persulcatus*, and 6 (10.3%) hybrids (**Table 3**). *B. garinii* prevailed in all *Ixodes* species, being found in 17 *I. pavlovskyi*, 4 *I. persulcatus*, and 5 hybrids. *B. afzelii* was found only in two *I. pavlovskyi*, whereas *B. bavariensis* was detected in one *I. persulcatus* and a hybrid tick. *I. pavlovskyi* ticks were significantly more often infected with *B. garinii* than other *B. burgdorferi* s.l. genospecies ($\chi^2 = 12.72$, $df = 1$, $P < 0.001$). The difference in the pathogen prevalence among other tick species or between different tick species for the same pathogen was not significant ($p > 0.5$).

The identified *B. burgdorferi* s.l. samples were genetically characterized by the *clp*A gene. A total of 23 *B. garinii*, two B. bavariensis and one *B. afzelii clp*A gene fragments with lengths of 719-785 bp were successfully sequenced. Two *B. bavariensis clp*A sequences exactly matched to 56 and 72 *clp*A alleles from the PubMLST database, which were common for Russian Siberia and Asian countries. The only determined *clp*A sequence of *B. afzelii* corresponded to allele 36 from the PubMLST database, which also is typical for ticks from Siberia.

Table 3. Prevalence of *Borrelia* spp. in Ixodidae ticks collected from humans.

Tick species	No. of ticks	No. (%) of ticks containing DNA of tested agents*				
		Bg	Ba	Bb	all B. burgdorferi (s.l.)	Bm
I. pavlovskyi	137	17 (12.4)	2 (1.5)	0	19 (13.9)	5 (3.6)
I. persulcatus	58	4 (6.9)	0	1 (1.7)	5 (8.6)	5 (8.6)
Hybrids	58	5 (8.6)	0	1 (1.7)	6 (10.3)	1 (1.7)
Dermacentor spp.	43	0	0	0	0	0
All species	**296**	**26 (8.8)**	**2 (0.7)**	**2 (0.7)**	**30 (10.1)**	**11 (3.7)**

Abbreviations: Bg - *B. garinii*; Ba - *B. afzelii*; Bb - *B. bavariensis*; Bm – *B. miyamotoi*. *Including cases of mixed infection.

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Six *B. garinii* sequences contained polymorphic sites and were excluded from further analysis. Among remaining 17 *B. garinii* sequences, 12 different sequence variants were identified. The sequences from seven ticks exactly matched *clp*A alleles from the PubMLST database (192, 195, 196, 211, and 326), which were previously found only in Western Siberia. The sequences from five ticks were identical to three variants of *clp*A gene sequences, previously identified in ticks from the Novosibirsk province (KX980253, KX980226, and KX980260) but differed from known *clp*A alleles. The sequence from one *I. pavlovskyi* (Nov21-186_Ipavl) was novel and differed by one substitution from allele 196, common for Siberia. Two ticks (*I. persulcatus* and *I. pavlovskyi*) carried *B. garinii* variant corresponding to the *clp*A allele 112, which is widespread in European countries and Western Siberia. In addition, a novel for Siberia *B. garinii* sequence from *I. persulcatus* (Nov21-43_Iper) matched to *clp*A allele 45, which previously was found only in European countries. Another variant unusual for Siberia was detected in one hybrid (Nov21-185_Iper/Ipav); this variant exactly matched the *clp*A allele 185, which was found in *I. ureae* collected from seabirds from Canada and Norway, one *I. persulcatus* in Japan (CP075232) and one *I. pavlovskyi* in Novosibirsk province (KX980241) (Figure 3).

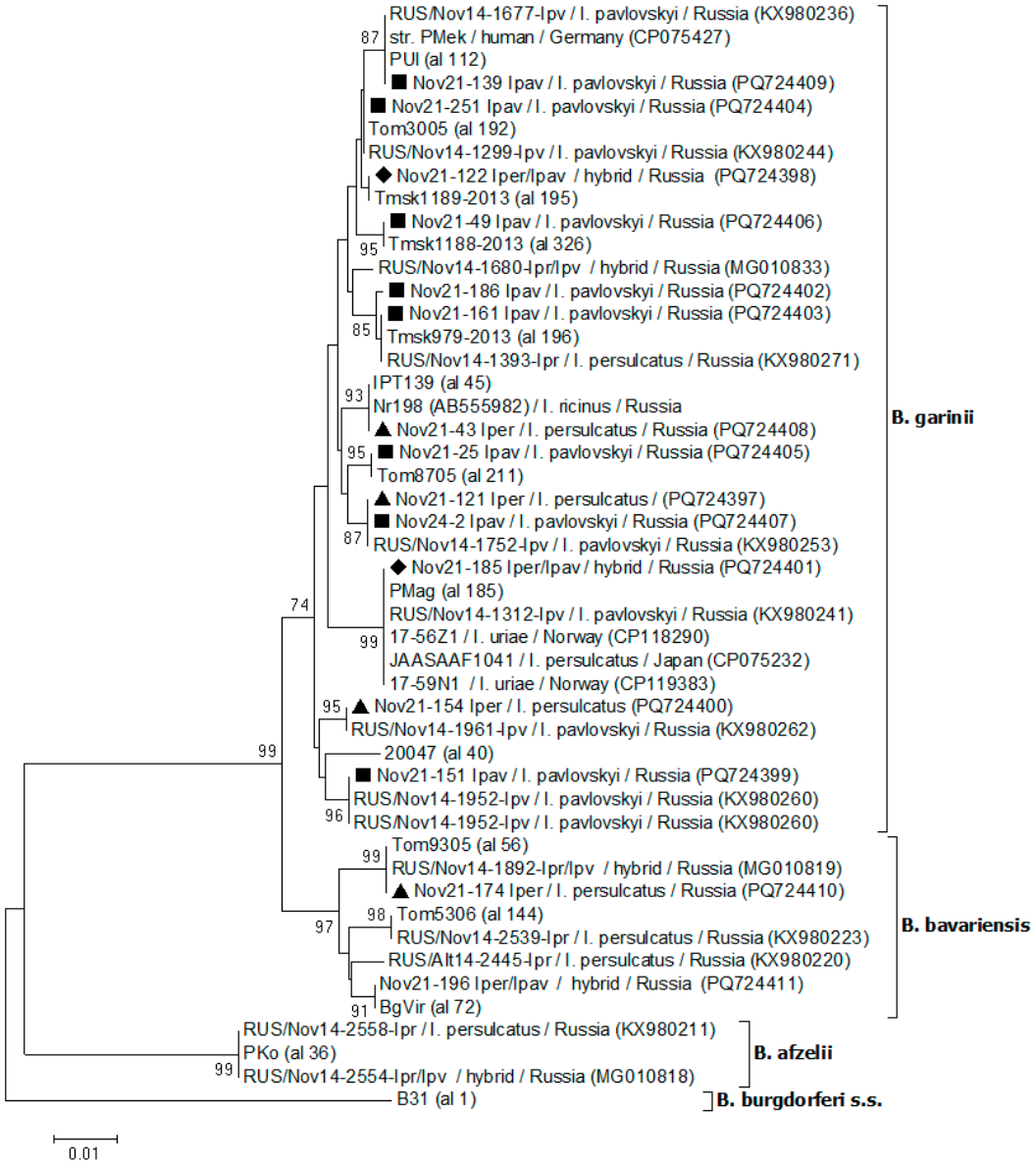

Figure 3. Phylogenetic trees constructed by the ML method based on nucleotide sequences of 579 bp fragment of *clp*A of *Borrelia* spp. The scale bar indicates an evolutionary distance of 0.001 nucleotide per position in the sequence. Significant bootstrapping values (>70%) are shown on the nodes. Legend: ■ – *I. pavlovskyi*; ▲ – *I. persulcatus*; ♦ – hybrids.

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Borrelia miyamotoi was found in 11 ticks, including five *I. pavlovskyi*, five *I. persulcatus* and one hybrid (Table 3). Based on *p66* gene sequence analysis, the determined samples of *B. miyamotoi* belong to the Siberian subtype.

3.4. Detection of Rickettsia spp. in Ticks

Rickettsial DNA was detected in 49/296 (16.6%) ticks: 9/137 (6.6%) *I. pavlovskyi*, 26/58 (44.8%) *I. persulcatus*, 3/58 (5.2%) hybrids, 10/38 (26.3%) *D. reticulatus*, and one *D. silvarum* (Table 4). Three rickettsial species were identified. "*Candidatus* R. tarasevichiae" was found in 26/58 (44.8%) *I. persulcatus*, 1/137 (0.7%) *I. pavlovskyi*, and 3/58 (5.2%) hybrids. *Rickettsia raoultii* was detected in 10/38 (26.3%) *D. reticulatus*, 4/137 (2.9%) *I. pavlovskyi*, and one *D. silvarum*, whereas *R. helvetica* was found in 3/137 (2.2%) *I. pavlovskyi*. In addition, *Rickettsia* sp. not belonging to valid species was identified in one *I. pavlovskyi*.

Positive samples were genotyped by the *gltA* and *ompB* genes. The determined "*Candidatus* R. tarasevichiae" sequences from different tick species were identical and corresponded to known sequences from *I. persulcatus* (KM288450, OP839041).

The *glt*A and *omp*B sequences of *R. raoultii* isolate from a single positive *D. silvarum* exactly matched to the sequences of *R. raoultii* isolate Am-650_Ds (MG545017; MG545018) previously found in *D. silvarum* from the Russian Far East. The determined *R. raoultii* sequences from eight *D. reticulatus* were identical by both examined genes; the *gltA* gene sequences exactly matched the sequence of the *R. raoultii* strain Marne from France (RpA4 genotype, DQ355803) and the *omp*B gene sequences corresponded to the sequence of the *R. raoultii* strain Khabarovsk from the Russian Far East (DnS14 genotype, DQ365798). For another two *R. raoultii* isolates from *D. reticulatus* and four isolates from *I. pavlovskyi*, only *ompB* gene sequences were obtained and all these sequences differed from each other. A sequence from *I. pavlovskyi* was identical to that of *Rickettsia raoultii* strain Khabarovsk, whereas another five sequences were most similar to the sequence of *R. raoultii* from Western Siberia (isolate Gorno-Altai-7, PP155665), differing from it by 2-3 substitutions (Figure 4).

For two of three *R. helvetica* isolates, the fragments of *glt*A and *omp*B genes were amplified and sequenced. The obtained sequences of each of the *glt*A and *omp*B genes were identical within the gene and corresponded to sequences of *R. helvetica* from *Ixodes ricinus* from Netherlands (OY974080) and *Ixodes apronophorus* from Russian Siberia (OQ866615 and OQ866619) and differed by one substitution in the *omp*B gene sequence from *R. helvetica* strain C9P9 from *I. ricinus* from Switzerland (NZ_CM001467). For the third *R. helvetica* isolate, only a *omp*B gene fragment was amplified; the obtained sequence differed from the other two determined sequences by one substitution.

Table 4. Prevalence of *Rickettsia* spp. in Ixodidae ticks collected from humans.

Tick species	No. of ticks	No. (%) of ticks containing DNA of tested agents				
		Rt	Rr	Rh	Rsp	All *Rickettsia* spp.
I. pavlovskyi	137	1 (0.7)	4 (2.9)	3 (2.2)	1 (0.7)	9 (6.6)
I. persulcatus	58	26 (44.8)	0	0	0	26 (44.8)
Hybrids	58	3 (5.2)	0	0	0	3 (5.2)
D. reticulatus.	38	0	10 (26.3)	0	0	10 (26.3)
D. nuttalli / *D. silvarum*	3	0	1	0	0	1
D. marginatus	2	0	0	0	0	0
All species	**296**	**30 (10.1)**	**15 (5.1)**	**3 (1.0)**	**1 (0.3)**	**49 (16.6)**

Abbreviations: Rt - "*Candidatus* R. tarasevichiae"; Rr - R. raoultii; Rh – *R. helvetica*; Rsp – *Rickettsia* sp.

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A new *Rickettsia* sp. (isolate *Rickettsia* sp. Nov21-51_Ipav) was characterized by the *omp*B gene and the obtained sequence was most similar (99.2 % similarity) to the corresponding sequence of the *R. raoultii* isolate Gorno-Altai-7 (PP155665), differing from it by six substitutions.

3.5. Detection of Anaplasmataceae Bacteria in Ticks

Three species from the Anaplasmataceae family were found in the examined ticks; however, their prevalence was rather low. The agent of HGA, *Anaplasma phagocytophilum*, was found in 3/137 (2.2%) *I. pavlovskyi*; *Ehrlichia muris* was detected in 2/137 (1.5%) *I. pavlovskyi,* and *Neoehrlichia mikurensis* was identified in 4/137 (2.9%) *I. pavlovskyi* and 2/58 (3.4%) *I. persulcatus*. Nor *Dermacentor* spp., nor hybrids were infected with bacteria from this family.

Table 5. Prevalence of Anaplasmataceae bacteria in Ixodidae ticks collected from humans.

Tick species	No. of ticks	No. (%) of ticks containing DNA of tested agents			
		Aph	Em	Nm	All Anaplasmataceae
I. pavlovskyi	137	3 (2.2)	2 (1.5)	4 (2.9)	9 (6.6)
I. persulcatus	58	0	0	2 (3.4)	2 (3.4)
Hybrids	58	0	0	0	0
Dermacentor spp.	43	0	0	0	0
All species	**296**	**3 (1.0)**	**2 (0.7)**	**6 (2.0)**	**11 (3.7)**

Abbreviations: Aph - *A. phagocytophilum*; Em - *E. muris*; Nm - *N. mikurensis.*

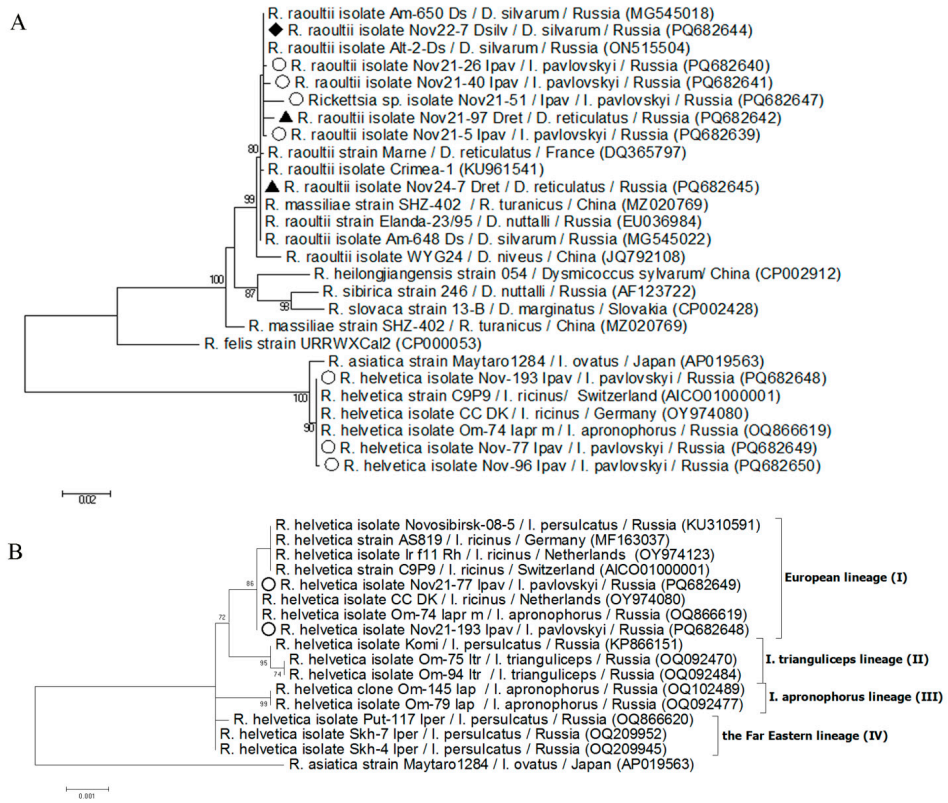

Figure 4. Phylogenetic trees constructed by the ML method based on nucleotide sequences of A) 716 bp fragment of *omp*B gene of *Rickettsia* spp. and B) 3097 bp fragment of *omp*B gene of *Rickettsia helvetica*. The scale bar indicates an evolutionary distance of 0.001 nucleotide per position in the sequence. Significant bootstrapping values (>70%) are shown on the nodes. Legend: ○ – *I. pavlovskyi*; ♦ – *D. silvarum*; ▲ – *D. reticulatus.*

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4. Discussion

The composition of tick population in the southern regions of Western Siberia significantly changed in recent decades. In some locations, especially in the suburbs of large cities (Novosibirsk and Tomsk), *I. pavlovskyi* almost completely displaced *I. persulcatus* (Rar et al., 2017; Romanenko et al., 2015). Moreover, natural *I. persulcatus* / *I. pavlovskyi* hybrids were identified throughout the sympatric areas of *I. persulcatus* and *I. pavlovskyi* (Kovalev et al., 2015; Rar et al., 2019). The distribution area of *D. reticulatus* ticks also expanded in last decades and their prevalence near large cities significantly increased (Kartashov et al., 2019; Romanenko et al., 2017). The reasons for such rapid changes in the tick population are not entirely clear, but may be related to climate change or anthropogenic impact. We cannot exclude that hybridization events, including backcrossing of hybrids with parental species, also influenced the ability of *I. pavlovskyi* to spread rapidly.

However, the comparative epidemiological significance of different *Ixodes* spp. remains to be established. Unlike previous investigations (Kartashov et al., 2022; Romanenko and Kondratyeva, 2011, we genetically identified not only valid tick species but also *I. persulcatus* / *I. pavlovskyi* hybrids in this study. *I. pavlovskyi* was shown to be the predominant tick species in the surroundings of Novosibirsk, accounting for 46% of ticks attacking humans. The prevalence of other ticks was lower than that amounting to 20% for both *I. persulcatus* and hybrids and only 13% for *D. reticulatus* (Table 2). *Dermacentor marginatus, D. nuttalli* and *D. silvarum* were found in rare cases. The obtained results are in good agreement with the data of a recent study of Kartashov et al. (2022), in which *I. pavlovskyi* dominated among ticks attacking humans with a frequency of 43%. Notably, in our previous study a similar prevalence of *Ixodes* spp. was observed in ticks collected from vegetation; the proportion of *I. pavlovskyi*, *I. persulcatus* and hybrids was 50%, 17%, and 26%, respectively (Rar et al., 2019). This close correspondence was unexpected, since questing ticks were collected from vegetation in only five randomly selected locations in the Novosibirsk province, whereas ticks taken from humans could have inhabited anywhere in the region.

The fact that *I. pavlovskyi* and hybrids readily attack humans does not mean that they are able to feed on humans effectively. To compare the ability of different tick species to feed on humans, we estimated the amount of human blood in the engorged ticks that was received during feeding. Certainly, this is a relative estimation, because the DNA content in human blood varies among individuals. Another limitation is the inability to distinguish whether the small amount of human material was obtained from the skin during movement and attachment or it was obtained during blood feeding. To exclude false positive results, we did not consider ticks with small amounts of human DNA as engorged and set the threshold at a level corresponding to 5.5 µl of blood. As expected, the number of engorged ticks was small, as in most cases people removed the ticks before or immediately after attachment.

Despite all the above limitations, engorged ticks were found among all tick species, with the maximum amount of human blood being approximately 106 µl for *I. pavlovskyi*, 78 µl for *I. persulcatus*, 22 µl for hybrids, and only 9 µl for *D. reticulatus*. The obtained results first demonstrated that *I. pavlovskyi* and hybrids can effectively feed on humans. However, the prevalence of engorged hybrids (3.4%) and *Dermacentor* spp. (2.3%) was lower compared to *I. pavlovskyi* (5.8%) and I. *persulcatus* (10.3%) (Table 2). Although the observed difference between the species was not statistically significant, it suggests that hybrids are less adapted to feeding on humans compared to *I. persulcatus*, possibly because they need more time for attachment. The low number of engorged *Dermacentor* spp. is likely due to the larger size of these ticks, which allows people to notice them.

In Western Siberia, LB and BMD are the most common and severe bacterial tick-borne infections. The main agents of LB in Siberia are *B. afzelii*, *B. bavariensis*, and *B. garinii*; these genospecies were most frequently identified in *Ixodes* spp. ticks and clinical samples (Rar et al., 2017, Tkachev et al., 2008). Notably, a new species "*Candidatus* Borrelia sibirica" was recently discovered in *Ixodes* spp. in the neighboring Omsk province; however, the pathogenic properties of this species are unknown (Sabitova et al., 2023). Previous studies of ticks collected in various regions of Siberia have demonstrated the association of *I. persulcatus* with *B. afzelii* and *B. bavariensis* and *I. pavlovskyi* with *B. garinii* (Rar et al., 2017; Sabitova et al., 2023; Mukhacheva and Kovalev, 2014). Unexpectedly, in ticks

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tested in this study *B. garinii* almost completely displaced *B. afzelii* and *B. bavariensis* and was dominant not only in *I. pavlovskyi* but also in hybrids and *I. persulcatus* (Table 3). This discrepancy may be due to the significant dominance of *I. pavlovskyi* in the tick population and the ability of *I. pavlovskyi* to transmit *B. garinii* to other *Ixodes* species via infected small mammals or by co-feeding.

Borrelia garinii is a genetically variable species associated with terrestrial and marine birds, whereas *B. afzelii* and *B. bavariensis* are associated with small mammals. Because of host specificity, *B. garinii* can be transmitted over long distances, and different *B. garinii* genovariants do not cluster by geography or tick species (Rollins et al., 2023). Since the analyzed ticks could attack humans anywhere, we expected to find new *Borrelia* genovariants.

Indeed, we found one novel *B. garinii* variant and two variants, corresponded to *clp*A alleles, which were widespread only in *I. ricinis* distributive area in Europe (allele 45) or in both European countries and Western Siberia (allele 112). Another unusual for Siberia variant exactly matched the *clp*A allele 185, closely associated with *I. ureae* and seabirds from Canada and Norway (Margos et al., 2023). Despite the close association with marine birds, several *B. garinii* isolates, containing the *clp*A allele 185, were found in single *I. persulcatus*, *I. pavlovskyi*, and *I. persulcatus* / *I.pavlovskyi* hybrid in Japan and Novosibirsk province (Rar et al., 2017 and this study). These findings clearly demonstrate the adaptation of the specialized *B. garinii* variant to a broader host range.

In this study, *Borrelia miyamotoi*, a spirochete of the relapsing fever group, was detected in all *Ixodes* spp., including hybrids, with a prevalence of 3.7% among all examined ticks and 4.3% among *Ixodes* spp. (Table 3). The observed prevalence in ticks attacking humans was consistent with the *B. miyamotoi* prevalence in ticks collected from vegetation in the Novosibirsk province, which ranged from 3.9% to 6.7% for various *Ixodes* species (Fomenko et al., 2010; Rar et al., 2019). The stable and relatively high prevalence of *B. miyamotoi* in ticks explains the consistently high incidence of BMD in Novosibirsk province, which is only twice as rare as LB and accounts for 10% of hospitalized patients (Savel'eva et al., 2018).

In Western Siberia, rickettsioses can be caused by several *Rickettsia* spp.; most cases were caused by *R. sibirica*, followed by *R. raoultii*. In rare cases, "*Candidatus* R. tarasevichiae", *Rickettsia aeschlimannii*, and *Rickettsia slovaca* were recorded as causative agents of infections in Novosibirsk province (Igolkina et al., 2022). For *Rickettsia* spp., the main route of transmission is transovarial; thus, their association with certain tick species should be more specific compared to *B. burgdorferi* s.l. The study of ticks taken from humans demonstrated a close association of *I. persulcatus* with "*Candidatus* R. tarasevichiae" and *D. reticulatus* with *R. raoultii* (Table 3); a similar association was shown for questing ticks collected from various locations in the Western Siberia (Rar et al., 2017, 2019; Yakubovsky et al., 2023). Despite the high prevalence of "*Candidatus* R. tarasevichiae" and *R. raoultii* in ticks removed from humans, cases of infections with these pathogens are quite rare, which can be explained by the low pathogenicity of these agents.

Both *R. raoultii* and *R. helvetica* are genetically variable species. This study demonstrated higher genetic variability of *R. raoultii* samples obtained from *I. pavlovskyi* compared to samples from *D. reticulatus*. These results correspond to our previous findings that *R. raoultii* isolates from *Ixodes* spp. ticks are more variable than isolates from *Dermacentor* spp. (Rar et al., 2017, 2019; Yakubovsky et al., 2023). Notably, *Ixodes* spp. ticks infected with *R. raoultii* were taken from humans, consistent with the high genetic variability of *R. raoultii* in clinical samples (Igolkina et al., 2022).

It has recently been shown that *R. helvetica* isolates are reliably subdivided into four genetic lineages (Igolkina et al., 2023b). The European lineage is the most numerous and includes all genotyped *R. helvetica* isolates from *I. ricinus* from European countries (Silaghi et al., 2011) and from *I. persulcatus* from Western Siberia (Kartashov et al., 2022; Igolkina et al., 2023b). In this study, *R. helvetica* isolates from *I. pavlovskyi* were first genotyped by a long fragment of the *omp*B gene, which showed that these isolates also belong to the European lineage. Despite the presence of pathogenic *R. helvetica* in *Ixode*s spp. ticks, no cases of *R. helvetica* infection have been registered in Novosibirsk province. This may be due to the fact that *R. helvetica* infection has symptoms atypical for rickettsioses (Nilsson et al., 1999, 2010) and was not properly diagnosed.

Rickettsia sibirica, which is the causative agent of widespread STT, was not detected among the examined ticks. The main vectors of *R. sibirica* are *D. nuttalli*, *D. silvarum*, and *D. marginatus* (Shpynov et al., 2006, 2009), which, as shown in this and other studies (Kartashov et al., 2022), rarely attack humans in the surroundings of Novosibirsk. Due to the high pathogenicity of *R. sibirica*, even rare cases of human infection with this agent can manifest as severe infection.

Three potentially pathogenic members of Anaplasmataceae family, *A. phagocytophilum*, *E. muris* and *N. mikurensis*, were found in ticks attacking humans; the prevalence of each species was low and did not exceed 2%. There are no confirmed cases of anaplasmosis and ehrlichiosis in humans in Siberia (Tkachev et al., 2008), so the epidemiological significance of the identified Anaplasmataceae bacteria is probably insignificant.

In conclusion, the obtained results indicated that *I. pavlovskyi* currently has the greatest epidemic significance for residents of Novosibirsk; these ticks attack humans more than 2-3 times as often as *I. persulcatus*, hybrids, and *D. reticulatus* and are able effectively feed on humans. It was first shown that *I. persulcatus* / *I. pavlovskyi* hybrids can readily attack humans and feed on them effectively. Ticks attacking humans were infected with three genospecies of *B. burgdorferi* s.l. species complex, *B. miyamotoi* from the relapsing fever group, three species of *Rickettsia*, and three species from Anaplasmataceae family. Notably, *B. garinii* almost completely displaced *B. afzelii* and *B. bavariensis* from tick population. The obtained results indicate a high risk of infection humans with causative agents of LB, primarily *B. garinii.*

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