

Detection and Identification of Bacterial Agents in *Ixodes persulcatus* Schulze Ticks from the North Western Region of Russia

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ABSTRACT

Ixodes persulcatus Schultz ticks are traditionally associated with transmission of Lyme disease, babesiosis, and tick-borne encephalitis. Here we compared the prevalence of infection with *Borrelia burgdorferi*, and rickettsial and ehrlichial agents in *I. persulcatus* ticks collected in different locations of the North Western administrative region of Russia. Altogether, 27.7% of ticks were infected with at least one organism, while the DNA of two or more bacteria was found in 11.8% of ticks tested. The highest average prevalence of Anaplasmataceae (20.8%) was detected in ticks from Arkhangel'sk province, while the prevalence in ticks from Novgorod province and St. Petersburg, respectively, was 7.3% and 12.2%. Only *Ehrlichia muris* DNA was identified by DNA sequencing. In comparison, the prevalence of *B. burgdorferi* DNA was 16.6%, 5.8%, and 24.5% in the respective locations. The 382-bp amplicon of *gltA* from *Candidatus Rickettsia tarasevichiae* was detected in 2.75% and 1.6%, respectively, of ticks from Arkhangel'sk and Novgorod provinces, extending further west and north the area where this rickettsia is known to be present. DNA of the rickettsia-like endosymbiont Montezuma was primarily associated with female ticks, 8–28% of which were infected. Since *I. persulcatus* is so commonly infected with multiple agents that may cause human diseases, exposure to these ticks poses significant risk to human health in this region. **Key Words:** *Ixodes persulcatus*—*Borrelia burgdorferi*—*Ehrlichia muris*—*Candidatus Rickettsia tarasevichiae*—Multiplex PCR—Quantitative PCR. Vector-Borne Zoonotic Dis. 7, 426–436.

INTRODUCTION

IXODID TICKS transmit a great variety of viral, bacterial, and parasitic pathogens to different mammalian hosts, including humans, and domestic and wild animals. These organisms have different types of relationships with their arthropod and mammalian hosts. For many microorganisms, ixodid ticks are an important part of their natural maintenance since most of

their life cycle and multiplication occurs within the tick. They can be transmitted to mammals with infected saliva during tick feeding. Many of these organisms exhibit transovarial transmission and horizontal infection during co-feeding (Balashov 1995). Other microorganisms primarily have intimate symbiotic relationships with their tick hosts, but the effect of these organisms on tick survival and fecundity is not completely understood.

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Because different microorganisms may share the same animal hosts and tick vectors, the ticks can be infected with more than one agent (Belongia 2002). Closely related *Ixodes* tick species harbor similar sets of pathogens worldwide. The *Ixodes scapularis* Say ticks are often found to be co-infected with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia* in North America (Adelson et al. 2004, Holman et al. 2004, Schulze et al. 2005). Its Euro-Asian relatives—*I. persulcatus* Schulze, 1930 and *I. ricinus* (L., 1758)—were implicated as the vectors of Lyme disease since the discovery of *Borrelia* in the Old World (Liz 2002). Subsequently, it was also shown that *I. persulcatus* and *I. ricinus* harbor different rickettsial and ehrlichial agents in many locations (Alekseev et al. 2001, Hildebrandt et al. 2003, Rar et al. 2005, Halos et al. 2006, Piccolin et al. 2006, Smetanova et al. 2006).

The North Western administrative region of Russia includes Arkhangel'sk, Kaliningrad, Leningrad, Novgorod, Pskov, and Vologda provinces, and Komi and Karelia Republics, and covers over 1,700,000 km². Its population is over 15 million people, primarily found in urban locations. A significant part of the territories is covered by forests comprised of a mixture of southern taiga and deciduous trees, with large numbers of animals and birds. Outside the industrial zones and large cities, this region offers ample opportunities for historic sightseeing, hiking, fishing, and hunting, and is experienced by millions of local and foreign visitors every year. The climate changes from coastal to moderate continental from the west to the east of the region and from continental to forest-tundra and tundra at the north part of the Arkhangel'sk region. This region covers the most western and northern ends of the distribution of *I. persulcatus* taiga ticks (Vansulin et al. 1976, 1981, Kovalevskii and Korenberg 1995, Korenberg et al. 2002). Furthermore, *I. ricinus* ticks are also found in Leningrad and Novgorod provinces (Fedorova et al. 1984, Kovalevskii and Korenberg 1995). Typically, the active tick questing season starts after the snow melts and lasts from the second week of April through the middle of May, while June–July is characterized by a relatively low level of tick activity. However, depending on seasonal temperature fluctuations, questing ticks may be found as late as Septem-

ber (Vansulin et al. 1981). The density of *I. persulcatus* ticks collected in these areas varied from 30 to 400 ticks per hectare (Vansulin et al. 1981, Fedorova et al. 1984). The prevalence of *I. persulcatus* ticks infected with *B. burgdorferi* in these areas ranged from 12% to 33.2% (median 29%), and the prevalence of infected *I. ricinus* ranged from 11.1% to 21% (median 16.7%) as determined by microscopic detection of spirochetes (Korenberg et al. 2002). Ten percent to 30% of the ticks from the resort areas of St. Petersburg and from Kaliningrad region of Russia were infected with *Borrelia* sensu lato, mainly *Borrelia afzelii* and *B. garinii* (Alekseev et al. 1998). A significant portion of these ticks were co-infected with several species of spirochetes (Dubinina et al. 1999) and with *Babesia* (Alekseev et al. 2003). Furthermore, recent studies conducted in other regions of Russia have detected *Ehrlichia* and *Anaplasma phagocytophilum*-like bacteria in the same collections of ticks (Alekseev et al. 2001, Semenov et al. 2001); these studies suggest that these new etiological agents need to be considered in the differential diagnosis of febrile illnesses associated with exposures to *I. persulcatus* ticks in these areas (Korenberg 2004).

The purpose of this study was to determine the prevalence of rickettsial and ehrlichial pathogens in *I. persulcatus* ticks collected in different sites in the North Western administrative region of Russia. Their prevalence was compared to that of *B. burgdorferi* to estimate the prevalence of mixed infections in them.

METHODS

Tick collection, identification, and DNA preparation

Questing ticks were collected from vegetation during the spring and summer months in parks of St. Petersburg and from several collection sites in Novgorod and Arkhangel'sk provinces of Russia (Fig. 1). In addition, a collection from Arkhangel'sk province also included ticks hand picked from people and a stray cat. All ticks were identified as *I. persulcatus* using standard taxonomic keys, categorized by sex and life stage, and preserved in ethanol or frozen prior to DNA extraction. Ticks were surface disinfected using a series of

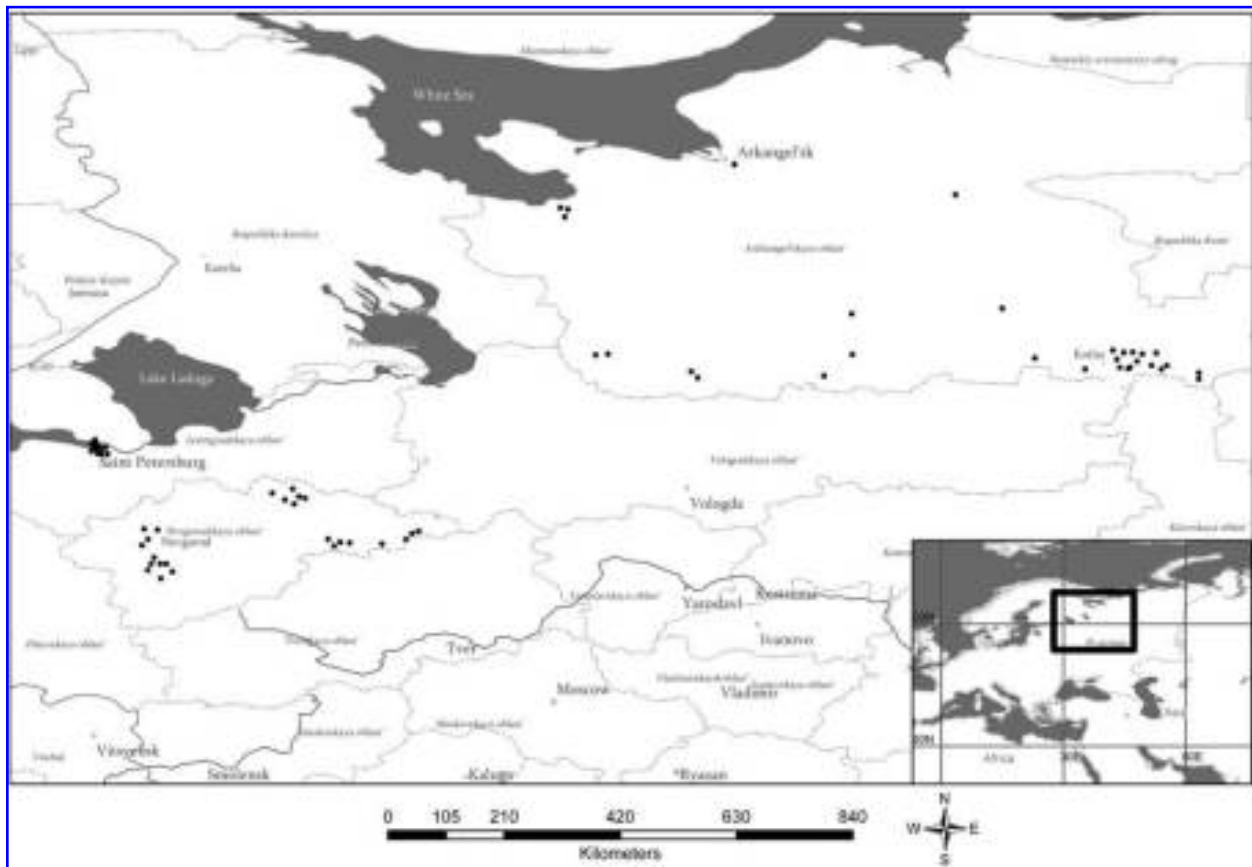


FIG. 1. Geographic map of tick collection sites. The position of each collection site was mapped according to their latitude and longitude using ArcGIS 9.2 software. The positions of some collection sites are overlapped due to the scale of the map. (Map courtesy of Adam D. Koon and Karoyle Colbert.)

washes with 10% bleach for 1–2 min, 70% ethanol for 3–5 min, and three times with distilled water, and excess water was removed using filter paper. The ticks were frozen in liquid nitrogen, and crushed into powder using Kontes pestles (Kimble-Kontes, Fisher, USA). The powder was resuspended in lysis buffer supplemented with 1 mg/mL of Proteinase K (Qiagen, Valencia, CA) and incubated overnight at 56°C. DNA was extracted using Promega Wizard DNA Extraction Kit (Promega, Madison, WI) and processed on a Biomek 2000 Laboratory Automation workstation (Beckman, Fullerton, CA). DNA was eluted with 100 μ L of sterile nuclease free water and stored at 4°C prior to further analysis.

Screening procedures

Tick DNA was tested using several polymerase chain reaction (PCR)-based methods

(Table 1). Conventional single-step PCR assay was used to detect the fragments of the citrate synthase gene (*gltA*) of *Rickettsia* (Regnery et al. 1991), or the 16S rRNA gene of the rickettsia-like endosymbiont *Montezuma* (Mediannikov et al. 2004) using Taq MasterMix (Qiagen). The original PCR cycling conditions, concentration of magnesium chloride, primer concentration, and primer sequences were modified to increase the specificity of each test. Amplicons of the expected sizes were detected by electrophoresis in 1% agarose gel supplemented with 0.5 μ g/mL of ethidium bromide. The 16S ribosomal RNA gene of Anaplasmataceae was detected with a quantitative PCR assay (Li et al. 2002) using SYBR Green PCR Reagent kit (PE Applied Biosystems, Foster City, CA) on an *i*-Cycler with real-time PCR detection system (BioRad, Hercules, CA). Automatic data acquisition and subsequent data analyses were performed using the *i*-Cycler software. A Taq-

TABLE 1. PRIMERS AND PROBES USED FOR PCR DETECTION AND IDENTIFICATION OF BACTERIAL AGENTS IN *I. PERSULCATUS*

Target gene	Organism detected	Primer sequence	Assay	Reference
<i>rrs</i>	<i>E. chaffeensis</i>	SYBR-F: AACACATGCAAGTCCGAACGG	SYBR Green quantitative PCR	Li et al. 2001, Eremeeva et al. 2006
	<i>E. muris</i>	SYBR-R: CCCCCGACGGGATTATACA		
<i>msp2</i>	<i>A. phagocytophilum</i>	ApMSP2f: ATGGAAGGTAGTGTGGTTATGGTATT	TaqMan	Courtney et al. 2004
	<i>N. mikurensis</i>	ApMSP2r: TTGGTCTTGAAAGCGCTCGTA		
	<i>A. phagocytophilum</i>	ApMSP2p-HEX ^a : TGGTGCTAGGTTGAGCTTGAGATTG		
	<i>A. phagocytophilum</i>	Bb23Sf: CGAGTCITAAAAGGGCGATTAGT		
<i>rfl</i>	<i>B. burgdorferi</i>	Bb23Sr: GCTTCAGCCTGGCCATAAATAG	TaqMan	Courtney et al. 2004
	<i>Rickettsia</i>	Bb23Sp-FAM ^b : AGATGTGGTAGACCCGGAAGCCGAGTG		
<i>gltA</i>	<i>Rickettsia</i>	RpCS877F: GGGGACCTGCTACGGCGG	PCR, sequencing	Regnery et al. 1991, this study
	<i>Rickettsial</i>	RpCS1258R: ATTGCAAAAAGTACAGTGAACA		
<i>rfs</i>	endosymbiont	GATTTATCGCTACAAGATGAGCCCATGCA	PCR, sequencing	This study
	Montezuma	GGAAITCCGGCAATCCCTCTGAT		
<i>rfs</i>	<i>A. phagocytophilum</i>	GE3a: CACATGCAAGTCCGAACGGATTATTC	Primary PCR	Massung et al. 1998
	<i>A. phagocytophilum</i>	GE10r: TTCCGTTAAGAAGGATCTAAATCTCC		
	<i>A. phagocytophilum</i>	GE9f: AACGGATTATCTTTATAGCTTGCT		
	<i>A. phagocytophilum</i>	GE2: GGCAGTATAAAAGCAGCTCCAGG		
	<i>A. phagocytophilum</i>	HE11: AAGGTCGTATCCCTCCTAAATAGG		
<i>rfs</i>	<i>E. chaffeensis</i>	HE14: GAGAACAATCCAAACTGAGACA	Conventional PCR, sequencing	Designed by R.F. Massung
	<i>E. muris</i>			
	<i>N. mikurensis</i>			

^aApMSP2p-HEX probe is labeled at the 5' and 3' ends with hexachloro-6-carboxy-fluorescein (HEX) and 6-carboxyl-tetramethyl-rhodamine (TAMRA).

^bBb23Sp-FAM probe is labeled at the 5' and 3' ends with 6-carboxy-fluorescein (6-FAM) and TAMRA.

Man multiplex assay was used to test tick DNAs simultaneously for the presence of *A. phagocytophilum* and *B. burgdorferi* (Courtney et al. 2004). The PCR reaction was performed using the Brilliant Quantitative PCR Core Reagent Kit with SureStart™ Taq polymerase (Stratagene, La Jolla, CA). The reactions were run on the 7900HT TaqMan Instrument (Applied Biosystems). Quantitative assays were run in duplicate; the results were considered positive if both replicates were positive and their Ct values did not differ by more than 10%.

DNA sequencing

Sequence reactions were performed using the ABI PRISM BigDye™ Terminator Cycle 3.1 Sequencing kit as recommended by the manufacturer (Applied BioSystems, Foster City, CA). The sequenced products were purified with Qiagen Dye Removal Kit (Qiagen, Valencia, CA) and run on an Applied Biosystems 3100 Sequencer and data analyzed with Nucleic Acid Sequence Analyzer (Applied Biosystems), CAP sequence assembler (www.infobiogen.fr), and ClustalW multiple sequence alignment (<http://clustalw.genome.jp/>, Kyoto University Bioinformatics Center, Kyoto, Japan). Sequence identities were determined using NCBI BLAST search engine. Prototypical sequences generated during this study were deposited at NCBI GenBank under following accession numbers: *Candidatus Rickettsia tarasevichiae gltA* EF445981, *Ehrlichia muris* 16S rDNA EF445982 and EF445983, rickettsial endosymbiont Montezuma 16S rDNA EF445984 and EF445985, *Acinetobacter sp. gltA* EF445980.

Statistical analysis

Statistical analysis of prevalence results was performed using the chi-square test.

RESULTS

Detection of *B. burgdorferi* and *A. phagocytophilum* by multiplex PCR

The DNA of *B. burgdorferi* was detected in 13.8% (median 16.5%) of ticks examined with prevalence ranging from 24.5% in St. Petersburg

and to 5.8% in Novgorod province (Table 2). The average prevalence was 13.6% in ticks from Arkhangel'sk province, but the DNA of *Borrelia* was detected at higher frequency in questing ticks compared to ticks found on people ($p = 0.00015$). The presence of *A. phagocytophilum msp2* was not detected in any tick specimens tested during this study.

Detection of Anaplasmataceae agents using SYBR Green PCR assay

A set of primers binding a conserved region of the 16S rRNA gene of Anaplasmataceae was used. These primers enable detection of DNA from at least *E. chaffeensis*, *E. muris*, *A. phagocytophilum*, and *Candidatus Neoehrlichia mikurensis* (Eremeeva et al. 2006). By this SYBR-Green assay 90 of 632 ticks (14.2%) collected from three regions were positive by SYBR-Green PCR assay (Table 2). However, the prevalence of positive ticks differed in each province studied, with a range of 7.3–23.7% (Table 2). Ticks found within the city limits of St. Petersburg exhibited similar prevalence of ehrlichial infection between male and female ticks ($p = 0.723$). A twofold greater prevalence of positive female ticks than male ticks was detected in Novgorod province ($p = 0.294$), and positive male ticks were more prevalent than female questing ticks from Arkhangel'sk province ($p = 0.166$). However, a higher portion of positive female than male ticks was found among ticks collected from people in the same areas of Arkhangel'sk province ($p = 0.0089$). Only *E. muris* DNA was identified by sequencing in 25 ticks, including representative samples from each of the regions studied. Species identification of the ehrlichial organisms associated with other ticks could not be done due to the very low DNA copy numbers detected which made preparation of amplicons for DNA sequencing difficult.

Detection of *Rickettsia* and rickettsia-like endosymbiont Montezuma

The presence of 382-bp amplicon of *gltA* from *Rickettsia* was detected in 0–7.5% of ticks collected in three regions (Table 2). All but one of the *gltA* sequences from the ticks were identical to that of *gltA* of *Candidatus Rickettsia tarasevichiae*.

TABLE 2. PREVALENCE OF TICK-BORNE BACTERIA IN *Ixodes persulcatus* FROM THE NORTH WESTERN REGION OF RUSSIA

Region, province	Positive for											
	Ticks collected		<i>Borrelia, rrl</i>		<i>Anaplasmataceae, rrs</i>		<i>Rickettsia, gltA</i>		<i>Rickettsia-like endosymbiont Montezuma, rrs</i>			
	Male/Female	Total	Male/Female	Prevalence	Male/Female	Prevalence	Male/Female	Prevalence	Male/Female	Prevalence	Male/Female	Prevalence
Arkhangel'sk	43/50	93	13/10	24.7%	13/9	23.7%	2/5	7.5%	4/17	22.6%		
Questing ticks	17/184	201	1/16	8.5%	7/29	17.9%	1/7	4.0%	4/53	28.4%		
Attached to people	80/111	191	4/7	5.8%	4/10	7.3%	1/2	1.6%	2/14	8.4%		
Novgorod	62/83	147 ^a	13/23	24.5%	7/11	12.2%	0/0	0%	10/31	27.9%		
St. Petersburg, city	202/428	632	31/56	13.8%	31/59	14.2%	4/14	2.8%	20/115	21.4%		
Total												

^aCollection of ticks from St. Petersburg also included 2 flat nymphs. One nymph was negative in every assay used; the other nymph was positive for DNA of *Borrelia* and the *Rickettsia*-like endosymbiont *Montezuma*.

sevichiae (NCBI AF503167). No nucleotide sequence differences were detected among fragments of *gltA* of *Candidatus* R. tarasevichiae from different collection sites. The nucleotide sequence of one *gltA* amplicon from tick 220 found on a human in the Vinogradov region of Arkhangel'sk province had its closest sequence similarity (90%) to a homologous *gltA* fragment from *Acinetobacter* sp. (NCBI M33037).

DNA from the rickettsia-like endosymbiont Montezuma (NCBI AF493952) was primarily detected in association with 8.4–27.6% of female questing ticks. A significantly lower prevalence of Montezuma organism (2.5–16%) was found in adult male *I. persulcatus* than females in all three regions, Novgorod, Arkhagell'sk, and St. Petersburg ($p = 0.00451$, $p = 0.0128$, $p = 0.005$, respectively). Ticks collected from people and a cat in Arkhagel'sk had a sim-

ilar prevalence of Montezuma-like organisms in male and female ticks.

Sequences generated for selected amplicons were identical to the nucleotide sequence of the homologous *rrs* fragment established for the endosymbiont previously detected in *I. persulcatus* collected from the Russian Far East (Mediannikov et al. 2004).

Prevalence of co-infection with bacterial agents in I. persulcatus

The prevalence of infections with single or multiple bacterial agents per tick was also analyzed (Table 3). Overall 11.8% of ticks contained DNA from two or more agents, while 27.7% of ticks were infected with only a single agent. Ticks from Novgorod province had the lowest prevalence of mixed infections (2.5%)

TABLE 3. PREVALENCE OF SINGLE AND MIXED INFECTIONS IN *I. PERSULCATUS* TICKS FROM THE NORTH WESTERN REGION OF RUSSIA

DNA detected from	Region			
	Arkhangel'sk	Arkhangel'sk	Novgorod	St. Petersburg
	Attached ticks	Questing ticks		
<i>B. burgdorferi</i>	4.5%	19.4%	4.2%	6.8%
Ehrlichiae	10.9%	11.8%	6.3%	3.4%
<i>Rickettsia</i>	1.0%	2.2%	1.0%	0%
<i>Rickettsia</i> -like endosymbiont				
Montezuma	17.4%	11.8%	5.8%	11.6%
Subtotal ticks infected with only one organism	33.8%	45.2%	17.3%	21.8%
<i>B. burgdorferi</i> + ehrlichiae	0.5%	7.5%	0%	2.7%
<i>B. burgdorferi</i> + rickettsia-like endosymbiont Montezuma	2.5%	4.3%	1.0%	9.5%
<i>Rickettsia</i> + <i>Rickettsia</i> -like endosymbiont Montezuma	1.5%	1.1%	0.5%	0%
Ehrlichiae + <i>Rickettsia</i> -like endosymbiont Montezuma	6.0%	2.2%	0.5%	0.7%
<i>B. burgdorferi</i> + ehrlichiae + <i>Rickettsia</i> -like endosymbiont Montezuma	0%	1.1%	0.5%	5.4%
<i>B. burgdorferi</i> + rickettsiae + <i>Rickettsia</i> -like endosymbiont Montezuma	1.0%	3.2%	0%	0%
Ehrlichiae + rickettsiae + <i>Rickettsia</i> -like endosymbiont Montezuma	0.5%	0%	0%	0%
<i>B. burgdorferi</i> + ehrlichiae + <i>Rickettsia</i> + <i>Rickettsia</i> -like endosymbiont Montezuma	0%	1.1%	0%	0%
Subtotal ticks infected with two or more organisms	12.0%	20.5%	2.5%	18.3%

consistent with the overall low prevalence of the organisms examined at this site. For all sites, none of the rates of co-infection with two or more agents were different from those expected by random chance based on the overall prevalence of the individual agents in these ticks.

DISCUSSION

This study reports for the first time detection of *E. muris* in *I. persulcatus* ticks from Arkhagel'sk and Novgorod provinces. We also detected the presence of *Candidatus R. tarasevichiae* and the rickettsia-like endosymbiont *Montezuma* in the same cohorts of ticks, thus greatly extending the western and northern limits where these organisms have been found in association with *I. persulcatus* (Shpynov et al. 2003, 2004, Mediannikov et al. 2004, Ereemeeva et al. 2006).

The prevalence of Anaplasmataceae infections in the current study was 14.2%. A similar prevalence range of 8.6–14% was found in *I. persulcatus* from the neighboring Baltic regions and in 16.6% ticks from Vologda province, respectively (Alekseev et al. 2001, Ereemeeva et al. 2006). The prevalence of ehrlichiae in eastern populations of *I. persulcatus* was similar (Rar et al. 2005). Only *E. muris* DNA was detected in *I. persulcatus* in this study, as no ticks were found to contain DNA of *E. chaffeensis* or *A. phagocytophilum*. In contrast, Alexeev et al. (2001) reported detection of DNA from an *A. phagocytophilum*-like organism in four out of 295 (1.4%) *I. ricinus* ticks from Baltic regions but no *A. phagocytophilum* was detected in 336 *I. persulcatus* (Alekseev et al. 2001). A total of 2.1–8% of *I. persulcatus* ticks from South Western Siberia were infected with *A. phagocytophilum* (Morozova et al. 2002, Rar et al. 2005). DNA of *E. chaffeensis* or a closely related organism was found in 8.6% of *I. persulcatus* ticks from Baltic regions (Alekseev et al. 2001). Shpynov et al. (2004) reported the presence of *E. muris* DNA in 3.1% of *I. persulcatus* ticks collected across Omsk, Novosibirsk and Tymen provinces and Altay region, and *A. phagocytophilum* DNA in 2.1–4.5% of *I. persulcatus* ticks found on territories extending from Altay to Prymorye regions

of Russia (Shpynov et al. 2004). Finally, 1.6% of *I. persulcatus* ticks from Omsk province were also infected with *Ehrlichia*-like Schotti variant, presently referred to as *Candidatus N. mikurensis* (Kawahara et al. 2004). We were able to identify retrospectively a similar genotype (EF445979) in one of the *I. persulcatus* ticks from Vologda region described previously (Ereemeeva et al. 2006). Variations in the prevalence of *Ehrlichia* and *Anaplasma* in *I. persulcatus* reported by different investigators may be due in part to differences in the specificity and sensitivity of the PCR assays used for each study.

Since *Ehrlichia* and *Anaplasma* species are generally not thought to be transmitted transovarially in ticks, their acquisition occurs from bacteremic vertebrate hosts or perhaps by tick co-feeding. DNA of *Ehrlichia* and *Anaplasma* has been detected in blood specimens from several species of small rodents (*Apodemus sylvaticum*, *Cleothionomys glareolus*, *Cl. rutilus*, *Cl. rufocanus*, *Sorex araneus*, and *Microtus agrestis*) trapped from areas endemic for *I. persulcatus* (Telford et al. 2002, Rar et al. 2006). However, it appears that bacteremic animals are found only during a short period correlating with seasons of questing tick activity. Passerine birds were also shown to be successful hosts for mediating co-feeding infections (Alekseev et al. 2001). Since blood meal sizes of male and female larvae and nymphs probably do not differ, the prevalence of Anaplasmataceae and *Borrelia* agents in adult flat ticks would be expected to be similar as was found.

Candidatus R. tarasevichiae was first described in *I. persulcatus* ticks from several regions spanning territories from the Southern Ural to Western and Eastern Siberia (Shpynov et al. 2003) where this rickettsia was found in 10% of ticks (median for three areas). This rickettsia has its closest sequence homology of *gltA* and 16S rDNA to *R. canadensis* which is found in association with North American *Haemaphysalis leporispalustris* Packard (McKiel et al. 1967) and to the typhus group-like rickettsia found in ladybird beetles in Europe (Werren et al. 1994). Here we report the most western and northern limits of this *Rickettsia* with a prevalence of 2.8%. Its potential for causing human disease has not been determined.

Similarly, there is only a speculative sugges-

tion that the rickettsial endosymbiont *Montezuma* causes human infections (Mediannikov et al. 2004). This organism has been found in every collection of *I. persulcatus* ticks analyzed for it and preferentially in female ticks (Eremeeva et al. 2006). Consequently it appears that *Montezuma* bacterium is transovarially transmitted by female lineages of *I. persulcatus* ticks while it may disappear or decline in amount in males. Phylogenetically, *Montezuma* most closely relates to the alpha-Proteobacterium endosymbionts of *Acanthamoeba* UWC36 and UWC8 (Fritsche et al. 1999). The biological significance of *Montezuma* bacterium in ticks and its tissue localization has not been established. Another microorganism, IricES1 with a similar phylogenetic position and similar association with female lineages has been detected in *I. ricinus* ticks (Beninati et al. 2004). The IricES1 was detected in almost 100% of wild-caught female ticks in many sites throughout Europe (Lo et al. 2006). The IricES1 bacterium was demonstrated to reside in the mitochondria of tick ovarian cells thus explaining its maternal inheritance with mitochondria (Beninati et al. 2004). The DNA of a genetically closely related bacterium was also detected in *Ixodes brunneus* Koch ticks collected in Mississippi (Goddard et al. 2003).

The relevance of these findings for evaluating the risk of tick-borne infections in these areas needs further study. The overall tick infectivity with *B. burgdorferi* and frequency of Lyme disease in persons bitten by *I. persulcatus* ticks ranges from 16:1 in Arkhangel'sk province to a ratio of 3:1 in St. Petersburg city (Tokarevich et al. 2002). However, no confirmed cases of ehrlichial or rickettsial infections were reported from these areas despite very similar prevalence of *Ehrlichia* and *Borrelia* in these ticks. Rickettsial prevalence was low but that for *Montezuma* exceeded that of all other agents. Serological tests for detecting infections with either *Candidatus* *R. tarasevichiae* or *Montezuma* are not available and it is unknown whether they readily cause human infections. On the other hand, there is limited serological evidence for human exposures to *Ehrlichia* and *Anaplasma* in other areas where *I. persulcatus* is present (Ravyn et al. 1999; Vorobyeva et al. 2002; Sidel'nikov et al. 2003; Korenberg 2004),

but neither PCR detection of these organisms nor isolation of the agents from clinical specimens has been reported. The significance of *E. muris* as a potential human pathogen is not understood completely; however, it causes persistent infection in immunocompetent laboratory mice (Olano et al. 2004). Only 1% of humans from metropolitan Tokyo were found to be seropositive for *E. muris* antibodies while the seroprevalence in mice was 63%; five *E. muris* isolates inducing splenomegaly were obtained from 10 *Haemaphysalis flava* Neum ticks caught in the neighboring areas (Kawahara et al. 1999).

At least 2 genetic variants of *A. phagocytophilum* have been infrequently detected in *I. persulcatus* ticks and small rodents from Western Siberia and Ural regions (Rar et al. 2006), but their pathogenic potential has not been established. *I. scapularis* ticks from the Eastern US were found to be infected with two different genotypes of *A. phagocytophilum*, Ap-ha and Ap-variant 1; however, only the Ap-ha genotype has been found to cause human disease (Massung et al. 2002, 2003). In contrast, Ap-variant 1 genotype is commonly detected in ticks and wild animals. It has been suggested that interference between these variants of *Anaplasma* may affect the maintenance and transmission of the agents pathogenic for humans and thus affect the prevalence of human infections in particular areas. Whether a compatible situation occurs in Eastern Europe is unknown but very few human infections with *Anaplasma* in Western Europe have been detected and isolates similar to the North American Ap-variant 1 appear to be common there. However, physicians in areas endemic for *I. persulcatus* should maintain a high index of suspicion for diagnosing rickettsial diseases in patients with tick exposures as these infections are likely to be readily treated with antibiotics effective against other rickettsial agents.

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REFERENCES

- Adelson, ME, Rao, RV, Tilton, RC, Cabets, K, et al. Prevalence of *Borrelia burgdorferi*, *Bartonella* spp., *Babesia microti*, and *Anaplasma phagocytophila* in *Ixodes scapularis* ticks collected in Northern New Jersey. *J Clin Microbiol* 2004; 42:2799–2801.
- Alekseev, AN, Dubinina, HV, Antykova, LP, Dzhivanyan TI, et al. Tick-borne borrelioses pathogen identification in *Ixodes* ticks (Acarina, Ixodidae) collected in St. Petersburg and Kaliningrad Baltic regions of Russia. *J Med Entomol* 1998; 35:136–142.
- Alekseev, AN, Dubinina, HV, Semenov, AV, Bolshakov, CV. Evidence of ehrlichiosis agents found in ticks (Acari: Ixodidae) collected from migratory birds. *J Med Entomol* 2001; 38:471–474.
- Alekseev, AN, Dubinina, HV, Van De Pol, I, Schouls, LM. Identification of *Ehrlichia* spp. and *Borrelia burgdorferi* in *Ixodes* ticks in the Baltic regions of Russia. *J Clin Microbiol* 2001; 39:2237–2242.
- Alekseev, AN, Semenov, AV, Dubinina HV. Evidence of *Babesia microti* infection in multi-infected *Ixodes persulcatus* ticks in Russia. *Exp Appl Acarol* 2003; 29:345–353.
- Balashov, Y. The interrelationships of ixodid ticks (Ixodidae) with the causative agents of transmissible vertebrate infections. *Parazitologiya* 1995; 29:337–352.
- Belongia, EA. Epidemiology and impact of coinfections acquired from *Ixodes* ticks. *Vector-Borne Zoonotic Dis* 2002; 2:265–273.
- Beninati, T, Lo, N, Sacchi, L, Genchi, C, et al. A novel alpha-Proteobacterium resides in the mitochondria of ovarian cells of the tick *Ixodes ricinus*. *Appl Environ Microbiol* 2004; 70:2596–2602.
- Courtney, JW, Kostelnik, LM, Zeidner, NS, Massung, RF. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *J Clin Microbiol* 2004; 42:3164–3168.
- Dubinina, EV, Alekseev AN. The biodiversity dynamics of the causative agents of diseases transmitted by ticks in the genus *Ixodes*: an analysis of multiyear data. *Med Parazitol (Mosk)* 1999; 2:13–19.
- Eremeeva, ME, Oliveira, A, Robinson, JB, Rybakova, N, et al. Prevalence of bacterial agents in *Ixodes persulcatus* ticks from Vologda province of Russia. *Ann N Y Acad Sci* 2006; 1078:291–298.
- Fedorova, VG, Alekseev, AN, Chunikhin, SP, Kurenkov, VB. Relation between the population count of taiga ticks (*Ixodes persulcatus* P. Sch.) and their virophoricity in the tick-borne encephalitis foci of Novgorod Province. *Med Parazitol (Mosk)* 1984; 1:37–39.
- Fritsche, TR, Horn, M, Seyedirashti, S, Gautom, RK, et al. In situ detection of novel bacterial endosymbionts of *Acanthamoeba* spp. phylogenetically related to members of the order Rickettsiales. *Appl Environ Microbiol* 1999; 65:206–212.
- Goddard, J, Sumner, JW, Nicholson, WL, Paddock, CD, et al. Survey of ticks collected in Mississippi for *Rickettsia*, *Ehrlichia*, and *Borrelia* species. *J Vector Ecol* 2003; 28:184–189.
- Halos, L, Vourc'h, G, Cotte, V, Gasqui, P, et al. Prevalence of *Anaplasma phagocytophilum*, *Rickettsia* sp. and *Borrelia burgdorferi* sensu lato DNA in questing *Ixodes ricinus* ticks from France. *Ann NY Acad Sci* 2006; 1078:316–319.
- Hildebrandt, A, Schmidt, KH, Wilske, B, Dorn, W, et al. Prevalence of four species of *Borrelia burgdorferi* sensu lato and coinfection with *Anaplasma phagocytophila* in *Ixodes ricinus* ticks in central Germany. *Eur J Clin Microbiol Infect Dis* 2003; 22:364–367.
- Holman, MS, Caporale, DA, Goldberg, J, Lacombe, E, et al. *Anaplasma phagocytophilum*, *Babesia microti*, and *Borrelia burgdorferi* in *Ixodes scapularis*, southern coastal Maine. *Emerg Infect Dis* 2004; 10:744–746.
- Kawahara, M, Ito, T, Suto, C, Shibata, S, et al. Comparison of *Ehrlichia muris* strains isolated from wild mice and ticks and serologic survey of humans and animals with *E. muris* as antigen. *J Clin Microbiol* 1999; 37:1123–1129.
- Kawahara, M, Rikihisa, Y, Isogai, E, Takahashi, M, et al. Ultrastructure and phylogenetic analysis of “*Candidatus* Neoehrlichia mikurensis” in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. *Int J Syst Evol Microbiol* 2004; 54:1837–1843.
- Korenberg, EI. Problems in the study and prophylaxis of mixed infections transmitted by ixodid ticks. *Int J Med Microbiol* 2004; 293:80–85.
- Korenberg, EI, Gorelova, NB, Kovalevskii, YV. Main features of natural focality of ixodid tick-borne borreliosis in Russia. *Parazitologiya* 2002; 36:177–191.
- Kovalevskii, YV, Korenberg, EI. Differences in *Borrelia* infections in adult *Ixodes persulcatus* and *Ixodes ricinus* ticks (Acari: Ixodidae) in populations of north-western Russia. *Exp Appl Acarol* 1995; 19:19–29.
- Li, JS, Yager, E, Reilly, M, Freeman, C, et al. Antibodies highly effective in SCID mice during infection by the intracellular bacterium *Ehrlichia chaffeensis* are of picomolar affinity and exhibit preferential epitope and isotype utilization. *J Immunol* 2002; 169:1419–1425.

- Liz, J. Ehrlichiosis in *Ixodes ricinus* and wild mammals. *Int J Med Microbiol* 2002; 291:104–105.
- Lo, N, Beninati, T, Sasser, D, Bouman, EA, et al. Widespread distribution and high prevalence of an alpha-proteobacterial symbiont in the tick *Ixodes ricinus*. *Environ Microbiol* 2006; 8:1280–1287.
- Massung, RF, Slater, K, Owens, JH, Nicholson, WL, et al. Nested PCR assay for detection of granulocytic ehrlichiae. *J Clin Microbiol* 1998; 36:1090–1095.
- Massung, RF, Mauel, MJ, Owens, JH, Allan, N, et al. Genetic variants of *Ehrlichia phagocytophila*, Rhode Island and Connecticut. *Emerg Infect Dis* 2002; 8:467–472.
- Massung, RF, Mather, TN, Priestley, RA, Levin, ML. Transmission efficiency of the AP-variant 1 strain of *Anaplasma phagocytophila*. *Ann NY Acad Sci* 2003; 990:75–79.
- McKiel, J, Bell, EJ, Lackman, DB. *Rickettsia canada*: a new member of the typhus group of rickettsiae isolated from *Haemaphysalis leporispalustris* ticks in Canada. *Can J Microbiol* 1967; 13:503–510.
- Mediannikov, OYu, Ivanov, LI, Nishikawa, M, Saito, R, et al. Microorganism “Montezuma” of the order Rickettsiales: the potential causative agent of tick-borne disease in the Far East of Russia. *Zh Mikrobiol Epidemiol Immunobiol* 2004; 1:7–13.
- Morozova, OV, Dobrotvorsky, AK, Livanova, NN, Tkachev, SE, et al. PCR detection of *Borrelia burgdorferi* sensu lato, tick-borne encephalitis virus, and the human granulocytic ehrlichiosis agent in *Ixodes persulcatus* ticks from Western Siberia, Russia. *J Clin Microbiol* 2002; 40:3802–3804.
- Olano, JP, Wen, G, Feng, HM, McBride, JW, et al. Histologic, serologic, and molecular analysis of persistent ehrlichiosis in a murine model. *Am J Pathol* 2004; 165:997–1006.
- Piccolin, G, Benedetti, G, Doglioni, C, Lorenzato, C, et al. A study of the presence of *B. burgdorferi*, *Anaplasma* (previously *Ehrlichia*) *phagocytophilum*, *Rickettsia*, and *Babesia* in *Ixodes ricinus* collected within the territory of Belluno, Italy. *Vector-Borne Zoonotic Dis* 2006; 6:24–31.
- Rar, VA, Fomenko, NV, Dobrotvorsky, AK, Livanova, NN, et al. Tickborne pathogen detection, Western Siberia, Russia. *Emerg Infect Dis* 2005; 11:1708–1715.
- Rar, VA, Livanova, NN, Panov, VV, Astanin, VB, et al. The study of *Anaplasma* and *Ehrlichia* genetic variability in parasitic systems in the South-Western Siberia and Ural. *Bull Siberian Med* 2006; 5:116–120.
- Ravyn, MD, Korenberg, EI, Oeding, JA, Kovalevskii, YV, et al. Monocytic *Ehrlichia* in *Ixodes persulcatus* ticks from Perm, Russia. *Lancet* 1999; 353:722–723.
- Regnery, RL, Spruill, CL, Plikaytis, BD. Genotypic identification of rickettsiae and estimation of intraspecific sequence divergence for portions of two rickettsial genes. *J Bacteriol* 1991; 173:1576–1589.
- Schulze, TL, Jordan, RA, Schulze, CJ, Mixson, T, et al. Relative encounter frequencies and prevalence of selected *Borrelia*, *Ehrlichia*, and *Anaplasma* infections in *Amblyomma americanum* and *Ixodes scapularis* (Acari: Ixodidae) ticks from central New Jersey. *J Med Entomol* 2005; 43:450–456.
- Semenov, AV, Alekseev, AN, Dubinina, EV, Kaufmann, U, et al. Detection of the genotypic heterogeneity of *Ixodes persulcatus* Schulze (Acari: Ixodidae) of the North-West region of Russia and characteristics of distribution of tick-borne pathogens causing Lyme disease and *Ehrlichia* infections in various genotypes. *Med Parazitol (Mosk)* 2001; 3:11–15.
- Shpynov, SN, Parola, P, Rudakov, NV, Samoilenko, IE, et al. Genotyping of spotted tick fever rickettsiae, detected in Russia and Kazakhstan. *Med Parazitol (Mosk)* 2003; 3:20–24.
- Shpynov, SN, Rudakov, NV, Yastrebov, VK, Leonova, GN, et al. New evidence for the detection of ehrlichia and anaplasma in *Ixodes* ticks in Russia and Kazakhstan. *Med Parazitol (Mosk)* 2004; 2:10–14.
- Sidel'nikov, YN, Mediannikov, OYu, Ivanov, LI, Zdanovskaia, NI. The first case of granulocytic ehrlichiosis in the Far East of the Russian Federation. *Klin Med (Mosk)* 2003; 81:67–68.
- Smetanova, K, Schwarzova, K, Kocianova, E. Detection of *Anaplasma phagocytophilum*, *Coxiella burnetii*, *Rickettsia* spp., and *Borrelia burgdorferi* s. l. in ticks, and wild-living animals in western and middle Slovakia. *Ann NY Acad Sci* 2006; 1078:312–315.
- Telford, SR 3rd, Korenberg, EI, Goethert, HK, Kovalevskii, IV, et al. Detection of natural foci of babesiosis and granulocytic ehrlichiosis in Russia. *Zh Mikrobiol Epidemiol Immunobiol* 2002; 6:21–25.
- Tokarevich, N, Stoyanova, N, Chaika, N, Kozarenko, A, et al. Lyme disease in the Arkhangelsk Province of the Russian Federation. *Epi North* 2002; 3:35–37.
- Vansulin, SA, Malakhov, IV. Ixodid ticks in the health resort zone of Leningrad. *Med Parazitol (Mosk)* 1976; 45:94–95.
- Vansulin, SA, Smyslova, TO, Solina, LT. Distribution and biological characteristics of *Ixodes persulcatus* (Ixodidae) ticks in the health resort zone of Leningrad. *Parazitologiya* 1981; 15:498–505.
- Vorobyeva, NN, Korenberg, EI, Grigoryan, YV. Diagnostics of tick-borne diseases in the endemic region of Russia. *Wien Klin Wochenschr* 2002; 114:610–612.
- Werren, JH, Hurst, GD, Zhang, W, Breeuwer, JA, et al. Rickettsial relative associated with male killing in the ladybird beetle (*Adalia bipunctata*). *J Bacteriol* 1994; 176:388–394.

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