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Short Communication

Ticks and Tick-borne Infections in the Far East of Russia

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Abstract

The vector-borne zoonosises remain public health concern especially in the Far East of Russia because of high population density of ixodid ticks and their biodiversity rearrangements due to anthropogenic pressure. *Ixodes persulcatus* Schulze, 1930 remains predominant in the isolated populations of the Sakhalin island and in continental wildlife reserves with its gradual displacement with other tick species near agro landscapes and towns. The infection rate of *I. persulcatus* with *Rickettsia* spp. (up to 100%) and *Borrelia burgdorferi* sensu lato (up to 69%) exceeded the corresponding frequencies of the tick-borne encephalitis virus (TBEV), *Borrelia miyamotoi*, *Anaplasma*, *Ehrlichia*, *Francisella tularensis* and *Babesia* spp. Phylogenetic analysis of the nucleotide sequences of the PCR products permitted to reveal genetic diversity of the tick-borne bacteria including *Borrelia garinii*, *B. afzelii*, *B. miyamotoi*, *Rickettsia helvetica*, *Candidatus Rickettsia tarasevichiae*, *A. phagocytophilum*, *E. muris*, *Candidatus Neoehrlichia mikurensis*, *Francisella tularensis* found in *I. persulcatus* ticks and only *B. burgdorferi* s. l. complex and *Rickettsia heilongjiangensis* - in *H. concinna*. The TBEV isolates from ticks and mosquitoes of the predominant Far Eastern subtype appeared to be very stable since 1937. Besides the monoinfection the real time PCR with subtype specific fluorescent probes showed mixed infection of the TBEV Far Eastern subtype with Siberian subtype in the ixodid ticks. Molecular epidemiological monitoring allowed us to estimate risks of the tick-borne infections in the Far East of Russia and to reveal their genetic diversity necessary for the diagnostic systems and vaccines.

INTRODUCTION

Ixodid ticks are second only to mosquitoes as vectors of viral, bacterial and protozoan agents. Ticks are known to be main carriers (so-called vectors) and reservoir hosts of the numerous pathogens. Their ability to feed on a variety of vertebrate animals, intracellular digestion of blood and their long life cycle up to 3-6 years at each stage of development make them ideal vectors for many tick-borne infectious agents. The ixodid ticks parasitize more than 100 different species of mammals, birds, reptiles and amphibians thus providing the vertebrate reservoir hosts involvement into epizootic process [1]. Ticks are able to survive during extended starvation up to 16 years [1,2]. Tick saliva lacks hemolytic enzymes, therefore, the ingested vertebrate blood remain undigested in the midgut for long periods with viable viruses and bacteria. Intracellular digestion occurs entirely within epithelial cells of tick midgut, except for hemolysis of the blood cells in the midgut lumen. To concentrate the diluted blood meal, ticks use their salivary glands to periodically secrete excess water from the blood meal back into the host. The ability of ticks to produce thousands of eggs is important for their population dynamics. In nature, once infected ticks at each developmental stage remain permanently infected [1,2].

Little is currently known about innate immunity in ticks. Tick cellular receptors recognize pathogen associated molecular patterns and their binding induces secretion of antimicrobial peptides, proteases and protease inhibitors, lectins, coagulation factors, oxidative stress reducing proteins, hydrolases, protein/lipid binding agents and others [3]. Lack of a specific immunity in arthropods is believed to facilitate the reproduction of the tick-borne infectious agents.

Ticks and their vertebrate hosts maintain more than 11 tick-borne bacteria, at least 5 viruses, a variety of protozoan and helmintide human pathogens in the same habitats, and as a consequence, they may be infected with two or more infectious agents [4-6]. Closely related *Ixodes* tick species harbor similar sets of pathogens in America and Eurasia [4,5,7]. Until 1987, only tick-borne encephalitis virus (TBEV) was thought to be associated with taiga ticks *I. persulcatus*, but later extensive studies have shown their competence in the transmission of pathogenic spirochetes, *Borrelia garinii* and *Borrelia afzelii*

[4-6,8,9]. Other known pathogenic bacteria transmitted by ticks are intracellular alpha proteobacteria, which includes the families *Anaplasmataceae*, *Bartonellaceae*, and *Rickettsiaceae*. Members of the genera *Anaplasma* and *Ehrlichia*, from the family *Anaplasmataceae*, infect mainly monocytes and granulocytes and cause human and animal anaplasmoses and ehrlichioses. The main *Ehrlichia* species found in *I. persulcatus* ticks from the Western Siberia is *Ehrlichia muris* [4-6]. The etiologic agent of human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, has also been identified there in *I. persulcatus* ticks [5-6]. The tick-borne protozoa of the genus *Babesia* reproduce in erythrocytes, thus causing babesiosis among humans as well as wild and domestic animals. Infection of different ixodids with *Babesia* species pathogenic for immunocompromised humans was previously described [5]. However, the diversity of pathogens associated with ixodid ticks in the Far East of Russia has not been well studied.

The objectives of the present research were to estimate the infection rate of different ixodid ticks with the TBEV, *Borrelia*, *Rickettsia, Anaplasma*/*Ehrlichia*, *Francisella* and *Babesia* spp. in the Far East of Russia.

MATERIALS AND METHODS

Adult questing ticks were flagged from vegetation during May and June of 1999–2014 in the Far East of Russia (Table 1). Ticks species were determined on the base of their morphological traits according to [1].Totally, 2008 individual ixodid ticks were collected, identified and examined by means of RT-PCR.

TBEV RNA detection

Reverse transcription (RT) was carried out by using "Reverta L" ("InterLabService", Russia). PCR was proceeded using "VectoTBE-amply" (Vector Best, Novosibirsk, Russia) from 1999 to 2006; PCR «AmpliSensPCR» ("InterLabService", Moscow, Russia) and primers [10] in 2007; test-system for detection TBE "IzoGen" (Moscow, Russia) in 2008; "AmpliSensTBE-FRT" from 2009 to 2010 and "AmplySens*TBE, B. burgdorferi, A. phagocytophila, E. muris/E. chaffensis*-FL" ("InterLabService", Russia) from 2011 to 2014 according to the manufacturer's instructions. Molecular typing with subtype-specific fluorescent probes was performed according to [11]. The TBEV strains were isolated from tick pools (10-30 ticks in each) in newborn mice after both intracerebral and subcutaneous infection as well as in the porcine embryo kidney (PS) cells.

Borrelia DNA detection

PCR was proceeded using primers SL [12] from 1999 to 2006; PCR test-system for detection *Borrelia burgdorferi* s.l. «IzoGen» ("IzoGen", Moscow, Russia) from 2007 to 2009; "AmpliSens *B. burgdorferi* sensu lato" in 2010 and "AmplySens *TBE, B. burgdorferi, A. phagocytophila, E. muris/E. chaffensis*-FL" ("InterLabService", Russia) from 2011 to 2014 according to the manufacturer's instructions. *Borrelia miyamotoi* DNA was detected using primers for p66 gene [9] in 2011-2013 and "Vecto *Borrelia miyamotoi* - FL" (Vector Best, Novosibirsk, Russia) in 2014.

Table 1: Geographic locations of the ixodid tick collection places.

Site №	District	Collection place	Biotop	Geographic coordinates of the site		Year
				N	E	
Amur region						
1	Magdagachinsky	Ductui	larch-birch forest	53°22'	126°08'	2011
2	Shimanovsky	Belovezh	larch-birch forest	52°19'	127°24'	2011
3	Svobodnensky	Kosmodrom	broadleaved forest	51°53'	128°20'	2011
4	Selemdzhinsky	Norsk	mixed deciduous forest	52°20'	129°53'	2010
5	Blagoveshchensky	Raduga camp	deciduous forest	50°40'	127°42'	2007
Jewish autonomous region						
6	Oktyabrsky	Stolbovoye	broadleaved forest	47°55'	131°03'	2013
7	Leninsky	Churki	coniferous–broadleaved forest	48°04'	132°39'	2013
8	Birobidzhansky	Birshosse, 17th km	coniferous–broadleaved forest	48°41'	132°48'	2013
Khabarovsk territory						
9	Lazo	Kiinsk	deciduous forest	47°59'	134°49'	2014
10	Khabarovsky	Khekhtzir	coniferous–broadleaved forest	48°15'	135°00'	1999-2014
11	Nanaisky	Troitzkoe	mixed broadleaved forest	49°22'	136°36'	2014
12	Vaninsky	Toki	larch-small-leaved forest	49°07'	140°18'	2011
Sakhalin region						
13	Kholmsky	Pionery	mixed coniferous--small-leaved forest	47°16'	142°02'	2011
14	Yuzhno-Sakhalinsky	Isvestkovy	mixed coniferous--small-leaved forest	46°50'	142°56'	2011

Anaplasma phagocytophilum and *Ehrlichia muris* DNA detection was proceeded using primers [5] from 2002 to 2010 and "AmplySens *TBE, B. burgdorferi, A. phagocytophila, E. muris/E. chaffensis*-FL" ("InterLabService", Russia) from 2011 to 2014 according to the manufacturer's instructions.

Rickettsia DNA detection and *Francisella tularensis* DNA detection was proceeded using PCR test-system for detection *Rickettsia spp.* and *Francisella tularensis* «IzoGen» ("IzoGen", Moscow, Russia) and *Rickettsia* - specific primers [13,14]. For the identification of *Candidatus Rickettsia tarasevichiae* species-specific primers were used [15].

Babesia DNA detection was performed by PCR as earlier described [16].

Nucleotide sequences of the PCR products were determined using BigDye 3.1 Terminator Cycle Sequencing Kit and DNA analyzer ABI 3500 (Applied Biosystems, USA).

Phylogenetic analysis was performed using MEGA 6.06 [17]. GenBank (http://www.ncbi.nlm.nih.gov) accession numbers of the tick-borne infectious agents nucleotide sequences determined in our study are the followings: the TBEV complete CDS KF880803-KF880805, KP869172, KP844724-KP844727, KT001070-KT001073; *B. garinii* 16S rRNA gene KY312010-KY312015, KY312118, KY346888–KY346892, KY346970-KY346973, KY348800; *B. garinii* 5S-23S ITS KY924779, KY937676 - KY937682, KY963154 - KY963161*; B. afselii* 16S rRNA gene KX622580-KX622852, KX688604*; B. afselii* 5S-23S ITS KX685726 - KX685729*; B. miyamotoi* 16S rRNA gene KX769848 - KX769851*; B. miyamotoi* p66 KX812709 - KX812712*; B. miyamotoi* glpQ KX898133*; A. phagocytophilum* 16S rRNA gene HM366588*; A. phagocytophilum groESL* gene HM366575-HM366577*; F. tularensis* 16S rRNA gene EF121555, EF121557; *Babesia* 18S rRNA gene GU057380- GU057382.

RESULTS

Four ixodid tick species *Ixodes persulcatus* P. Schulze, 1930, *Haemaphysalis concinna* Koch, 1844, *Haemaphysalis japonica douglasi* Nuttall et Warburton, 1915, *Dermacentor silvarum* Olenev, 1932 are currently prevailing in the Far East of Russia. Both total ixodid population densities (up to 220 imago collected on a "flag" per hour) and distribution of the tick species appeared to depend on the anthropogenic pressure. On the Sakhalin island monodominant type of ixodid populations with the *I. persulcatus* only remained. In continental wildlife reserves *I. persulcatus* proportions within a range 87-92% exceeded those of other tick species. In the coniferous–broadleaved forests the prevalent *I. persulcatus* was observed together with *H. japonica, H. concinna* and *D. silvarum* in different proportions during the tick activity seasons. In the southern parts of the Amur region and on the Khabarovsk territory with agro landscapes replaced the original broad-leaved and coniferous forests after long-term settlements the total ixodid populations were less (upto 40 ticks/hour) with *H. concinna* dominance.

Therefore, the tick-borne infections remain the public health concern. Among them the most important are the tick-borne encephalitis, Lyme disease and rickettsiosis.

The TBEV RNA was detected in prevailing tick species *I. persulcatus* (up to 10.3%) and *H. concinna* (up to 14.3%) in all regions (Table 2). Molecular typing using RT-real time PCR with the subtype-specific fluorescent probes revealed the TBEV predominant Far Eastern (FE) subtype as mono- and mixed infection with the Siberian (Sib) subtype in *I. persulcatus* pools. The TBEV strains of the FE subtype were isolated from ixodid ticks *I. persulcatus*, *H. concinna* and from a pool of mosquitoes *Aedes vexans* in newborn mice and in the porcine embryo kidney cells. Ten TBEV strains isolated from *I. persulcatus* from the Khabarovsk territory and the Jewish autonomous region during 1985-2013 (GenBank accession numbers KP869172, KF880804, KF880805, KT001070-KT001072, and KP844724-KP844727) form a cluster with the TBEV vaccine strain Sofjin (JN229223) isolated from a patient brain in 1937 near Khabarovsk. The TBEV strain from *H. concinna* collected in the Amur region (KF880803) is relative to the vaccine strain 205 isolated in 1973 from *I. persulcatus* collected in the Jewish autonomous region. The TBEV strain Lazo MP36 of the FE subtype isolated from a pool of mosquito *Aedes vexans* in Khabarovsk territory in 2014 (KT001073) differs from strains: 1) isolated from *Ixodes persulcatus* and *Haemaphysalis concinna* ticks; 2) from mosquitoes [strain Malyshevo (KJ744034) isolated in 1978 from *Aedes vexans nipponii* on Khabarovsk territory]; and 3) from patient brain [the vaccine strain Sofjin (JN229223) and others].

The tick-borne bacteria found in the ixodid ticks in the Far East of Russia include *Borrelia burgdorferi* sensu lato (s.l.), *Borrelia miyamotoi, Rickettsia* spp., *Anaplasma phagocytophilum, Ehrlichia muris* and *Francisella tularensis* (Table 2).

Borrelia DNA was detected in ticks, collected in the Amur region, the Jewish autonomous region, the Khabarovsk territory and the Sakhalin region. *Borrelia* DNA was revealed in *I. persulcatus* (up to 69%) and in a few samples of *H. concinna* by PCR (Table 2). Phylogenetic analysis of both 16S rRNA gene and 5S-23S intergenic spacer sequences revealed two species: *Borrelia garinii* (KY312010, KY346890-KY346892, KY346970, KY346971, KY346973, KY348800, KY312011-KY312015, KY312118; KY937679-KY937682, KY963154-KY963161) and *Borrelia afzelii* (KX622580-KX622852, KX688604; KX685726-KX685729) in *I. persulcatus* ticks throughout the examined territories besides the Sakhalin island with the *B. garinii* only (KY346888, KY346889, KY346972; KY924779, KY937676-KY937678). Both borrelia species are known to dominate in surrounding regions of Siberia, China, Japan and Korea [7]. Intraspecies differentiation of the *B. burgdorferi* s.l. appeared to occur on the base of their geographic location. Thus, the majority of the *B. garinii* isolated in the Far East of Russia belong to NT29 group, whereas all the studied *B. afzelii* isolates correspond to VS461. Moreover, *B. miyamotoi* DNA was detected in several *I. persulcatus* ticks from the Jewish autonomous region, Khabarovsk territory and Sakhalin island. *B. miyamotoi* sequences (KX769848- KX769851; KX812709-KX812712) were identical to each other and to *B. miyamotoi* FR64b (CP004217) from Japan. The single nucleotide polymorphism (SNP) was observed in *B. miyamotoi* glpQ gene (KX898133) with the same sequences for all isolates from the Far East and Siberia (FJ940729).

PCR detection of *Rickettsia* DNA was successful in up to

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Table 2: PCR detection of the tick-borne pathogens in the Far East of Russia.

Site№	TBEV				*Borrelia burgdorferi* s.l.				*Borrelia* miyamotoi		*Anaplasma phagocytophilum*				*Ehrlichia muris*				*Rickettsia* spp.				*Francisella tularensis*	
	I. persulcatus		*H. concinna*		*I. persulcatus*		*H. concinna*		*I. persulcatus*		*I. persulcatus*		*H. concinna*		*I. persulcatus*		*H. concinna*		*I. persulcatus*		*H. concinna*		*I. persulcatus*	
	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)	N+/N	Rate (%)
Amur region																								
1	1/48	2.1	0/51		33/48	68.8	2/51	3.9	0/48	0	2/48	4.2	0/51	0	5/48	10.4	0/51	0	30/48	62.5	19/51	37.3	7/48	14.6
2	0/9	0	1/89	1.1	2/9	22.2	4/89	4.5	0	0	0/9		0/89	0	0/9	0	0/89	0	9/9	100.0	36/89	40.5	0/9	0
3	0/12	0	1/85	1.2	2/12	16.7	3/85	3.5	0	0	2/12	16.7	0/85	0	0/12	0	0/85	0	4/12	33.3	26/85	30.6	0/12	0
4	0	0	1/56	1.8	0	0	0/56	0	0	0	0/24	0	0/56	0	0/24	0	0/56	0	0	0	6/56	10.7	0	0
5	2/30	6.7	2/70	2.9	8/30	26.7	0/70	0	0	0	0	0	0	0	0	0	0	0	15/30	50.0	22/70	31.4	0	0
1-5	**3/99**	**3.0**	**5/351**	**1.4**	**45/99**	**45.5**	**9/351**	**2.6**	**0/48**	**0**	**4/93**	**4.3**	**0/281**	**0**	**5/93**	**5.4**	**0/281**	**0**	**58/99**	**58.6**	**109/351**	**31.1**	**7/69**	**10.1**
Jewish autonomous region																								
6	2/20	10.0	0/2	0	2/20	10.0	0/2	0	1/20	5.0	0/20	0	0/2	0	0/20	0	0/2	0	4/20	20.0	1/2	50.0	0/20	0
7	0/23	0	0	0	6/23	26.1	0	0	1/23	4.3	0/23	0	0	0	0/23	0	0	0	15/23	65.2	0	0	0/23	0
8	0/50	0	0/13	0	27/50	54.0	0	0	3/50	6.0	6/50	12.0	0/13	0	2/50	4.0	0/13	0	37/50	74.0	1/13	7.7	0/50	0
6-8	**2/93**	**2.2**	**0/15**	**0**	**35/93**	**37.6**	**0/2**	**0**	**5/93**	**5.4**	**6/93**	**6.5**	**0/15**	**0**	**2/93**	**2.2**	**0/15**	**0**	**56/93**	**60.2**	**2/15**	**13.3**	**0/93**	**0**
Khabarovsk territory																								
9	0/15	0	3/21	14.3	3/15	20.0	1/21	4.8	0		0/15	0	0/21	0	1/15	6.7	0/21	0	0/15	0	8/21	38.1	0/15	0
10	109/1054	10.3	0	0	288/780	36.9	0	0	1/50	2.0	77/550	14.0	0	0	36/480	7.5	0	0	269/550	48.9	0	0	16/550	2.9
11	0/26	0	0/21	0	9/26	34.6	4/21	19.0	3/26	11.5	0/26		0/21	0	1/26	3.8	0/21	0	1/26	3.8	4/21	19.0	0	0
12	4/100	4.0	0/13	0	69/100	69.0	0/13		1/50	2.0	8/100	8.0	0	0	1/100	1.0	0	0	30/100	30.0	0	0	5/100	5.0
8-12	**113/1195**	**9.5**	**3/55**	**5.5**	**369/921**	**40.1**	**5/55**	**9.1**	**5/126**	**4.0**	**85/691**	**12.3**	**0/42**	**0**	**39/621**	**6.3**	**0/42**	**0**	**300/691**	**43.4**	**12/42**	**28.6**	**21/655**	**3.2**
Sakhalin region																								
13	4/100	4.0	0	0	19/100	19.0	0	0	5/50	10.0	0/100	0	0	0	3/100	3.0	0	0	64/100	64.0	0	0	7/100	7.0
14	0/100		0	0	24/100	24.0	0	0	0	0	1/100	1.0	0	0	1/100	1.0	0	0	36/55	65.5	0	0	0/100	0
13, 14	**4/200**	**2.0**	**0**	**0**	**43/200**	**21.5**	**0**	**0**	**5/50**	**10.0**	**1/200**	**0.5**	**0**	**0**	**4/200**	**2.0**	**0**	**0**	**100/155**	**64.5**	**0**	**0**	**7/200**	**3.5**
In total, Far East																								
1-14	**122/1587**	**7.7**	**8/421**	**1.9**	**492/1313**	**37.5**	**14/408**	**3.4**	**15/317**	**4.7**	**96/1077**	**8.9**	**0/338**	**0**	**50/1077**	**4.6**	**0/338**	**0**	**514/1038**	**49.5**	**123/408**	**30.1**	**35/1017**	**3.4**

Note: N+/N means a ratio of the tick's positive in PCR with specific primers and a total number of the analyzed ticks.

100.0% *I. persulcatus* (Table 2). Species-specific PCR revealed *Candidatus Rickettsia tarasevichiae* in 95.0% of them on the Khabarovsk territory. *Rickettsia* DNA was also detected in up to 40.5% *H. concinna*. The *R. heilongjiangensis* sequences from *H. concinna* collected in Amur region and on Khabarovsk territory were identical to previously described AY285776 and *R. helvetica* sequences from *I. persulcatus* from Khabarovsk territory and Sakhalin island were the same as U59723. *Candidatus R. tarasevichiae* identical to AF503167 appeared to be the predominant rickettsia species in *I. persulcatus* ticks from the Khabarovsk territory, while *R. helvetica* significantly prevailed in *I. persulcatus* ticks in Sakhalin region. *Rickettsia raoultii* was found in *D. silvarum* ticks in the Amur region.

Anaplasma phagocytophilum and *Ehrlichia muris* DNA were detected in *I. persulcatus* ticks up to 16.7% and 10.4%, respectively, on the continental part of the Russian Far East (Table 2). On contrary, in the isolated population of ticks from Sakhalin island only a few *Anaplasmatacea/Ehrlichia* positive samples were found. Genetic variants of *A. phagocytophilum* from Khabarovsk territory were closely related to the isolates detected in Siberia (KF745748) and China (DQ342324), *E. muris* isolates were identical to *E. muris* from Japan (AB196302). Besides that on the Khabarovsk territory a few isolates of "Candidatus *Neoehrlichia mikurensis*" were identical to *Candidatus Neoehrlichia mikurensis* Nagano21 (AB196305) from Japan.

Francisella tularensis DNA was found in 0-14.6% *I. persulcatus* ticks in the Amur region, in 0-5.0% ticks from the Khabarovsk territory and in 0-7.0% on Sakhalin Island (Table 2).

Protozoa *Babesia* DNA was revealed in a few samples of *I. persulcatus* and *H. japonica* collected from the Khabarovsk territory (GU057384, GU057380-GU057382).

DISCUSSION

Molecular genetics methods allowed us to reveal a framework for vector-borne zoonosises in the Far East of Russia. Natural populations of ixodid ticks remain numerous and diverse with 22 earlier registered ixodid species [18] and 4 currently prevailing species such as *Ixodes persulcatus* P. Schulze, 1930, *Haemaphysalis concinna* Koch, 1844, *Haemaphysalis japonica douglasi* Nuttall et Warburton, 1915 and *Dermacentor silvarum* Olenev, 1932. Extrinsic cues (anthropogenic pressure and climate change) and intrinsic dynamics rearranged the ixodid tick population structure with the gradual displacement of the previously dominant *Ixodes persulcatus* with *Haemaphysalis concinna,* currently prevailing near agro landscapes and towns. The tick infection rate with *Rickettsia* spp. (up to 100%) and *Borrelia burgdorferi* sensu lato (up to 69%) essentially exceeded the corresponding frequencies of the TBEV, *Borrelia miyamotoi*, *Anaplasma*, *Ehrlichia, Francisella tularensis* and *Babesia* spp. All the tick-borne pathogens do not seem to interfere with each other in ticks without specific immunity [4,5]. The retrospective analysis of chronological rows revealed the genetic stability of the tick-borne pathogens despite the ixodid ticks population dynamics and rearrangements. Thus, the RNA-containing tick-borne flavivirus - TBEV of the prevailing FE subtype remained stable since 1937 in the continental endemic regions of the Far East of Russia. Both available Russian vaccines against the tick-borne encephalitis based on the FE strains correspond well to the new viral isolates and, consequently, can protect against the viral infection. Distribution of *Borrelia* species in the Far East of Russia was similar to previously described diversity in flanking areas of Siberia, China, Japan and Korea [7]. Previously *Rickettsia sibirica* and *Rickettsia heilongjiangensis* were found in the Far East, later *Rickettsia raoultii*, *Rickettsia helvetica*, and *Candidatus Rickettsia tarasevichiae* from the *Rickettsia canadensis* group were detected [19] but during last years the first of them was not found there. However, despite the enormous tick numbers, the tick-borne pathogens persistence in wild nature and the absence of any vaccines against all the bacterial and protozoan tick-borne infections the total corresponding human disease rate, except rickettsiosis, remains less 10 clinical cases per 100,000 populations.

REFERENCES

1. Filippova NA. Taiga tick *Ixodes persulcatus* Schulze (*Acarina, Ixodidae*). Leningrad, "Nauka". 1985; 416.
2. Nutall PA, Labuda M. Dynamics of infection in tick vectors and at the tick-host interface. Adv Virus Res. 2003; 60: 233-272.
3. Sonenshine DE, Hynes WL. Molecular characterization and related aspects of the innate immune response in ticks. Front Biosci. 2008; 13: 7046-7063.
4. Morozova OV, Dobrotvorsky AK, Livanova NN, Tkachev S, Bakhvalova VN, Beklemishev AB. 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.
5. Rar VA, Fomenko NV, Dobrotvorsky AK, Livanova NN, Rudakova SA, Fedorov EG, et al. Tickborne pathogen detection, Western Siberia, Russia. Emerg Infect Dis. 2005; 11: 1708-1715.
6. Rar V, Livanova N, Tkachev S, Kaverina G, Tikunov A, Sabitova Y, et al. Detection and genetic characterization of a wide range of infectious agents in Ixodes pavlovskyi ticks in Western Siberia, Russia. Parasit Vectors. 2017; 10: 258.
7. Kurtenbach K, Hanincová K, Tsao JI, Margos G, Fish D, Ogden NH. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nat Rev Microbiol. 2006; 4: 660-669.
8. Fomenko NV, Livanova NN, Chernousova NY. Diversity of *Borrelia burgdorferi* sensu lato in natural foci of Novosibirsk region. Int J Med Microbiol. 2008; 298: 139-148.
9. Fomenko NV, Livanova NN, Borgoiakov VI, Kozlova IV, Shulaĭkina IV, Pukhovskaia NM. Detection of Borrelia miyamotoi in ticks Ixodes persulcatus from Russia. Parazitologiia; 2010; 44: 201-211.
10. Tkachev SE. Genetic Variability of the Tick-Borne Encephalitis Virus in Natural Foci of Novosibirsk and its Surrounding Regions, Diss. Novosibirsk (in Russian). 2015.
11. Morozova OV, Grishechkin AE, Karan LS, Isayeva EI, Shchuchinova LD, Loginova NV, et al. Detection of tick-borne encephalitis virus in Ixodes ticks collected in natural focus of Gonyi Altai. Voprosi virusologii. 2011; 2: 23-26.
12. Demaerschalck I, Ben Messaoud A, De Kesel M, Hoyois B, Lobet Y, Hoet P, et al. Simultaneous presence of different Borrelia burgdorferi genospecies in biological fluids of Lyme disease patients. J Clin Microbiol. 1995; 33: 602-608.
13. Livanova NN, Rar VA, Livanov SG, Igolkina IP. Diversity of parasite systems with participation of small mammals and *Ixodes persulcatus*

Schulze on the Northern Ural. Siberian Ecological. 2005; 6: 1079-1084.

14. Lee JH, Park HS, Jang WJ, Koh SE, Kim JM, Shim SK. Differentiation of *Rickettsiae* by groEL gene analysis. J Clin Microbiol. 2003; 41: 2952-2960.

15. Pukhovskaia NM, Rar VA, Ivanov LI, Vysochina NP, Igolkina IaP, Fomenko NV, et al. PCR detection of the causative agents of infections transmitted by ticks on the Kamchatka Peninsula. Med Parazitol (Mosk). 2010; 4: 36-39.

16. Rar VA, Epikhina TI, Livanova NN, Panov VV, Pukhovskaya NM, Visochina NP, et al. Detection of *Babesia* DNA in small mammals and ixodic ticks in North Urals, Western Siberia, and Far East of Russia. Mol Gen Micro Virol. 2010; 25: 118-123.

17. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725-2729.

18. Volkov VI. Medical ecological atlas of Khabarovsk territory and Jewish autonomous region. Khabarovsk (in Russian). 2005.

19. Shpynov SN, Fournier PE, Rudakov NV, Samoilenko IE, Reshetnikova TA, Yastrebov VK, et al. Molecular identification of a collection of spotted fever group rickettsiae obtained from patients and ticks from Russia. Am J Trop Med Hyg. 2006; 74: 440-443.

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