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A Serological Survey About Zoonoses in the Verkhoyansk Area, Northeastern Siberia (Sakha Republic, Russian Federation)

Jean-François Magnaval,^{1,2} Isabelle Leparc-Goffart,³ Morgane Gibert,² Alla Gurieva,⁴ Jonathan Outreville,⁵ Praskovia Dyachkovskaya,⁶ Richard Fabre,² Sardana Fedorova,⁷ Dariya Nikolaeva,⁸ Damien Dubois,⁹ Olga Melnitchuk,¹⁰ Pascale Daviaud-Fabre,¹¹ Marie Marty,² Anatoly Alekseev,¹² and Eric Crubezy²

Abstract

In 2012, a seroprevalence survey concerning 10 zoonoses, which were bacterial (Lyme borreliosis and Q fever), parasitic (alveolar echinococcosis [AE] and cystic echinococcosis [CE], cysticercosis, toxoplasmosis, toxocariasis, and trichinellosis), or arboviral (tick-borne encephalitis and West Nile virus infection), was conducted among 77 adult volunteers inhabiting Suordakh and Tomtor Arctic villages in the Verkhoyansk area (Yakutia). Following serological testing by enzyme-linked immunosorbent assay and/or western blot, no positive result was found for cysticercosis, CE, toxocariasis, trichinellosis, and both arboviral zoonoses. Four subjects (5.2%) had anti-*Toxoplasma* IgG, without the presence of specific IgM. More importantly, eight subjects (10.4%) tested positive for Lyme borreliosis, two (2.6%) for recently acquired Q fever, and one (1.3%) for AE. Lyme infection and Q fever, whose presence had not been reported so far in Arctic Yakutia, appeared therefore to be a major health threat for people dwelling, sporting, or working in the Arctic area of the Sakha Republic.

Key Words: Arctic—Seroepidemiology—Verkhoyansk—Yakutia—Zoonoses

Introduction

WHATEVER THEIR STATUS, worldwide or restricted to particular areas on the planet, zoonoses are recognized as an important source of disease (van Doorn 2014) and an increasing major threat for human health in the future (Learner 2013). Zoonoses are often acquired by contact with either domestic or pet animals or with wildlife, and this feature is enhanced by the permanently growing need of resources that leads humans to increase the pressure on the environment (Karesh et al. 2012). Moreover, climate change

may participate in the modification of natural foci of zoonotic diseases (Revich et al. 2008). This epidemiological situation prevails in Arctic and subarctic regions (Jenkins et al. 2013), including Siberia.

In 2007, a survey about the seroepidemiology of nine zoonoses was conducted in Vilújsk, Yakutia, by researchers from the Northeastern Federal University in Yakutsk and from University Toulouse III/CNRS UMR 5288 (Magnaval et al. 2011). The results from this field work prompted French and Russian scientific authorities to include research on zoonoses in a larger program entitled COSIE (Coevolution of

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Man and environment in Eastern Siberia). The Vilûjsk survey had been carried out in a subarctic area, ~325 km south of the Arctic Circle. Therefore, future investigations were intended to concern a more boreal zone and be performed in the administrative region of Verkhoyansk (latitude: 67°33'13.9" [67.5539°] North—longitude: 133°23'23.1" [133.3898°] East). Field information provided by archeologists also working inside the COSIE program selected two villages, Suordakh (latitude: 66°40'34.5" [66.6762°] North—longitude: 131°46'16.4" [131.7712°] East—elevation: 216 meters—325 inhabitants) and Tomtor (latitude: 67°12'17.9" [67.205°] North—longitude: 132°8'10.4" [132.1362°] East—elevation: 177 meters—282 inhabitants). Both villages are located on the Dulgalakh River on the eastern rim of the Khrebet Orulgan Mountains that are part of the Verkhoyansk Range. Climatic conditions are the harshest in the Northern Hemisphere, and Verkhoyansk has been nicknamed the Pole of Cold. According to the Climats™ database provided by Météo France (Toulouse, France), data concerning the Verkhoyansk weather station (elevation: 136 meters) indicate that the mean annual temperature is -15.9°C, the annual thermal amplitude ranges on average from -55°C in January to +20°C in July, and the average number of days with freeze is 285 a year.

The present article reports the results from a survey that was conducted from 27 March, 2012, to 4 April, 2012, in Suordakh and Tomtor villages to determine the seroprevalence of 10 agents of zoonoses and to identify possible risk factors for seropositivity. It should be recalled that such serological investigations detect only a contact with a given infectious agent, and not the related disease. However, the names of the zoonoses were used throughout the text due to a better convenience.

Materials and Methods

Ethical consideration

The study protocol and consent forms were approved by the Ethics Committee of the Siberian Branch of the Russian Academy of Sciences in Yakutsk (file no. 2012/4). Written informed consent was obtained from subjects for their enrollment in the study.

Study population

One hundred eleven apparently healthy adult volunteers gave their written informed consent to be enrolled in the study. To avoid any cluster effect, only one record per family was randomly selected at the statistical step. After the subjects completed a questionnaire inquiring about age, sex, family affiliations, occupation (present or past), ethnicity, housing conditions, and food habits, a 10 mL venous blood sample was taken in a vacuum glass vial. After clotting, the samples were centrifuged using a hand asset (Hettig AG, Tuttingen, Germany). Sera were collected in sterile vials and stored outside, where the temperature was never higher than -30°C. They were subsequently conveyed to Yakutsk and then shipped to France on dry ice.

Immunodiagnoses

In the Department of Parasitology of the University Hospitals in Toulouse, immunodiagnosis of four bacterial or parasitic zoonoses was conducted using exclusively

commercial reagents, either on an automated immunoassay system for toxoplasmosis (Architect® system, anti-*Toxoplasma* IgG/IgM; Abbott Labs, Wiesbaden, Germany) or by conventional microplate enzyme-linked immunosorbent assay (ELISA) for cysticercosis (*Taenia solium* IgG; DRG, Inc., Springfield Township, NJ), for Q fever (ELISA IgG classic *Coxiella burnetii* Phase II; Serion Immunodiagnostica GmbH, Würzburg, Germany), and for Lyme borreliosis (Enzygnost® Lyme link VlsE/IgG; DADE Behring, Marburg, Germany). Behring's assay was based upon a detergent extract from cultured *Borrelia afzelii* mixed with recombinant VlsE antigen from *Borrelia burgdorferi sensu stricto*, *B. afzelii*, and *Borrelia bavariensis* and was found efficient for the detection of long-standing Lyme infections (Marangoni et al. 2008). However, any serum that was found positive for Lyme borreliosis was subsequently tested for the presence of anti-*Treponema* antibodies using the TPHA kit (Lab21 Healthcare Ltd., Suffolk, United Kingdom).

In-house assays concerned the immunodiagnosis of four helminthozoonoses. For human toxocariasis, the detection of specific antibodies was based upon a western blotting (WB) procedure using in-house produced *Toxocara canis* larval excretory–secretory antigens (Magnaval et al. 1991). Serology of both alveolar echinococcosis (AE) and cystic echinococcosis (CE), and also of trichinellosis, relied upon a similar in-house ELISA. Briefly, 96-well flat-bottomed titration plates (Nunc Maxisorb®; Life Technology, Cergy Pontoise, France) were sensitized with a commercial soluble extract of *Echinococcus granulosus* protoscoleces or *Trichinella spiralis* larvae (SR2B, Avrillé, France), and the antigen concentration was 5 mg/mL for CE/AE and 6 mg/mL for trichinellosis. For both methods, antihuman immunoglobulin serum (anti IgG+A+M) conjugated with peroxidase (Bio-Rad, Hercules, CA) was used in addition to a kit for complementary reagents, for example, buffers and substrate (Enzygnost; DADE Behring). Detection of AE was therefore ensured by cross-reactivity between *E. granulosus* and *Echinococcus multilocularis* antigens. ELISA using such an *E. granulosus* protoscoleces extract has been found to have a sensitivity rate >99.9% for the detection of AE (Schweiger et al. 2012).

A two-tiered testing protocol was applied to the serodiagnosis of cysticercosis, AE, and CE. ELISA results exhibiting an optical density (OD) ≥ 0.100—namely threefold lower than the cutoff value—were verified using commercial WB kits, which were Cysticercosis WB IgG™ and Echinococcus WB IgG™ (LDBIO Diagnostics, Lyon, France). The latter uses an *E. multilocularis* whole larval extract and proved able to discriminate between AE and CE (Liance et al. 2000).

In the French National Reference Centre for Arboviruses (IRBA) in Marseilles, the detection of IgG specific for tick-borne encephalitis (TBE) and infection by West Nile virus (WNV) was achieved by in-house IgG ELISA. Microplates were coated with specific viral antigens prepared on Vero cells and inactivated by betapropiolactone (Sigma-Aldrich, St Quentin-Fallavier, France). A specific binding was demonstrated by using a peroxidase-labeled goat anti-human IgG conjugate (Interchim, Montluçon, France). A result was considered positive if the ratio, OD of the sample/mean OD of negative test sera, was ≥ 3.0.

TABLE 1. RESPECTIVE SENSITIVITY AND SPECIFICITY OF THE ASSAYS USED FOR THE SEROLOGICAL SURVEY

Infectious agent (disease)	Test	Sensitivity (%)	Specificity (%)
Bacteria			
<i>Borrelia burgdorferi</i> s.l. (Lyme disease)	ELISA VIsE ^a	72.4	98.3
<i>Coxiella burnetii</i> (Q fever)	ELISA (Phase II) ^a	94.2	96.2
Parasites			
<i>Echinococcus multilocularis</i> (alveolar echinococcosis)	ELISA Western blot ^a	NA (two-tiered protocol) 96.7	97
<i>Echinococcus granulosus</i> (cystic echinococcosis)	ELISA Western blot ^a	NA (two-tiered protocol) 98	98
<i>Taenia solium</i> (cysticercosis)	ELISA Western blot ^a	NA (two-tiered protocol) 74	100
<i>Toxoplasma gondii</i> (toxoplasmosis)	ELISA-IgG ^a ELISA-IgM ^a	97.5 89.9	99.1 99.8
<i>Toxocara</i> spp. (toxocarasis)	Western blot ^b	97	100
<i>Trichinella</i> spp. (trichinellosis)	ELISA ^b	89	96
Viruses			
Tick-borne encephalitis	ELISA ^b	99.8	95.6
West Nile infection	ELISA ^b	93.7	99.9

^aAccording to commercial sources.

^bInternal assessment.

ELISA, enzyme-linked immunosorbent assay; NA, not applicable.

Respective sensitivity and specificity of these serological assays are displayed in Table 1.

Statistical analysis

Records from only 77 volunteers of 111 (21 at Suordakh and 56 at Tomtor) were analyzed (see explanation above). The effects of the investigated factors on the seroprevalence of 10 zoonoses were assessed using χ^2 and Fisher's exact tests to analyze the distributions and Mann-Whitney and Spearman's tests to search possible correlations between continuous variables. All tests were implemented by the Statistica[®] package (StatSoft, Inc., Tulsa, OK). The significance level for *p*-value was <0.05.

Results

The demographic and occupational characteristics of the study subjects are displayed in Table 2, and Table 3 shows the environmental features and the food habits of the surveyed population. Because of a similar environment and way of life in both villages, the criterion, place of residence, was not entered.

Table 4 shows the results of the serological investigations. No positive result was observed for cysticercosis, CE, toxocarasis, trichinellosis, and both arboviral zoonoses. The positivity rate was 10.4% for Lyme borreliosis, 5.2% for toxoplasmosis (only specific IgG were detected), 2.6% for recently acquired Q fever, and 1.3% for AE. All patients' sera exhibiting a positive result for Lyme serology tested negative by *Treponema* TPHA.

Bivariate analysis of the data set using χ^2 , Fisher's, and Mann-Whitney tests did not find any correlation between the outcome variables, namely the serology results stratified as positive or negative, and the possible exposure variables listed in Tables 2 and 3. No correlation was found between age and serology results considered as continuous variables (Spearman's test).

Discussion

First, the study population was essentially a convenience sample rather than representative of the entire population of Suordakh and Tomtor villages, so results concerning the observed diseases must be cautiously considered. Then, Russian literature was used when the articles were written in English and available through Internet.

A prominent finding concerning bacterial zoonoses was a 10.4% seroprevalence rate for Lyme borreliosis. Although Lyme disease in western Siberia may be caused by *B. afzelii* or *Borrelia garinii* (Rar et al. 2005), ELISA using VIsE recombinant antigen can detect antibodies directed against these species belonging to the *B. burgdorferi* sensu lato complex (Wilske 2005). According to Russian sources, northeastern Siberia was considered as free of Lyme borreliosis (Malkhazova et al. 2014). However, specimens of the tick *Ixodes persulcatus*, specifically the vector for *B. burgdorferi* s.l. in Siberia (Eisen and Lane 2002), have been recently found in Yakutia (Revich et al. 2012). Moreover, during the 2007 Vil'jusk survey, Lyme serology using indirect immunofluorescence detected 9 positive results in 90 subjects (Magnaval et al. 2011), and a survey carried out among 41 rural volunteers from the same Vil'jusk area found a 19.5% positivity rate using ELISA, followed by WB (Storch et al. 2008). These findings suggest that infection by *B. burgdorferi* s.l. would have spread to the Arctic areas of the Sakha Republic from not officially recognized foci that pre-existed in subarctic Yakutia. This could be due to climate change, since the level of ambient temperature regulates tick density, and the periods of the year that ticks are active (Eisen and Lane 2002); in Yakutia, the annual average temperature has increased by 1.1°C between 1955 and 2000 (Revich et al. 2008).

A similar discrepancy between available data and the results from the present survey arose about Q fever. Human cases were reported from eastern Siberia during the USSR era (Revich et al. 2012), but more recent epidemiological

TABLE 2. DEMOGRAPHIC AND OCCUPATIONAL CHARACTERISTICS OF 77 ADULTS FROM SUORDAKH AND TOMTOR VILLAGES, SAKHA REPUBLIC

	Females (n=53)	Males (n=24)	
Ethnicity, <i>n</i>			
Even	5	1	
Kyrgyz	0	1	
Yakut	48	22	
Age group (years), <i>n</i>			Occupation ^{a,b} [n]
18–29	8	3	<i>Unemployed</i> [3], <i>B</i> [1], <i>C</i> [4], <i>F</i> [1], <i>J</i> [1], <i>N</i> [1]
30–49	19	11	<i>Unemployed</i> [1], <i>A</i> [1], <i>B</i> [2], <i>C</i> [7], <i>E</i> [2], <i>G</i> [1], <i>H</i> [1], <i>J</i> [1], <i>K</i> [1], <i>L</i> [3], <i>M</i> [1], <i>N</i> [7], <i>O</i> [1], <i>P</i> [1]
≥ 50	26	10	<i>Retired</i> [13], <i>unemployed</i> [1], <i>A</i> [1], <i>B</i> [3], <i>C</i> [3], <i>E</i> [1], <i>F</i> [1], <i>I</i> [3], <i>J</i> [1], <i>L</i> [4], <i>N</i> [5]
Range of age values in females or males (groups)	(21–82)	(18–81)	

^aItalics represents occupation.

^bStandard occupational classification, according to the Bureau of Labor Statistics, US Department of Labor (www.bls.gov/soc/major_groups.htm).

A, management occupations; B, community and social service occupations; C, education, training, and library occupations; D, arts, design, entertainment, sports, and media occupations; E, healthcare practitioners and technical occupations; F, healthcare support occupations; G, protective service occupations; H, food preparation and serving-related occupations; I, personal care and service occupations; J, sales and related occupations; K, office and administrative support occupations; L, farming, fishing, and forestry occupations; M, construction and extraction occupations; N, installation, maintenance, and repair occupations; O, production occupations; P, transportation and material moving occupations.

TABLE 3. ENVIRONMENTAL CHARACTERISTICS AND FOOD HABITS OF 77 ADULTS FROM SUORDAKH AND TOMTOR VILLAGES, SAKHA REPUBLIC

	n (%)	Mean	SD	95% CI
Environment				
No. of rooms per house		3.58	1.59	3.22–3.94
No. of persons per house		3.92	2.05	3.46–4.38
Toilets outside house	75 (97.4)			91–99.3
Having a datcha ^a	30 (39)			28.8–50.1
Distance between datcha and home (km)		4.65	8.14	2.83–6.47
Having a kitchen garden				
In the field (summer)	63 (81.8)			71.8–88.8
In a greenhouse	52 (67.5)			56.5–76.9
Possession of cats	33 (42.9)			32.4–54
No. of cats per subject		0.44	0.52	0.32–0.56
Possession of dogs	58 (75.3)			64.6–83.6
No. of dogs per subject		1.42	1.61	1.06–1.78
Cattle breeding	38 (49.4)			
No. of cows per subject		3.0	3.6	2.2–3.8
Horse breeding	38 (49.4)			38.5–60.3
No. of horses per subject		5.6	10.5	3.25–7.95
Fishing	46 (59.7)			48.6–70
Hunting	24 (31.2)			21.9–42.2
Food habits				
Raw beef ^b	1 (1.3)			0.2–7.0
Raw horse meat ^b	71 (92.2)			84–96.4
Pork meat ^c	37 (48.1)			37.3–59
Reindeer meat ^c	59 (76.6)			66–84.7
Poultry ^c	67 (87.0)			77.7–92.8
Raw freshwater fish ^b	67 (87.0)			77.7–92.8
Game birds ^c	75 (97.4)			91–99.3
Wild game ^c	69 (89.6)			80.8–94.6
Bear meat ^c	15 (19.5)			12.2–29.7
Wild mushrooms ^c	67 (87.0)			77.7–92.8
Raw wild berries	76 (98.7)			93–99.8

^aSecondary house for farming, fishing, or hunting.

^bAll subjects also used this food well cooked.

^cAlways well cooked.

CI, confidence interval; SD, standard deviation.

TABLE 4. RESULTS OF SEROLOGICAL TESTING FOR 10 ZONOSSES AMONG 77 ADULTS FROM SUORDAKH AND TOMTOR VILLAGES, SAKHA REPUBLIC

Zoonosis	<i>Lyme borreliosis</i>	<i>Q fever</i>	AE	
Method	ELISA IgG	ELISA IgG	ELISA+WB	
Cutoff value	0.160 (OD)	20 (IU)	0.100 (OD) NA	
No. of positive	8	2	1	
Prevalence (%)	10.4	2.6	1.3	
95% confidence interval	5.4–19.2	0.7–9	0.2–7	
Zoonosis	CE		Cysticercosis	
Method	ELISA+WB		ELISA+WB	
Cutoff value	0.100 (OD) NA		0.100 (OD) NA	
No. of positive	0.0		0.0	
Prevalence (%)	0.0		0.0	
95% confidence interval	0.0–4.8		0.0–4.8	
Zoonosis	<i>Toxocariasis</i>	<i>Toxoplasmosis</i>		<i>Trichinellosis</i>
Method	WB	ELISA IgG	ELISA IgM	ELISA
Cutoff value	NA	3.0 (IU)	0.6 (OD)	0.5 (OD)
No. of positive	0.0	4.0	0.0	0.0
Prevalence (%)	0.0	5.2	0.0	0.0
95% confidence interval	0.0–4.8	2.0–12.6	0.0–4.8	0.0–4.8
Zoonosis	<i>Tick-borne encephalitis</i>		<i>West Nile infection</i>	
Method	ELISA IgG		ELISA IgG	
Cutoff value	3.0 (Index)		3.0 (Index)	
No. of positive	0.0		0.0	
Prevalence (%)	0.0		0.0	
95% confidence interval	0.0–4.8		0.0–4.8	

AE, alveolar echinococcosis; CE, cystic echinococcosis; IU, international unit; NA, not available; OD, optical density; WB, western blot.

information from this area is not currently available in the English literature. The presence of IgG antibodies against Phase II antigens is evocative of an acute or recent infection, so the finding of two positive results (2.6%) suggests that Q fever has a rather high incidence rate in the studied rural population. An analysis of the official Russian statistics for the period 1985–2005 yielded a mean annual incidence in Russia of ~1 case per million of population (Tokarevich et al. 2006), but it was stated that Q fever was obviously underdiagnosed.

Among parasitic zoonoses, specific immunodiagnoses tested negative for cysticercosis, toxocariasis, and trichinellosis. Concerning toxocariasis, the Vilûjsk survey (Magnaval et al. 2011), which also used WB, found a 4.4% seroprevalence rate among 90 subjects, a result which was not significantly different from the 0% recorded in this study (Fisher’s exact test). In Canada, in the Arctic region of Nunavik, which extends from 55° N to 62° N, the seroprevalence rate by ELISA was 1.7% among 2213 volunteers (Goyette et al. 2014). According to Russian official statistics, the incidence rate for toxocariasis would be very low, ranging from 0.26 per 100,000 in the Sakha Republic to 0.44 per 100,000 in the Arctic district of Arkhangelsk (Dudarev et al. 2013). However, the assessment of the incidence rate is not appro-

priate to study the epidemiology of this long-standing zoonosis—because it is quite difficult to distinguish between current and past infections (Smith et al. 2009)—and was actually never used (Lee et al. 2014). Undoubtedly, the harsh Arctic climate destroyed *Toxocara* sp. eggs in the soil and therefore dampened the diffusion of this mostly soil-transmitted helminthozoonosis.

For cysticercosis, the international literature did not provide any information about the prevalence of this helminthiasis in Arctic areas in America or Europe or in Siberia (Dudarev et al. 2013). In the present study, where no positive result was observed, only 48.1% of the subjects reported a food habit of pork meat that was always well cooked. This factor was further circumstantial evidence that any human infection with the adult form of *T. solium* was absent.

The food-borne route, through ingestion of meat from various domestic or wild carnivorous or omnivorous mammals, and also from horses, is the major way of contamination for human trichinellosis. The absence of any positive serological result in a population that has a food habit of meat from hunted bears or from locally slaughtered horses (Table 3) was therefore surprising. In Vilûjsk (Magnaval et al. 2011), the seroprevalence was 4.4%, but did not significantly differ from the 0% rate found in the present survey (Fisher’s exact test). In Alaska, only sporadic cases or small epidemics have been reported (Jenkins et al. 2013). In Nunavik, the seroprevalence of trichinellosis in the Inuit population was as high as 18.6% (Goyette et al. 2014), but the prominent risk factor was a food habit of raw meat from freshly killed walruses (Larrat et al. 2012), a culinary tradition, which is unknown in the Verkhoyansk area. Russian official statistics reported not only a 0.31 per 100,000 incidence rate for trichinellosis in the entire Sakha Republic but also very low incidence values from Arctic Russia, ranging from nil in the Murmansk district to 0.01 per 100,000 in the Arkhangelsk district (Dudarev et al. 2013), all facts which were in accordance with the zero seroprevalence found in the Verkhoyansk area. Explanation of this result could have a climatic origin: in the Arctic or subarctic areas of Yakutia, people used to store meat from game or domestic animals outside the home for weeks, in pantries that are therefore exposed for at least 8 months a year to temperatures under –20°C, and this storage habit could achieve natural sanitization.

Concerning echinococcoses, seroprevalence was found to be zero for CE, similar to the Vilûjsk survey (Magnaval et al. 2011), but one AE case was detected (1.3%). In Alaska, only 13 CE human cases have been diagnosed by imaging and/or surgery between 1987 and 2009 (Jenkins et al. 2013) among a population that had grown from 540,000 up to 700,000 residents. In Nunavik, the seroprevalence, as assessed by a commercial ELISA kit using a crude antigenic extract of *E. granulosus*, was 6.3% among 2213 subjects (Goyette et al. 2014). For AE, the presence of only one positive result of 77 should be cautiously considered because reliable elements of comparison with other Arctic areas are lacking. In western Alaska, the seroprevalence ranged in 1980 from <1% to 1.6% in the villages located on the Alaskan shore of the Chukchi Sea or on the small islands in the Bering Strait (Jenkins et al. 2013). However, the sensitivity and the specificity of the immunodiagnostic methods used in the late 1970s are questionable. No data are available for Nunavik, and concerning Europe, AE in humans has not been detected in northern

Scandinavia (Wahlström et al. 2012). The combination of these facts with the finding of this single Yakutian-positive result suggests that AE transmission would occur at a low level in Arctic Yakutia. Unfortunately, the Russian official statistics do not distinguish between AE and CE and report only the incidence for echinococcoses, which ranged from 0.04 per 100,000 to 1.21 per 100,000 (Dudarev et al. 2013). Because *Echinococcus* sp. eggs that would be spread in a humid soil are then extremely resistant to extreme temperatures (Jenkins et al. 2013), the Arctic environment cannot explain such a low level of seroprevalence for CE and AE, and therefore this finding requires further investigation.

For toxoplasmosis, the 5.2% seroprevalence found in the present study did not significantly differ from the 8.9% rate reported from Viljusk (Magnaval et al. 2011). In Alaska, a 16% seroprevalence rate was found in 1975 from 1572 natives (Peterson et al. 1974). However, the expression of the serology results in dilution, and not in international units, made any comparison with the current result from Yakutia impossible. In Nunavik, a 27.2% seroprevalence was found among 2209 Inuit subjects, a result that significantly differed (Fisher's exact test: 0.000001, $p=0.000002$) from the 5.2% rate found in the Verkhoyansk area. Consumption of meat from marine mammals, a food habit that was absent in the Verkhoyansk area, was identified as the only risk factor in Nunavik (Goyette et al. 2014). In the studied Yakutian communities, classical risk factors for toxoplasmosis transmission, namely the presence of domestic cats at home (42.9%) and the food habit of raw horse meat (92.2%), were prominent. These facts made the observed 5.2% seroprevalence surprising. Again, the rough climatic conditions can be suspected to play a major role in the reduction of toxoplasmosis transmission. The long Siberian cold season with a deep freeze certainly affects the survival of oocysts in the environment and therefore the infection rate in meat-producing animals (Hill and Dubey 2002). Moreover, freeze sanitizes meat by destruction of the cysts containing bradyzoites. For example, tissue cysts in pork meat were rendered nonviable following exposure at -12°C for 3 days (Dubey 1988). Similar to trichinellosis, the local habit of storing meat in external pantries during the cold season or, during summer, in cellars deeply dug in the permafrost certainly drastically lowers toxoplasmosis transmission in Arctic Yakutia.

No seroprevalence was recorded for TBE and WNV infections, and the difference with the results from the Viljusk survey (Magnaval et al. 2011) was not significant (Fisher's exact test). Currently, the main endemic area of TBE is a belt extending from eastern Europe to China and Japan (Lindquist and Vapalahti 2014) with the 60° N parallel as the northern limit (Estrada-Peña and de la Fuente 2014). However, due to a patchy distribution of active foci and also to the climate change, this zoonosis has to be considered outside this traditional North border (Revich et al. 2008). In the Arctic Russia, TBE has been reported on a regular basis, for example, from the Arkhangelsk district or from the northern part of the Komi Republic (Revich et al. 2008). This epidemiological fact, combined with the likely presence of *I. persulcatus* ticks in the Verkhoyansk area, as suspected from the Lyme seroprevalence found in the present study, suggests that foci of TBE could exist in northern Yakutia. Further surveys, including a molecular-based search of TBE viruses in ticks, should therefore be envisaged. WNV infection oc-

curs mostly in temperate climates, but several strains of WNV have been isolated from ticks, birds, and mosquitoes in the southern area of European Russia and western Siberia (Platonov 2001), and occasional foci of human infection have been reported in northern Europe and Siberia (Platonov et al. 2014). These findings indicate that the lack of positive results in the present study should be considered similarly to TBE.

In conclusion, in the Verkhoyansk district, Lyme borreliosis and Q fever appeared to be transmitted to humans on a regular basis. In the case of a possible presence outside this area, these zoonoses would represent a major threat to the health of people living, sporting, or working in Arctic Yakutia. The epidemiological situation of AE and CE in the Verkhoyansk region has to be clarified. Climate change can be incriminated in the spreading of Lyme borreliosis in Yakutia and particularly toward upper latitudes in this country (Revich et al. 2012), whereas the recognized epidemiology of AE and Q fever suggests that both zoonoses have been present in Arctic Yakutia for a long time.

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