








A serological survey of echinococcosis, toxocariasis and trichinellosis among rural inhabitants of Central Yakutia

Sergey S. Nakhodkin ^a, Vera G. Pshennikova ^{a,b}, Paraskovia S. Dyachkovskaya ^c, Nikolay A. Barashkov ^{a,b}, Alena A. Nikanorova ^{a,b}, Fedor M. Teryutin ^{a,b}, Olga A. Melnichuk ^d, Eric Crubézy^e, Sardana A. Fedorova^{a,b} and Jean-François Magnaval^f

^aLaboratory of Molecular Biology, Institute of Natural Sciences, Maksim K. Ammosov Northeastern Federal University, Yakutsk, Russia; ^bDepartment of Molecular Genetics, Yakut Scientific Center of Complex Medical Problems, Yakutsk, Russia; ^cInstitute of Medicine, Maksim K. Ammosov Northeastern Federal University, Yakutsk, Russia; ^dInstitute of Foreign Philology and Regional Studies, Maksim K. Ammosov Northeastern Federal University, Yakutsk, Russia; ^eCNRS UMR 5288 “AMIS”, Université Paul-Sabatier, Toulouse, France; ^fDepartment of Medical Parasitology, Université Paul-Sabatier, Toulouse, France

ABSTRACT

In 2018, a seroepidemiological survey was carried out in 3 *ulus*, or districts (Churapchinsky, Megino-Kangalassky and Ust-Aldansky) in Central Yakutia (Sakha Republic, Russian Federation) about 3 helminth zoonoses, namely, echinococcosis (alveolar or cystic), toxocariasis and trichinellosis. Ninety rural volunteers agreed to answer a questionnaire that inquired about demographic and environmental parameters along with food habits. Then they were asked to provide a venous blood sample. Serological investigations were carried out by enzyme-linked immunosorbent assay. Four subjects tested positive for echinococcosis, 1 for toxocariasis and 2 for trichinellosis. No demographic or environmental or dietary possible risk factor was found to be associated with these positive results. In conclusion, only echinococcosis and trichinellosis appeared to be in Yakutia as health threats among the 3 investigated zoonoses.

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Introduction

In Eastern Siberia, Yakutia, or the Sakha Republic, is a large country (3,083 523km²) in which 959,689 inhabitants are in close contact with the environment and particularly with domestic or wild animals, even in the outskirts of the capital Yakutsk. Therefore, zoonoses (bacterial, parasitic or viral) are considered to be a major health threat in this Republic. Concerning helminth zoonoses, more than 20 million individuals in Russia have been found to suffer from helminthiases, mostly of zoonotic origin, according to Rospotrebnadzor, the Federal Service for Supervision of Consumer Rights Protection and Human Welfare [1]. In the Sakha Republic, the incidence of zoonotic helminthiases between 1995 and 2017 ranged from 3,500 to 5,000 cases per year [2]. Fish tapeworm infection due to 1 of the 3 *Diphyllobothrium* species present in the country was the most common helminth zoonosis. It was followed by echinococcosis (no distinction was made between alveolar and cystic forms). However, it should be noted that the reports to the Ministry of Health are based upon clinical observations, or radiological or surgical findings. Consequently, the prevalence of tissue-dwelling helminth

zoonoses such as toxocariasis or trichinellosis might have been underestimated.

In 2007 and 2012, 2 serological surveys of various bacterial, parasitic or viral zoonoses were conducted in the subarctic Vilyuysky *ulus*—approximately, district—in the northwestern part of the country [3], and in the northern arctic Verkhoyansky *ulus* [4]. Rather surprisingly, the results showed the seroprevalence rates for partially or totally soil-transmitted helminth zoonoses, namely, alveolar echinococcosis (AE), cystic echinococcosis (CE) or toxocariasis, ranged from nil to very low values. To improve the knowledge of the epidemiology of tissue-dwelling helminthiases in Yakutia, a serological survey was therefore carried out in the central part of the Republic during the year 2018.

Materials and methods

Ninety adult volunteers of both sexes were investigated. Twenty-seven lived in the Churapchinsky *ulus*, in Maralay village (61°59' N 131°55' E, 837 inhabitants), 39 in the Megino-Kangalassky *ulus*, in Pavlovsk village (61°52' N 129°53' E, 2091 inhabitants) and 24 in the Ust-

CONTACT Jean-François Magnaval  jean-francois.magnaval@univ-tlse3.fr  Department of Medical Parasitology, Faculty of Medicine, Université Paul-Sabatier, 37 allées Jules-Guesde, Toulouse 31000, France

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Aldansky ulus, in Borogontsy town (62°40' N 131°08' E, 5,222 inhabitants) or the surrounding area. Every volunteer had to complete a questionnaire inquiring about age, sex, ethnicity, housing conditions and food habits. Then, a 10-mL venous blood sample was taken. The samples were transported to the Laboratory of Molecular Biology, Institute of Natural Sciences, Maksim K. Ammosov Northeastern Federal University in Yakutsk where serodiagnostic tests for echinococcosis, toxocariasis and trichinellosis were performed by ELISA. All kits (Echinococcus IgG, Toxocara canis IgG and Trichinella spiralis IgG) were purchased from DRG Diagnostics GmbH, Marburg, Germany, and assays were carried out according to the manufacturer's instructions using a Victor X5 Multilabel plate reader (Perkin-Elmer, Waltham, MA). The cut-off value (a positive result) was ≥ 11 DRG units for the Echinococcus kit, and ≥ 0.5 optical density (OD) units for both the Toxocara and Trichinella kits. DRG claimed that the sensitivity was 97% and the specificity was 100% for the Echinococcus ELISA, 87.5% and 93.3 for the Toxocara ELISA, 100% and 100% for the Trichinella ELISA, respectively. It should be noted that the Echinococcus IgG assay is not species-specific and cannot differentiate between alveolar or cystic echinococcosis infections. Statistical analysis of the data from the questionnaire and serology results used Statistica (TIBCO Software Inc., Palo Alto, CA). χ^2 test was mostly used to compare the frequency distribution for every variable, and Fisher's exact test was employed when an outcome value in a contingency table was lesser than 5. Finally, the results from the serological testing were given individually to every volunteer. Subjects who were found positive, whatever the tested zoonosis, were urged to get in touch with a medicine doctor in order to perform further medical investigations.

Ethical standards

The study protocol and consent forms were approved by the Committee on Biomedical Ethics at the Yakut Scientific Center for Complex Medical Issues (file no. 30/4–2012). Written informed consent was obtained from all individual volunteers included in the study.

Results

Table 1 displays the demographic characteristics of the study subjects, and Table 2 shows their environmental features and their food habits. The sex ratio was 0.53, and the age range (years) was 21–80 for women and 21–74 for men. The results of the serological investigations showed that the seroprevalence rate was 4.4%

Table 1. Demographic characteristics of 90 adults from Churapchinsky, Megino-Kangalassky and Ust-Aldansky ulus,* Sakha Republic (Russian Federation).

	n	%	95% CI [†]
Sex			
Women	59	65.6	54.7–75.1
Men	31	34.4	25.0–45.3
Classes of age (years)			
21–29			
Women	6	6.7	2.7–14.5
Men	4	4.4	1.4–11.6
30–49			
Women	26	29.0	20.1–39.6
Men	11	12.2	6.6–21.2
≥ 50			
Women	27	30	21.0–40.7
Men	16	17.8	10.8–27.6
Ethnicity			
Even	2	2.2	0.4–8.6
Yakut	83	92.2	84–96.6
Yakut/Even ‡	3	3.3	0.9–10.1
Yakut/Evenk [‡]	2	2.2	0.4–8.6
Place of residence (ulus*)			
Churapchinsky	27	30	21.0–40.7
Megino-Kangalassky	39	40.3	33.1–54.2
Ust-Aldansky	24	26.7	18.2–37.2

*(approximately) district.

[†]confidence interval of the proportion.

[‡]mixed blood.

(95% confidence interval [CI]: 1.4–11.6) for echinococcosis (4 positive subjects), 1.1% (95% CI: 0.6–6.9) for toxocariasis (1 positive) and 2.2% (95% CI: 0.4–8.6) for trichinellosis (2 positive). Bivariate analysis of the data set 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 1 and 2.

Discussion

In the Russian Federation, both AE and CE are endemic [5] and the overall incidence of echinococcosis was 0.28 per 100,000 in 2016 [1]. Concerning specifically CE, this situation likely originates in the globally high infection level of dogs, which ranges from 67% up to 100% in most parts of the Federation [6]. Echinococcosis is considered to be a health problem in Yakutia where the incidence was 0.94 per 100,000 in 2017 [7].

From the USSR era to the turn of the 2000s, Central Yakutia had been considered as a focus of transmission for echinococcosis [5] and the results of the present study confirm this status. No significant difference was found (Fisher's exact test) between the 4.4% rate in Central Yakutia and the 0% rates (AE or CE) in Vilyuysk [3] or the 0% (CE)/1.1% (AE) in Verkhoyansk [4]. However, these findings need to be confirmed by larger studies. Moreover, discrepancy exists between the high degree of *Echinococcus* sp. infection in Yakut dogs and the relatively low seroprevalence rate in humans. Such low levels of

Table 2. Environmental characteristics and food habits of 90 adults from Churapchinsky, Megino-Kangalassky and Ust-Aldansky ulus,* Sakha Republic (Russian Federation).

	n	%	95% CI†
Environment			
Having a kitchen garden	70	77.9	67.6–85.6
Toilets outside house	72	80.00	70.0–87.4
Cattle breeding	40	44.4	34.1–55.3
Horse breeding	31	34.4	25.0–45.3
Possession of pet animals	59	65.6	54.7–75.1
Possession of cats	22	24.4	16.3–34.8
Possession of dogs	44	48.9	38.3–59.6
Fishing	35	38.9	29.0–49.8
Gathering	81	90.0	81.4–95.0
Hunting	24	26.7	18.2–37.2
Food habits			
Beef	85	94.4	86.9–97.9
Raw beef	9	10.00	5.0–18.6
Horse meat	79	87.8	78.8–93.5
Raw horse meat	67	74.4	64.0–82.8
Pork	70	77.8	67.6–87.6
Raw pork	5	5.6	2.1–13.1
Reindeer meat‡	20	22.2	14.4–32.5
Bear meat	6	6.7	2.7–14.5
Raw bear meat	1	1.1	0.6–6.9
Raw venison	2	2.2	48.0–69.0
Game birds	53	59.9	50.2–71.0
Raw game birds	3	3.3	0.9–10.1
Freshwater fish	80	88.9	80.1–94.3
Raw freshwater fish	45	50	39.4–60.7
Wild berries	84	93.3	85.5–97.3
Raw wild berries	61	67.8	57.0–77.0
Wild mushrooms	76	84.4	74.9–90.9
Wild raw mushrooms	9	10	5.0–18.6
Imported fruits	85	94.4	86.9–97.9
Home grown vegetables	85	94.4	86.9–97.9

*(approximately) district.

†confidence interval of the proportion.

‡always cooked.

human infection have been observed in endemic countries where control programmes for canine infection, based upon periodic administration of praziquantel, have been implemented [8]. This is not the case in Yakutia. Since *Echinococcus* sp. eggs which would be spread in humid soil are very resistant to extreme temperatures [9] the harsh Yakut climate cannot explain this discrepancy. An explanation may lie in the genetic diversity of *Echinococcus* sp. in Russia. A recent study has demonstrated that the species predominant in Yakutia, where husbandry concerns mainly cattle or horses, but not sheep, is *E. canadensis* including the genotypes G6, G8 and G10 [10]. In the human intermediate host, this species elicits predominantly pulmonary cysts [11] that are less detectable by serology than hepatic involvement [12]. Interestingly, data from Canada, another large subarctic country where *E. canadensis* is predominant, indicated a lesser incidence of CE, since only 108 cases have been reported between 2001 and 2005. A lesser infection rate of dogs and wolves (from 20% to 30%) could explain this discrepancy [9].

In 2013, in the Federation of Russia, the incidence of toxocariasis, based on clinical reports, was 2.12 per

100,000 for adults and 4.88 per 100,000 for children and teenagers under 17 years old [13]. In 2016, the rate was 0.31 per 100,000 in the Sakha Republic [7], or approximately 3 cases a year. The present survey found a 1.1% seroprevalence rate, not significantly different (Fisher's exact test) from the previous findings in Vilyuysk (4.4%) or Verkhoyansk (0%). However, children were not included for ethical reasons in these studies and in the present work. Perhaps this recruitment bias has influenced the seroprevalence result. Human toxocariasis is mostly a benign, asymptomatic, self-limiting helminthiasis that leaves residual antibodies in self-cured subjects [14]. Therefore, seroprevalence rather than incidence is a marker of the level of transmission, which appears to be very low in Yakutia. Nonetheless, this finding is surprising due to the high prevalence in Russia of dogs harbouring adult *Toxocara* roundworms—from 30.5% to 81.8% [6]—and the proportion of subjects in this study who owned dogs (48.9%). Toxocariasis is primarily a soil-transmitted zoonosis, and the presence of *Toxocara*-infected dogs in the close environment is a well-established major risk factor [15]. However, viability of *Toxocara* sp. ova is affected by desiccation or freezing [16]. Therefore, it is likely that the long Siberian winter (in Yakutsk, 8 months per year with a minimum temperature of -42°C in January) would sanitise the soil by destroying roundworms' propagules, and thus would dampen the infection rate. In the Cree communities of the Subarctic area of James Bay, in Canada (around 54°N), the seroprevalence of toxocariasis ranged from 0% to 10% [17]. According to the 95% CI values, these results were similar to the rate found in Central Yakutia.

According to Rospotrebnadzor, in 2016 the incidence of trichinellosis in the European part of the Russian Federation was 0.09 per 100,000 [1]. In Yakutia, the incidence was 0.2 per 100,000 in 2015, and nil in 2016 [7]. The seroprevalence rate in Vilyuysk (4.44%) or in Verkhoyansk (0%) did not significantly differ (Fisher's exact test) from the 2.2% result found in the present survey. In Eastern Siberia, the most frequent source of infection is meat from brown (*Ursus arctos collaris*) or polar (*Ursus maritimus*) bears that accounted for 60.2% of the outbreak cases recorded between 1998 and 2002 [18]. Consumption of pork was the second major risk factor, followed by badger meat (*Meles leucurus sibiricus*). Interestingly, both cases detected by ELISA in the present survey had consumed pork but not cooked or raw bear meat. Since *Trichinella nativa* likely is the predominant species in Yakutia [19], it could be feared an ELISA using *T. spiralis* excretory-secretory antigens gave false negative results. However, the use of such a commercial

assay to investigate patients during a recent outbreak of trichinellosis due to *T. nativa* [20] found an excellent sensitivity (7 positive results from 8 convalescent sera).

As a general conclusion, it should be underlined first that the study population was a convenience group that is likely not representative of the Central Yakutia population. Particularly, the overrepresentation of female gender in the present study represented a significant bias, so the results must be cautiously considered. In addition, no firm tie exists between the annual incidence such as those reported by Rospotrebnadzor and the seroprevalence rates found in the present study. However, no blatant discrepancy appeared between both indexes, regardless of the considered helminth zoonosis. According to the results of the present and previous studies [3,4] and the Rospotrebnadzor data, clearly toxocarasis does not represent a health problem in the Sakha Republic. Finally, echinococcosis (AE or CE) and trichinellosis seem to be the major health threats in Central Yakutia. Concerning echinococcosis, the results of the present study should be confirmed by field surveys combining serology and ultrasonography in the human intermediate host [12]. Respective parts of AE or CE will have to be clarified by checking by computerised tomography any subject found positive by ultrasonography and by using Western blot to confirm the positive ELISA results [21]. These future studies also will have to investigate concomitantly the canine definitive hosts. The search for *E. granulosus* DNA in the faeces will yield crucial information for assessing the transmission pressure. Moreover, genotype studies will have to be carried out on this faecal material in order to clarify the role of *E. canadensis*.

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Disclosure statement

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ORCID

Sergey S. Nakhodkin  <http://orcid.org/0000-0002-6917-5760>
 Vera G. Pshennikova  <http://orcid.org/0000-0001-6866-9462>
 Paraskovia S. Dyachkovskaya  <http://orcid.org/0000-0002-7818-7018>
 Nikolay A. Barashkov  <http://orcid.org/0000-0002-6984-7934>
 Alena A. Nikanorova  <http://orcid.org/0000-0002-7129-6633>
 Fedor M. Teryutin  <http://orcid.org/0000-0002-8659-0886>
 Olga A. Melnichuk  <http://orcid.org/0000-0002-5864-3434>

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