Genetic diversity of *Echinococcus* spp. in Russia

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SUMMARY

In Russia, both alveolar and cystic echinococcoses are endemic. This study aimed to identify the aetiological agents of the diseases and to investigate the distribution of each *Echinococcus* species in Russia. A total of 75 *Echinococcus* specimens were collected from 14 host species from 2010 to 2012. Based on the mitochondrial DNA sequences, they were identified as *Echinococcus granulosus* sensu stricto (s.s.), *E. canadensis* and *E. multilocularis*. *E. granulosus* s.s. was confirmed in the European Russia and the Altai region. Three genotypes, G6, G8 and G10 of *E. canadensis* were detected in Yakutia. G6 was also found in the Altai region. Four genotypes of *E. multilocularis* were confirmed; the Asian genotype in the western Siberia and the European Russia, the Mongolian genotype in an island of Baikal Lake and the Altai Republic, the European genotype from a captive monkey in Moscow Zoo and the North American genotype in Yakutia. The present distributional record will become a basis of public health to control echinococcoses in Russia. The rich genetic diversity demonstrates the importance of Russia in investigating the evolutionary history of the genus *Echinococcus*.

Key words: Echinococcosis, *Echinococcus*, Russia.

INTRODUCTION

Echinococcoses are serious parasitic zoonoses caused by the members of genus *Echinococcus* Rudolphi, 1801 (Cestoda: Taeniidae). The genus consists of 9 species: *Echinococcus multilocularis, E. oligarthrus, E. vogeli, E. granulosus* sensu stricto (s.s.), *E. equinus, E. ortleppi, E. canadensis* (including the genotypes G6, G7, G8 and G10) and *E. felidis* (Lymbery 1995; Eckert *et al.* 2001; Lavikainen *et al.* 2003; Xiao *et al.* 2005; Hüttner *et al.* 2008; Nakao *et al.* 2010*b*; Knapp *et al.* 2011). The members of the *E. granulosus* sensu lato (s.l.) species complex morphologically resemble each other and are only distinguishable by molecular analysis. The causative agents of cystic echinococcosis (CE) referred to as *E. granulosus* s.l. utilize ungulates (sheep, goat, cattle, horse, etc.) as intermediate hosts, and dogs and wolves as definitive hosts (Abuladze, 1964; Foreyt *et al.* 2009). On the other hand, *E. multilocularis* causes alveolar echinococcosis (AE), and exploits rodents as intermediate hosts and foxes, dogs and wolves as definitive hosts (Abuladze, 1964; Ito *et al.* 2013). Since the dissolution of the Soviet Union in 1991, human infections with *Echinococcus* spp. in Russia increased rapidly. According to the official website of Rospotrebnadzor (www.rospotrebnadzor.ru), only 190 cases were reported in 1992 but the recorded cases reached 553 in 2001. Among these 553 cases, only 30 cases were registered as AE. Based on serological studies,

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the Rospotrebnadzor also pointed out that the number of cases was underestimated and it could be as much as three times greater. Therefore, an extensive epidemiological survey is needed to understand the current situation of echinococcoses in Russia. For the epidemiology of echinococcoses, a reliable molecular method to identify the aetiological agents is necessary because of the morphological similarity among *Echinococcus* species. However, molecular identification of *Echinococcus* species and genotypes was rarely made in Russia until recently.

In all Russian territories, *E. granulosus* s.l. is widespread and biological strains using different host animals have been described (Yastreb, 2010). Molecular identification studies revealed the distribution of *E. granulosus* s.s., *E. ortleppi* and *E. canadensis* (G6 and G7). The identification of species and genotypes has been conducted mainly by restriction fragment length polymorphism (Nikulina *et al.* 2003; Lukmanova *et al.* 2008) and the nucleotide sequence data of mitochondrial DNA (mtDNA) are available only for some cases (Konyaev *et al.* 2012*a*,*b*; Nakao *et al.* 2013*b*). *E. multilocularis* is also widespread in most parts of Russia. Based on the incidence records of human AE cases, nine endemic areas were identified (Lukashenko, 1975), and the largest focus was situated in Yakutia (Alperovich, 1972). Another part of endemic area is located in western Siberia and most of the cases were distributed in the southern part of the region – Altai Krai and the Altai Republic (Gaenko, 1958). For more than 50 years, researchers have paid special attention to the significant differences in the incidences of inhabitants in different territories. Although *E. multilocularis* infection is common in intermediate and definitive hosts, human AE cases are very rare in Yakutia and Taimyr and Yamal Peninsulas (Gubanov, 1964; Gubanov and Fedorov, 1970; Martynenko *et al.* 1984; Bessonov, 2002, 2003). One hypothesis to explain the difference in the incidence rate is that there are pathogenic (red fox strain) and low-pathogenic or non-pathogenic (Arctic fox strain) strains (Shakhmatova, 1981). The recent phylogeographic analyses defined the division of *E. multilocularis* into European, Asian, Mongolian and North American genotypes (Nakao *et al.* 2009; Ito *et al.* 2010). Among them, only one human AE case has been confirmed as the North American genotype until now (Yamasaki *et al.* 2008). To evaluate the pathogenicity of the genotypes to humans, extensive sampling of *E. multilocularis* from both humans and animals in Russia is essential.

The present study aimed to identify the species and genotypes of *Echinococcus* spp. from humans and wild/domestic animals in Russia based on comparison of mtDNA sequences. For this purpose, results of our previous reports (Konyaev *et al.* 2012*a*, *b*; Nakao *et al.* 2013*b*) were combined with this study to overview the species and genotype compositions. Consequently we analysed 75 parasite samples from 14 host species include humans collected including human from a wide geographic range. The distribution, lifecycle and public health importance of each parasite species and genotype were discussed.

MATERIALS AND METHODS

From 2010 to 2012, both adult and larval stages of *Echinococcus* spp. were collected from various host species in Russia (Table 1). Adult worms were obtained from wolves (*Canis lupus*), red foxes (*Vulpes vulpes*) and arctic foxes (*Vulpes lagopus*). Metacestode larvae were collected from humans, a domestic cat, sheep (*Ovis aries*), elk (*Alces alces*), reindeer (*Rangifer tarandus*), a captive Senegal bushbaby (*Galago senegalensis*) and arvicoline rodents (*Microtus gregalis, Microtus oeconomus, Myodes rufocanus, Alticola strelzowi* and *Alticola olchonensis*). The detailed information of most of the human cases in the Altai region (Altai Krai and the Altai Republic) and the CE case of domestic cat in Saint Petersburg have been published previously (Konyaev *et al.* 2012*a*, *b*).

The genomic DNA of each parasite isolate was extracted from the ethanol-fixed adult worms and metacestodes by DNeasy blood and tissue kit (Qiagen), and subsequently used as templates for polymerase chain reaction (PCR). Only one adult worm or an isolated metacestode from each host animal was used for the analysis. For a mtDNA genetic marker, the entire cytochrome *c* oxidase subunit I (*cox1*) was amplified by PCR as reported previously (Hüttner *et al.* 2008). The PCR products obtained were treated with illustra ExoStar (GE Healthcare) to remove excess primers and dNTPs, and directly sequenced with a BigDye™ Terminator v3.1 and a 3500 DNA sequencer (Life Technologies). The *cox1* sequences of Russian *Echinococcus* spp. published in our previous reports (Konyaev *et al.* 2012*a*, *b*; Nakao *et al.* 2013*b*), were also added to the following analysis.

By using ClustalW 2.0 (Larkin *et al.* 2007), sequences of each *cox1* haplotype in Russian *Echinococcus* spp. were aligned with representative *cox1* sequences of *Echinococcus* spp. available in the GenBank database. A phylogenetic tree was constructed by neighbour-joining (NJ) method with Kimura's two-parameter model (Kimura, 1980), using the integrated software PAUP 4.0b10 (Swofford, 2002). The robustness of the phylogenetic tree was tested by bootstrapping with 1000 replicates. In the tree construction, *Versteria mustelae* was used as an outgroup because the species is sister to *Echinococcus* (Knapp *et al.* 2011; Nakao *et al.* 2013*a*). To evaluate the relationship among *E. multilocularis* genotypes, a haplotype network was drawn by TCS 1.2 software (Clement *et al.* 2000) using statistical parsimony (Templeton *et al.* 1992).

RESULTS

In the present study, 29 isolates of *Echinococcus* spp. were collected from various host species. The information on the hosts and localities of each isolate is shown in the Table 1 and Fig. 1. Nucleotide sequences of mitochondrial *cox1* gene (1608–1609 bp) were determined for 4 adult worms and 24 metacestodes, and consequently 12 haplotypes were obtained. Only a partial (1053 bp) sequence was determined for an adult worm from a wolf in the Altai Republic. Together with the previous studies (Konyaev *et al*. 2012*a*, *b*; Nakao *et al*. 2013*b*), a total of 75 isolates of *Echinococcus* spp. including 37 *cox1* haplotypes could be analysed. Three species of *E. granulosus* s.s., *E. canadensis* (G6, G8 and G10) and *E. multilocularis* were found in these isolates (Fig. 2), and the Russian isolates of *E. multilocularis* were further separated into four genotypes (Figs. 2 and 3).

As shown in Table 1, metacestodes of *E. granulosus* s.s. were confirmed from three human CE patients in Altai Krai and the Republic of Bashkiria and from four sheep in Permskiy Krai. Five *cox1* haplotypes (EgRUS7-11) were confirmed among these seven isolates. A basic local alignment search tool (BLAST) revealed that the sequence of the haplotype EgRUS9 was the same as those of isolates from sheep in Iran (the database accession nos. JQ219962 and JQ250811), but the other haplotypes had no identical sequences in the GenBank database. Together with the previous studies (Konyaev *et al*. 2012*a*, *b*), a total of 12 *cox1* haplotypes have been confirmed from 14 isolates of *E. granulosus* s.s. in Russia.

The distributional records of Russian isolates of *E. canadensis* have already been summarized (Konyaev *et al*. 2012*a*; Nakao *et al*. 2013*b*). The detailed information was as follows. The adult worms of *E. canadensis* G6 were collected from two wolves in Altai Krai and the Altai Republic. These G6 isolates were divided into two *cox1* haplotypes (EcRUS1 and EcRUS2). The sympatric distribution of *E. canadensis* genotypes (G6, G8 and G10) was confirmed, especially in areas (Yakutsk and the Districts of Verkhoyansky, Ust-Maysky and Oymyakonsky) of Yakutia. The metacestode of G6 identified as the haplotype EcRUS1 was collected from a domestic reindeer in Yakutsk. Both adults and metacestodes of G8 were obtained from a wolf in Verkhoyansky District and two elk in Oymyakonsky District, respectively. Only one haplotype (EcRUS3) was found from the G8 isolates. The adult worms of G10 were obtained from a wolf in Verkhoyansky District, and the metacestodes were isolated from the lung and liver of an elk in Oymyakonsky District and a wild reindeer in Oleneksky District. G10 was also obtained from a CE patient in Oleneksky District. The cysts were removed from the lung of a young Yakutian boy. Four haplotypes (EcRUS4-7) were detected from these G10 isolates.

The adult worms of *E. multilocularis* Asian genotype were obtained from a red fox in Ryazan Oblast, southeast of Moscow. The partial (1053 bp) *cox1* gene sequence of the adult worm from a wolf collected in the Altai republic was 100% identical to the Asian genotype (AB688125), but slightly different from the other genotypes. The metacestodes of the Asian genotype were found from seven human AE cases in Altai Krai, a narrow-headed vole (*M. gregalis*) in Novosibirsk Oblast and a root vole (*M. oeconomus*), and a grey red-backed vole (*M. rufocanus*) in Kemerovo Oblast. Although a total of 40 isolates of *E. multilocularis* Asian genotype have been obtained in Russia, 62·5% (25/40) of them had the same haplotype designated as EmRUS1 (Fig. 3). A BLAST search revealed this haplotype to be 100% identical to those from Japan (AB018440, AB385610 and AB461416). We detected *E. multilocularis* Mongolian genotype from a flat-headed vole (*A. strelzowi*) in Kosh-Agachsky District of Altai Republic and from two Lake Baikal mountain voles (*A. olchonensis*) in the Olkhon Island of Lake Baikal, Irkutsk Oblast. The metacestode of *E. multilocularis* European genotype was obtained from a captive Senegal bushbaby, small African primate belonging to the family Galagidae, reared in Moscow Zoo. In 2012, ten Senegal bushbabies died over short period in the zoo and were diagnosed as AE by morphological observation of histopathological specimens. One of the tissue specimens was subjected to molecular diagnosis and then identified as the European genotype. The adult worms of *E. multilocularis* North American genotype were found from three Arctic foxes in the Lower Kolyma, (Nizhnekolymsky District), northeast Yakutia. The metacestodes of the North American genotype were also found from narrow-headed voles around Yurung-Haya village in Anabarsky District, northwest Yakutia.

DISCUSSION

E. granulosus s.s.

Seven isolates of *E. granulosus* s.s. were collected and five *cox1* haplotypes were obtained in the present study. Together with the previous reports (Konyaev *et al*. 2012*a*, *b*), a total of 12 haplotypes have been found from 14 isolates in Russia. These haplotypes were geographically derived from European and Asian Russia. Other previous studies also suggest that *E. granulosus* s.s. is mainly distributed in western and central parts of Russia (Nikulina *et al*. 2003; Lukmanova *et al*. 2008). *E. granulosus* s.s. was originally recognized as the 'sheep strain' within *E. granulosus* s.l. and sheep farming strongly affects the distribution, although the involvement of cattle, goats and camels as intermediate hosts has also

Table 1. Russian isolates of *Echinococcus* spp. used in this study.

Species (genotypes)	Hosts (parasite stages)[a]	Localities (position numbers)[b]	*cox1* haplotypes	Accession numbers	References
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS1	AB688136	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS2	AB688137	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS3	AB688138	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Novosibirsk Oblast (6)	EgRUS4	AB688139	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS5	AB688140	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS6	AB688141	Konyaev *et al*. 2012*a*
E. granulosus s.s.	Human (L)	Republic of Bashkiria (5)	EgRUS7	AB777904	This study
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS10	AB777907	This study
E. granulosus s.s.	Human (L)	Altai Krai (8)	EgRUS11	AB777908	This study
E. granulosus s.s.	Domestic cat (L)	Saint Petersburg (1)	EgRUS12	AB622277	Konyaev *et al*. 2012*b*
E. granulosus s.s.	*Ovis aries* (L)	Permskiy Krai (4)	EgRUS8	AB777905	This study
E. granulosus s.s.	*Ovis aries* (L)	Permskiy Krai (4)	EgRUS9	AB777906	This study
E. granulosus s.s.	*Ovis aries* (L)	Permskiy Krai (4)	EgRUS9	AB777906	This study
E. granulosus s.s.	*Ovis aries* (L)	Permskiy Krai (4)	EgRUS9	AB777906	This study
E. canadensis (G6)	Human (L)	Altai Krai (8)	EcRUS1	AB688142	Konyaev *et al*. 2012*a*
E. canadensis (G6)	*Rangifer tarandus* (L)	Yakutsk, Yakutia (14)	EcRUS1	AB688142	Nakao *et al*. 2013*b*
E. canadensis (G6)	*Canis lupus* (A)	Altai Krai (8)	EcRUS1	AB688142	Nakao *et al*. 2013*b*
E. canadensis (G6)	*Canis lupus* (A)	Altai Republic (9)	EcRUS2	AB777909	Nakao *et al*. 2013*b*
E. canadensis (G8)	*Alces alces* (L)	Oymyakonsky, Yakutia (16)	EcRUS3	AB777910	Nakao *et al*. 2013*b*
E. canadensis (G8)	*Alces alces* (L)	Oymyakonsky, Yakutia (16)	EcRUS3	AB777910	Nakao *et al*. 2013*b*
E. canadensis (G8)	*Canis lupus* (A)	Verkhoyansky, Yakutia (13)	EcRUS3	AB777910	Nakao *et al*. 2013*b*
E. canadensis (G10)	*Alces alces* (L)	Oymyakonsky, Yakutia (16)	EcRUS4	AB777911	Nakao *et al*. 2013*b*
E. canadensis (G10)	*Rangifer tarandus* (L)	Oleneksky, Yakutia (12)	EcRUS6	AB777913	Nakao *et al*. 2013*b*
E. canadensis (G10)	Human (L)	Ust-Maysky, Yakutia (15)	EcRUS7	AB777914	This study
E. canadensis (G10)	*Canis lupus* (A)	Verkhoyansky, Yakutia (13)	EcRUS5	AB777912	Nakao *et al*. 2013*b*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*

E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Republic (9)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Republic (9)	EmRUS1	AB688125	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS2	AB688126	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS3	AB688127	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS4	AB688128	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS5	AB688129	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS6	AB688130	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS7	AB688131	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS8	AB688132	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS8	AB688132	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS9	AB688133	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS10	AB688134	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS10	AB688134	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS10	AB688134	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS11	AB688135	Konyaev *et al*. 2012*a*
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS11	AB777914	This study
E. multilocularis (Asia)	Human (L)	Altai Krai (8)	EmRUS12	AB777915	This study
E. multilocularis (Asia)	*Microtus gregalis* (L)	Novosibirsk Oblast (6)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	*Microtus oeconomus* (L)	Kemerovo Oblast (7)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	*Myodes rufocanus* (L)	Kemerovo Oblast (7)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	*Vulpes vulpes* (A)	Ryazan Oblast (3)	EmRUS1	AB688125	This study
E. multilocularis (Asia)	*Canis lupus* (A)	Altai Republic (9)	ND[c]	ND[c]	This study
E. multilocularis (Mongolia)	*Alticola strelzowi* (L)	Altai Republic (9)	EmRUS17	AB777920	This study
E. multilocularis (Mongolia)	*Alticola olchonensis* (L)	Irkutsk Oblast (10)	EmRUS18	AB777921	This study
E. multilocularis (Mongolia)	*Alticola olchonensis* (L)	Irkutsk Oblast (10)	EmRUS18	AB777921	This study
E. multilocularis (N America)	*Vulpes lagopus* (A)	Nizhnekolymsky, Yakutia (17)	EmRUS13	AB777916	This study
E. multilocularis (N America)	*Vulpes lagopus* (A)	Nizhnekolymsky, Yakutia (17)	EmRUS13	AB777916	This study
E. multilocularis (N America)	*Vulpes lagopus* (A)	Nizhnekolymsky, Yakutia (17)	EmRUS13	AB777916	This study
E. multilocularis (N America)	*Microtus gregalis* (L)	Anabarsky, Yakutia (11)	EmRUS14	AB777917	This study
E. multilocularis (N America)	*Microtus gregalis* (L)	Anabarsky, Yakutia (11)	EmRUS15	AB777918	This study
E. multilocularis (Europe)	*Galago senegalensis* (L)	Moscow (2)	EmRUS16	AB777919	This study

[a] L, larva; A, adult.

[b] Position numbers are shown in Fig. 1.

[c] *Cox1* haplotype was not determined because of the incomplete sequence, and the sequence was not deposited to the GenBank database.

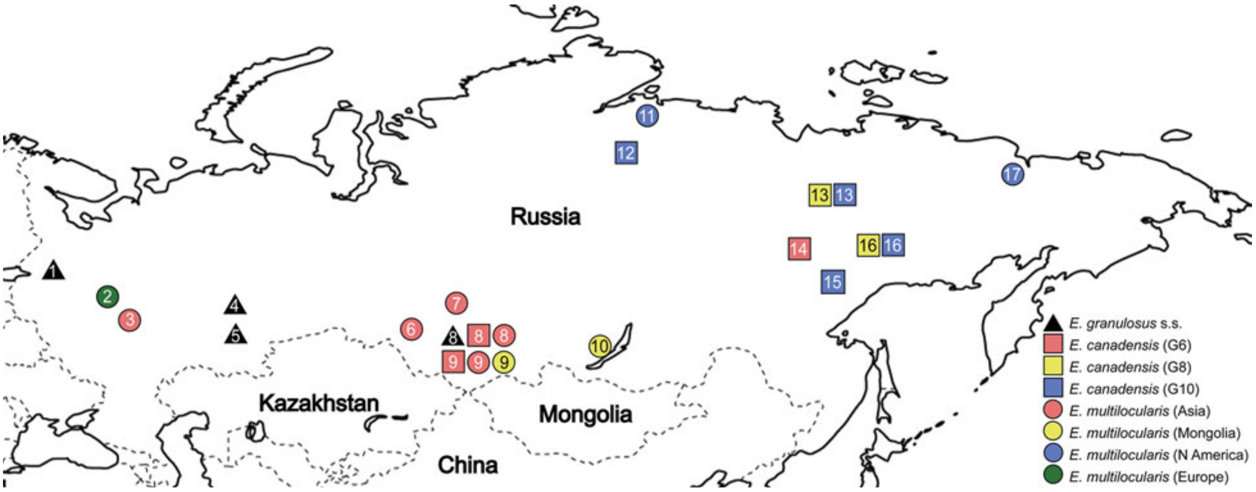

Fig. 1. Spatial distribution of *Echinococcus* spp. in Russia. Localities are shown in serial numbers as follows: 1, Saint Petersburg; 2, Moscow; 3, Ryazan Oblast; 4, Permskiy Krai; 5, Republic of Bashkiria; 6, Novosibirsk Oblast; 7, Kemerovo Oblast; 8, Altai Krai; 9, Altai Republic; 10, Irkutsk Oblast; 11, Anabarsky District; Yakutia, 12; Oleneksky District, Yakutia; 13, Verkhoyansky District, Yakutia; 14, Yakutsk, Yakutia; 15, Ust-Maysky District, Yakutia; 16, Oymyakonsky District, Yakutia; 17, Nizhnekolymsky District, Yakutia.

been confirmed (Cardona and Carmena, 2013). In Yakutia, the sheep farming is almost non-existent because the weather and landscape are unsuitable. On the other hand, the indigenous people have a close contact with dogs which are often fed or scavenge on discarded offal of hunted or domestic cervids. Therefore, it is likely that most of the human CE cases are not caused by *E. granulosus* s.s. but by the 'cervid strain' of *E. canadensis* in Yakutia.

Recent phylogeographic studies have demonstrated the existence of a cosmopolitan *cox1* haplotype, which is dominant in Europe, the Middle East (Iran and Jordan), China and Peru (Nakao *et al.* 2010*a*; Casulli *et al.* 2012; Yanagida *et al.* 2012), suggesting that *E. granulosus* s.s. has rapidly dispersed worldwide through the anthropogenic movement of domestic animals. The cosmopolitan haplotype corresponded to EgRUS1 in Russia from a human CE case in Altai Krai (Konyaev *et al.* 2012*a*). Russia has a large territory with the distribution of *E. granulosus* s.s., and is thus an important place to clarify the dispersal history of this cosmopolitan parasite. Further extensive epidemiological studies in the different part of Russia are needed.

E. canadensis

In Altai Krai, *E. canadensis* G6 has been found in a human CE patient (Konyaev *et al.* 2012*a*). The present study confirmed the natural distribution of G6 from a wolf in the Altai region. However, the larval stage was not yet confirmed from any domestic or wild animals in the region. Therefore, the infection cycle of *E. canadensis* G6 in the Altai region is still unknown. Although the G6 genotype has traditionally been considered as 'camel strain', less than 500 individuals of semi-free Bactrian camels (*Camelus bactrianus*) are distributed in the Altai region. Thus, it is unlikely that the G6 genotype is maintained solely by a wild wolf-camel life-cycle. Besides, the CE patient and the wolf infected with G6 were found in a region in which camels are absent (Konyaev *et al.* 2012*a*). Therefore, it is reasonable to assume the existence of the infection cycle not involving camels. Indeed, sheep, cattle and goats are also known to be susceptible to the G6 genotype (Omer *et al.* 2010; Soriano *et al.* 2010; Hailemariam *et al.* 2012). In addition, this genotype was found from a domestic reindeer in Yakutia. This is the first report of G6 infection in cervids. The present study indicates that the transmission cycle of *E. canadensis* G6 in Russia can be maintained by both domestic and wild animals.

The G8 and G10 genotypes of *E. canadensis* are considered as a 'cervid strain' (Bowles *et al.* 1994) and a 'Fennoscandian cervid strain' (Lavikainen *et al.* 2003), respectively. The sympatric distribution of these genotypes has been demonstrated in Estonia and North America (Thompson *et al.* 2006; Moks *et al.* 2008; Bryan *et al.* 2012; Schurer *et al.* 2013). In this study, both G8 and G10 genotypes were collected from wolves and cervids in Yakutia. These results indicate that the two genotypes are sympatrically distributed through northern Eurasia and North America. Interestingly, *E. canadensis* G6 was also confirmed in Yakutia as mentioned above. The genotypes of *E. canadensis* have originally been recognized by the differences in their life-cycles and/or geographical distributions. However, sympatric distribution of different genotypes with the utilization of same definitive host species has been demonstrated. Furthermore, the recent phylogenetic studies have revealed that they are genetically closely

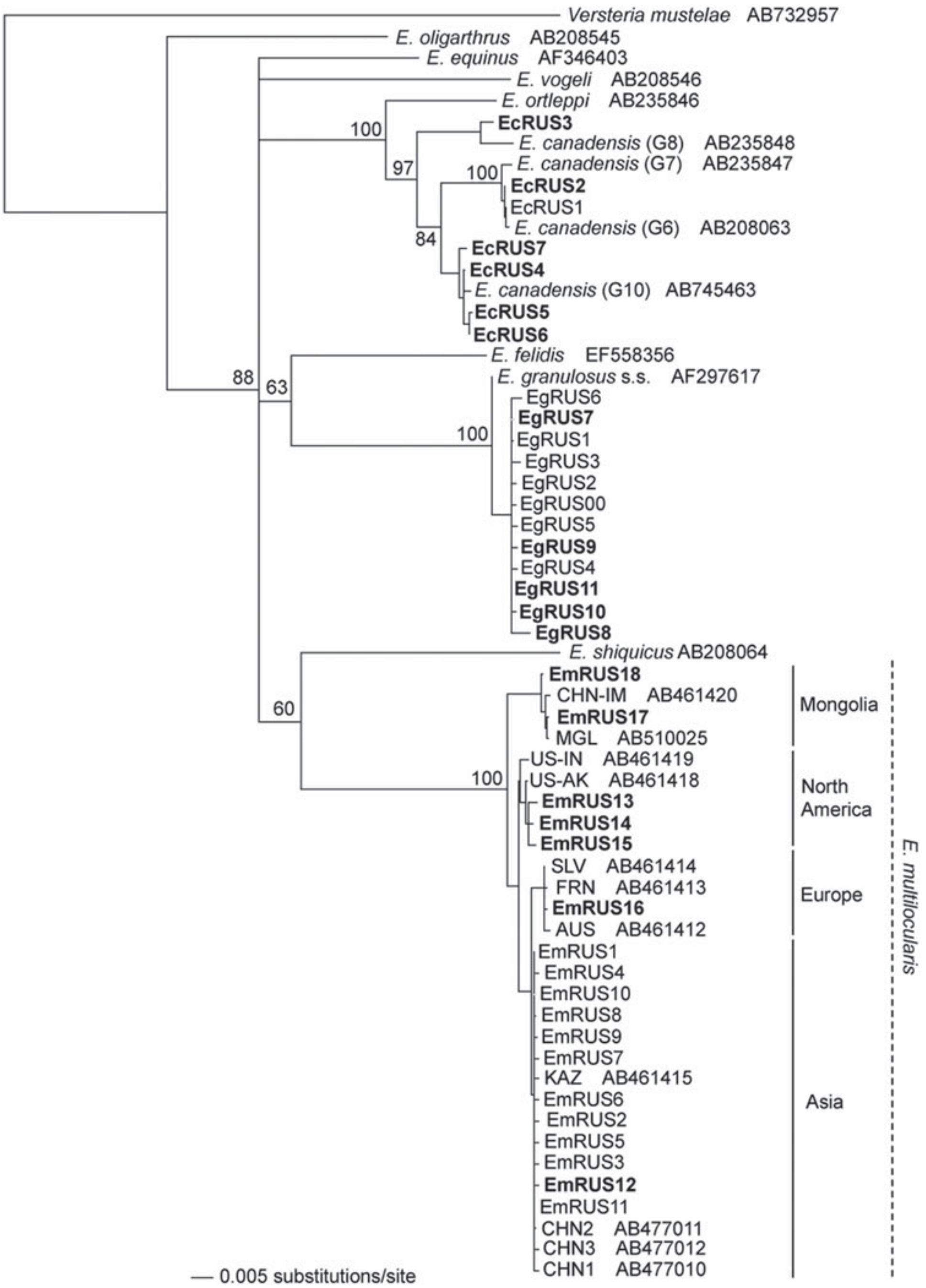

Fig. 2. A neighbour-joining tree of *Echinococcus* spp. constructed from the nucleotide sequences of mitochondrial *cox1* gene. Numbers on the nodes are bootstrap values. The names of the haplotypes obtained in the present study are shown in bold. CHN-IM, Chinese Inner Mongolia; MGL, Mongolia; US-AK, Alaska (St Lawrence Island); US-IN, Indiana; US-SD, South Dakota; SLV, Slovakia; AUS, Austria; FRN, France; KAZ, Kazakhstan; CHN, China (Sichuan).

related (Nakao *et al*. 2013*b*). Therefore, it is reasonable to assume that these genotypes can mate and produce hybrid offspring. A population genetic study in Yakutia where the three genotypes (G6, G8 and G10) are sympatrically distributed will shed light on the species status of *E. canadensis*. Instead of a maternally inherited mtDNA marker, the use of nuclear DNA markers is necessary to examine gene flow among the genotypes.

In this study, a human CE patient in Yakutia was demonstrated to be infected with *E. canadensis* G10. As far as we know, this is the second confirmed human case of the G10 infection after the first report from Mongolia (Jabbar *et al*. 2011). In a Canadian indigenous community, 11% of people were serologically positive for *E. granulosus* s.l. infection and the eggs of *E. canadensis* G10 were detected in 6% of environmentally collected canine faeces (Himsworth *et al*. 2010). This result suggests that the human infection of G10 is not rare. As shown in the present study, the life-cycles of G8 and G10 are maintained in Yakutia by wild wolves and cervids

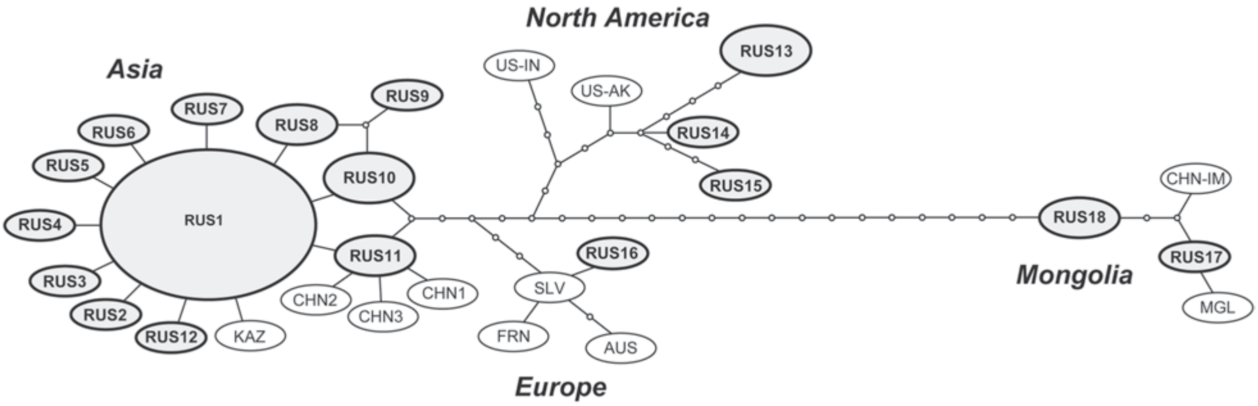

Fig. 3. The parsimonious network of *cox1* haplotypes of *E. multilocularis*. The haplotypes obtained in Russia are shown in circles with names in bold. CHN-IM, Chinese Inner Mongolia; MGL, Mongolia; US-AK, Alaska (St Lawrence Island); US-IN, Indiana; US-SD, South Dakota; SLV, Slovakia; AUS, Austria; FRN, France; KAZ, Kazakhstan; CHN, China (Sichuan).

(elk and reindeer), and the indigenous people having close contact with the wildlife have a risk of CE caused by *E. canadensis*. Another risk of CE is the raising of reindeer. Indigenous people often feed shepherd dogs with offal of slaughtered reindeer, and sleep with the dogs in the traditional tent house ‘chum’. In Nizhnekolymsky District, 2·3% of people had antibody against *Echinococcus* spp. (Martynenko *et al*. 1984). In Chukotka Autonomous Okrug located in the Russian Far East, 11·5% of hunters and 12·3% of herders were seropositive to *Echinococcus* spp. (Vol’fson, 1968). Wilson *et al*. (1968) observed 101 CE cases of Alaskan indigenous people and reported the differences in pathogenicity between the European (‘pastoral’) and the Alaskan (‘sylvatic’) forms of *E. granulosus* s.l. While the liver CE was most frequent (48–78%) in Europe, Australia, South America and the Middle East, lung CE was most frequent (66%) in Alaska. Similarly, 40% of echinococcosis patients in the Chukotka Autonomous Okrug had hydatid cysts in the lungs (Boĭtsov *et al*. 1992). There was also a significant difference in the degree of symptoms; the ‘pastoral form’ was often (80–95%) symptomatic, while the ‘sylvatic form’ was rarely (6–8%) symptomatic (Wilson *et al*. 1968). In fact, most of the patients in Alaska were admitted to hospital for the examination or treatment of tuberculosis, and the parasite cysts were incidentally found by routine roentgenography. Although there is no direct evidence, it is likely that the ‘sylvatic form’ of *E. granulosus* s.l. in Alaska was *E. canadensis* G8 or G10. Based on this assumption, it is possible that the CE patients infected with *E. canadensis* are easily overlooked because of the lack of typical symptoms. Therefore, extensive epidemiological surveys using modern sensitive immunodiagnostic methods for the human infection with *E. canadensis* are necessary to better understand the current situation of CE in Russia, especially in Yakutia. Also, molecular identification of the causative agent of human CE is essential to evaluate the difference in pathogenicity among species and/or the genotypes.

E. multilocularis

In the present study, all four geographic genotypes of *E. multilocularis* were confirmed in Russia. The Asian genotype was obtained in the Altai region bordering with Asian countries. As the Asian genotype was also found in European Russia, this genotype is expected to be widely distributed in North Eurasia. A wolf collected in the Altai republic was infected with the Asian genotype. The infection of wolves with *E. multilocularis* Mongolian genotype has also been found in Mongolia (Ito *et al*. 2013). These results imply the importance of wolves in the transmission ecology of *E. multilocularis*. Two Lake Baikal mountain voles infected with the Mongolian genotype, were captured in Olkhon Island in Lake Baikal. The vole is an endemic species inhabiting rocky steppes in Olkhon and several other islands in the lake. This is the first record of a helminth parasite from the Lake Baikal mountain vole whose biology is poorly understood. The infection cycle is assumed to be maintained with red foxes as the definitive host, because the traces of foxes were found near the colony of the voles and only red foxes inhabit in the islands of Lake Baikal. The foxes can migrate freely from the mainland to the islands and *vice versa* across the ice bridge in winter. Another isolate of the Mongolian genotype was found in the Altai Republic. The sympatric distribution of the Asian and Mongolian genotypes in the Altai Republic indicates possible gene flow between the two genotypes. The Mongolian genotype was once described as a new species of *Echinococcus russicensis* from corsac fox (Tang *et al*. 2007) and later regarded as a synonym of *E. multilocularis* based on the comparative genetic studies (Nakao *et al*. 2009, 2010*b*; Ito *et al*. 2010). The human case caused by the Mongolian genotype has not been found yet in Russia, although its pathogenicity to humans was demonstrated in Mongolia (Ito *et al*. 2010).

A Senegal bushbaby reared in Moscow Zoo was demonstrated to be infected with *E. multilocularis* European genotype. Until now, *E. multilocularis* infections have been reported in zoo's captive primates including the Old World monkeys, *Macaca* spp. (Brack *et al*. 1997; Bacciarini *et al*. 2004; Sato *et al*. 2005; Boufana *et al*. 2012), the gorilla, *Gorilla gorilla* (Kondo *et al*. 1996; Rehmann *et al*. 2003), the orangutan, *Pongo pygmaeus* (Taniyama *et al*. 1996) and the ring-tailed lemur, *Lemur catta* (Kondo *et al*. 1996). In the present study, the infected bushbaby was reared in the zoo located in the centre of Moscow, and there were no stray dogs or wild foxes inside. All canids regularly had deworming twice in every year. Therefore, it is not likely that there were carrier animals as the infectious source of echinococcosis in the zoo. However, the infected bushbabies were born and bred in the zoo, and thus they must have become infected there. The most probable source of *Echinococcus* eggs was mulch used as ground cover in the cage. Mulch is made of different natural materials such as woods and straws that came from Yaroslavl Oblast and occasionally from Baltic countries (Latvia or Estonia). These Baltic countries are known to be endemic for *E. multilocularis* (Moks *et al*. 2005; Bagrade *et al*. 2009) and it is possible that the mulch was contaminated with eggs of the parasite.

In this study, the North American genotype was found in northeast and west Yakutia. The result indicates that this genotype has trans-Beringian or circumpolar distribution. Martynenko (1984) suggested that there were three different patterns of the transmission cycle of *E. multilocularis* in Yakutia; 'Arctic fox-lemming' cycle in tundra, 'red fox-vole' cycle in taiga and 'dog-mouse/rat' cycle in towns. Retrospective analyses of *E. multilocularis* infections in definitive hosts in Yakutia from 1961 to 2006 indicated that the prevalence could vary quite widely, but was always relatively high in Arctic foxes and low in the red fox (Gubanov, 1964; Martynenko, 1984; Odnokurtsev and Sedalishchev, 2012). Although up to 100% of the foxes were infected in the Lower Kolyma region of Yakutia, only a single human AE case was recorded from 1972 to 1981 (Martynenko *et al*. 1984). Moreover, human AE has never been found in Taimyr and Yamal Peninsulas, where infected Arctic foxes were confirmed (Bessonov, 2003). Interestingly, human AE cases are also very uncommon in North America (excepting Alaska), even though *E. multilocularis* is highly prevalent in rodents and canids (Rausch, 1995; Storandt *et al*. 2002). One of the only two reported human AE cases in the USA was analyzed retrospectively and was demonstrated to be caused by the North American genotype of *E. multilocularis* (Yamasaki *et al*. 2008). To date, this is the only confirmed human AE case caused by this genotype. Based on these epidemiological observations, it is speculated that *E. multilocularis* North American genotype has a low infectivity to humans, and this might correspond to the non-pathogenic 'Arctic fox strain' postulated by Shakhmatova (1981). Further epidemiological surveys in Siberia and North America are needed to clarify the difference of pathogenicity among *E. multilocularis* genotypes.

CONCLUSIONS

The present molecular survey revealed the rich genetic diversity of *Echinococcus* spp. in Russia, using 75 isolates from 14 host species including humans. The species and genotype compositions were disclosed as follows: three species (*E. granulosus* s.s., *E. canadensis* and *E. multilocularis*), three genotypes of *E. canadensis* (G6, G8 and G10) and four genotypes of *E. multilocularis*. The nationwide survey also suggests that distributions of these taxa overlap in Europe, Asia and far eastern Russia, depending on their complicated ecosystems. The present distributional records of *Echinococcus* spp. and their genotypes will become the basis of public health strategy to control cystic and alveolar echinococcoses in Russia. This study demonstrated the importance of Russia in investigating the biological and clinical features of *Echinococcus* spp. Further extensive epidemiological surveys with molecular identification of the causative agents are needed to clarify the distribution, life cycle and pathogenicity of each species and genotype.

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