Characteristic non-synonymous SNP in *cox*I mtDNA of Russian isolates of *Trichinella spiralis*

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Summary. Non-synonymous nucleotide restitution in the *cox*I mitochondrial gene of Russian isolates of *Trichinella spiralis* can serve as a genetic marker to distinguish these isolates from West European and North American ones.

Key words: East Asia**,** genetic variability, wild and domesticated hosts, *Trichinella*, trichinellosis.

Trichinella spiralis (Owen, 1985) is a dangerous parasitic nematode of humans and animals. This parasite is capable of successfully spreading in anthropogenic habitats (Rosenthal, 2008). Typically, pigs and rats are the hosts in such sites. Infestation of wild animals with this nematode throughout Eurasia was also reported (Pozio *et al*., 2009). Every year, outbreaks of trichinellosis caused by *T. spiralis* are reported from different Eurasian countries, thus demanding a deeper understanding of *T. spiralis* epidemiology. To have a molecular tool to distinguish between separate intraspecific groups of this cosmopolitan parasite would be useful; however, the very low genetic variability reported for *T. spiralis* (Rosenthal *et al*., 2008) is the obvious obstacle for the design of such a tool. Nearly complete genetic homogeneity, expressed as a high level of fixation in microsatellite and mitochondrial markers, was demonstrated for the *T. spiralis* strains isolated in Western Europe, North Africa and the Americas (Rosenthal *et al.*, 2008). Unlike these similar *T. spiralis* isolates, the isolates from East Asia (China, Thailand) display a more pronounced genetic variability (Rosenthal *et al*., 2008). The territory of the Russian Federation is situated just between East Asian and West European parts of *T. spiralis* distribution area with different levels of genetic variability. The question of which *T. spiralis* haplotypes inhabit this vast territory is of importance from both scientific and applied points of view. Below we have proposed a marker to distinguish between West European and Russian isolates of this species.

MATERIAL AND METHODS

Isolates studied. Five isolates of *T. spiralis* were available, collected in the Czech Republic and different regions of Russia from different hosts, including sable, hunted in Lazo district of Primorskii krai (Table 1). The samples of muscular tissue positive for *Trichinella* juveniles were transported to the laboratories in Moscow (I.M. Odoyevskaya) or Kirov (L. A. Bukina), where the

Table 1. Origin of *Trichinella spiralis* isolates used for DNA extraction and sequencing.

	Host	Geographic origin	Laboratory label	Deposition N
1	Domestic pig (Sus scrofa)	Czech Republic	Tri74	KU321693
2	Wolf (Canis lupus)	South-east part of Siberia	Tri84	KU321694
3	Dog (Canis lupus familiaris)	South-east part of Siberia	Tri40	KU321695
4	Fox (Vulpes vulpes)	Primorskii Region	Tri109	KU321696
5	Sable (*Martes zibellina*)	Primorskii Region	Tri117	-

Table 2. Nucleotide differences between *Trichinella spiralis* isolates in the partial *cox*1 mtDNA sequence.

Species, isolate designation, NCBI accession number, Locality of origin	1	2	3	4	5	6	7	8
1 T. spiralis AF293969 - France	--							
2 T. spiralis ISS31 GU386314 - USA	**0**	--						
3T. spiralis ISS3 KM357422 - Poland	**0**	**0**	--					
4 T. spiralis T74 - Czech Republic	**0**	**0**	**0**	--				
5T. spiralis T40 - South-East of Siberia	**2**	**2**	**2**	**2**	--			
6 T.spiralis T84 - South –East of Siberia	**2**	**2**	**2**	**2**		--		
7 T spiralis T109 - Primorskii region	**4**	**4**	**4**	**4**	**2**	**2**	--	
8 T spiralis T117 - Primorskii region	**4**	**4**	**4**	**4**	**2**	**2**	**0**	--

detected *Trichinella* juveniles were used to establish the laboratory cultures in mice and hamsters. *Trichinella* juveniles were obtained from the laboratory inoculated animals through the digestion of muscular tissues with artificial gastric juice (8-10 g hydrochloric acid of 1,175 density and 3% pepsin l^{-1}). *Trichinella* juveniles were filtered from the digest, concentrated on the bottom of the tube and used for DNA extraction with Wizard® SV Genomic DNA Purification System columns (Promega Corp., USA).

PCR protocol, sequencing and analysis. A pair of primers of original design were used to obtain a partial sequence of mitochondrial gene of cytochrome oxidase I (*cox*I). The composition of primers was based on primers 37F and 42R (Hu *et al*., 2002) with some nucleotides replaced to meet the restitution characteristics for trichinellid *cox*I mtDNA: 37F_Tri GCA GTA AAT TTA GAA TTT AAA C and 42R_Tri CCT AAT ATT CAT GGT GTT CAT A. DNA was extracted from juvenile suspensions with Promega® Wizard. The size of amplicon obtained with these primers was about 1300 bp. PCR products were purified through gel electrophoresis and precipitation and sequenced with Genotech, Moscow. Four sequences were obtained for each amplicon (twice with both forward and reverse primers). The sequences of other species and genotypes of *Trichinella* were downloaded from GenBank and used to compare with the obtained ones. The sequences were aligned using Clustal X with default values for gap opening and gap extension penalties. Different methods of alignment analysis (maximum parsimony, neighbour joining and maximum likelihood) were performed with PAUP 4.0b.10 (Swofford, 1998) and MEGA5 (Tamura *et al*., 2011).

RESULTS

Approximately 1300 bp long amplicon was obtained with designed primers for 4 *Trichinella spiralis* isolates from Russia and one from Czech Republic. Nucleotide base calling of approximately 1310-1320 bp for each isolate was obtained and resulted in approximately 1320 bp long alignment. Pairwise nucleotide differences between the studied isolates were calculated with PAUP 4.0b.10 and are presented in Table 2. All four studied Russian isolates of *T. spiralis* (Tri40, Tri84, Tri109 and Tri117) differed from the European isolates by 2-4 bp. Thus, three *Cox*I mtDNA haplotypes of this species were detected. Analysis of the amino acid sequences for this gene obtained after translation of nucleotide sequences revealed the presence of one non-synonymous nucleotide substitution in *T. spiralis*: alanine in the Russian isolates instead of threonine in the European ones (Fig.1.). This nucleotide substitution was present in all studied Russian isolates of *T. spiralis*.

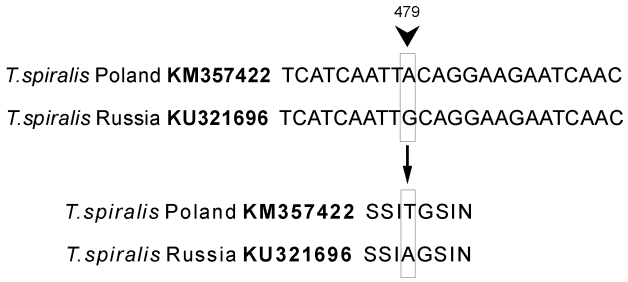

Fig.1. The position of non-synonymous nucleotide substitutions in the *cox*I mtDNA nucleotide and amino acid sequences of the Russian isolate of *Trichinella spiralis.*

Arrowhead is indicating the position of this SNP in the complete sequence of mitochondrial genome of *T. spiralis* from Poland.

DISCUSSION

Despite intensive molecular studies on *Trichinella*, only three complete sequences of the *cox*I mtDNA gene were available in the NCBI GenBank. Several other sequences of *T. spiralis* deposited are quite short (approx. 400 bp) and correspond to the 3' end of this *cox*I mtDNA sequence. As primers of our own design amplify a

5' portion of this gene and the site with non-synonymous substitution was at the beginning of this sequence, only three deposited sequences of *T. spiralis* were available for comparison. Our study showed that the sequence obtained for the Czech isolate of *T. spiralis* was identical with sequences deposited of this species from France, Poland and USA. The similarity of West European and North American strains was demonstrated earlier and considered to be a result of the introduction of this pathogenic nematode to North America by European colonists (Rosenthal *et al*., 2008). All the Russian isolates have several characteristic nucleotide differences with the West European/American isolates. Among them, one nucleotide substitution was non-synonymous, i.e. resulting in the mutation of a single amino acid in the encoded protein. Genetic isolation of *T. spiralis* strains of East Asian origin was demonstrated earlier by Rosenthal *et al*., 2008, but their conclusion was based on the analysis microsatellite repeats and the 3100 bp long sequence of several spanning mtDNA genes (*cyt*b, tRNA-Ser, SSU rDNA, tRNA-Val, LSU rDNA, *atp*6, and *cox*3). As the mtDNA fragment studied and those used in the cited paper are not overlapping, we are unable to predict whether East Asian isolates of *T. spiralis* do contain the described non-synonymous SNP, but we believe that the genetic marker discovered can be used in wider studies of intraspecific groups of *T. spiralis* throughout Eurasia. The finding of *T. spiralis* in the sables in Primorskii Region of Russian Far East is indicative of the presence of *T. spiralis* in non-anthropogenic habitats in East Asia.

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Спиридонов С.Э., Букина Л.А., Середкин И.В., Одоевская И.М. Характерный не-синонимичный однонуклеотидный полиморфизм последовательности *cox*I митохондриальной ДНК российских изолятов *Trichinella spiralis*.

Резюме. Обнаруженный не-синонимичный однонуклеотидный полиморфизм в последовательности *cox*I митохондриальной ДНК четырех российских изолятов *Trichinella spiralis* из Южной Сибири и Дальнего Востока Российской Федерации может быть использован как генетический диагностический маркер. С его помощью возможно отличать изоляты *Trichinella spiralis*, характерные для Западной Европы и Северной Америки, от изолятов из восточной части Российской Федерации.