Molecular taxonomic study of *Trichinella spp.* from mammals of Russian Arctic and subarctic areas

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Abstract

Analysis of taxonomic affiliation of *Trichinella* species circulating in the Chukotka Autonomous Region and some subarctic areas of the Russian Federation showed that the representatives of *T. spiralis* and the Arctic trichinellas - *T. nativa* (genotype T2) and *Trichinella* sp. (genotype T6) can be found there. The partial sequences of *Cox*b (704 bp) of these Arctic *Trichinella* spp. from Russia differ from *Cox*b sequences of those genotypes (T2 and T6) deposited in NCBI GenBank (1-3 bp). The cultivated larvae of *Trichinella* sp., which were established from muscular tissue sample of stray cat (shot on the fur farm in Chukotka peninslula) differ at molecular level (*Cox*b) even more significantly; 21-24 bp difference between *Trichinella* sp. and *T. nativa* and 46-47 bp difference between the same isolate and *T. spiralis* were recorded.

Key words: mammals, parasitic nematodes, phylogenetic tree, taxonomy, *Trichinella* sp.T6

DOI: 10.5817/CPR2014-1-4

Introduction

The presence of parasitic nematodes of the genus *Trichinella* Railliet 1895 in Artic ecosystems and their importance as epizootic and epidemiological factor in these areas were reported from different regions, *e.g.* Chukotka peninsula in Russia, Northern Canada and Alaska, USA (Lukashenko et al. 1971, Forbes 2000, Gajadhar et Forbes 2010, Seymour 2012). The connection of human infection to the consumption of the meat of several terrestrial and marine mammals (walruses, bears, bearded seals, ringed seals) was demonstrated (Rausch 1970, Serhir et al. 2001, Leclair et al. 2004, Pozio 2007, Seymour 2012). Nowadays, molecular taxonomical methods used

Received April 29, 2014, *accepted* August 1, 2014.
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Acknowledgements: This study was supported by the grant № 14-04-01064 from the Russian Foundation for Basic Researches.

for the identification of parasitic organisms, including also nematodes are more and more common, but up to now no study was particularly focused on the molecular determination of *Trichinella* species from Russian Arctic region.

It is known that two genotypes of *Trichinella*, *Trichinella nativa* (T2) and not-yet-described as an independent species *Trichinella* sp. (T6) are found at the Arctic coasts, *e.g.* of Greenland, Canada and Alaska (Dunams-Morel et al. 2012). *Trichinella* of these two genotypes were found in animals common for Arctic ecosystems, such as wild white arctic foxes (*Alopex lagopus*), red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), brown bears (*Ursus arctos*), polar bears (*Ursus maritimus*) and wolverines (*Gulo gulo*). Similarly, *Trichinella* genotypes *T. nativa* (T2) and T6 have been found in a number of marine mammals: walruses (*Odobenus rosmarus rosmarus*, *Odobenus rosmarus divergens*), ringed seals (*Phoca hispida*), spotted seals (*Phoca largha*), bearded seals (*Erignathus barbatus*), harp seals (*Phoca groenlandica*), Steller sea lions (*Eumetopias jubatus*), as well as representatives of the order Cetacea (*Cetacea*), the suborder of toothed whales (*Odontoceiti*), beluga (*Delphinapterus leucas*) (Dunams-Morel et al. 2012, Forbes 2000, Gajadhar et Forbes 2010, Leclair et al. 2004, Møller et al. 2005, Murrell et al. 2000, Pozio et Zarlenga 2005, Serhir et al. 2001). The results of molecular taxonomic study of *Trichinella* spp. collected in the Russian arctic and subarctic territories are presented below.

Material and Methods

In our study, we have carried out molecular genetic studies of 5 isolates of *Trichinella* spp. isolated from the muscles of terrestrial and marine mammals of Chukotka: sledge dog (*Canis familiaris*), stray cat (*Felis familiaris*), a wild fox (*Alopex lagopus*), a seal (*Phoca hispida*), and a brown bear (*Ursus arctos*). Several samples of *Trichinella* collected from mammals outside of Chukotka were also studied, inlcuding samples from polar bears (*U. maritimus*) and wolverine (*G. gulo*) from Sakha-Jakutia republic, Strays cats (*F. familiaris*) and dogs (*C. familiaris*) from Kirov and Voronezh regions, brown bears from Kirov and Primorskii (Vladivostok area) regions. The *Trichinella* isolates from a rat (Ossetia) were used as a control group. Laboratory culture of *Trichinella nelsoni* (obtained from Central Helminthological Laboratory in Sophia, Bulgaria) was used as outgroup for phylogenetic analysis. All these samples were kept in the collection of living *Trichinella* strains and frozen samples at the K. I. Skryabin All-Russian Institute of Helminthology.

DNA was extracted from a suspension of *Trichinella* larvae L1, which were isolated from experimentally infected animals. The laboratory animals (mice or Syrian hamsters), were per-orally fed with the suspension of larvae obtained through digestion of the muscle tissue of hunted animals with artificial gastric juice. Laboratory animals were euthanized with ether on 45-60 day post infection to obtain samples of muscle tissue for digestion. DNA was extracted from suspension of larvae by using of columns system of Wizard ® SV Genomic DNA Purification System according to the protocol of Promega company.

Partial sequence of mitochondrial *Cox*b gene was obtained with primers Tricob F1 (forward) CAA TCC ATT AGG TAC ACA CTC AC and Tricob R3 (reverse) TAA GTA AGA TTT CAA TGG CG

(Rosenthal et al. 2008). The following protocol was used for polymerase chain reaction (PCR): primary denaturation (94°C) – 4 min, then 35 cycles of denaturation at 94°C – 10 sec, annealing at 55°C – 30 sec, elongation at 72°C – 30 sec. Upon completion of 35 cycles, a final elongation was performed at 72°C – 3 min. The separation of PCR amplicons was performed in 1% agarose gel and the profiles were visualized on Gel Imager. The PCR amplicons were cuted from the agarose gels and the isolation of DNA from the gel cubes was carried out by using a kit of Promega company - Wizard ® SV Gel and PCR Clean-Up System. Eluted DNA was precipitated with 96% ethanol with 5M ammonium acetate and re-diluted in 30-50 µl of water. The concentration of DNA was estimated with spectrophotometer Nanodrop 2000. Direct sequencing was performed with the same primers as used for the primary PCR. Obtained chromatograms were read with Chromas 1.45., exported to FASTA-format and used to obtain alignments with Clustal X 1.81. The programme Genedoc 2.5. was used to remove flanking parts to obtain rectangular alignment and export the data into Nexus-format. Such alignments were analysed with PAUP 4.0b10 and MEGA 5.0 (Swofford 1998, Tamura et al. 2011). Phylogenetic analysis was conducted by the combination of several methods including maximum parsimony, maximum likelihood and Bayesian inference. All these methods demonstrated similar topologies of resulting phylogenetic trees. Thus, only maximum parsimony tree is presented below.

Results and Discussion

The alignment of the *Cob*1 mtDNA sequences obtained for the *Trichinella* samples from Chukotka and other Arctic and subarctic areas of the Russian Federation resulted in the partial sequence of length 704 bp. The phylogenetic tree obtained with the method of maximum parsimony obtained after the analysis of this alignment was generated and it is presented on Fig. 1. According to the phylogenetic tree the studied *Trichinella* samples are divided into two main groups. Samples from the seal, husky, polar fox (sample 54) from Chukotka as also from polar bear from Jakutia and from stray cat and dog of Kirov and Voronezh regions are clustering together with the sequences of the species *Trichinella nativa* and *Trichinella* sp. 'T6" deposited in NCBI GenBank (Dunams-Morel et al. 2012), whereas samples from polar bear and polar fox (sample 24) from Chukotka, as also from brown bear from Vladivostok area, polar bear and wolverine are demonstrating the close relationships with *Trichinella spiralis*. Only one sequence – sample 51 from stray cat (Chukotka) was out of obvious relationship with known *Trichinella* sequences (Fig. 1.), clustering with basal node for *T. nativa*.

More detailed overview of revealed nucleotide differences is presented in the Table 1. In accordance to the phylogenetic tree the studied trichinellids are divided into two main groups. One group, consisting of three Chukotka *Trichinella* samples from: seal, husky dog and polar fox from fur farm (sample 54), together with several samples from other areas of Russia (brown bear, Kirov region; stray cat, Voronezh region; stray dog, Kirov region). There is demonstrated low number of differences in nucleotide sequence to those of *Trichinella nativa* (JQ430661) and *Trichinella* sp., genotype T6 (JQ430674) observed in database. Obviously, all ana-

lyzed isolates belong to the complex of species "*nativa*-T6". There are no significant differences in the studied *Cob1* mtDNA sequence according to which is possible to discriminate between *T. nativa* and T6. Remaining sequences of studied *Trichinella* samples are clustering with the sequence of *T. spiralis* (GU339148). The sequential difference of *T. spiralis* and *T. nativa Cob1* genes (JQ430661 and GU339148) is 50 bp – approximately 8%. The sample of *Trichinella* sp. from stray cat, hunted at the fur-farm, demonstrated 21-24 bp difference (about 3%) with all *T. nativa* sequences, and 46-47 bp difference (7%) with *T. spiralis* (Table 1). *Trichinella* sample from stray dog (Kirov region) demonstrated the difference in 3 bp (less than 1%) to other *T. nativa* samples.

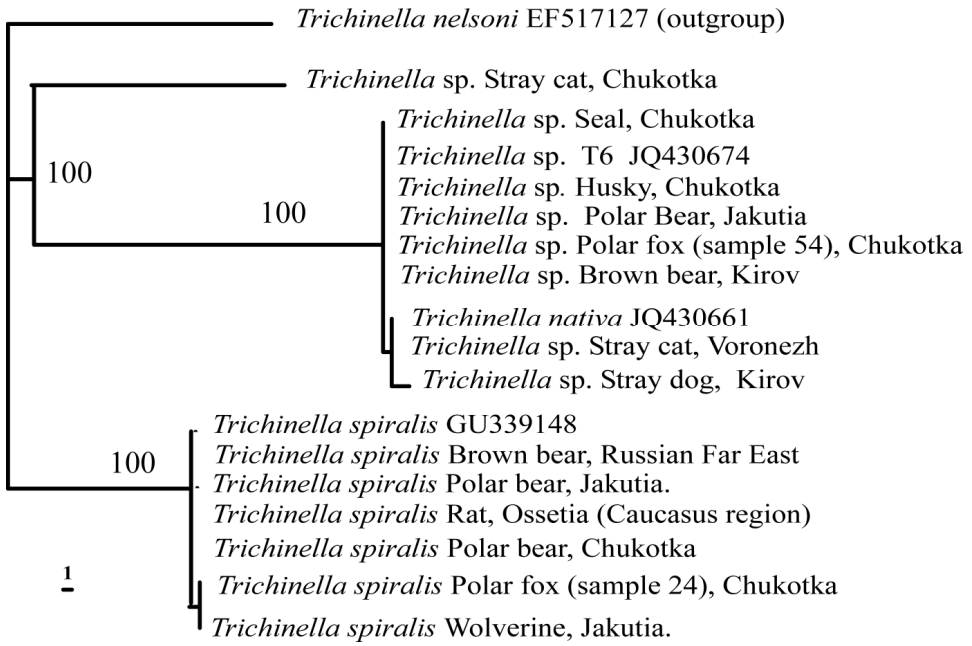

Fig. 1. Phylogenetic tree of partial *Coxb* gene sequences of studied *Trichinella* spp. samples from the Russian Arctic and those of other *Trichinella* species - *Trichinella* sp. T6, *T. nativa*, *T. spiralis*, *T. nelsoni* presented in public databases. Maximum parsimony analysis, 1000 bootstrap repeats. Bootstrap values are presented near appropriate nodes. Scale equals to the nucleotide difference in 1 bp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *Trichinella* sp. stray cat, Chukotka	–	3%	3%	3%	3%	3%	3%	3%	3%	3%	7%	7%	7%	7%	7%	7%	7%	9%
2. *Trichinella* sp. seal, Chukotka	21	–	<1%	0	0	0	0	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
3. *Trichinella* sp. 'T6' JQ430674	21	1	–	0	0	0	0	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
4. *Trichinella* sp. husky dog, Chukotka	21	0	0	–	0	0	0	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
5. *Trichinella* sp. polar bear, Jakutia	21	0	0	0	–	0	0	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
6. *Trichinella* sp., polar fox, sample 54, Chukotka	21	0	0	0	0	–	0	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
7. *Trichinella* sp. brown bear, Kirov region	21	0	0	0	0	0	–	0	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
8. *Trichinella nativa* JQ430661	21	0	0	0	0	0	0	–	<1%	<1%	8%	8%	8%	8%	8%	8%	8%	8%
9. *Trichinella* sp. stray cat, Voronezh region	22	1	1	1	1	1	1	1	–	<1%	8%	8%	8%	8%	8%	8%	8%	8%
10. *Trichinella* sp. stray dog, Kirov region	24	3	3	3	3	3	3	3	2	–	8%	8%	8%	8%	8%	8%	8%	8%
11. *Trichinella spiralis* GU339148	46	57	57	57	57	57	57	58	58	60	–	8%	8%	8%	8%	8%	8%	7%
12. *Trichinella* sp. brown bear, Vladivostok area.	46	57	57	57	58	57	57	58	578	60	0	–	8%	8%	8%	8%	8%	7%
13. *Trichinella* sp. polar bear, Jakutia	46	57	57	57	57	57	46	57	58	60	0	0	–	8%	8%	8%	8%	7%
14. *Trichinella* sp. rat, Ossetia (Caucasus)	46	57	57	57	57	57	57	58	58	60	0	0	0	–	8%	8%	8%	7%
15. *Trichinella* sp. polar bear, Chukotka	46	57	58	57	57	57	57	57	58	60	0	0	0	0	–	8%	8%	7%
16. *Trichinella* sp.polar fox (sample 24), Chukotka	47	58	58	58	58	58	58	58	59	61	1	1	1	1	1	–	8%	7%
17. *Trichinella* sp. wolverine, Jakutia	47	58	58	58	58	58	58	58	47	61	1	1	1	1	1	0	–	7%
18 *T. nelsoni*	62	59	59	59	59	59	59	59	60	62	51	51	51	51	51	52	52	–

Table 1. The differences in partial *coxb* gene sequences of studied *Trichinella* spp. samples from the Russian Arctic and those of other *Trichinella* species - *Trichinella* sp. T6, *T. nativa*, *T. spiralis*, *T. nelsoni* presented in public databases. Below diagonal - number of different nucleotides, above diagonal - percentage of differences in nucleotides (percentage of compared sequences is rounded to an integer).

Concluding remarks

Phylogenetic analysis of several *Trichinella* samples collected in Chukotka Autonomous Region of the Russian Federation revealed the presence of two main groups of trichinellids: *Trichinella spiralis* and representatives of the so-called Arctic complex *T. nativa* - *Trichinella* sp. T6. Both these genotypes are common in Greenland sector of Arctic as it was demonstrated by Dunam-Morel et al., (2012). Partial sequence of *Cob1* mtDNA did not provide the sufficient nucleotide differences for reliable discrimination between these two species of Arctic complex *T. nativa* and *Trichinella* sp.'T6'. Representatives of this species complex are found in Chukotka in seal, husky dog and polar fox, when *T. spiralis* is found in Chukotka in polar bear and polar fox. Besides of our Chukotka survey we also confirmed the presence of *T. nativa* - *Trichinella* sp.T6 complex in polar bear from Jakutia, brown bear from Kirov region, stray cat from Voronezh region and stray dog from Kirov region. *Trichinella spiralis* was reported from brown bear from Primorskii region (Vladivostok area), polar bear from Jakutia, rat from Ossetia (North slope of Caucasus) and wolverine from Jakutia. Low level of intraspecific nucleotide differences was reported for this species in our material, what corresponds to the soon reported uniformity of this species throughout its area of distribution (Rosenthal et al. 2008). One unique *Trichinella* sample significantly different from both main groups was also discovered – *Trichinella* sp. from stray cat, Chukotka. Taxonomic position of this *Trichinella* sample demands additional study. The *Trichinella* circulation in Arctic ecosystems is still unclear. An ability of *Trichinella* larvae to survive in the body tissues of different marine invertebrates and birds was demonstrated (Hulebak 1980, Odoyevskaya et al. 2013). The possibility of trichinellid juveniles' transfer between marine mammals cannot be excluded also, as *e.g.* the predation of walruses on seals was recently reported (Seymour 2012). The development of precise identification of *Trichinella* species at intraspecific level of can serve as an important pre-requisite for the investigation of trichinellosis circulation pathways in Arctic region.

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