Comparative analysis of excretory-secretory proteins of nematodes *Trichinella spiralis* and *Trichinella pseudospiralis* muscle larvae

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Summary. Analysis of excretory-secretory (E-S) proteins produced by muscle larvae of *Trichinella spiralis* strain MH 259593 and *Trichinella pseudospiralis* strain KU 357408 by liquid chromatography-tandem mass spectrometry revealed the presence of important functional proteins in both strains: deoxyribonuclease family proteins, 5'-nucleotidases and serine proteases. The revealed proteins showed different degree of identity with proteins previously found in E-S products of *Trichinella*, for which immunoreactivity was shown (Bien *et al.*, 2015), as well as with those predicted based on the results of DNA and RNA sequence analysis. The results of this work point to the importance of further investigation of the variety of E-S proteins, obtained from *Trichinella* domestic strains of different taxonomic origin, to find early diagnostic antigens or potential candidates for development of a polyvalent vaccine.

Key words: Trichinellosis, functional proteins, LC-MS/MS analysis.

Trichinellosis is an acute anthropozoonosis disease of humans and animals caused by parasitism in the intestine, migration in the body and encapsulation of larvae of nematodes (*Trichinella* larvae) in striated muscles of infected mammals. The causative agent of trichinellosis is a nematode of the family Trichinellidae, genus *Trichinella*, belonging to 12 genotypes/species. Some representatives of this genus are capable of encapsulation in host tissues, and can infect only mammals (*T. spiralis* = T1, *T. nativa* = T2, *T. britovi* = T3, *T. murrelli* = T5, *T. nelson* = T7, *T. patagoniensis* = T12 and genotypes T6, T8, and T9 with uncertain up to now species status). Three other species of *Trichinella* are also known, not forming a capsule in the tissues of the host (*T. pseudospiralis* = T4, infecting both mammals and birds; *T. papuae* = T10 and *T. zimbabwensis* = T11, infecting mammals and reptiles).

Morphological features of *Trichinella*. *Trichinella* is one of the smallest nematodes. Mature *Trichinella* (females are 1-3 mm in length, males 1-2 mm) are located in the mucosa of the small intestine. They are dioecious and viviparous. The development of *Trichinella* is an example of a shortened cycle, when one and the same individual of a mammal plays a role of a final, and then an intermediate host. *Trichinella* shows a steady parasitism, since none of the stages during development comes out into the environment. After fertilisation of the females the males die. On the 3^{d} day after infection fertilised females start to produce larvae, which spread *via* the lymphatic and vascular systems throughout the body and settle in striated muscles. On penetration into the muscle tissue, larvae of *Trichinella* transform cells of striated muscles into special 'feeding cells'. Gradually the myofibrils disappear and the muscle tissue of the host undergoes deep modification (Dupouy-Camet & Murrel, 2007). The long-term survival of the intracellular helminth in the host organism and successful accomplishment of its life stages are provided by a complex of proteins, released during ontogeny in succession by intestinal (adult) *Trichinella*, newborn larvae that migrate *via*

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the lymphatic and vascular systems and, finally, the larvae that have settled in striated muscle. Many of these complexes of proteins act as antigens; in response to their impact a humoral and cellular immunity is formed in infested individuals. Thus, these proteins may be used for the development of diagnostic products and vaccines against trichinellosis, drawing special attention of many researchers to investigation of the immunogenicity of such proteins-antigens produced by *Trichinella* muscle larvae. These are involved twice in the stimulation of the host immune system – the first time during four moults in the intestine, turning into a mature individual, and the second time during penetration inside the myofibrils, and then continuing to interact with the immune system of the macroorganism (the host) by secreting protein complexes throughout the rest of the nematode's life.

Large-scale identification of potentially immunoreactive proteins at all major ontogenetic stages of *T. spiralis* was conducted by Bien *et al.* (2015). Immunoreactive proteins in somatic extracts of muscle and intestinal *Trichinella*, as well as excretory-secretory (E-S) products of these worms were characterised by immunoblott/SDS-PAGE with sera of experimentally infected by *T. spiralis* pigs (AB to AG of *Trichinella*). Basing on the results of immunoblotting, 18 proteins in the range of molecular masses from 20 to 27, 41, 59 and 105 kDa were selected and finally identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The aim of the present work was to identify proteins in E-S products of muscle larvae of two species of *Trichinella*: *T. spiralis*, strain MH 259593, and *T. pseudospiralis*, strain KU 357408, and to perform comparative analysis of these proteins in the light of the results obtained by the other researchers (Robinson *et al.*, 2005, 2007; Bien *et al.*, 2013, 2015).

MATERIAL AND METHODS

Strains of *Trichinella*: *T. spiralis* MH 259593 (domestic pig) and *T. pseudospiralis* KU 357408 (raccoon) were used. To maintain strains in a viable state, passage on laboratory rodents was used during many years.

Animals. To produce preparative quantities of biomass of even-aged infective larvae of two species of *Trichinella*, infection was conducted in two groups (3 individuals each) of white mongrel rats at a dose of 1,000 larvae per rat. The slaughter of animals was carried out with diethyl ether 2 months after infection. During the experiment, classic methods were used, including biochemical digestion of muscle tissue in artificial gastric juice, the preparation and purification of E-S products, and determination of the final concentration of the protein. Carcasses of animals were subjected to biochemical digestion in artificial gastric juice in the unit 'Gastros', infective larvae were carefully washed with physiological solution with the addition of the antibiotics: ampicillin (5 μg ml^{-1}) and gentamycin (4 μg ml^{-1}); 10,000 larvae were placed in Petri dishes 5 cm in diameter with the DMEM medium and incubated at 39°C for 48 h. After incubation, larvae were allowed to sediment by settling in conic tubes and culture medium containing E-S products of *Trichinella* was used for preparation of samples for LC-MS/MS analysis.

Preparation of samples for LC-MS/MS analysis. The culture medium containing E-S products was centrifuged at 1,000 *g* for 5 min for removal of insoluble components. Collected supernatants were further dialysed. Dialysis of the samples was carried out against distilled water within a day with four changes of water to remove low molecular weight protein components and components of DMEM medium with glutamine (Paneko). Dialysed samples were kept frozen (–18°C).

Reduction, alkylation and digestion of the proteins. Sample preparation was performed as described previously with minor modifications (Kulak *et al.*, 2014). Sodium deoxycholate (SDC) reduction and alkylation buffer pH 8.5 were added to a sample of *Trichinella* E-S proteins containing 100 μg of protein so that the final concentration of protein, TRIS, SDC, TCEP and 2-chloroacetamide were 1 mg ml^{-1}, 100 mM, 1% (w/v), 10 mM and 40 mM, respectively. The solution was boiled for 10 min and after cooling down to room temperature, the equal volume of trypsin solution in 100 mM TRIS pH 8.5 was added in a 1:100 (w/w) ratio. Digestion was performed at 37°C overnight. Peptides were acidified to a final concentration of 1% trifluoroacetic acid (TFA) for SDB-RPS binding, and 20 μg was loaded on two 14-gauge StageTip plugs. Ethylacetate/1% TFA (125 ml) was added, and the StageTips were centrifuged at 200 *g*. After washing the StageTips using two wash steps of 100 μl ethylacetate/1% TFA and one of 100 μl 0.2% TFA consecutively, peptides were eluted by 60 μl of elution buffer (80% acetonitrile, 5% ammonia). The collected material was completely dried using a SpeedVac centrifuge (Savant, SpeedVac concentrator) and stored at –80°C before LC-MS/MS analyses. Before analyses, peptides were suspended in loading buffer (2% acetonitrile,

0.1% TFA) and sonicated for 2 min (Elma, Elmasonic S100).

Liquid chromatography and mass spectrometry. Approximately 1 μg of peptides was loaded for 2 h gradient. Peptides were separated on a 25-cm 75-μm inner diameter column packed in-house with Aeris Peptide XB-C18 2.6 μm resin (Phenomenex). Reverse-phase chromatography was performed with an Ultimate 3000 Nano LC System (Thermo Fisher Scientific), which was coupled to the Q Exactive HF mass spectrometer (Thermo Fisher Scientific) *via* a nanoelectrospray source (Thermo Fisher Scientific). Peptides were loaded in buffer A (0.2% (v/v) formic acid) and eluted with a linear 120-min gradient of 4-45% buffer B (0.1% (v/v) formic acid, 80% (v/v) acetonitrile) at a flow rate of 350 nl min^{-1}. After each gradient, the column was washed with 95% buffer B for 5 min and re-equilibrated with buffer A for 5 min. Column temperature was kept at 40°C. Peptides were analysed on a mass spectrometer, with one full scan (300-1,400 *m*/*z*, *R* = 60,000 at 200 *m*/*z*) at a target of 3×10^6 ions, followed by up to 15 data-dependent MS/MS scans with higher-energy collisional dissociation (HCD) (target 1×10^5 ions, max ion fill time 60 ms, isolation window 1.4 m/z, normalised collision energy (NCE) 28%, under fill ratio 2%), detected in the Orbitrap (*R* = 15,000 at fixed first mass 100 *m*/*z*). Other settings: charge exclusion – unassigned, 1, > 6; peptide match – preferred; exclude isotopes – on; dynamic exclusion – 30 s was enabled. Each sample was analysed by LC-MS/MS in three technical replicates and not less than two biological replicates.

Data analysis. MS/MS based qualitative proteome analysis of *Trichinella* E-S protein samples was made using PEAKS Studio 8.0 build 20160908 software (Ma *et al.*, 2003). Peptide lists generated by the PEAKS Studio were searched against the *T. spiralis* and *T. pseudospiralis* database (http:// parasite.wormbase.org) with cysteine carbamidomethylation as a fixed modification and N-terminal acetylation and methionine oxidations as variable modifications. The false discovery rate (FDR) for peptide-spectrum matches was set to 0.01 and was determined by searching a reverse database. Enzyme specificity was set to trypsin, and a maximum of three missed cleavages were allowed in the database search. Peptide identification was performed with an allowed initial precursor mass deviation up to 10 ppm and an allowed fragment mass deviation of 0.05 Da. A protein identified by no fewer than 10 unique peptides was considered to be reliably defined.

RESULTS AND DISCUSSION

The intensity of infestations in 90 days after the infection in the average was 789 larvae of *T. pseudospiralis* and 1208 larvae of *T. spiralis* per gram of muscle tissue. Differing indicators of capacity (IC) of reproduction for the studied genotypes T1 and T4 were associated with substantial morphological and physiological differences. Cosmopolitan species *T. spiralis* (T1) has a high degree of adaptation to the inhabitants of synanthropic biocenosis – pigs, rats, domestic carnivores and humans. High virulence, the severity of the pathological process characteristic for a person, infested by this type of *Trichinella*, are due to the high level of female fertility of *T. spiralis*. Very long uterus in females of this species (compared to other) is an important morphological feature, and, of course, it correlates with the index of fertility and the amount of newborn larvae produced by the female worms. Most of the deaths caused by infection by the above mentioned species of *Trichinella* are due to the introduction of a vast number of migrating larvae in the host tissue, fusion of muscle fibers, the formation of large infiltrates, allergic reactions. Distribution of the species *T. spiralis* in Northern latitudes is limited by relatively low resistance to freezing, although, according to the latest data, the manifestation of the physiological characteristics largely depends on the degree of mutual adaptation of parasite and host. Genotype T1 is often found in natural communities – many of the wild animals: badgers, foxes, raccoon dogs, lynxes, wolves, bears, rodents were registered as hosts for this species of *Trichinella* in different countries of the world. As a rule, the infection of this type occurs through the carcasses of dead animals, waste from pig farming and other anthropogenic factors. Typical configuration of the capsules is oval or ovate.

Non-encapsulating species of *Trichinella*, *T. pseudospiralis* (genotype T4), is a cosmopolitan, found in many countries of Europe, Asia, North America. To date 14 species of mammals and seven species of birds were registered around the world as the hosts of *T. pseudospiralis*. In Russia outbreaks of trichinellosis caused by this species of *Trichinella* have been registered among the population of Kamchatka, Krasnodar territory, Far East, Tula region. The main biological features of *T. pseudospiralis* are the absence of a collagen capsule around the muscle larva, the ability to infect not only mammals but also birds, about 1/3 smaller new born larvae and mature worms in comparison with other species of *Trichinella*. Reproduction potential

of this species of *Trichinella* is lower than that of *T. spiralis*, resistance to freezing is also low.

It should be noted that the viability of the North Ossetian isolates of *T. spiralis* from domestic pigs and Krasnodar isolate of *T. pseudospiralis* from raccoon were maintained in laboratory rodents (white out bred rats and mice) for more than ten years. Probably various processes that modify immunological parameters of both surface and E-S antigens of *Trichinella* determine adaptability of these tissue helminths to diverse spectrum of animal hosts.

Identification of *Trichinella* proteins that play an important role in the development of infection caused by these parasites, as well as those most crucial for the immunological identification is drawing increasing attention of researchers now days.

One of the problems complicating such investigations arises from relatively low protein content in the E-S products (Robinson *et al.*, 2007). Proteomic analysis has proved to be effective in this way and was successfully used to identify E-S proteins, including those recognised by antibodies present in the serum of infected host animal (Robinson *et al.*, 2007; Bien *et al.*, 2015; Wang *et al.*, 2017).

Recently, it has been shown (Bien *et al.*, 2013, 2015) that three proteins revealed in the E-S products of *Trichinella spiralis* strain ISS-003 possessed high immunoreactivity, when detected with antibodies obtained from pigs infected by *Trichinella*. These proteins were identified as P49 antigen (M64242.1, cloned cDNA sequence, partial), 5'-nucleotidase (XM_003374516.1, predicted full-length mRNA) and serine proteinase (AY028974.1, cloned cDNA sequence). One of these proteins, namely 5'-nucleotidase, was proposed to be a potential antigen for early disease diagnosis, as well as a target protein for the development of a vaccine against trichinellosis (Bien *et al.*, 2015).

Each of these proteins has one or more homologues in other species and strains of *Trichinella* in current versions of protein databases like NCBI, WormBase, UniProt. Most of the records were derived from *in-silico* gene prediction and therefore rarely have any experimental evidences. The other possible drawback of such prediction methods is rather low accuracy of 5'-end prediction. Also homologues of P49 antigen, 5'-nucleotidase and serine proteinase have different levels of sequence identity between species of *Trichinella* and even between different strains. In connection with the above, it is very important to investigate how significant can be strain differences for these proteins in *Trichinella* and obtain experimental evidences at the protein level. To answer this question we have applied MALDI MS-MS analysis for identification of these proteins in the cultivation media of *T. spiralis*, strain MH 259593 and *T. pseudospiralis*, strain KU 357408 muscle larvae and conducted a comparative analysis of the identified proteins against proteins of *T. spiralis* and *T. pseudospiralis*, discovered previously (Robinson *et al.*, 2007; Bien *et al.*, 2015).

Table 1. E-S proteins of *Trichinella spiralis* strain MH 259593 muscle larvae identified by LC-MS/MS analysis, comparison with the reference E-S proteins of *T. spiralis* strain ISS-003.

Compared proteins, accession numbers[a] *T. spiralis*, strain MH 259593	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers*[a] *T. spiralis*, strain ISS-003**
43 kDa secreted glycoprotein Q27073[a]	91.5/100.0	P49 antigen partial, M64242.1*/Q27076[a]
5'-nucleotidase E5S553[a]	82.8/39.3	5'-nucleotidase, XM_003374516.1*/E5SH34[a]
5'-nucleotidase E5S554[a]	89.5/38.6	5'-nucleotidase, XM_003374516.1*/E5SH34a
Transmembrane serine protease 8 E5SBE6[a]	66.7/70.5	Serine proteinase, AY028974.1*/Q9BIC9[a]

[a] – accession number according to Uniprot database.

* – reference protein, accession number specified by Bien *et al.*, 2015 (NCBI database).

Analysing E-S protein preparations from *T. spiralis* strain MH 259593 by MALDI MS-MS method we have discovered protein products of at least three genes:

1. 43 kDa secreted glycoprotein, gene *Tsp_00874* (UniProt: Q27073).
2. 5'-nucleotidase, which is encoded by two genes *Tsp_06020* (UniProt: E5S553) and *Tsp_06021* (UniProt: E5S554) with very high level of sequence identity and are most likely paralogous genes resulting from recent tandem duplication.
3. Transmembrane serine protease 8, gene *Tsp_01070* (UniProt: E5SBE6).

Sequence identity of these proteins from MH 259593 strain with proteins identified in ISS-003 strain of *T. spiralis* and database accessions information are summarised in Table 1. Data presented show that identified proteins demonstrate a different degree of identity with E-S proteins of *T. spiralis* strain ISS-003. The only identical protein product found in both strains is 43 kDa secreted glycoprotein/p49 antigen (encoded by *Tsp_00874* gene), which has two records in UniProt: Q27073 for predicted gene model and Q27076 for sequenced mRNA. Both records are identical besides few additional 5' amino acids in Q27076 most likely due to misprediction of real start codon in Q27073, which is a common case to all automatically annotated genes. We also detected protein products of *Tsp_06020* and *Tsp_06021* genes both encoding 5'-nucleotidase (E5S553 and E5S554). It is interesting to note that in strain ISS-003 protein product of *Tsp_03760* (E5SH34, also 5'-nucleotidase), which is a more divergent paralogue of *Tsp_06020* and *Tsp_06021* was identified by Bien *et al.* (2015) and that can be due to strain-specific expression of these 5'-nucleotidases. Transmembrane serine protease 8 (E5SBE6) that we detect in strain MH 259593 differs clearly from that (Q9BIC9) detected in ISS-003 (Table 1).

These data suggest that we observe some differences in composition of E-S proteins fraction between strains of *T. spiralis*.

Analysing E-S proteins produced by muscle larvae of *T. pseudospiralis*, strain KU 357408 we also found proteins belonging to the groups of deoxyribonucleases, 5'-nucleotidases and serine proteinases. We compared these proteins with proteins of *T. spiralis* and *T.pseudospiralis* identified earlier (Robinson *et al.*, 2007; Bien *et al.*, 2015) (Table 2).

Table 2. E-S proteins of *Trichinella pseudospiralis* strain KU 357408 muscle larvae identified by LC-MS/MS analysis, comparison with the reference E-S proteins of two strains of *T. spiralis* and *T. pseudospiralis* strain ISS13.

Compared proteins, accession numbers[a] *T. pseudospiralis*, strain KU 357408	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers*[a] *T. pseudospiralis*, strain ISS13**	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers*[a] *T. spiralis*, strain ISS-390**	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers**[a] *T. spiralis*, strain ISS-003**
Deoxyribonuclease-2-alpha partial A0A0V1KA14[a]	21.5/94.1	38 kDa excretory/secretory protein, Y118168*/ Q8MTB3[a]	[b]	Was not found	21.4/80.3	P49 antigen partial, M64242.1**/Q27076[a]
Snake venom 5'-nucleotidase A0A0V1JN66[a]	[b]	Was not found	98.9/86.6	5'-nucleotidase, AAM97494*/ Q8MQS9[a]	88.5/40	5'-nucleotidase, XM_003374516.1**/ E5SH34[a]
5'-nucleotidase A0A0V1JM22[a]	[b]	Was not found	56.5/93.2	5'-nucleotidase, AAM97494*/Q8MQS9[a]	0[c]	5'-nucleotidase, XM_003374516.1**/ E5SH34[a]
Transmembrane protease serine 9, A0A0V1K369[a]	[b]	Was not found	99.8/71.3 0[c]	TpSerP, AF331156*/Q9BJM1[a] Serine proteinase, AY028974.1*/**Q9BIC9**[a]	0[c]	Serine proteinase, AY028974.1**/ **Q9BIC9**[a]
Serine protease 30 A0A0V1K7X8[a]	[b]	Was not found	0[c] 39.3/62.0	TpSerP, AF331156*/Q9BJM1[a] Serine proteinase, AY028974.1*/**Q9BIC9**[a]	39.3/62	Serine proteinase, AY028974.1**/ **Q9BIC9**[a]

[a] – accession number according to Uniprot database.
* – reference protein, accession number specified by Robinson *et al.*, 2007 (NCBI database).
** – reference protein, accession number specified by Bien *et al.*, 2015 (NCBI database).
[b] – no data in the absence of a reference protein.
[c] – not found in the specified search conditions.

Table 3. E-S proteins of *Trichinella spiralis* strain MH 259593 muscle larvae identified by LC-MS/MS analysis, comparison with reference E-S proteins in *T. pseudospiralis* and *T. spiralis* strain ISS-003.

Compared proteins, accession numbers[a] *T. spiralis*, strain MH 259593	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers[a] *T. pseudospiralis*, strain KU 357408**	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers*[a] *T. spiralis*, strain ISS-003**	**Query coverage (%) /alignment identity (%)**	**Reference proteins, accession numbers*[a] *T. pseudospiralis*, strain ISS13**
43 kDa secreted glycoprotein Q27073[a]	19.6/80.3	Deoxyribonuclease-2-alpha partial, A0A0V1KA14[a]	(b)	Was not found	100.0/84.8	38 kDa excretory/secretory protein, Y118168*/ Q8MTB3[a]
5'-nucleotidase E5S553[a]	97.0/86.6 58.7/93.2	Snake venom 5'-nucleotidase, A0A0V1JN66[a] 5'-nucleotidase, A0A0V1JM22[a]	96.0/100.0	5'-nucleotidase, AAM97494*/ Q8MQS9[a]	(b)	Was not found
5'-nucleotidase E5S554[a]	98.9/86.6 56.5/92.9	Snake venom 5'-nucleotidase, A0A0V1JN66[a] 5'-nucleotidase, A0A0V1JM22[a]	100.0/98.5	5'-nucleotidase, AAM97494*/ Q8MQS9[a]	(b)	Was not found
Transmembrane serine protease 8 E5SBE6[a]	0[c]	Transmembrane protease serine 9, A0A0V1K369[a]	66.74/70.5	Serine proteinase, AY028974.1*/ Q9BIC9[a]	(b)	Was not found
	27.1/86.4	Serine protease 30, A0A0V1K7X8[a]	0[c]	TpSerP, AF331156*/Q9BJM1[a]	(b)	Was not found

[a] – accession number according to Uniprot database.
***** – reference proteins, accession numbers specified by Robinson *et al.*, 2007 (NCBI database).
(b) – no data in the absence of a reference protein.
[c] – not found in the specified search conditions.

The results presented in Table 2 suggest that *T.pseudospiralis* strain KU 357408 has a greater repertoire of proteins in E-S fraction and also confirm the observation that there are inter-strain differences in composition of E-S fraction. In addition, these results demonstrate inter-species differences of the proteins, identified in this fraction.

For the next step of analyses we compared proteins identified by us in *T. spiralis* strain MH 259593 with the proteins identified in *T. pseudospiralis*, strain KU 357408, and proteins identified by Robinson *et al.* (2007), in *T. pseudospiralis* strain ISS13 and *T. spiralis* strain ISS390 (data presented in Table 3).

Special attention should be drawn to the fact that proteins in the analysed strains of *Trichinella* may show a rather high degree of identity with the proteins more remote in systematic relation (Table 3), for example, proteins with deoxyribonuclease activity – 43 kDa secreted glycoprotein Q27073 and 38 kDa excretory-secretory protein Q8MTB3. At the same time, it is difficult to draw a similar conclusion regarding deoxyribonucleases identified in *T. spiralis* strain MH 259593 and in *T. pseudospiralis*, strain KU 357408, since the length of the compared proteins varies greatly. In another case, the nucleotidases found in *T. spiralis* strain MH 259593 5'-nucleotidase E5S553 and E5S554 showed a high degree of identity both with one of nucleotidases identified in E-S products in *T. pseudospiralis* strain KU 357408 – A0A0V1JN66 and with 5'-nucleotidase Q8MQS9 revealed by Robinson *et al.* (2007) (Table 3), but the degree of identity was less obvious when compared with 5'-nucleotidase E5SH34 of *T. spiralis* strain ISS-003 (Bien *et al.*, 2015) (see Table. 1). As we have noted above, immunoreactivity of some of these proteins has been demonstrated by Bien *et al.* (2015). A greater or lesser degree of identity of the proteins identified in this work and proteins, possessing high immunoreactivity, in all probability can correlate, although it is possible that such correlation is not unconditional. For example, even with very high (> 90%) or full identity of aligned sequences, it is not possible to claim that these proteins are identical polypeptide chains. It is important to note that the length of protein products in *Trichinella* of different systematic position may, apparently, differ, so that in this case 100% alignment identity of the two proteins

may be valid only for part of the polypeptide chain, while the other part may be absent.

Summarising data presented in the article we can emphasise that protein composition in E-S fraction of *Trichinella* can vary greatly between species and also between strains. Two main sources of such variability can be pointed out on the level of genome organisation. First is the presence of paralogous genes in the same genome that can be differentially expressed in strains or even in different environment conditions. The example here is 5'-nucleotidase that clearly has at least three paralogous genes in *T. spiralis*. Second source is activation of non-orthologous genes belonging to the same functional group. That could be the case of serine proteases: level of amino acid sequence divergence is much higher here. In some cases, it is not even possible to find a homologous protein in other species.

In general, it can be concluded that the diversity of the identified proteins in different strains (even of the same species) of *Trichinella* does not allow us to state unambiguously the prospects of using one or two of them as target strains for production of proteins suitable for development of a polyvalent vaccine. It is obvious, however, that it is more expedient to use sets of proteins, obtained from domestic strains of *Trichinella* of different taxonomic origin, which could be a promising approach to solve this problem. A similar conclusion can be drawn in relation to protein antigens for early diagnosis of the disease. Obviously, in this respect it is necessary to clone proteins with the highest immunoreactivity and in the highest degree identical in different *Trichinella*. This approach requires large-scale proteomic studies of E-S products in different species and strains of *Trichinella* at the first stages and later – cloning of the selected sequences.

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И.М. Одоевская, И.Б. Кудряшова, О.П. Курносова, В.В. Рекстина, Ю.А. Руденская, Р.Х. Зиганшин и Т.С. Калебина. Сравнительный анализ экскреторно-секреторных белков мышечных личинок *Trichinella spiralis* и *Trichinella pseudospiralis*.

Резюме. Анализ экскреторно-секреторных (Э-С) белков мышечных личинок *Trichinella spiralis* штамма MH 259593 и *Trichinella pseudospiralis* штамма KU 357408, проведённый с помощью тандемной масс-спектрометрии, сопряженной с высокоэффективной жидкостной хроматографией, показал в обоих штаммах наличие важных функциональных белков, принадлежащих к семейству дезоксирибонуклеаз, 5'-нуклеотидазам и сериновым протеазам. Выявленные белки показали различную степень идентичности с белками, обладающими иммунореактивностью, ранее обнаруженными в Э-С продуктах трихинелл (Bien *et al.*, 2015), а также с белками, прогнозируемыми по результатам анализа последовательностей ДНК и РНК. Результаты данной работы указывают на важность дальнейшего изучения разнообразия Э-С белков, полученных из отечественных штаммов трихинелл различного таксономического происхождения, для выявления ранних диагностических антигенов и потенциальных кандидатов на разработку поливалентной вакцины.