Herald of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences. – Astana: S. Seifullin Kazakh Agrotechnical Research University, 2025. – № 1 (009). – P. 72-84. - ISSN 2958-5430, ISSN 2958-5449

doi.org/ 10.51452/kazatuvc.2025.5(009).1874
UDC 576.8 **Research article**

Analysis of fatp1 and px-domain genes to investigate the possibility of using them in species-specific diagnosis of *Trichinella nativa* infection

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Received: 26-02-2025 **Accepted:** 17-03-2025 **Published:** 31-03-2025

Abstract

Background and Aim. Trichinellosis, caused by nematodes of the genus *Trichinella*, is a zooanthroponotic infection which importance is steadily increasing due to its widespread distribution and significant impact on human and animal health. This study focuses on the species of *Trichinella nativa*, one of the most resistant species adapted to cold conditions, which frequently infects various wild mammalian species.

Materials and Methods. Recent molecular studies have helped to clarify the genetic structure of *T. nativa,* but significant gaps remain in understanding its immunogenic profile and biological characteristics. A comprehensive bioinformatics analysis using the BepiPred 3.0 tool of two key *T. nativa* proteins, the long-chain fatty acid transport protein (FATP1) and the phox-homology (PX) domain, to identify potential B-cell epitopes and understand their interaction with the host immune system has identified several significant immunogenic regions in both proteins. A more detailed study of these proteins and their analysis may be relevant for the development of diagnostic and therapeutic agents.

Results. Our results emphasize the need for further research on the immunobiology of *T. nativa*, especially in the context of increasing cases associated with climate and environmental changes.

Conclusion. This study provides important insights that can contribute to the development of specific diagnostic methods and effective control strategies for trichinellosis, improving public health in the affected Kazakhstan regions.

Keywords: bioinformatic analysis; diagnosis; FATP1; protein; PX-domain gene; trichinellosis.

Introduction

Trichinella nativa is one of the most cold-resistant species within the genus *Trichinella*, which causes its distribution in the Arctic and subarctic regions of the world, including Kazakhstan [1]. This species has adapted to harsh climatic conditions and is found in a variety of wild animals including bears, foxes and wolves, indicating its wide ecological range [2].

The study of *Trichinella nativa* faces several significant challenges, among which the limited data on its biological and immunological characteristics stand out. Although studies in recent years have shed light on general aspects of the morphology and genetics of *Trichinella* species, the lack of data on its immunoactive proteins remains one of the main obstacles in understanding the pathogenesis and host-parasite interactions [3, 4]. Modern omics technologies, such as proteomics and genomics, are gradually beginning to resolve this gap, revealing the molecular mechanisms underlying the host immune response to *T. nativa* infection [5].

The study of *Trichinella nativa* immunoactive proteins is a key element for the development of effective diagnostic tests that will allow more accurate infection presence identification at early stages. One promising area is research on serine proteases, which have shown to be useful in the context of early diagnosis of trichinellosis at the intestinal larval development stage [6]. While data on these markers for *Trichinella spiralis* are already beginning to be integrated into serologic tests, information on *T. nativa* is still scarce [7].

The development of specific antigens for serological diagnosis could greatly accelerate the detection of infection and minimize the risk of disease spreading in the population [7]. Although exosecretory products of muscle larvae show promising results, more research is needed to adapt and utilize them in the diagnosis of T. nativa [8].

In the present study, analysis of bioinformatic resources highlights two key proteins of high potential relevance in the context of the *T. nativa* immune response. Long-chain fatty acid transport protein 1 (FATP1) plays an important role in lipid metabolism, as highlighted in studies on *T. spiralis*, where this protein was detected in the serum of infected mice and showed potential as a secreted protein [9]. Other studies also indicate the importance of this class of proteins in linking to immune responses by participating in phosphoinositide signaling and modifying immune cell functions [10].

Moreover, the phox-homology (PX) domains have been shown in various studies to play a significant role in biological signaling and interactions with cell membranes, allowing them to participate in immunomodulation and interactions with immunomodulatory proteins in parasites [11, 12].

The choice of these proteins for analysis is based not only on their presumed functional significance in the life cycle of *T. nativa*, but also on the limited study of their role in the immune response. It is the unique function of these proteins in cellular pathways that led to the hypothesis that they may be determinants in *T. nativa* immune activation.

Thus, the aim of the present study is to perform bioinformatic analysis of potential epitopes of these proteins and to evaluate their interactions with components of the immune system. This approach can increase the understanding of the molecular mechanisms of *Trichinella nativa* pathogenesis and provide new opportunities for the development of more accurate diagnostic and therapeutic tools.

Materials and Methods

Ethical approval.

The study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology, S. Seifullin Kazakh Agrotechnical Research University (KATRU) and was performed in accordance with the "Guidelines for Animal Housing and Care: Species Specific Provisions for Laboratory Rodents and Rabbits" (Interstate Standard, GOST 33216-2014) [13]. All protocols were performed in accordance with the "International Guidelines for Biomedical Research Using Animals" [14].

Experiments were conducted at the Research Platform of Agricultural Biotechnology, KATRU from August 2024 to February 2025. Four male Soviet Chinchilla rabbits aged 7-8 months with a live weight of 4100-4600 g were used in the study. Rabbits were kept in proper hygienic conditions in the vivarium of KATRU, Astana, Kazakhstan.

Larvae isolation. T. nativa larvae were obtained by isolating them from muscle tissue samples of spontaneously infected wild animals. The isolation from samples of muscle tissue of animals was carried out by the method of compressor trichinoscopy and digestion in artificial gastric juice (AGJ) in accordance with the methods of Methodological Guidelines 4.2.2747-10 "Methods of sanitary-parasitological examination of meat and meat products". Detected and isolated helminthological material was preserved in 70% ethanol solution [15].

RNA isolation. Total RNA was isolated using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA concentration was measured using NanoDrop 2000 (Thermo Scientific, USA). Total RNA was reverse transcribed into first strand cDNA using ProtoScript II First Strand cDNA Synthesis Kit (New England BioLabs, England).

Setting up PCR. The reaction was performed on a VerityPro amplifier (Applied Biosystems, USA):

The composition of the reaction mixture included:

Compounds	Volume, µl
DreamTaq Buffer	2
MgCl2	1
dNTPs	2
Primers (F, R)	2
DreamTaq polymerase	0.25
DNA	2 (100 ng)
MilliQ (H2O)	Up to 25

Procedure PCR:

Steps	Temperature (°C)	Time	Number of cycles
Initialization	95	5 min	1
Denaturation	95	30 sec	30
Primer annealing	58	30 sec	30
Elongation	72	60 sec	30
Final elongation	72	5 min	1

Sequencing. DNA sequencing was performed using BigDye Terminator v3.1 kit (Thermo Fisher, USA) according to the manufacturer's protocol. SeqStudio genetic analyzer (Thermo Fisher, Applied Biosystems, USA) was used to analyze amplified fragments.

Bioinformatic analysis. Epitope analysis. The BepiPred 3.0 tool was used to predict potential B-cell epitopes of PX-domain and FATP1 proteins. This method is based on a machine learning algorithm that predicts linear epitopes based on amino acid sequence. The input data consisted of amino acid sequences of proteins obtained from the NCBI database. The results of the analysis were presented graphically, where regions with high probability value (>0.5) were considered as potential epitopes.

Three-dimensional structure modeling. The PHYRE 2.2 program was used to build three-dimensional models of the PX-domain and FATP1 proteins. The methodology included amino acid sequence analysis using a homology modeling approach. The modeling results were evaluated for amino acid sequence coverage and model validity. Visualization of 3D structures was performed in PyMOL software for further interpretation of protein structural features.

Phylogenetic analysis was performed to study the evolutionary relationships of the PX-domain and FATP1 proteins *of T. nativa*. Amino acid sequences were aligned using the MAFFT program. Phylogenetic tree construction was performed using the IQ-TREE program using the maximum likelihood method with automatic model selection. The tree was visualized using the FigTree program version 1.4.3. The obtained data allowed us to estimate the evolutionary position of proteins in comparison with their homologs from other *Trichinella species.*

Statistical analysis. Statistical analysis was performed using GraphPad Prism 7.0 and Microsoft Excel 2010. Values of $p<0.05$ were considered statistically significant.

Results

During the initial stages of research, 1520 larvae were isolated from the iris of foxes captured in the territory of Akmola region. Morphological and molecular analysis confirmed their belonging to the species *T. nativa.*

During the experiment, a total RNA with a concentration of 150-200 ng/µl was isolated, after which cDNA synthesis on the matrix of isolated RNA was performed. Specific primer pairs were selected for amplification of target genes encoding FATP1 and PX domain using Primer BLAST tool:

For FATP1:

T.n. FATP1_PrF: CGTCATGGGTTGATTGTTTT

T.n. FATP1_PrR: GTCTTTGTACTTCAGTGCGTCA

For PX domain:

T.n. PXdp_PrF: GTTATTGGCGAAGGCAGCAGTG

T.n. PXdp_PrR: TTTGTTCGCGGGAAGGCTAG

PCR analysis successfully detected the target fragments, which was confirmed by agarose gel electrophoresis. The detected bands coincided with the expected sizes, indicating the presence of the desired sequences in the samples (Figure 1).

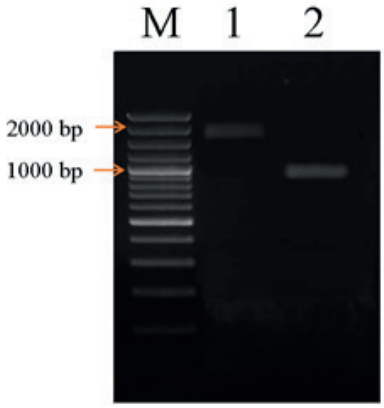


Figure 1 – Electrophoresis of PCR products amplified using specific primers for fatty acid transport protein 1 (FATP1) and PX domain genes (Note: M – molecular marker (Thermo Scientific GeneRuler 100 bp Plus DNA Ladder, ready-to-use), 1 – FATP1 amplification product, 2 – PX do-main amplification product)

After PCR reaction, the amplified fragments were subjected to sequencing for further analyses.

The sequencing of the FATP1 gene (Long-chain fatty acid transport protein 1) with a length of 1920 nucleotide pairs was performed by the Sanger method. Since this method allows us to read a sequence of up to 1000 nucleotide pairs in length, we used three-step sequencing with three short primers for complete analysis. The sequencing scheme is presented in figure 2, which shows the amplification sites, and the location of the primers used (PrF1, PrR1, PrF2, PrR2, PrF3, PrR3) that ensure sequential reading of the entire nucleotide sequence.

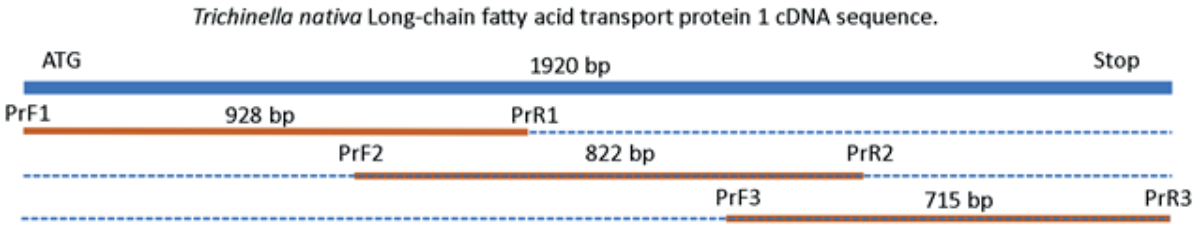


Figure 2 – Sequencing scheme of the FATP1 (Long-chain fatty acid transport protein1) gene of *T. nativa*

At the same time, the size of the PCR product of the PX domain was 969 nucleotide pairs, which allowed its sequencing using the standard method without additional fragmentation. For further bioinformatic analysis of the proteins, the obtained nucleotide sequences were converted into amino acid sequences using a standard genetic code.

The bioinformatic study of these proteins began with the analysis of epitope regions (Figure 3), which play a key role in understanding their immunogenicity and potential use in diagnostics and vaccine development.

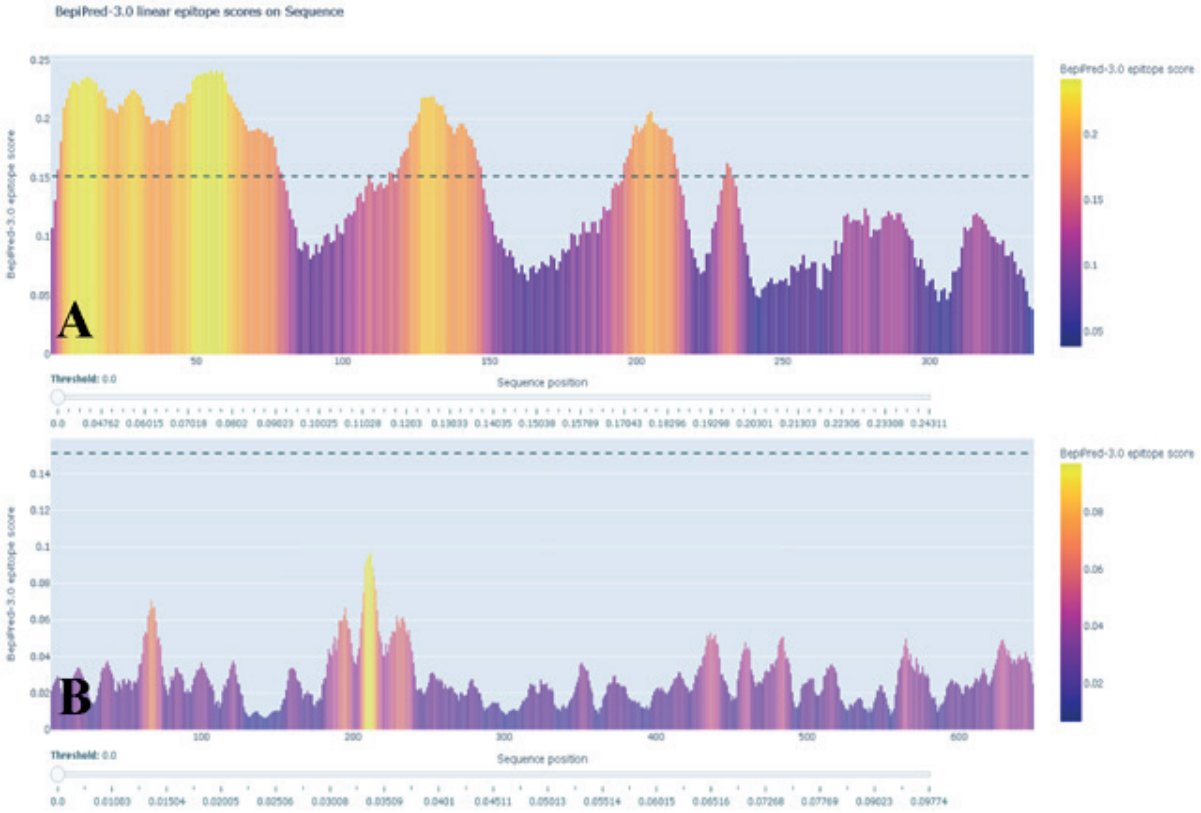


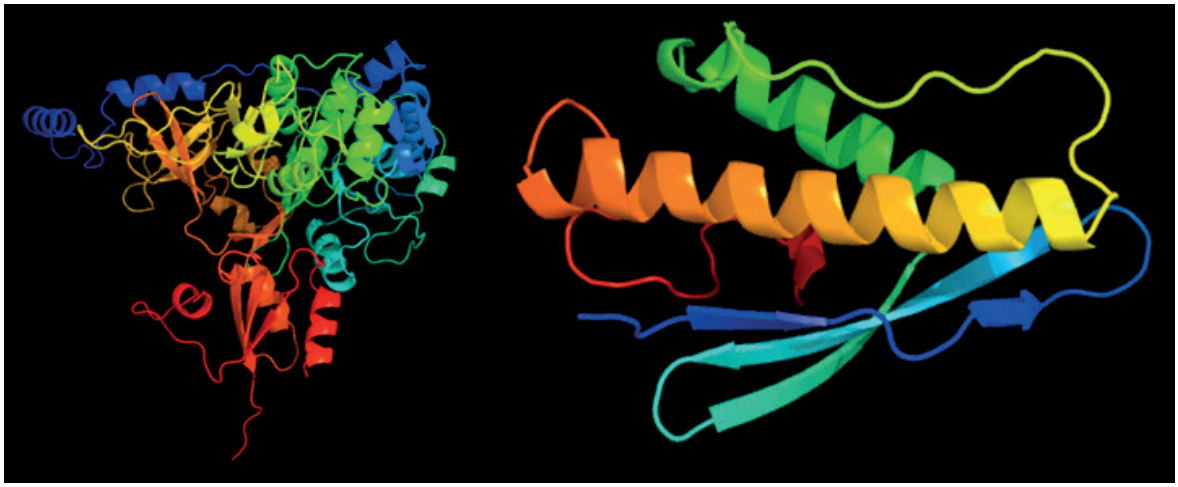
Figure 3 – Linear B-cell epitope analysis of the PX domain (A) and FATP1 (B) of *T. nativa* using BepiPred-3.0 (Threshold-0.1512)

Linear epitope analysis of PX domain protein using BepiPred-3.0 revealed several key immunogenic regions highlighting its significant role in the immune response. Epitope areas above the threshold value (0.1512) are clearly identified in the graph. The first significant region, spanning positions 30-60, shows pronounced peaks with a maximum value of about 0.23. This region is probably critical for recognition by the immune system because it is in a domain that is involved in interactions with other proteins or lipids. The second region, located between positions 120-160, is also characterized by high immunogenic activity. The consistently high epitope score confirms the importance of this region as a promising antibody target. The third region (positions 200-240) shows similar immunogenicity, which strengthens the arguments in favor of a significant role of PX do-main protein in the pathogenesis of *T. nativa.*

Compared to PX domain protein, Long-chain fatty acid transport protein 1 showed less pronounced immunoactive results by explicit epitope analysis. However, they are also of diagnostic interest. Longchain fatty acid transport protein 1 showed moderate immunogenicity, emphasizing its importance in the body's immune response. Using BepiPred-3.0, we were able to identify several regions with potential epitope potential. The first region, covering positions 90-110, shows a stable exceeding of the threshold value (0.09774), indicating the presence of a linear epitope capable of interacting with antibodies. Of particular interest is the second region (positions 180-200), where a peak epitope score of ~0.14 is observed. This site may be functionally significant and involved in immune interactions. The third region, located between positions 450-470, although characterized by a more moderate epitope score, may complement the overall immune picture of the protein.

After performing epitope region analysis of proteins, it becomes clear that it is necessary to study their three-dimensional structure to better understand the biological functions. Epitope analysis provides important information about potentially immunoactive regions. However, to more accurately interpret

protein interactions with other molecules and to develop more effective diagnostic and therapeutic strategies, further investigation of their spatial organization is needed. This will provide a more complete understanding of their functional mechanisms and role in biological processes (Figure 4).



FATP1 protein PX domain

Figure 4 – Predicted three-dimensional structure of *T. nativa* proteins

FATP1 has been significantly studied due to its important role in the transport of long-chain fatty acids across cell membranes. The protein covers a substantial portion of its amino acid sequence with high model accuracy (81% coverage, 100% confidence). Its structural organization includes both alpha-helices and beta-sheets, which allows FATP1 to function not only as a transporter but also as an aquilizing fatty acid linker. Accordingly, its complex configuration makes it a promising target for diagnostic methods and antigenic domain studies [16, 17].

In contrast to FATP1, the structure of the PX-domain has a coverage of only 34% amino acids, but it retains full model fidelity. The PX-domain is a membrane-binding region involved in the insolation of proteins to cell membranes through binding to phosphoinositide. Despite its compact structure, understanding its functional aspects requires further investigation. This limitation in studying structural configuration makes epitope analysis more challenging [18]. FATP1 has demonstrated a more complete and detailed structural characterization, which makes it more suitable for diagnosis and therapeutic interventions at the molecular level. In contrast, the PX-domain, with less coverage, is still the subject of further research.

Both proteins show significant differences in their functions; FATP1 is involved in fatty acid transport, whereas the PX-domain is involved in membrane binding. These functional differences determine their biological relevance and directions in research developments. Precise knowledge of the structure of FATP1 opens opportunities to study antigenic determinants, which may improve the efficiency of development based on it. For the PX domain, further studies are essential to identify and detail its functional utilization. These protein patterning results demonstrated the relevance and need for detailed studies of FATP1 and PX domain. Further advances in understanding their structures and functions will facilitate the development of novel treatment and diagnostic strategies at the molecular level.

Next, the reference sequences of this protein were analyzed among different *Trichinella* species. For this purpose, amino acid sequences were aligned to identify similarities and differences among the species studied. This alignment process helps to identify conserved and variable regions of the protein that may be important for its functional activity or interaction with other molecules (Figure 5).

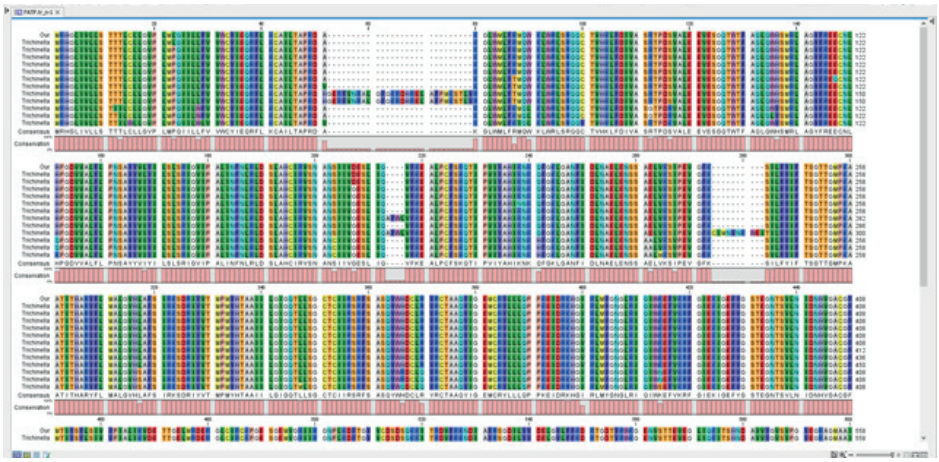


Figure 5 – Alignment of the amino acid sequence of Long-chain fatty acid transport protein 1 (FATP1) of *Trichinella spp*

The alignment showed that the amino acid sequence in different *Trichinella* species is highly conserved. However, sufficiently variable regions (90.3%) were identified and showed differences between species, which may indicate specificities. Based on the amino acid sequence data obtained, a phylogenetic tree was constructed. This allowed visualizing the evolutionary relationships between different *Trichinella* species and assessing the degree of their genetic difference (Figure 6).

Figure 6 – Phylogenetic tree of the Long-chain fatty acid transport protein 1 (FATP1) protein in *Trichinella spp.* (Bootstrap = 10,000)

The phylogenetic tree was constructed with 10,000 bootstrap replications to assess the reliability of clustering. The amino acid sequences of FATP1 obtained from the NCBI database were used in the analysis. The results of phylogenetic analysis demonstrate a clear separation of the studied sequences into evolutionarily valid clusters corresponding to known species of the genus *Trichinella*. High bootstrap support values confirm the reliability of the obtained topology. In particular *T. spiralis, T. nativa,* and *T. murrelli* form separate groups, indicating the divergent evolution of the FATP1 protein in different ecological niches and geographic ranges.

Of particular interest is the phylogenetic position of *T. patagoniensis* and *T. nelsoni*, which form distinct, well-supported clusters. This may indicate specific adaptive changes in the structure and function

of FATP1 in these species. The identification of *T. pseudospiralis* as a distinct group is consistent with previously published data on its unique biological features and the absence of cuticles in adults.

Analysis of the obtained data indicates that the key motifs of the FATP1 protein are conserved among *Trichinella* representatives, which is confirmed by their high phylogenetic similarities. However, certain species-specific differences are also observed, which may indicate differences in the mechanisms of fatty acid transport caused by adaptation to different conditions of existence. This study deepens the understanding of the evolutionary history of the FATP1 protein in *Trichinella spp*. and lays the foundation for further functional studies aimed at identifying the molecular mechanisms of parasitism and metabolic adaptation in this nematode genus.

Similar studies were performed with the PX domain (Figure 7).

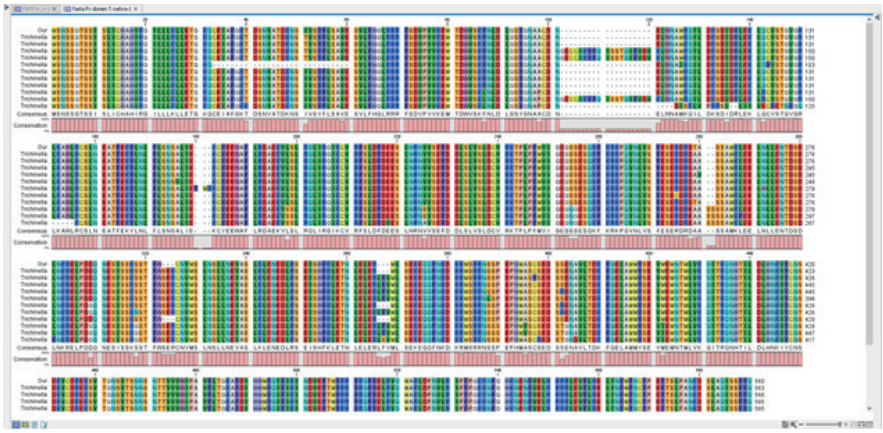


Figure 7 – Amino acid sequence alignment of the Peroxisomal targeting signal receptor (PX) domain in *Trichinella spp*

PX domain alignment, as for FATP1, showed high conservation (89.3%). However, in contrast to the previous protein, slightly more variable sites were found in the PX domain, indicating more pronounced differences between species in this region (Figure 8).

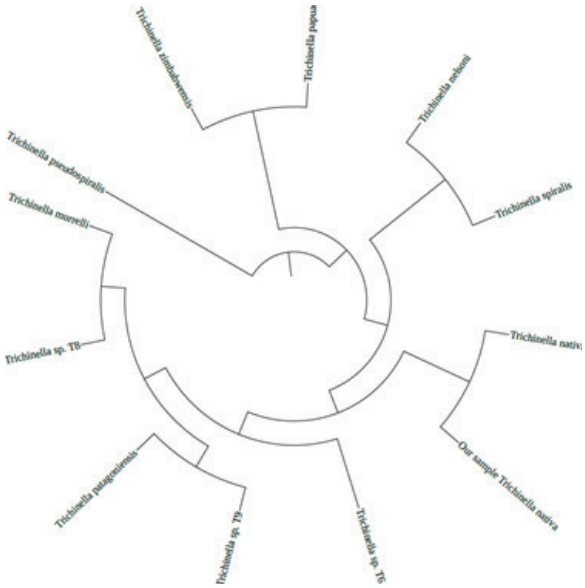


Figure 8 – Phylogenetic tree of the Peroxisomal targeting signal receptor (PX) domain in *Trichinella spp.* (Bootstrap = 10,000)

The amino acid sequences of the Peroxisomal targeting signal receptor domain (PX) obtained from the NCBI database were used in the analysis. The PX domain plays a key role in peroxisomal protein transport, which is critical for maintaining cellular metabolism and adaptation in parasitic nematodes.

The results of phylogenetic analysis demonstrate a clear divergence of *Trichinella* species, reflecting their evolutionary differences. *T. spiralis* forms a distinct cluster, confirming its phylogenetic isolation within the genus. *T. nativa, T. nelsoni,* and *T. murrelli* group into close clusters, indicating that conserved structural features of the PX domain are conserved in North American and Arctic isolates. This analysis emphasizes the importance of the PX domain in the physiology of *Trichinella spp.* and points to species-specific adaptations associated with the evolution of this parasitic genus.

The results obtained may be useful for further studies in parasite molecular biology, including the development of novel therapeutic targets based on peroxisomal transport proteins.

Discussion and Conclusion

Nematodes of the genus *Trichinella* are parasites capable of infecting a wide range of mammals, including humans, through the consumption of infected meat, especially pork and game that has not been adequately heat-treated [19]. According to recent epidemiologic studies, Trichinellosis is prevalent on all continents, including both industrialized regions and developing countries, exhibiting unique patterns of transmission and showing significant differences in clinical symptoms [20]. Such differences underscore the importance of in-depth research to investigate Trichinella natural focal areas and develop comprehensive trichinellosis control and prevention strategies that will be effective on a global scale [21].

Recent molecular studies have helped to further understand the genetic structure of *T. nativa*, allowing the identification of unique genetic markers that distinguish it from other *Trichinella* species [22]. These discoveries have important implications for epidemiologic monitoring and the development of species-specific diagnostic methods. Moreover, recent studies show that climate change may influence the expansion of *T. nativa's* range, which requires continuous surveillance and revision of existing control strategies to prevent new outbreaks of Trichinellosis in vulnerable regions [23].

The results of our study confirm that the FATP1 gene and the PX domain are promising molecules for further study in the context of diagnosis of *T. nativa* infestation. Sequencing and amino acid sequence analysis revealed high conservation between different parasite species, indicating the importance of these proteins in biological functions and their possible role in diagnosis [9, 24].

Initial work with RNA and successful sequencing demonstrated the activity and stability of these sequences, which is critical for further studies at the molecular level. The unique immunogenic regions identified, particularly in the PX domain, provide comprehensive information for the development of specific diagnostic tools and vaccines. It should be noted that despite the less pronounced immunogenic characteristics of FATP1, its role in fatty acid metabolism and transport is no less significant and requires further study [9, 16, 17, 22, 25].

Phylogenetic analyses highlighting evolutionary relationships between different *Trichinella* species emphasize the divergent evolution of FATP1 and the PX domain. Conserved regions in these proteins indicate their importance, while sequence variability may open new avenues for understanding the mechanisms of parasite adaptation to different ecological niches [26].

Despite significant progress in the development of Trichinellosis diagnostics, the problem of creating test systems specific to *T. nativa* remains unsolved. Currently, the most available serologic diagnostic methods are focused on *T. spiralis*, the most common trichinella species, which reduces their effectiveness in areas where *T. nativa* predominates [27]. These methods do not consider the unique antigenic structures of *T. nativa*, resulting in low specificity and sensitivity of the tests in arctic and subarctic regions where this species is most prevalent [28].

Recent studies emphasize the need to develop new antigenic panels that can detect *T. nativa* specific immunological responses [29].

Given the data obtained, it can be assumed that further studies of the structural organization and functional activity of FATP1 and the PX-domain may lead to the creation of new biomarkers for diagnosis and therapeutic methods, which will significantly increase the effectiveness of trichinellosis control. Research in this area is highly relevant and promising, given the importance of investigating the mechanisms of pathogenesis, impact on the host organism and the development of new antigenic targets for vaccine development [6].

This study provides significant insights into the molecular characteristics of *T. nativa* genes, particularly focusing on the immunogenic potential of FATP1 and PX-domain proteins. The bioin-

formatics analysis identified several B-cell epitopes in both proteins, suggesting their potential as diagnostic targets. Structural modeling and phylogenetic analysis further highlighted the evolutionary relationships and functional significance of these proteins within the *Trichinella* genus. The findings emphasize the need for further research to validate these molecular markers through experimental approaches, including serological testing and functional assays. Given the expanding geographical distribution of *T. nativa* due to climate change, developing specific diagnostic tools is crucial for effective surveillance and control of trichinellosis. The results of this study contribute to a better understanding of the parasite's immune interactions and provide a foundation for future advancements in diagnostic and therapeutic strategies.

Authors' Contributions

AZh: Investigation and drafted the manuscript. NG, FT: Investigation (bioinformatic analysis). NA: Investigation (isolation of trichinella larvae). AA: Conceptualized the study. AG: Data analysis. ZS: Investigation and edited the manuscript. OA: Conceptualized the study, investigation, data analysis, and drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgements

The study was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan to frame the Grant Financing for 2024-2026 the project No. AP23489156 "Identification of alternative genes of *Trichinella nativa* immunospecific proteins for latex diagnosticum producing".

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