Herald of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences. – Astana: S. Seifullin Kazakh Agrotechnical Research University, 2024. – № 4 (008). – P. 4-15. - ISSN 2958-5430, ISSN 2958-5449

VETERINARY SCIENCES

doi.org/ 10.51452/kazatuvc.2024.4(008).1780
UDC 576.8 **Research article**

Study of the Immunologic Value of Trichinella spiralis recombinant Protein Tsp-LE

Orken S. Akibekov[1], Fabio Tosini[2], Alfiya S. Syzdykova[3], Aissarat M. Gajimuradova[3], Dariga D. Zhumalina[3], Fariza S. Zhagipar[3], Nurtai N. Gubaidullin[3], Nasipkhan A. Askarova[3], Saltanat S. Yesseneyeva[4]

[1]Department of Microbiology and Biotechnology, Faculty of Veterinary and Livestock Technology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[2]European Union Reference Laboratory for Parasites, «Istituto Superiore di Sanità», Roma, Italy
[3]Research platform for agricultural biotechnology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan
[4]Department of Veterinary Sanitation, Faculty of Veterinary and Livestock Technology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan

Corresponding author: Orken S. Akibekov: orken.a.s@mail.ru
Co-authors: (1: FT) fabio.tosini@iss.it; (2: AS) halik.kz@mail.ru; (3: AG) aisarat3878@mail.ru; (4: DZh) dariga.ast@gmail.com; (5: FZh) zhagipar.fariza@gmail.com; (6: NG) nur-tai.kz@mail.ru; (7: NA) nasiko.askarova@bk.ru; (8: SYe) eseneeva.saltanat@mail.ru
Received: 01-10-2024 **Accepted:** 26-12-2024 **Published:** 30-12-2024

Abstract

Background and Aim. Trichinellosis is a serious zoonotic disease, with frequent outbreaks reported across various regions in Europe and Asia. However, current diagnostic tests based on excretory-secretory antigens (ES-Ag) and somatic antigens (S-Ag) lack adequate specificity and sensitivity. Moreover, the production of these antigens is both labor-intensive and costly.

Materials and Methods. The immunodiagnostic potential of a recombinant 11 kDa serine protease protein, previously characterized in earlier studies was assessed. This study detected infection in *Balb/c* mice as early as 14 days post-infection with *T. spiralis* larvae, yielding no false-positive results and an average infection level of 61±5.3 larvae per mouse.

Results. Comparative indirect ELISA analysis on mouse sera after the 3rd and 5th immunizations, alongside tests on S-Ag and S-Ag using sera from infected rabbits and pigs, demonstrated the high diagnostic accuracy of the recombinant protein. The protein revealed strong specificity for trichinellosis detection, showing no cross-reactivity with echinococcosis.

Conclusion. These findings support using rTsp-LE as a reliable component in developing ELISA test systems for trichinellosis detection.

Keywords: ELISA; Es-Ag; immunization; recombinant protein; S-Ag; *Trichinella spiralis.*

Introduction

Trichinellosis is a zoonotic disease caused by nematodes of the family *Trichinellidae,* affecting humans and a range of animals. The disease is registered in domestic animals such as pigs, dogs, and cats, as well as in numerous wild carnivores (including wolves, foxes, and bears), rodents (such as rats and mice), and humans [1]. Approximately 11 million people worldwide are estimated to be chronically infected with *Trichinella spiralis* [2].

In 2020, 181 cases of trichinellosis were reported across nine EU/EEA countries, with 117 cases confirmed and 64 classified as probable [3]. In China, from 2009 to 2020, cases of human trichinellosis have been primarily concentrated in southwestern regions, with eight outbreaks resulting in 479 cases and two fatalities [4]. Southeast Asia also shows variable seroprevalence rates of human trichinellosis, particularly in Laos and Vietnam, where rates range from 0% to 10.5% in certain villages. Additionally, in Cambodia, Laos, Malaysia, Thailand, and Vietnam, 13 outbreaks involving 1,604 cases and a mortality rate of less than 1% have been recorded over the past 21 years [5].

Given the epidemiological context, effective early diagnosis of trichinellosis in potentially high-risk domestic and wild animals is essential. However, routine testing of meat and animals for trichinellosis remains limited, primarily due to inflated costs, which can reach up to $3 per animal [6].

Early clinical diagnosis of trichinellosis is challenging due to the lack of pronounced clinical symptoms. Moreover, diagnosing the disease in its chronic stages proves even more difficult [7].

The detection of anti-Trichinella IgG via enzyme-linked immunosorbent assay (ELISA) using muscle excretory-secretory antigens (Es-Ag) from *Trichinella spiralis* larvae is the most used serologic method for diagnosing trichinellosis. However, a major limitation of current serologic tests is the potential for false negatives during early infection stages [8, 9, 10]. Immunoenzyme methods utilizing recombinant antibodies promotes the diagnostic process, eliminating the need for animal slaughter, reducing reliance on antigen availability, standardizing components, and enhancing both the quality and specificity of ELISA-based diagnostics [11, 12].

Sufficient literature supports the high sensitivity and specificity of ELISA with recombinant antigens. For instance, Wang et al. (2015) studied a recombinant 31 kDa protein and found a sensitivity of 96.67% and 96.87%, compared to 100% and 98.44% for ELISA using ES antigens. ELISA with recombinant antigens was able to detect antibodies in mice with heavy (500 larvae), moderate (300 larvae), and light (100 larvae) infections at 8, 12, and 14 days post-infection (dpi), respectively, whereas detection using ES antigens occurred at 10, 8, and 10 dpi, respectively [13]. In mice infected with 200 muscle larvae (ML) of *T. spiralis,* the rTsTryp-ELISA demonstrated a natural muscle protease sensitivity of 98.1%, compared to 94.2% for the ES antigen-ELISA. Additionally, the specificity of rTsTryp-ELISA was significantly higher than that of the ES antigen-ELISA, at 98.7% versus 95.4%, respectively [12]. Somatic antigens with molecular masses of 43 kDa (TsCSAg-43), 79 kDa (TsCSAg-79), and 101 kDa (TsCSAg-101), isolated by Supcharoengoon et al. (2022) from the sera of trichinellosis-infected pigs, showed IgG-ELISA sensitivities of 100%, with specificities of 97.77%, 95.54%, and 90.63%, respectively [14].

Y. Liu et al. (2021) developed a novel competitive enzyme-linked immunoassay (rCLP-cELISA) based on recombinant cystatin-like protein and monoclonal antibodies. This method demonstrated high sensitivity and specificity with human serum field samples and showed robust applicability with porcine and murine serum [15]. The expression of *Trichinella* antigens encoding proteins of 53 kDa [16, 17], 49 kDa [18, 13], 35.5 kDa [19], and 21 kDa [20] in heterologous systems has become a standard approach for generating antigens used in diagnostic test systems.

Recombinant antigens maintain great promise for several reasons: they exhibit high specificity, minimizing the risk of false negatives and cross-reactions. Also, the expression of recombinant antigens enables standardized production with high product purity and stability, while also reducing the risk of infection for personnel handling parasites.

The aim of our study was to evaluate the immunodiagnostic potential of recombinant *Trichinella spiralis* serine protease protein at various stages of infection in experimentally infected laboratory mice.

Materials and Methods

Ethical approval. All animal procedures were conducted following exacting standards of biosafety and animal welfare. Protocols adhered to the International Guidelines for Biomedical Research Involving Animals [21].

The care and use of laboratory animals were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry Technology at S. Seifullin Kazakh Agrotechnical Research University (KATRU), Astana, Kazakhstan (Protocol No. 1, dated February 23, 2023).

Experiments were conducted at the Research Platform of Agricultural Biotechnology, NCJSC: "S. Seifullin Kazakh Agrotechnical Research University."

Infestation of animals T. spiralis larvae were maintained through serial passage in Soviet Chinchilla breed rabbits at the Joint Kazakh-Chinese Laboratory of Biological Safety.

Larvae were collected through artificial digestion following the standard protocol described previously [22]. For each species of trichinellosis, seven mice, aged three to four months, were selected for the experiment at 3, 5, 7-, 14-, 21-, and 30-days post-infection, with three mice forming the control group. Using the principle of analogs for *T. spiralis,* an experimental group of three mice was formed for each day of selection (18 in total), along with three control mice. The infection dose was 250 larvae per mouse, and the animals were orally infected using a disposable pipette containing *Trichinella larvae.*

Scheme of experiment. On the 3rd, 5th, 7th, 14th, 21st, and 30th days, three mice from each group were euthanized for pathological autopsy. Euthanasia was performed via sequential intramuscular injection of xylazine (Bioveta, Czech Republic) at a dose of 1.5 mg/kg, followed by intravenous injection of Anestofol ('VIK' LLC, Russia) at a dose of 7.5 mg/kg. Muscles were examined for the presence of parasites according to the guidelines of H.R. Gamble et al. (2019) [23]. The small intestine was dissected longitudinally, washed three times with ice-cold saline solution, then cut into 2-cm fragments and cultured in saline at 37 °C for 2.5 hours. Larvae that emerged from the small intestine into the physiological solution were collected using the Berman method [24].

Larvae isolation. Trichinella larvae were isolated from animal muscle tissue samples using compressor trichinelloscopy and digestion in artificial gastric juice (AGJ), following the methods outlined in Methodological Guidelines 4.2.2747-10 "Methods of Sanitary-Parasitological Examination of Meat and Meat Products" [25]. The detected and isolated helminthological material was preserved in a 70% ethanol solution.

Antiserum testing by indirect ELISA. ELISA was performed as previously described [13]. Briefly, 96-well ELISA plates (Corning, USA) were coated overnight at 4 °C with 100 µL of purified recombinant antigens (1 µg/mL) or ES antigens (1 µg/mL) in bicarbonate buffer (pH 9.6). After blocking with PBS containing 0.1% BSA for 1 hour at 37 °C, the following reagents were added sequentially and incubated for 1 hour at 37 °C: (1) mouse serum diluted 1:100 in PBST, and (2) HRP-conjugated anti-mouse IgG (Sigma, USA) diluted 1:5000. Reactions were detected by adding a one-component TMB substrate (tetramethylbenzidine, CJSC "HBO Immunotech", Russia), and the reaction was stopped by adding 50 µL/well of 2M H2SO4. Optical density (OD) values at 450 nm were measured using a microplate reader (BioSan, Lithuania). All samples were analyzed in duplicate. A ratio of test sample OD to negative control OD (S/N ratio) < 2.1 was considered negative, while S/N ≥ 2.1 was considered positive.

Statistical analysis. Mean and standard deviation were calculated using Microsoft Excel 2010. For each value, Student's r was calculated, and a p-value < 0.05 was considered statistically significant. Serological studies were carried out using the method described by T.S. Saiduldin (1981) [26].

Results

During experiments infecting laboratory Balb/c mice with *T. spiralis* larvae at a dose of 250 larvae, the phase of larval migration from the intestine to the muscle was observed. The results indicate that larval migration begins as early as day 14, which is consistent with multiple studies. Figure 1 shows the numerical values of larvae detected through postmortem examination of the mice.

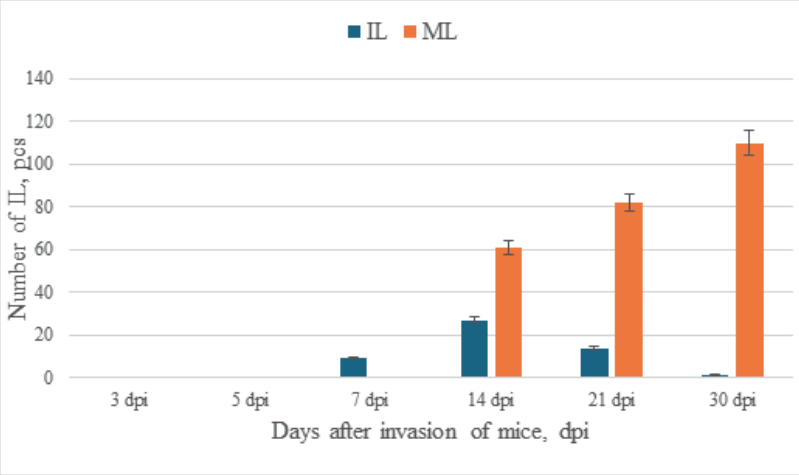
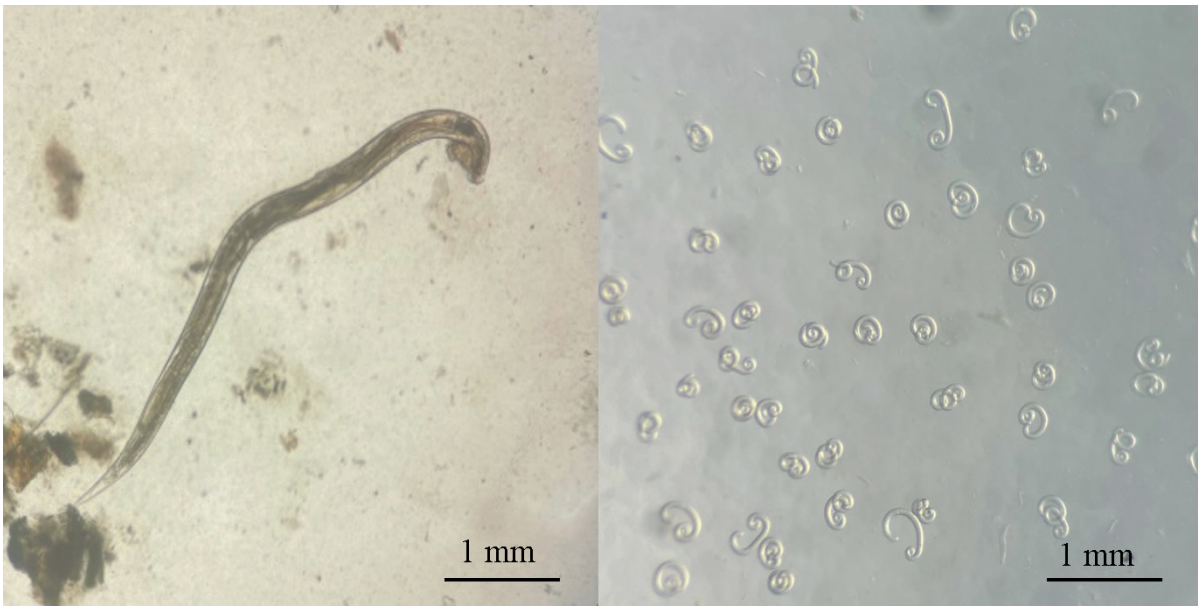


Figure 1 – Development of trichinella larvae in intestine and muscle

As shown in Figure 1, larvae were first detected in the intestines of infected mice at 7 dpi. However, by 14 dpi, active migration of T. spiralis larvae to the muscle was observed, with an average of 61±5.3 larvae. A significant decrease in the number of larvae in the intestine was noted by 21 dpi (14±2.08) ($p<0.01$), accompanied by a 34.4% increase in muscle larvae ($p<0.05$). By 30 dpi, the number of muscle larvae reached 110±4.3 ($p<0.01$), while the number of intestinal larvae decreased to 1.6±0.88 ($p<0.01$). Figure 2 shows muscle larvae isolated at 21 days post-infection.

On day 14, muscle larvae found in the muscles were both spirally twisted and hairpin-shaped, characteristics typical of the early muscle stage. This suggests ongoing larval migration from the intestine. Figure 2 shows muscle larvae isolated at 21 days post-infection.



T. spiralis larvae at 21 dpi found in mouse intestines

T. spiralis larvae at 21 dpi found in mouse muscles

Figure 2 – *Trichinella larvae* in the muscles of BALB/c mice (21 dpi)

Blood serum was collected from mice at each stage of infestation for antibody analysis using indirect ELISA. The results are shown in Table 1.

Table 1 – Antibody titer of mice infected with T.spiralis against ES-Ag measured by indirect ELIS AInventory /number of mice	Days after invasion of animals						Mean antibody titers
	3 days	5 days	7 days	14 days	21 days	30 days	
1	RA	RA	RA	1:800	1: 1600	1:3200	1:1600 (+18.9; -15.9)
2	RA	RA	1:100	1:800	1:3200	1:6400	1:3200 (+20.6; -17.1)
3	RA	RA	RA	1:400	1:1600	1:3200	1:980 (+12.5; -11.1)
Control group							
4	RA	RA	RA	RA	RA	RA	-
5	RA	RA	RA	RA	RA	RA	-
6	RA	RA	RA	RA	RA	RA	-

Note: Reaction absence

The tested method showed low efficiency in detecting trichinellosis during the intestinal phase of infection, despite the presence of larvae in the intestine. Antibodies were first detected at 14 days post-infection (dpi) with titers of 1:400 to 1:800 in some mice within the group. By day 30, titers had increased to 1:3200 and 1:6400, confirming a positive result. However, results obtained between days 7 and 14 could be interpreted as either positive or potentially false positive.

The recombinant serine protease protein Tsp-LE 11 kDa (PP099881.1), based on the cDNA sequence of the large exon of the *T. spiralis* serine protease gene (data not shown), was also evaluated for its diagnostic value. Studies were conducted on mouse, rabbit, and pig sera using Es-Ag, S-Ag, and the test antigen rTsp-LE.

An initial ELISA was performed using sera from mice immunized with the recombinant protein. The results are summarized in Table 2.

Table 2 – ELISA results using Es-Ag and rTsp-LE antigens in mice following two consecutive immunizations

Antigen	Average antibody titer	
	after the 3rd immunization	after the 5th immunization
ES-AG	1:300 (+41.4; -29.3)	1:570 (+23.1; -18.7)
Recombinant protein	1:570 (+23.1; -18.7)	1:2260 (+14.9; -13.0)
P	0.002	0.0001

During the first two immunizations, specific IgG was first detected in the infected mice serum after the third immunization with S-Ag, with OD 450 values of 0.324 and 0.234 at dilutions of 1:200 and 1:400, respectively. When using the recombinant antigen, a positive result was also observed after the third immunization at nearly the same titers of 1:400-1:800, with OD 450 values of 0.277 and 0.205, respectively. After the fifth immunization, detection occurred at titers of 1:400-1:800 and 1:1600-1:3200, with mean OD 450 values of 0.326 and 0.231 for ES-Ag and rTsp-LE, respectively. Despite the high specificity of ES-Ag, the ELISA values for detecting *trichinellosis* in mice using rTsp-LE are only slightly lower. However, it should be noted that the 11 kDa recombinant protein binds specifically

to the serine protease protein of *Trichinella* larvae, as evidenced by the absence of cross-reaction with *Echinococcus*, a commonly detected disease. Furthermore, this antigen was successfully used to diagnose animals infected with T. nativa, yielding results analogous to those obtained with *T. spiralis* (1:3200).

To study species specificity and the reaction of the obtained protein with antibodies from other animal species, indirect ELISA was performed using S-Ag, Es-Ag, and the recombinant protein. The results are presented in the table below.

Table 3 – Determination of antibody titer in sera of infected rabbits and pigs using Es-Ag, S-Ag and rTsp-LE

Antigens	Average antibody titer	
	Infested rabbits	Positive swine serum
S-Ag	1:20 480 (+14.1; -12.3)	1:6 400 (+32.0; -24.2)
ES-Ag	1:20 480 (+14.1; -12.3)	1:3 940 (+39.5; -28.2)
Recombinant protein	1:2 260 (+14.9; -13.0)	1:800 (+41.4; -29.3)
P	0.003	0.001

When sera from infected rabbits and pigs were tested using the three types of antigens, S-Ag showed a positive reaction with a mean titer of 1:20,480 in rabbits and 1:6,400 in pigs, with mean OD 450 values of 0.631 and 0.332, respectively. The results for ES-Ag were nearly identical to those for S-Ag, with titers of 1:20,480 in rabbits and 1:3,940 in pigs, and OD 450 values of 0.581 and 0.456, respectively. The recombinant Tsp-LE protein yielded positive results at mean titers of 1:2,600 in rabbits and 1:800 in pigs, with mean OD 450 values of 0.225 and 0.226, respectively.

Based on the results, the recombinant 11 kDa rTsp-LE protein demonstrates sufficient diagnostic value for detecting trichinellosis infection in different animal species. This protein can be utilized for the further development of a highly specific ELISA test.

Discussion and Conclusion

ES antigens derived from the muscle tissue of *T. spiralis* larvae are widely used as serodiagnostic agents for trichinellosis and are recommended by the International Commission on Trichinellosis (ICT) [27]. However, the process of obtaining ES-Ag requires harvesting muscle tissue larvae from experimentally infected laboratory animals, which can be resource-intensive and labor-intensive. Recombinant proteins offer a promising alternative to ES antigens, as they can be efficiently produced in large quantities using bacterial expression systems and used as antigens in sensitive, specific, and standardized ELISA tests for trichinellosis serodiagnosis. This approach could enhance the accuracy and convenience of diagnosing the disease. Previous studies [28, 29] have suggested that the serine protease of adult *T. spiralis* larvae plays a role in capsule formation and may help protect newborn larvae in the host bloodstream. These findings imply that serine proteases may be involved in intestinal mucosal invasion and could serve as potential targets for early detection of parasitic invasion, as well as for the development of vaccines against trichinellosis infection.

In our study, larvae were detected in the gut starting at 7 dpi, with their numbers increasing from 12 to 91 larvae by 30 dpi. Transformation into muscle tissue was observed between 21 and 30 dpi, and the number of larvae in the gut significantly decreased from 14 to 1.6 larvae. These findings suggest that signs of the disease can be detected between days 7 and 14 using the recombinant serine protease, enabling earlier diagnosis.

These findings are consistent with studies by O.N. Andreyanov, who demonstrated that at a dose of 100 T. spiralis larvae, survival in the intestine persists for 18-24 days [30]. Similarly, Pereverzeva et al. observed no intestinal, sexually mature *Trichinella* in mice after day 21 of infection. In their control group, the survival rates of intestinal *Trichinella* were: 54% after 4 days, 44% after 7 days, 21-30% after 15 days, 16.5% after 21 days, and 4-5% between 28-30 days [31], which aligns with our results.

No encapsulated larvae were observed by day 30 post-infection. These findings align with those of Pereverzeva, who reported the presence of semi-formed larvae in muscle tissue by day 21, suggesting ongoing transport from the intestine. Notably, even by day 35 post-infection, encapsulation in *Trichinella spiralis* was not observed in mice [32].

Serine protease can be expressed at high levels in encapsulated species (such as *T. spiralis, T. nativa, T. britovi,* and *T. nelsoni*) but at lower levels in non-encapsulated species (e.g., *T. pseudospiralis*) [33]. Previous studies have shown that both ES-Ag and S-Ag detect infection between days 8 and 14, with recombinant antigens exhibiting similar sensitivity [33, 34, 36]. However, recombinant antigens offer significantly higher specificity, and the more straightforward and standardized production process compared to ES-Ag and S-Ag makes them a preferable alternative for diagnostic applications.

In the present study, the recombinant rTsp-LE antigen demonstrated its diagnostic potential across three animal species (mice, rabbits, and pigs), with average antibody titers of 1:570, 1:2260, and 1:800, respectively. In comparison, when ES-Ag and S-Ag were used, the mean titers were much higher: 1:20480 for ES-Ag and S-Ag in rabbits, and 1:6400 and 1:3940 for pigs, respectively. These findings suggest that the recombinant 11 kDa *T. spiralis* antigen could serve as a viable alternative to ES-Ag and S-Ag for diagnosing trichinellosis. Additionally, no significant difference in antibody detection rates was observed when sera from mice infected with *T. nativa* were tested using the recombinant antigen, and there was no cross-reactivity with sera from *Echinococcosis*-infected animals.

According to the literature, antibodies are typically detectable 4-6 weeks after humans are infected from wild animals. A diagnostically significant result is a fourfold or greater increase in antibody titers in paired sera collected 14-20 days apart. Antibody levels peak between the 2nd and 4th months post-infection and then gradually decline, but can persist for up to 10 years or longer [34].

Garkavi et al. reported that in experimentally infected mice, total antibody concentrations peak at 6 weeks and again at 2-3 months, with IgM peaking on days 30 and 90, and IgA peaking on days 90 and 120 post-infection [35].

The recombinant serine protease protein has recently been actively studied as a component in vaccination. Mice vaccinated with the purified rTspSP-1.3 protein [36] showed an average 39% reduction in muscle larval load compared to the control group.

Recombinant rTsSP protein combined with cholera toxin subunit B (CTB) [37] induced a significant intestinal local sIgA response and a systemic *TsSP*-specific antibody response in vaccinated mice. Moreover, an increased number of goblet cells, acid mucins, and IgA-secreting cells were observed in the small intestine of vaccinated mice. Immune serum against rTsSP specifically recognized the cuticle of various stages of the worm, including muscle larvae, intestinal infective larvae, and adult worms. Vaccinated mice showed a 71.10% reduction in adult worm numbers at 9 dpi and a 62.10% reduction in muscle larvae at 42 dpi. Furthermore, rTsSP vaccination also inhibited the development of intestinal *T. spiralis* and reduced female fecundity.

These findings align with the study by Wang L. et al., who examined the 31 kDa antigen of T. spiralis and observed a slightly lower result compared to ES-Ag, with ELISA sensitivity and specificity of 96.67% and 96.87% for the recombinant protein, versus 100% and 98.44% for ES antigens [13]. In a study of four recombinant antigens for *Trichinella*, the r-P1 protein detected antibodies as early as 8 dpi, followed by the r-P2, r-P3, and r-P4 proteins at 10, 14, and 16 dpi, respectively, with antibody levels remaining elevated up to 45 dpi [38]. Similarly, the recombinant serine protease of *T. spiralis* (rTsSP-ZH68) was recognized by sera from infected mice at 8-10 dpi and by sera from early trichinellosis patients at 19 dpi [39].

The recombinant protein rTsp-LE, developed in this study, showed its effectiveness in detecting infection in experimentally infected animals. This antigen demonstrates promise not only for the development of ELISA-based diagnostic tests but also as a potential component for vaccines aimed at reducing the invasive load in animals.

Detection of the intestinal stage of *T. spiralis* on the 7th day post-infection (dpi) allows for the early identification of antibodies, enabling detection of the disease at an early stage. The studied recombinant protein demonstrates high diagnostic value, showing reliable results as early as after the third immunization. The absence of cross-reaction with echinococcosis, along with the ability to detect antibodies to *T. nativa,* supports the potential use of this protein in the development of an ELISA test. Future studies should focus on exploring the use of this protein not only in diagnostic applications but also as a component of a vaccine, or independently, to reduce the parasitic load during infection.

Authors' Contributions

OA: Designed and supervised the study and drafted the manuscript. AG: Statistical analysis and drafted the manuscript. AS, NA: Designed and conducted the study. DhZ: Bioinformatic analysis. FZ: Conducted the study and drafted the manuscript. NG, SYe: Conducted the study and bioinformatic analysis. 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".

Conflicts of Interest

The authors declare that they have no competing interests.

References

1 Клинков, АВ, Одоевская, ИМ. (2010). Сравнительный анализ препаратов защитного действия на основе рекомбинантных и экскреторно-секреторных антигенов *Trichinella spp.* При экспериментальном трихинеллезе лабораторных животных. *Российский паразитологический журнал,* 3.

2 Murrell, KD, Pozio, E. (2011). Worldwide occurrence and impact of human trichinellosis, 1986-2009. *Emerging Infectious Diseases,* 17(12), 2194-2202. DOI: 10.3201/eid1712.110896.

3 European Centre for Disease Prevention and Control. Trichinellosis. In: ECDC. Annual Epidemiological Report for 2020. (2022). Stockholm: ECDC.

4 Zhang, XZ, Wang, ZQ, Cui, J. (2022). Epidemiology of trichinellosis in the People's Republic of China during 2009-2020. *Acta Tropica,* 229, 106388. DOI: 10.1016/j.actatropica.2022.106388.

5 Year, H., Bory, S., Khieu, V., Caron, Y. (2022). Human trichinellosis in Southeast Asia, 2001-2021. *Food Waterborne Parasitology*, 16, 28. DOI: 10.1016/j.fawpar.2022.e00171.

6 Zhang, N., Li, W., Fu, B. (2018). Vaccines against Trichinella spiralis: Progress, challenges and future prospects. *Transboundary and Emerging Diseases*, 65(6), 1447-1458. DOI: 10.1111/tbed.12917.

7 Centers of Disease control and prevention (CDC) Clinical Overview of Trichinellosis (2024). https://www.cdc.gov/trichinellosis/hcp/clinical-overview/index.html#:~:text=Diagnosis,or%20specific%20antibody%20in%20serum.

8 Gamble, HR, Patrascu, IV. (1996). Whole blood, serum, and tissue fluids in an enzyme immunoassay for swine trichinellosis. *Journal of Food Protection,* 59(11), 1213-1217. DOI: 10.4315/0362-028X-59.11.1213.

9 Gottstein, B., Pozio, E., Nöckler, K. (2009). Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews,* 22(1), 127-145. DOI: 10.1128/CMR.00026-08.

10 Rawla, P., Sharma, S. (2024). *Trichinella spiralis* Infection. P. Rawla, StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

11 Wang, ZQ, Shi, YL, Liu, RD, Jiang, P., Guan, YY, Chen, YD, Cui, J. (2017). New insights on serodiagnosis of trichinellosis during window period: early diagnostic antigens from *Trichinella spiralis* intestinal worms. *Infectious Diseases of Poverty*, 6(1), 41. DOI: 10.1186/s40249-017-0252-z.

12 Han, LL, Lu, QQ, Li, YL, Zheng, WW, Ren, P., Liu, RD, Cui, J. Wang, ZQ. (2024). Application of a recombinant novel trypsin from *Trichinella spiralis* for serodiagnosis of trichinellosis. *Parasites Vectors*, 17(1), 9. DOI: 10.1186/s13071-023-06067-7.

13 Wang, L., Tian, XY, Sun, GG, Liu, RD, Liu, LN, Zhang, X., Jiang, P., Wang, ZQ, Cui, J. (2015). The Use of Recombinant 31 kDa Antigens of *Trichinella spiralis* for Serodiagnosis of Experimental Trichinellosis. *Iranian Journal of Parasitology*, 10(2), 230-237.

14 Supcharoengoon, U., Reamtong, O., Dekumyoy, P., Watthanakulpanich, D., Limpanont, Y., Zhiyue, L., Chaimon, S., Martviset, P., Adisakwattana, P. (2022). Evaluation of indirect-ELISA using eluted antigens from *Trichinella spiralis* muscle larvae for diagnosis of swine trichinellosis. *Acta Tropica,* 235, 106644. DOI: 10.1016/j.actatropica.2022.106644.

15 Liu, Y., Xu, N., Li, Y., Tang, B., Yang, H., Gao, W., Liu, M., Liu, X., Zhou, Y. (2021). Recombinant cystatin-like protein-based competition ELISA for *Trichinella spiralis* antibody test in multihost sera. *PLoS Neglected Tropical Diseases,* 15(8). DOI: 10.1371/journal.pntd.0009723.

16 Jung, D., Teifke, JP, Karger, A., Michael, K., Venz, S., Wittmann, W., Kindermann, K., Nöckler, K., Mundt, E. (2007). Evaluation of baculovirus-derived recombinant 53-kDa protein of *Trichinella spiralis* for detection of Trichinella-specific antibodies in domestic pigs by ELISA. *Parasitology Research*, 100(3), 429-437. DOI: 10.1007/s00436-006-0296-7.

17 Yang, C., Liu, H., Li, X., Sui, S., Liu, Y. (2016). Protective effect of recombinant Trichinella 53-kDa protein in sepsis and the effect on macrophages. *Experimental and Therapeutic Medicine*, 12, 965-968. DOI:10.3892/etm.2016.3354.

18 Su, XZ, Prestwood, AK, McGraw, RA. (1991). Cloning and expression of complementary DNA encoding an antigen of *Trichinella spiralis*. *Molecular and Biochemical Parasitology*, 45(2), 331-336. DOI: 10.1016/0166-6851(91)90101-b.

19 Wang, B., Wang, ZQ, Jin, J., Ren, HJ, Liu, LN, Cui, J. (2013). Cloning, expression and characterization of a *Trichinella spiralis* serine protease gene encoding a 35.5 kDa protein. *Experimental Parasitology,* 134(2), 148-54. DOI: 10.1016/j.exppara.2013.03.004.

20 Nagano, I., Wu, Z., Nakada, T., Matsuo, A., Takahashi, Y. (2001). Molecular cloning and characterization of a 21 kDa protein secreted from *Trichinella pseudospiralis*. *Journal of Helminthology,* 75(3), 273-278. DOI:10.1079/JOH200158.

21 The Development of Science-based Guidelines for Laboratory Animal Care: Proceedings of the November 2003 International Workshop. (2004). National Academies of Sciences, Engineering, and Medicine. Washington, DC: The National Academies Press. DOI:10.17226/11138.

22 Akibekov, OS, Syzdykova, AS, Lider, LA, Zhumalin, AK, Baibolin, ZK, Zhagipar, FS, Akanova, ZZ, Ibzhanova, AA, Gajimuradova, AM. (2022). Hematological, biochemical, and serological parameters of experimentally infected rabbits with *Trichinella nativa* and *Trichinella spiralis* for early identification of trichinellosis. *Veterinary World,* 15(9), 2285-2292. DOI: 10.14202/vetworld.2022.2285-2292.

23 Gamble, HR, Alban, L., Hill, D., Pyburn, D., Scandrett, B. (2019). International Commission on Trichinellosis: Recommendations on pre-harvest control of *Trichinella* in food animals. *Food and Waterborne Parasitology*, 15. DOI: 10.1016/j.fawpar. 2019.e00039.

24 Tintori, SC, Sloat, SA, Rockman, MV. (2022). Rapid Isolation of Wild Nematodes by Baermann Funnel. *Journal of Visualized Experiments*, 179. DOI: 10.3791/63287.

25 Committee of Sanitary and Epidemiological Control of the Ministry of Healthcare of the Republic of Kazakhstan. (2020). Monitoring the Incidence of Parasitic Infections among the Population of the Republic of Kazakhstan. Available from: https://www.gov.kz/memleket/entities/ kkkbtu/press/news/details/262201?lang=ru. Retrieved on 10-05-2024.

26 Сайдулдин, ТС. (1981). Статистическая обработка результатов серологических реакций. *Ветеринария*,7, 62-66.

27 Liu, RD, Qi, X., Sun, GG, Jiang, P., Zhang, X., Wang, LA, Liu, XL, Wang, ZQ, Cui, J. (2016). Proteomic analysis of *Trichinella spiralis* adult worm excretory-secretory proteins recognized by early infection sera. *Veterinary Parasitology*, 231, 43-46. DOI: 10.1016/j.vetpar.2016.10.008.

28 Gamble, HR, Pozio, E., Bruschi, F., Nockler, K., Kapel, C.M., Gajadhar, A.A. (2004). International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of Trichinella infection in animals and man. Parasite, 11, 3- 13. DOI: 10.1051/parasite/20041113.

29 Xu, J., Bai, X., Wang, LB, Shi, HN, Giessen, JWB, Boireau, P., Liu, MY, Liu, XL. (2017). Immune responses in mice vaccinated with a DNA vaccine expressing serine protease-like protein from the new-born larval stage of *Trichinella spiralis*. *Journal of Parasitology*, 144, 712-719. DOI: 10.1017/S0031182016002493.

30 Андреянов, ОН. (2009). Биологические свойства изолята *Trichinella spiralis* обыкновенной лисицы, пассированного на лабораторных животных. *Теория и практика борьбы с паразитарными болезнями,* 10.

31 Переверзева, ЭВ, Озерецковская, Н., Веретенникова, НЛ. (1974). Реакция белой крови у мышей, зараженных различными штаммами трихинелл. *Wiadomosci Parazytologiczne,* 20(1), 67-80.

32 Переверзева, ЭВ. (1975). Особенности кишечной и мышечной фазы трихинеллеза у белых мышей при экспериментальном угнетении иммунитета. *Wiadomosci Parazytologiczne*, 21, 601-619.

33 Xue, Y., Zhang, B., Wang, N., Huang, HB, Quan, Y., Lu, HN, Zhu, ZY, Li, JY, Pan, TX, Tang, Y., Jiang, YL, Shi, CW, Yang, GL, Wang, CF. (2022). Oral Vaccination of Mice With *Trichinella spiralis* Putative Serine Protease and Murine Interleukin-4 DNA Delivered by Invasive *Lactiplantibacillus plantarum* Elicits Protective Immunity. *Frontiers in Microbiology*, 13. DOI: 10.3389/fmicb.2022.859243.

34 Cui, J., Han, Y., Yue, X., Liu, F., Song, YY, Yan, SW, Lei, JJ, Zhang, X., Jiang, P., Wang, ZQ. (2019). Vaccination of mice with a recombinant novel cathepsin B inhibits Trichinella spiralis development, reduces the fecundity and worm burden. *Parasites Vectors*, 12(1), 581. DOI: 10.1186/s13071-019-3833-9.

35 Гаркави, БЛ. (2007). Трихинеллез, вызываемый *Trihinella pseudospiralis* (морфология и биология возбудителя, эпизоотология и эпидемиология, диагностика, меры борьбы и профилактика). *Российский паразитологический журнал,* 2, 35-116.

36 Li, X., Yao, P., Pan, AH, Liu, W., Hu, XC, Wu, ZD, Zhou, XW. (2013). An antigenic recombinant serine protease from *Trichinella spiralis* induces protective immunity in *BALB/c* mice. *Parasitology Research*, 112(9), 3229-3238. DOI: 10.1007/s00436-013-3500-6.

37 Sun, GG., Lei, JJ, Ren, HN, Zhang, Y., Guo, KX, Long, SR, Liu, RD, Jiang, P., Wang, ZQ, Cui, J. (2019). Intranasal immunization with recombinant Trichinella spiralis serine protease elicits protective immunity in BALB/c mice. *Experimental Parasitology,* 201, 1-10. DOI: 10.1016/j.exppara.2019.04.006.

38 Zhai, CC, Liu, XL, Bai, X., Jia, ZJ, Chen, SH, Tian, LG, Ai, L., Tang, B., Liu, MY, Wu, XP, Chen, JX. (2021). Bioinformatic Prediction and Production of Four Recombinant Proteins from Different Developmental Stages of *Trichinella spiralis* and Testing of Their Diagnostic Sensitivity in Mice. *Iranian Journal of Parasitology,* 16(1), 122-135. DOI: 10.18502/ijpa.v16i1.5531.

39 Gao, H., Tang, B., Bai, X., Wang, L., Wu, X., Shi, H., Wang, X. Liu, X., Liu, M. (2018). Characterization of an antigenic serine protease in the *Trichinella spiralis* adult. *Experimental Parasitology*, 195, 8-18. DOI: 10.1016/j.exppara.2018.09.009.

References

1 Klinkov, AV, Odoevskaya, IM. (2010). Sravnitel'nyj analiz preparatov zashchitnogo dejstviya na osnove rekombinantnyh i ekskretorno-sekretornyh antigenov *Trichinella spp.* Pri eksperimental'nom trihinelleze laboratornyh zhivotnyh. *Rossijskij parazitologicheskij zhurnal,* 3. [*in Russ*].

2 Murrell, KD, Pozio, E. (2011). Worldwide occurrence and impact of human trichinellosis, 1986-2009. *Emerging Infectious Diseases*, 17(12), 2194-2202. DOI: 10.3201/eid1712.110896.

3 European Centre for Disease Prevention and Control. Trichinellosis. In: ECDC. Annual Epidemiological Report for 2020. (2022). Stockholm: ECDC.

4 Zhang, XZ, Wang, ZQ, Cui, J. (2022). Epidemiology of trichinellosis in the People's Republic of China during 2009-2020. *Acta Tropica*, 229, 106388. DOI: 10.1016/j.actatropica.2022.106388.

5 Year, H., Bory, S., Khieu, V., Caron, Y. (2022). Human trichinellosis in Southeast Asia, 2001-2021. *Food Waterborne Parasitology*, 16, 28. DOI: 10.1016/j.fawpar.2022.e00171.

6 Zhang, N., Li, W., Fu, B. (2018). Vaccines against *Trichinella spiralis:* Progress, challenges and future prospects. *Transboundary and Emerging Diseases,* 65(6), 1447-1458. DOI: 10.1111/tbed.12917.

7 Centers of Disease control and prevention (CDC). Clinical Overview of Trichinellosis (2024). https://www.cdc.gov/trichinellosis/hcp/clinical-overview/index.html#:~:text=Diagnosis,or%20specific%20antibody%20in%20serum.

8 Gamble, HR, Patrascu, IV. (1996). Whole blood, serum, and tissue fluids in an enzyme immunoassay for swine trichinellosis. *Journal of Food Protection,* 59, 1213-1217. DOI: 10.4315/0362-028X-59.11.1213.

9 Gottstein, B., Pozio, E., Nöckler, K. (2009). Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews,* 22(1), 127-145. DOI: 10.1128/CMR.00026-08.

10 Rawla, P., Sharma, S. (2024). *Trichinella spiralis* Infection. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

11 Wang, Z, Shi, YL, Liu, RD, Jiang, P., Guan, YY, Chen, YD, Cui, J. (2017). New insights on serodiagnosis of trichinellosis during window period: early diagnostic antigens from *Trichinella spiralis* intestinal worms. *Infectious Diseases of Poverty*, 6(1), 41. DOI: 10.1186/s40249-017-0252-z.

12 Han, LL, Lu, QQ, Li, YL, Zheng, WW, Ren, P., Liu, RD, Cui, J., Wang, ZQ. (2024). Application of a recombinant novel trypsin from *Trichinella spiralis* for serodiagnosis of trichinellosis. *Parasites Vectors,* 17, 9. DOI: 10.1186/s13071-023-06067-7.

13 Wang, L., Tian, XY, Sun, GG, Liu, RD, Liu, LN, Zhang, X., Jiang, P., Wang, ZQ, Cui, J. (2015). The Use of Recombinant 31 kDa Antigens of *Trichinella spiralis* for Serodiagnosis of Experimental Trichinellosis. *Iranian Journal of Parasitology*, 10(2), 230-237.

14 Supcharoengoon, U., Reamtong, O., Dekumyoy, P., Watthanakulpanich, D., Limpanont, Y., Zhiyue, L., Chaimon, S., Martviset, P., Adisakwattana, P. (2022). Evaluation of indirect-ELISA using eluted antigens from *Trichinella spiralis* muscle larvae for diagnosis of swine trichinellosis. *Acta Tropica*, 235, 106644. DOI: 10.1016/j.actatropica.2022.106644.

15 Liu, Y., Xu, N., Li, Y., Tang, B., Yang, H., Gao, W., Liu, M., Liu, X., Zhou, Y. (2021). Recombinant cystatin-like protein-based competition ELISA for *Trichinella spiralis* antibody test in multihost sera. *PLoS Neglected Tropical Diseases,* 15(8). DOI: 10.1371/journal.pntd.0009723.

16 Jung, D., Teifke, JP, Karger, A., Michael, K., Venz, S., Wittmann, W., Kindermann, K., Nöckler, K., Mundt, E. (2007). Evaluation of baculovirus-derived recombinant 53-kDa protein of Trichinella spiralis for detection of *Trichinella*-specific antibodies in domestic pigs by ELISA. *Parasitology Research*, 100(3), 429-437. DOI: 10.1007/s00436-006-0296-7.

17 Yang, C., Liu, H., Li, X., Sui, S., Liu, Y. (2016). Protective effect of recombinant Trichinella 53-kDa protein in sepsis and the effect on macrophages. *Experimental and Therapeutic Medicine*, 12, 965-968. DOI: 10.3892/etm.2016.3354.

18 Su, XZ, Prestwood, AK, McGraw, RA. (1991). Cloning and expression of complementary DNA encoding an antigen of *Trichinella spiralis. Molecular and Biochemical Parasitology*, 45(2), 331-336. DOI: 10.1016/0166-6851(91)90101-b.

19 Wang, B., Wang, ZQ, Jin, J., Ren, HJ, Liu, LN, Cui, J. (2013). Cloning, expression and characterization of a *Trichinella spiralis* serine protease gene encoding a 35.5 kDa protein. *Experimental Parasitology,* 134(2), 148-154. DOI: 10.1016/j.exppara.2013.03.004.

20 Nagano, I., Wu, Z., Nakada, T., Matsuo, A., Takahashi, Y. (2001). Molecular cloning and characterization of a 21 kDa protein secreted from *Trichinella pseudospiralis. Journal of Helminthology,* 75(3), 273-278. DOI:10.1079/JOH200158.

21 The Development of Science-based Guidelines for Laboratory Animal Care: Proceedings of the November 2003 International Workshop. (2004). *National Academies of Sciences, Engineering, and Medicine. Washington, DC: The National Academies Press.* DOI: doi.org/10.17226/11138.

22 Akibekov, OS, Syzdykova, AS, Lider, LA, Zhumalin, AK, Baibolin, ZK, Zhagipar, FS, Akanova, ZZ, Ibzhanova, AA, Gajimuradova, AM. (2022). Hematological, biochemical, and serological parameters of experimentally infected rabbits with *Trichinella nativa* and *Trichinella spiralis* for early identification of trichinellosis*. Veterenary World*, 15(9), 2285-2292. DOI: 10.14202/vetworld.2022.2285-2292.

23 Gamble, HR., Alban, L., Hill, D., Pyburn, D., Scandrett, B. (2019). International Commission on Trichinellosis: Recommendations on pre-harvest control of *Trichinella* in food animals. *Food and Waterborne Parasitology,* 15. DOI: 10.1016/j.fawpar. 2019.e00039.

24 Tintori, SC, Sloat, SA, Rockman, MV. (2022). Rapid Isolation of Wild Nematodes by Baermann Funnel. *Journal of Visualized Experiments,* 179. DOI: 10.3791/63287.

25 Komitet sanitarno-epidemiologicheskogo nadzora Ministerstva zdravoohraneniya Respubliki Kazahstan. Monitoring zabolevaemosti parazitarnymi infekciyami sredi naseleniya Respubliki Kazahstan. (2020). [*in Russ*].

26 Saiduldin TS. (1981). Statistichekaia obrabotka rezultatov serologicheskikh reaktcii. *Veterinariya*,7, 62-66.

27 Liu, RD, Qi, X., Sun, GG, Jiang, P., Zhang, X., Wang, LA, Liu, XL, Wang, ZQ, Cui, J. (2016). Proteomic analysis of Trichinella spiralis adult worm excretory-secretory proteins recognized by early infection sera. *Veterinary Parasitology*, 231, 43-46. DOI: 10.1016/j.vetpar.2016.10.008.

28 Gamble, HR, Pozio, E., Bruschi, F., Nockler, K., Kapel, CM, Gajadhar, AA. (2004). International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. *Parasite,* 11, 3-13. DOI: 10.1051/parasite/20041113.

29 Xu, J., Bai, X., Wang, LB, Shi, HN, Giessen, JWB, Boireau, P., Liu, MY, Liu, XL. (2017). Immune responses in mice vaccinated with a DNA vaccine expressing serine protease-like protein from the new-born larval stage of *Trichinella spiralis. Journal of Parasitology*, 144, 712-719. DOI: 10.1017/S0031182016002493.

30 Andreyanov, ON. (2009). Biologicheskie svojstva izolyata *Trichinella spiralis* obyknovennoj lisicy, passirovannogo na laboratornyh zhivotnyh. *Teoriya i praktika bor'by s parazitarnymi boleznyami,* 10.

31 Pereverzeva, EV, Ozereckovskaya, NN, Veretennikova, NL. (1974). On the developmental particularities of *T.spiralis* larvae isolated from the racoon (procyon lotor) muscles – in albino mice. *Wiadomosci Parazytologiczne*, 20(1), 67-80.

32 Pereverzeva, EV. (1975). Osobennocti kishechnojii myshechnoi fazy trihinelleza u belyh myshei pri eksperimental'nom ugnetenii immuniteta. *Wiadomosci Parazytologiczne*, 21, 601-619. [In Russ].

33 Xue, Y., Zhang, B., Wang, N., Huang, HB, Quan, Y., Lu, HN, Zhu, ZY, Li, JY, Pan, TX, Tang, Y., Jiang, YL, Shi, CW, Yang, GL, Wang, CF. (2022). Oral Vaccination of Mice With *Trichinella spiralis* Putative Serine Protease and Murine Interleukin-4 DNA Delivered by Invasive Lactiplantibacillus plantarum Elicits Protective Immunity. *Frontiers in Microbiology*, 13. DOI: 10.3389/fmicb.2022.859243.

34 Cui, J., Han, Y., Yue, X., Liu, F., Song, YY, Yan, SW, Lei, JJ, Zhang, X., Jiang, P., Wang, ZQ. (2019). Vaccination of mice with a recombinant novel cathepsin B inhibits *Trichinella spiralis* development, reduces the fecundity and worm burden. *Parasites Vectors*, 12(1), 581. DOI: 10.1186/s13071-019-3833-9.

35 Garkavi, BL. (2007). Trihinellez, vyzyvaemyj *Trihinella pseudospiralis* (morfologiya i biologiya vozbuditelya, epizootologiya i epidemiologiya, diagnostika, mery bor'by i profilaktika). *Rossiiskii parazitologicheskii zhurnal,* 2, 35-116. [*In Russ*].

36 Li, X., Yao, JP, Pan, AH, Liu, W., Hu, XC, Wu, ZD, Zhou, XW. (2013). An antigenic recombinant serine protease from *Trichinella spiralis* induces protective immunity in *BALB/c* mice. *Parasitology Research*, 112, 3229-3238. DOI: 10.1007/s00436-013-3500-6.

37 Sun, GG, Lei, JJ, Ren, HN, Zhang, Y., Guo, KX, Long, SR, Liu, RD., Jiang, P., Wang, ZQ, Cui, J. (2019). Intranasal immunization with recombinant *Trichinella spiralis* serine protease elicits protective immunity in *BALB/c* mice. *Experimental Parasitology*, 201, 1-10. DOI: 10.1016/j.exppara.2019.04.006.

38 Zhai, CC, Liu, XL, Bai, X., Jia, ZJ, Chen, SH, Tian, LG, Ai, L., Tang, B., Liu, MY, Wu, XP, Chen, JX. (2021). Bioinformatic Prediction and Production of Four Recombinant Proteins from Different Developmental Stages of *Trichinella spiralis* and Testing of Their Diagnostic Sensitivity in Mice. *Iranian Journal of Parasitology*, 16(1), 122-135. DOI: 10.18502/ijpa.v16i1.5531.

39 Gao, H., Tang, B., Bai, X., Wang, L., Wu, X., Shi, H., Wang, X., Liu, X., Liu, M. (2018). Characterization of an antigenic serine protease in the *Trichinella spiralis* adult. *Experimental Parasitology*, 195, 8-18. DOI: 10.1016/j.exppara.2018.09.009.