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GENERATION OF MONOCLONAL ANTIBODIES AGAINST TRICHINELLA SPIRALIS AND DETERMINATION OF THEIR IMMUNOCHEMICAL PROPERTIES

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Abstract

Diagnosis of trichinellosis in humans and animals is still in the developmental stage of the most effective and sensitive test for early detection. Serological methods are the most sensitive for detecting trichinella invasion. However, there is a high risk of non-specific reactions with other parasitological diseases. To address this issue, monoclonal antibodies (mAbs) against excretory-secretory antigens (ES-Ag) of trichinella muscle larvae are employed. In this study, hybridomas secreting mAbs against ES-Ag of *Trichinella spiralis* muscle larvae were generated. Out of 79 colonies, 5 mAb producer monoclonal clones (1D5, 1E7, 3C10, 4D6, 4F7) were obtained, and further stability testing selected 3 clones (1D5, 1E7, and 4F7) that yielded 14 to 40% active subclones. Western blotting using T. spiralis ES antigens demonstrated that the mAbs recognized a single band at 75 kDa. The productivity of the clones ranged from 2.0 to 4.0 mg/ml of protein. The obtained results indicate that the high activity, specificity of mAbs, and productivity of the clones recommend these mAbs for application in diagnostic purposes.

Key words: diagnostic activity; immunochemical properties; monoclonal antibodies; trichinellosis.

Introduction

Trichinellosis represents a foodborne parasitic zoonosis instigated by the muscle larvae (ML) of *Trichinella spp*. Human incidences of this malady are consistently documented on a global scale, primarily attributed to the ingestion of inadequately cooked or raw pork, along with the consumption of meat from wild animals, including canines [1]. Trichinella infection unfolds in two distinct phases: the initial enteric phase involves the residence of adult worms in the intestines. The clinical manifestations during the first week post-infection manifest as gastroenteritis, diarrhea, and abdominal pain. Subsequently, in the second or parenteral phase, larvae invade muscle tissue, provoking the formation of a nurse cell where they await a new host. Clinically, this stage is characterized by fever, myalgia, and arthralgia. Infections with a low parasite burden may be asymptomatic [2].

Timely detection of trichinellosis during the enteric phase is crucial to mitigate the infection, given the enhanced efficacy of anthelminthic drugs at this juncture. However, early clinical identification of human trichinellosis is challenging due to the absence of pathognomonic signs or symptoms [3]. Diagnosis relies on three principal criteria: patient infection history, clinical assessment, and laboratory investigations, including serological tests [4]. The primary limitation of serological tests, such as ELISA, in detecting antibodies to trichinella lies in the elevated rate of false-negative outcomes during the early stages of infection. Research indicates that the optimal positive dynamics of ELISA for detecting antibodies to trichinella are not attained until at least 1-3 months post-infection with the

parasite [5]. Hence, the acquisition of monoclonal antibodies (mAbs) capable of facilitating the development of serodiagnostic tools for detecting circulating antigens of trichinellosis is imperative.

Various mAbs targeting newborn larvae, muscle larvae, or adults of T. spiralis have been documented in the literature [6,7]. However, immunodiagnostic methods utilizing these mAbs have encountered challenges pertaining to sensitivity, specificity, and reproducibility. Antigens derived from the excretory-secretory (ES) complex of muscle larvae of *T.spiralis* have proven effective in generating mAbs for the development of immunodiagnostic approaches for trichinellosis [8,2].

The aim of this study was to create and characterize mAbs against ES antigens of muscle larvae of *T.spiralis*, providing a foundation for the development of specific serological methods for the early diagnosis of trichinellosis.

Materials and Methods

All stages of the project were conducted at the scientific and technical base of the "S. Seifullin Kazakh Agrotechnical Research University" (KATRU): at the Faculty of Veterinary Medicine and Animal Husbandry in the Professor N.T. Kadyrov Parasitology Laboratory, the "Joint Kazakhstan-China Laboratory for Biosafety," and the Scientific-Production Platform for Agricultural Biotechnology.

Ethics Approval:

All animal-related activities adhered to high biosafety standards and animal welfare. All protocols were in compliance with the International Guiding Principles for Biomedical Research Involving Animals. All animal care and use procedures were approved by the Ethics Committee on Animal Care of the Faculty of Veterinary Medicine and Animal Husbandry, S. Seifullin Kazakh Agrotechnical Research University (KATRU), Astana, Kazakhstan (Protocol No. 2, dated July 20, 2020).

Parasites:

The study utilized samples of muscle tissue from experimentally infected laboratory *Balb/c* mice with *Trichinella spiralis* larvae, generously provided by Dr. Anne Mayer-Scholl, a specialist from the Department of Diagnostics, Genetics, and Characterization of Pathogens at the Reference Center for Risk Assessment (BfR) in Berlin.

Experimental Animals and Cell Line:

Laboratory *Balb/c* mice were used for infection and monoclonal antibody production. The X63Ag8.653 cell line was employed as myeloma cells.

Immunization:

Monoclonal antibodies against trichinella ES antigens were obtained through mouse immunization. The immunization involved administering 100 μL of ES antigens with a concentration of 25 μg/mL on the first day, mixed with Freund's complete adjuvant at a 1:1 ratio. Subsequently, antigen immunization mixed with incomplete Freund's adjuvant continued on the 7 days post immunization (dpi), with additional doses on the 11 dpi, 12 dpi, and 13 dpi, and serum collection was conducted on the 17 dpi.

Hybridization and Monoclonal Antibody Production:

For the experiment, three groups of *Balb/c* mice of approximately the same age and weight were selected. Antigen used for hybridization was trichinella ES antigens. Animals with the highest antibody titers (at least 1:800) determined by ELISA on 4 dpi were sacrificed using cervical dislocation. Spleens were aseptically extracted, and splenocytes were obtained by perfusion. Hybridization of prepared splenocytes with X63Ag8.653 myeloma cells was performed following the method of Oi V. and Herzenberg L. [9].

Production of Monoclonal Antibodies in vitro and in vivo:

Following two rounds of cloning, hybrid cells were propagated to a substantial quantity in plastic culture flasks containing 25-50 mL of complete growth medium. The cells underwent a 3-4 day culture period in a CO2 incubator. Subsequently, detachment from the plastic substrate was achieved by pipetting, followed by centrifugation at 1000 rpm for 7-10 minutes. The resulting cell pellet was reconstituted in an incomplete growth medium, and 2x106 cells were intraperitoneally injected into *Balb/c* mice. These mice had received a prior injection of pristane (Sigma, USA) at a dose of 0.5 mL per mouse 7-10 days before the injection (dbi). The mAbs were then obtained from the culture supernatant of hybridoma cells for subsequent research purposes.

Upon the development of ascitic tumors within 12-15 days, mice were humanely euthanized through cervical dislocation. Ascitic fluid was extracted from the abdominal cavity using a

syringe equipped with a needle. Antibodies from the ascitic fluid were precipitated at 5000 rpm for 30 minutes at 4 °C. Purified monoclonal antibodies in the form of ascitic fluid were either stored at -70 °C without a preservative or at 4 °C with the addition of 0.1% sodium azide. The concentration of antibodies in both ascitic and culture fluids was quantified using the Bradford method [10].

SDS-PAGE and Western Blotting

Electrophoresis of ES antigens was performed on a 10% polyacrylamide gel using the Laemmli method with SDS-PAAG. The electrophoretic transfer of trichinella antigens from the gel to a nitrocellulose membrane and the detection of specific protein bands using sera from infected mice and/or hyperimmunized mice were carried out through standard procedures. The electrophoresis and blotting protocols followed the methods described by U.K. Laemmli et al. (1970) and H. Towbin et al. (1979), respectively [11,12].

Results

Immunization of Balb/c mice with Trichinella spiralis ES-Ag.

The ES antigen used in immunization was obtained by the research group in a previous study [13]. The initial phase of the current study involved generating a sufficient quantity of the ES antigen through mouse immunization. Subsequently, following the immunization protocol, the purified ES antigen was intraperitoneally injected into mice five times at a concentration of 50 μg/mL. Blood serum was collected on the 17 dpi. Serum samples were then tested to determine the maximum antibody titer using the indirect ELISA method (Table 1).

Table 1 – Investigations of Blood Sera from Immunized Balb/c mice

Antigenic preparat	Mouse Serial Numbers	Duration of Immunization	Number of Isolated Splenocytes (million cells)	Antibody Titers
Trichinella Excretory-Secretory Antigen	1	17 days	$7x10^6$	1:6 400
	2		$10x10^6$	1:6 400
	3		$5 x10^6$	1: 6 400

As evident from Table 1, the preparations demonstrated their antigenicity and immunogenicity in *Balb/c* mice. The maximum antibody titer in immunized animals reached 1:6400 for ES-Ag, indicating that the obtained preparations possess significant antigenicity and can be used for mAb production. Based on these results, it was decided to proceed with ES-Ag for further work, as it exhibited higher antigenicity and antibody titers. Several sources also note that ES antigens of *Trichinella spiralis* muscle larvae are valuable antigens for obtaining mAbs for use in developing trichinellosis immunodiagnostic methods [14,15].

Hybridization of Myeloma Cells

The hybridization of myeloma cell line *X63Ag8.653* with immune B-lymphocytes from mice immunized with ES-Ag was performed at a ratio of 1:10. The number of splenocytes isolated from the spleens of immunized mice by perfusion was 80 million cells, while the myeloma cell count was 8×106. Hybridization was conducted in the presence of PEG-4000, and the cell suspension was resuspended in complete growth medium with 10% fetal bovine serum before being seeded on 96-well plates [16].

Twenty-four hours after hybridization, cultivation continued in selective HAT medium (Sigma, USA) containing hypoxanthine, aminopterin, and thymidine, with a switch to selective HT medium (Sigma, USA) on the 7th day, which contains hypoxanthine and thymidine, promoting the growth of hybrid cells exclusively. The formation of hybrid colonies was observed on the 10th day post-hybridization (Figure 1).

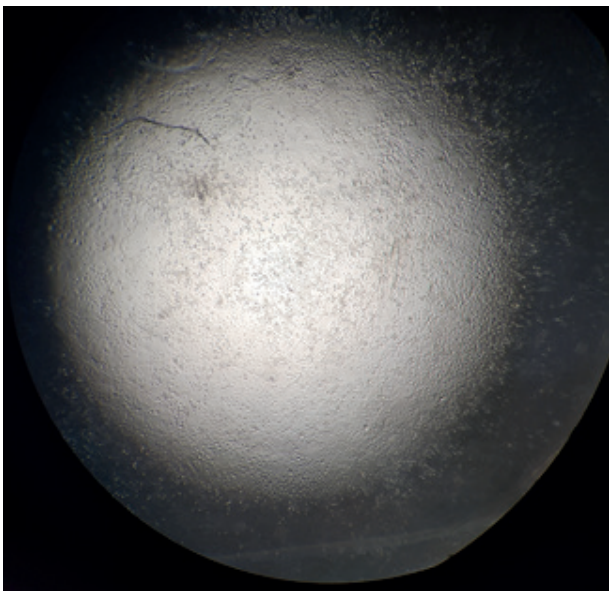

Figure 1 – Growth of hybrid cells

On the 21st day after hybridization, through continuous screening, the growth of hybrid cells was detected in 79 wells out of 384 seeded, or in 20.5% of cases. The ability of the formed hybrids to produce antibodies against trichinella ES preparations was determined by testing the culture fluid using the ELISA method. As a result, out of the 79 colonies, activity was observed in 5 strains: 1D5, 1E7, 3C10, 4D6, 4F7. Subsequent work was conducted with these 5 cell strains. The hybrid cells were then transferred to 24-well plates and subsequently to culture flasks. The hybrid cells exhibited growth as immobile colonies. When introduced into flasks, they exhibited a weak attachment to the substrate, presenting as spherical cells roughly comparable in size to the original myeloma. The hybrid cytoplasm displayed a slender perimeter surrounding the nucleus. When seeded at concentrations of 1-2 million cells/mL, the cells established a monolayer within an average period of 5-7 days, concurrently demonstrating mAb productivity in the range of 30 to 60 μg/mL. To obtain offspring from a single producing cell, the most active clones were further cloned using the limiting dilution method. Culture medium testing was performed on days 10-17 after cloning using the ELISA method. For testing, samples of culture fluid were taken only from wells where single colonies of cells were growing. The cloning results are presented in Table 2.

Table 2 – Activity of subclones of hybrids during the first cloning

Number of bores	Name of clones	Number of formed subclones	
		total	active of them
1	1D5	7	1 (14%)
2	4D6	11	3 (27.2%)
3	1E7	5	2 (40%)

As evident from Table 2, the activity of subclones during cloning was relatively low. For instance, among the 1E7 clone, 40% of subclones exhibited activity, while for the other two hybrids, 1D5 and 4D6, the proportion of active subclones was significantly lower, at 14% and 27.2%, respectively.

Subsequent work was conducted with the hybrid cells 1D5F3, 1E7D4, and 1E7B9, which demonstrated their activity. While obtaining a preparative quantity of mAbs typically requires only one clone, this does not guarantee its productivity and activity.

Monoclonal Antibody Production

Positive active clones were transferred to flasks for the production of preparative quantities of subclones 1D5F3, 1E7D4, and 1E7B9 under in vitro conditions. Based on the results of indirect ELISA, the most active hybrids, 1D5F3 and 1E7B9, were selected for further use in obtaining mAbs under both *in vitro* and *in vivo* conditions. For in vitro mAb production, hybrids were cultured in 50 ml polystyrene flasks. The culture fluid was tested by ELISA, collected in separate

sterile bottles, and preserved using a sodium azide solution. As a result, more than 250 ml of culture fluid containing mAbs from active clones of hybrids 1D5F3 and 1E7B9 was collected. The mAb titers were high, ranging from 1:8 to 1:16, and the overall protein concentration was 30-60 μg/ml. After harvesting the culture fluid, a portion of the hybrids in the logarithmic growth phase underwent cryopreservation.

To generate substantial quantities of mAbs through in vivo means, subclones 1D5F3 and 1E7B9 were introduced into mice. Hybrid cells were intraperitoneally injected into four linear mice (two hybrids per mouse) at a dose of 1 million cells in 0.5 ml, which were pre-treated with pristane at a dosage of 0.5 ml. Upon the formation of ascitic tumors, ascitic fluid was harvested from the peritoneal cavity on the 14th day. The collected ascitic fluid underwent centrifugation, and hybrid cells were cryopreserved in liquid nitrogen. The mAbs were subsequently isolated and purified from the supernatant using ammonium sulfate precipitation and dialysis against phosphate-buffered saline. The outcome yielded 12 ml of ascitic fluid from four mice, with 7 ml attributed to the 1E7B9 hybrid and 5 ml to the 1D5F3 hybrid. The mAb concentration in the ascitic fluid of both hybrids ranged from 2.0 to 4.0 mg/ml.

Despite the abundance of literature on the production of mAbs against trichinellosis for neonatal larvae, muscle larvae, or adult *T.spiralis* individuals, the immunodiagnostic methods developed using these mAbs encountered challenges related to sensitivity, specificity, and reproducibility.

Therefore, through cloning, two hybrid cultured cell clones (1D5F3 and 1E7B9) were selected, possessing all the necessary indicators. Moreover, based on the obtained and tested hybrids, a panel of hybrid cells stably producing mAbs against *Trichinella spiralis* was developed.

Determination of Immunohistochemical Properties of mAbs

The immunohistochemical properties of mAbs were assessed through SDS-PAGE and immunoblotting. The determination of the molecular mass of the obtained mAbs was conducted using vertical electrophoresis in 10% denaturing polyacrylamide gel. It was found that the molecular mass of the investigated mAbs was 65 and 25 kDa, aligning with widely accepted values for heavy and light chains of immunoglobulins.

Immunoblotting results demonstrated that the obtained mAbs reacted with the ES antigen of trichinella, forming a distinct band at approximately 75 kDa (Figure 2).

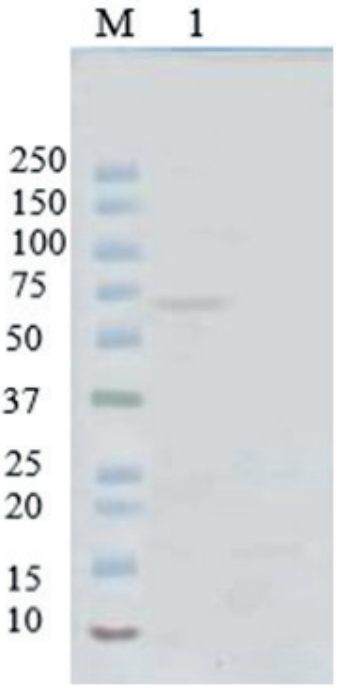

Figure 2 – The result of immunoblotting - binding of mAb to ES-Ag:
M - marker; 1 - monoclonal antibodies

The overall protein concentration in the ascitic fluid was 2.0 mg/ml for the 1D5F3 hybridoma and 4.0 mg/ml for 1E7B9. To determine the titration activity of monoclonal antibodies, an indirect ELISA based on trichinella ES-Ag was employed. The titers of mAbs were found to be high, ranging from 1:8 to 1:16 in the culture medium and 1:3200 to 1:6400 in the ascitic fluid.

The class and subclass of mAbs were identified through immunodiffusion reactions using standard reference sera for determining the class and subclass of immunoglobulins, provided by Sigma, USA. The results revealed that the obtained monoclonal antibodies belong to the IgG class, subclass G1. The immunochemical characteristics of mAbs from hybridoma cells 1D5F3 and 1E7B9 are presented in Table 3.

Table 3 – Characteristics of the mAb of hybrid cells 1E7B9 and 1D5F3

Name of clones	Isotype of mAbs	*In vitro* productivity of mAbs, mg/ml	*In vivo* productivity of mAbs, mg/ml	Titre of mAbs in culture medium	Titre of mAbs in ascitic fluid
1E7B9	IgG1	0.06	4.0	1:16	1:6400
1D5F3	IgG1	0.03	2.0	1:8	1:3200

As evident from Table 3, the hybridoma clones exhibited high productivity both in cell culture and ascitic fluid. Cultivating hybridomas in the abdominal cavity of mice yielded ascitic fluid with antibody titers against trichinella at levels ranging from 1:3200 to 1:6400.

Therefore, the monoclonal antibodies obtained in the course of this study demonstrated high activity and productivity. This suggests their potential application as components for developing serological test systems for diagnosing trichinellosis.

Discussion

The global prevalence of trichinellosis has instigated the development of diverse serological tools aimed at identifying trichinella invasion in both humans and animals. Serological techniques involve the identification of specific antibodies and circulating antigens of parasites present in serum or tissue fluids. In the context of diagnosing human trichinellosis, serological tests designed to detect trichinella-specific antibodies play a crucial role in diagnostic approaches. According to the guidelines set forth by the International Trichinella Commission, serological methods for identifying trichinella infection in animals are not recommended as a substitute for individual carcass meat inspection. Nevertheless, serological approaches for antibody detection are deemed suitable for the surveillance of both domestic and wild animals, contributing significantly to the comprehension of trichinella circulation [17,5].

In this study, the generated IgG mAbs against ES antigens of T.spiralis, as revealed by immunoblotting, exhibited a single band at 75 kDa, indicating specificity and purity of the reaction.

Srimanote et al. (2000) reported a high percentage of growing hybridomas secreting antibodies cross-reactive to many of the 23 tested heterologous parasites. Only six monoclonals (designated 3F2, 5D1, 10F6, 11E4, 13D6, and 14D11) secreted monoclonal antibodies specific to the ES antigen and/or crude extract (CE) of infectious larvae of *T.spiralis* [15].

Data are also available on the use of mAbs to detect circulating antigens and coproantigens using a sandwich-ELISA method without reactivity between mAb and common antigens of other helminths (e.g., *Angiostrongylus cantonensis, Ascaris suum, Echinococcus granulosus, Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Toxocara canis,* and *Trichuris trichiura*). IgM mAbs recognized antigens with masses of 45, 49, and 55 kDa in ES antigens [2].

Trichinella infection triggers a specific antibody response, with the timing of seroconversion influenced by factors such as the infection dose, trichinella species, and host species. The persistence of antibodies varies among different hosts. In humans, seroconversion typically occurs between the second and fifth weeks after primary infection with Trichinella larvae, and specific antibodies may endure for several years. However, antibody levels do not correlate with the severity of the disease or the clinical course during the acute phase of trichinellosis [18,19,20]. In animals infected with trichinella, detectable antibody levels are usually absent for 2-3 weeks or more after infection [21]. Antibodies against trichinella

can persist for at least 6 months after infection without diminishing ES-ELISA results. Yet, in horses, antibody levels decline a few months after infection, despite the presence of infectious larvae in the muscles. Consequently, serological methods are not recommended for detecting trichinella infection in horses [22,23].

In the context of human diagnosis, antibody detection tests serve as a valuable adjunct. Classes of specific antibodies to immunoglobulin G (IgG), immunoglobulin E (IgE), and immunoglobulin A (IgA) become evident only 2-3 weeks after infection with trichinellosis. The literature details diverse methods for detecting antibodies against Trichinella infection in humans and animals, including the indirect fluorescence antibody test (IFAT), Western blot analysis, and indirect ELISA [24,3]. The development of serological tests hinges on the availability of high-quality antigens, with a variety of antigens currently employed for this purpose [25].The mAbs obtained during this study showed a high titer in both culture medium and ascitic fluid, while the 1D5F3 and 1E7B9 subclones showed high productivity in vivo, producing from 2.0 to 4.0 mg/ml of protein.

Thus, obtaining mAbs against trichinella will increase the sensitivity and specificity of the designed serological test systems and will help reduce the level of non-specific reactions with other diseases.

Conclusion

The applied methods of obtaining and operating mAbs have shown their effectiveness in obtaining active and productive hybrid cells. The specific reaction of mAbs with ES-Ag of muscle larvae makes it possible to use these antibodies for the serodiagnosis of trichinellosis.

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References

1 Takumi, K., Within-host dynamics of Trichinella spiralis predict persistent parasite transmission in rat populations [Text] / Takumi, K., Franssen, F., Fonville, M., Grasset, A., Vallée, I., Boireau, P., Teunis, P., Giessen, J. // International Journal for Parasitology. - 2010. - Vol.40(11). - P. 1317-1324.

2 Zumaquero-Ríos, J-L, Trichinella spiralis: Monoclonal antibody against the muscular larvae for the detection of circulating and fecal antigens in experimentally infected rats [Text] / Zumaquero-Ríos, J-L, García-Juarez, J., de-la-Rosa-Arana, J.-L., Marcet, R., Sarracent-Pérez, J. // Experimental Parasitology, - 2012. -Vol.132(4). - P.444-449.

3 Dupouy-Camet, J., Opinion on the diagnosis and treatment of human trichinellosis [Text] / Dupouy-Camet, J., Kociecka, W., Bruschi, F., Bolas-Fernandez, F., Pozio, E. // Expert Opin Pharmacother, - 2002. - Vol.3(8). - P.1117-1130.

4 Gómez-Morales, M.A., A distinctive Western blot pattern to recognize Trichinella infections in humans and pigs [Text] / 4 Gómez-Morales, M.A., Ludovisi, A., Amati, M., Blaga, R., Zivojinovic, M., Ribicich, M., Pozio, E. // International Journal for Parasitology. - 2012. -Vol.42(11). - P.1017-1023.

5 Gamble, H.R., International commission on Trichinellosis: recommendations on the use of serological tests for the detection of Trichinella infection in animals and man [Text] / Gamble, H.R., Pozio, E., Bruschi, F., Noeckler, K., Kapel, C.M., Gajadhar, A. // Parasite. - 2004. - Vol.11. - P.3-13.

6 Romari, S. F., Appleto, N.J.A. Invasion of epithelial cells by Trichinella spiralis: in vitro observations [Text] / Parasite. - 2001. - Vol.8. - P.48-50.

7 de-la-Rosa-Arana, J-L., Moran-Tlatelpa, E., Medina, Yamir, Gomez-Priego, A., Correa, Dolores Detection of circulating and fecal Trichinella spiralis antigens during experimental infection using monoclonal antibodies against the new born larvae [Text] / Parasite. - 2001. - Vol.8. - P.123-125.

8 Li, C.K, Ko, R.C. Inflammatory response during the muscle phase of Trichinella spiralis and T. pseudospiralis infections [Text] / Parasitol Res. - 2001. -Vol.87(9). - P.708-714.

9 Oi, V., Herzenberg, L. Immunoglobulin – producing hybrid cell lines [Text] / Selected methods in cellular immunology. - 1980. - Vol.2. - P.351-352.

10 Bradford, M. A rapid and sensitive method fer the quantitation of microgram quantitaties of protein utilizing the principle of protein - due binding [Text] / Analytical Biochemistry. - 1976. - Vol.72. - P.248-254.

11 Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4 [Text] / Nature. - 1970. - Vol.227. - P.680-685.

12 Towbin, H., T Staehelin, J. Gordon Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications [Text] / Proc.Natl.Acad. Sci. USA. -1979. - Vol.76. - P.350-354.

13 Akibekov, O.S., Poluchenieekskretorno-sekretornogo i somaticheskogo antigenov Trichinella spiralis [Russian] [Text] / Akibekov, O.S., Zhagipar, F.S., Syzdykova, A.S., Gajimuradova, A.M., Akanova, Zh.Zh. // Vestnik nauki Kazahskogo agrotekhnicheskogo universiteta im. S. Sejfullina (mezhdisciplinarnyj). -2022. - No2 (113). - T.2. - P.133-145.

14 Duzhang, Zh., Robin, G.B. Trichinella spiralis: Murine strain variation in response to monoclonally defined, protective, nonstage-specific antigens [Text] / Experimental Parasitology. -1990. - Vol.70 (3). - P.330-343.

15 Srimanote, P., Ittiprasert, W., Sermsart, B., Chaisri, U., Mahannop, P., Sakolvaree, Y., Tapchaisri, P., Maleewong, W., Kurazono, H., Hayashi, H., Chaicumpa, W. Trichinella spiralis-specific monoclonal antibodies and affinity-purified antigen-based diagnosis [Text] / Asian Pac J Allergy Immunol. - 2000. -Vol.18(1). - P.37-45.

16 Escalante, M., Ubeira Evaluation of Trichinella spiralis Larva Group 1 Antigens for Serodiagnosis of Human Trichinellosis [Text] / Escalante, M., Romarís, F., Rodríguez, M., Rodríguez, E., José Leiro, M., Gárate, T., Florencio, M. // ASM Journals Journal of Clinical Microbiology. - 2004. - Vol. 42(9). - P.154-167.

17 Gómez-Morales, M. A. Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis [Text] / Clinical and Vaccine Immunology. - 2008. - Vol.15(11). - P.1723-1729.

18 Yang, Y., Serological tools for detection of Trichinella infection in animals and humans [Text] / Yang, Y., Cai, Y.N., Wei Tong, M., Sun, N., Xuan, Y.H., Kang, Y.J., Vallée, I., Boireau, P., Cheng, S., Ming, Y.L. // One Health. - 2016. - V.2. - P. 25-30.

19 Gómez-Priego, A., Crecencio-Rosales, L., de-La-Rosa, J.L. Serological evaluation of thin-layer immunoassay-enzyme-linked immunosorbent assay for antibody detection in human trichinellosis [Text] / Clin Diagn Lab Immunol. - 2000. - Vol.75. - P.2-810.

20 Ruangkunaporn, Y., Immunodiagnosis of trichinellosis: efficacy of somatic antigen in early detection of human trichinellosis [Text] / Ruangkunaporn, Y., Watt G., Karnasuta, C., Jongsakul, K., Mahannop, P., Chongsa-nguan, M., Chaicumpa, W. // Asian Pac. J. Allergy Immunol. - 2011. - Vol.12. - P.39-42.

21 Wang, L., Identification of early diagnostic antigens from major excretory-secretory proteins of Trichinella spiralis muscle larvae using immunoproteomics [Text] / Wang, L., Cui, J., Hu, D.D., Liu, R.D., Wang, Z.Q. // Parasit Vectors. - 2014. - Vol.7. - P.1-8.

22 Hill, D.E., Forbes, L., Gajadhar, A.A, Gamble, H.R. Viability and infectivity of Trichinella spiralis muscle larvae in frozen horse tissue [Text] / Vet Parasito. - 2007. - Vol.146. - P.6-102.

23 Gamble, H.R., Alvin, A.G., Morse B.S. Methods for the Detection of Trichinellosis in Horses [Text] / J Food Prot. - 1996. - Vol.59. - P.420-425.

24 Bruschi, F., Moretti, A., Wassom, D., Piergili Fioretti, D. The use of a synthetic antigen for the serological diagnosis of human trichinellosis [Text] / Parasite. - 2001. - Vol.8. - P.141-143.

25 Boireau, P., Characterization of eleven antigenic groups in Trichinella genus and identification of stage and species markers [Text] / Boireau, P., Vayssier, M., Fabien, J., Perret, C., Calamel, M., Soulé, C. // Parasitology. - 1997. - Vol.115. - P.641-651.

References

1 Takumi, K., Franssen, F., Fonville, M., Grasset, A., Vallée, I., Boireau, P., Teunis, P., Giessen, J. (2010). Within-host dynamics of Trichinella spiralis predict persistent parasite transmission in rat populations. International Journal for Parasitology, 40(11), 1317-1324. https://doi.org/10.1016/j.ijpara.2010.03.019

2 Zumaquero-Ríos, J-L, García-Juarez, J., de-la-Rosa-Arana, J.-L., Marcet, R., Sarracent-Pérez, J. (2012). Trichinella spiralis: Monoclonal antibody against the muscular larvae for the detection of circulating and fecal antigens in experimentally infected rats. Experimental Parasitology, 132(4),444-449. https://doi.org/10.1016/j.exppara.2012.09.016

3 Dupouy-Camet, J., Kociecka, W., Bruschi, F., Bolas-Fernandez, F., Pozio, E. (2002). Opinion on the diagnosis and treatment of human trichinellosis. Expert Opin Pharmacother, 3(8), 1117-1130. https://doi.org/10.1517/14656566.3.8.1117

4 Gómez-Morales, M.A., Ludovisi, A., Amati, M., Blaga, R., Zivojinovic, M., Ribicich, M., Pozio, E. (2012). A distinctive Western blot pattern to recognize Trichinella infections in humans and pigs. International Journal for Parasitology, 42(11), 1017-1023. https://doi.org/10.1016/j.ijpara.2012.08.003

5 Gamble H.R., Pozio E., Bruschi F., Noeckler K., Kapel C.M., Gajadhar 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.

6 Romari S F., Appleto N J.A. (2001). Invasion of epithelial cells by Trichinella spiralis: in vitro observations. Parasite, 8, S48-S50.

7 de-la-Rosa-Arana, J.-L., Moran-Tlatelpa, E., Medina, Y., Gomez-Priego, A., Correa, D. (2001). Detection of circulating and fecal Trichinella spiralis antigens during experimental infection using monoclonal antibodies against the newborn larvae. Parasite (Paris, France), 8, 123-125. https://doi.org/10.1051/parasite/200108s2123

8 Li CK, Ko RC. (2001). Inflammatory response during the muscle phase of Trichinella spiralis and T. pseudospiralis infections. Parasitol Res, 87(9), 708-714. https://doi.org/10.1007/s004360100420

9 Oi V., Herzenberg L. (1980). Immunoglobulin – producing hybrid cell lines. In: Selected methods in cellular immunology, Ed. By Mishell B and Shiigi. San Francisco. P. 351-352.

10 Bradford M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

11 Laemmli U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

12 Towbin H., T Staehelin, J Gordon. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc.Natl.Acad. Sci. USA, 76, 350-354.

13 Akibekov O.S., Zhagipar F.S., Syzdykova A.S., Gajimuradova A.M., Akanova ZH.ZH. (2022). Poluchenie ekskretorno-sekretornogo i somaticheskogo antigenov Trichinella spiralis. [Russian] Vestnik nauki Kazahskogo agrotekhnicheskogo universiteta im. S. Sejfullina (mezhdisciplinarnyj), 2(113), 133-145.

14 Zhu, D., Bell, R. G. (1990). Trichinella spiralis: Murine strain variation in response to monoclonally defined, protective, nonstage-specific antigens. Experimental Parasitology, 70(3), 330-343. https://doi.org/10.1016/0014-4894(90)90115-S

15 Srimanote P, Ittiprasert W, Sermsart B, Chaisri U, Mahannop P, Sakolvaree Y, Tapchaisri P, Maleewong W, Kurazono H, Hayashi H, Chaicumpa W. (2000). Trichinella spiralis-specific monoclonal antibodies and affinity-purified antigen-based diagnosis. Asian Pac J Allergy Immunol, 18(1), 37-45.

16 Escalante, M., Romarís, F., Rodríguez, M., Rodríguez, E., Leiro, J., Gárate, M. T., Ubeira, F. M. (2004). Evaluation of Trichinella spiralis Larva Group 1 Antigens for Serodiagnosis of Human Trichinellosis. ASM Journals Journal of Clinical Microbiology, 42(9),4060-4066. https://doi.org/10.1128/jcm.42.9.4060-4066.2004

17 Gómez-Morales M. A. et al. (2008). Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. Clinical and Vaccine Immunology, 15(11), 1723-1729.

18 Yang, Y., Cai, Y. N., Tong, M. W., Sun, N., Xuan, Y. H., Kang, Y. J., Vallée, I., Boireau, P., Cheng, S. P., & Liu, M. Y. (2016). Serological tools for detection of Trichinella infection in animals and humans. One Health, 2, 25-30.

19 Gómez-Priego, A., Crecencio-Rosales, L., de-La-Rosa, J. L. (2000). Serological evaluation of thin-layer immunoassay-enzyme-linked immunosorbent assay for antibody detection in human trichinellosis. Clinical and Diagnostic Laboratory Immunology, 7(5), 810. doi: 10.1128/CDLI.7.5.810

20 Ruangkunaporn, Y., Watt, G., Karnasuta, C., Jongsakul, K., Mahannop, P., Chongsa-nguan, M., & Chaicumpa, W. (2011). Immunodiagnosis of trichinellosis: efficacy of somatic antigen in early detection of human trichinellosis. Asian Pacific Journal of Allergy and Immunology, 12, 39–42.

21 Wang, L., Cui, J., Hu, D. D., Liu, R. D., & Wang, Z. Q. (2014). Identification of early diagnostic antigens from major excretory-secretory proteins of Trichinella spiralis muscle larvae using immunoproteomics. Parasites & Vectors, 7, 1-8.

22 Hill, D. E., Forbes, L., Gajadhar, A. A., & Gamble, H. R. (2007). Viability and infectivity of Trichinella spiralis muscle larvae in frozen horse tissue. Veterinary Parasitology, 146(1–2), 6–102.

23 Gamble, H. R., Gajadhar, A. A., Solomon, M. B. (1996). Methods for the Detection of Trichinellosis in Horses. Journal of Food Protection, 59(4), 420–425.

24 Bruschi, F., Moretti, A., Wassom, D., Piergili Fioretti, D. (2001). The use of a synthetic antigen for the serological diagnosis of human trichinellosis. Parasite, 8, 141-143.

25 Boireau, P., Vayssier, M., Fabien, J., Perret, C., Calamel, M., & Soulé, C. (1997). Characterization of eleven antigenic groups in Trichinella genus and identification of stage and species markers. Parasitology, 115(4), 641-651.