

ANNOTATION

of the dissertation by Tolepova Gulzat Kanibekovna on the topic «Parasitological monitoring of natural water bodies in opisthorchiasis-unfavorable regions of the Republic of Kazakhstan (Akmola, Pavlodar, North Kazakhstan, and West Kazakhstan Regions) for the purpose of determining the safety and quality of fish and fish products for humans», submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D09102 – «Veterinary Sanitation»

Relevance of the research topic. Opisthorchiasis, clonorchiasis, pseudamphistomiasis, metorchiasis, echinocasmus, metagonimiasis, nanophyetiasis, heterophyiasis, paragonimiasis, diphylobothriasis, anisakiasis, dioctophymiasis, gnathostomiasis, spiroidosis, and corynosomiasis are socially significant and widespread diseases, with fish being the main source of infection. These invasions are severe and challenging to treat. In Kazakhstan, opisthorchiasis is one of the most common invasions. Its distribution ranges from the eastern to the western borders of the republic and includes a large number of freshwater bodies in the Pavlodar, North Kazakhstan, Kostanay, Akmola, Karaganda, and West Kazakhstan regions.

Following the address by the Head of State, Kassym-Jomart Kemelevich Tokayev, «Kazakhstan in a New Reality: Time for Action», a special program for the development of fisheries until 2030 was approved. Its aim is to provide the population with high-quality and environmentally safe fish products. To implement this program, it is first necessary to ensure the epizootic well-being of natural water bodies in terms of fish parasitic diseases. Furthermore, to prevent human infections from opisthorchiasis, metorchiasis, diphylobothriasis, and other dangerous helminth infections, public health education should be conducted, and limits on the catch of carp species should be reviewed.

Given the social significance of zoonotic invasions, scientific research in parasitology remains relevant for veterinary science and practice worldwide.

Objective of the dissertation research. To conduct parasitological monitoring of natural water bodies in opisthorchiasis-unfavorable regions of the Republic of Kazakhstan (Akmola, Pavlodar, North Kazakhstan, and West Kazakhstan regions) to determine the safety and quality of fish and fish products.

Research objectives:

- Parasitological monitoring of natural water bodies in opisthorchiasis-unfavorable regions of the Republic of Kazakhstan (Akmola, Pavlodar, North Kazakhstan, and West Kazakhstan regions);
- Determine the infection rates of cyprinid fish species with *opisthorchis* and other zoonotic invasion pathogens;
- Study of the nutritional value of fish meat affected by *opisthorchis* metacercariae;
- Conducting microbiological studies of ide meat infected with *opisthorchis* metacercariae;

- Determination of the resistance of *Opisthorchis metacercariae* to various physical and chemical factors;
- Development of scientifically-based recommendations for enhancing the effectiveness of veterinary and sanitary measures.

Research methods:

The complete parasitological examination of fish was conducted according to the methodological recommendations developed by A.M.Abdybekova, G.S. Shabdarbayeva, and S.S.Tokpan for studying fish parasites.

Fish were caught using research nets (six nets, each 25 meters long) with a mesh size of 20–80 mm in the wings. The nets were placed in the water at night, and the catch was collected after 12 hours. The caught fish were weighed, measured for length, and scales were collected for biological analysis to determine the fish's age.

To detect *Opisthorchis* spp. metacercariae, fish meat was examined using a STAKE V trichinoscope (with 50–70x magnification) and a KRUSS MS Z 5000 stereomicroscope.

Species identification of the detected opisthorchiid agents was conducted by compressing the specimens and extracting DNA using the phenol-chloroform method after incubation in an extraction buffer. Specific primers were used in PCR to differentiate between the two opisthorchiid agents - *Opisthorchis felineus* and *Methorchis bilis*.

The amplification of marker genes was conducted in a final reaction volume of 25 µl, containing 10× DreamTaq buffer, 20 mM MgCl₂, 1U DreamTaq Hot Start DNA Polymerase (Thermo Scientific™), 2 mM dNTP (New England BioLabs Inc.), 10 pmol of each primer, and 20 ng of extracted DNA from one sample. PCR was performed with ITS1 primers under the following thermal cycling conditions: 95°C for 15 s, 62°C for 25 s, and final elongation at 72°C for 30 s. The amplified DNA products were analyzed by horizontal electrophoresis on a 1% agarose gel using 1× TAE buffer solution and EtBr. The Gene Ruler 100 bp Plus DNA Ladder (Thermo Scientific™) was used to estimate the base pair lengths of the obtained amplicons.

Organoleptic properties and chemical composition of the fish samples were determined according to the requirements of GOST 7631-2008. The fat content was measured using a Soxhlet apparatus, protein content was determined by the Kjeldahl method, moisture was measured by drying in an oven at 105°C until a constant dry mass was achieved. Mineral elements were determined according to GOST 31795-2017, GOST R 55484-2013, and GOST R 55503-2013, and toxic elements were analyzed according to GOST 30178-96. An Analytic Jena atomic absorption spectrophotometer №aa100831106 (Germany) was used in this study. Fatty acid content in the muscle tissue of ide was determined according to GOST R 55483-2013, and amino acid composition was determined by capillary electrophoresis according to M-04-38-2009 on a Capel M-105 device. Vitamin content was measured according to M-04-41-2005, GOST R 54635-2011, and GOST R 54634-2011.

Microbiological studies were conducted according to the «Instruction on Sanitary and Microbiological Control of Food Production from Fish and Marine Invertebrates» and the technical regulations of the Eurasian Economic Union «On the Safety of Fish and Fish Products» (TR EAEU 040/2016).

To determine the resistance of opisthorchiasis metacercariae to physical and chemical factors, infected ide fish were frozen at -18°C , -20°C , and -28°C , heated in a thermostat at $+60^{\circ}\text{C}$ for 1 hour, boiled at $+80^{\circ}\text{C}$ for 10 minutes (from the boiling point), and fried in open air with 100 g of meat at 150°C for 15 minutes. Salting was performed with sodium chloride solutions at concentrations of 50 g/L, 100 g/L, 140 g/L, and 150 g/L.

For drying the fish at a temperature of 20°C – 21°C , salting was carried out using a sodium chloride mass fraction of 100 g per 1 kg of fish, with control on days 3, 4, 5, and 10.

Main provisions for defense:

- Indicators of fish infection with zoonotic agents in natural water bodies in opisthorchiasis-unfavorable regions of the Republic of Kazakhstan (Akmola, Pavlodar, North Kazakhstan, West Kazakhstan, Karaganda, and Kostanay regions);

- Indicators of opisthorchiasis incidence among the population in the Republic of Kazakhstan;

- Nutritional value indicators of ide meat infected with *Opisthorchis metacercariae*;

- Resistance indicators of *Opisthorchis metacercariae* to various physical and chemical factors;

- Scientifically-based veterinary and sanitary measures for opisthorchiasis.

Description of main research results.

The study identified Korgalzhyn Lakes in the Akmola region as a natural focus of opisthorchiasis, metorchiasis, and pseudamphistomiasis. Other natural foci of opisthorchiasis and metorchiasis were the Irtysh River in the Pavlodar region, the Uly Zhylyanshik River in the Kostanay region, and the Irtysh-Karaganda canal in the Karaganda region. No cases of opisthorchiasis were found in the West Kazakhstan and North Kazakhstan regions.

Among the fish of the *Cyprinidae* family studied by PCR, *Opisthorchis felineus*, *Metorchis bilis*, and migrating trematodes of the *Diplostomatidae* family were found in ide. In the Pavlodar region, these trematodes were identified only in tench caught in the Irtysh River. In fish caught in the Karaganda region, the species *Holostephanus dubinini*, belonging to the family *Cyathocotylidae*, was detected.

An epidemiological analysis from the last 10 years (2014–2023) showed the highest cases of opisthorchiasis in West Kazakhstan (1,111 cases), Pavlodar (2,800 cases), Kostanay (365 cases), Akmola (403 cases), North Kazakhstan (293 cases), and Astana (1,255 cases). The average epidemiological rate in the country from 2014–2023 was 3.7 cases per 100,000 population. The highest rates were recorded in Pavlodar City - 37.02, Astana - 12.96, West Kazakhstan - 17.16, North Kazakhstan - 5.26, Akmola - 4.69, and Kostanay - 4.15 per 100,000 people.

The study results showed that the organoleptic characteristics of fish infected with *Opisthorchis felineus* metacercariae did not differ from those of uninfected

fish. Chemical composition analysis revealed that infected fish had increased moisture content and decreased protein levels. Mineral substances and toxic elements in the examined fish samples did not exceed permissible levels. In the muscle tissue of ide infected with opisthorchiasis, the levels of fatty acids, amino acids, and vitamins were lower than in uninfected fish. Bacteriological studies indicated that the fish meat samples were not contaminated with pathogenic microflora, and the levels of contamination with conditionally pathogenic microorganisms did not exceed the regulatory standards established by technical regulations.

For the first time, freezing at low temperatures (-18°C, -20°C, and -28°C) was used to study the effects of physical and chemical factors on opisthorchiasis metacercariae. Results showed that freezing fish at -28°C for 32 hours completely destroyed metacercariae (SanPiN 321333-03).

Salting at a sodium chloride concentration of 5% for 30 days, 10% for 21 days, and 15% for 15 days proved effective. Instead of the 40-day period recommended by Veterinary regulations, salting with a 14% sodium chloride concentration for 15 days was sufficient.

At a temperature of 20–21°C and a salt concentration of 100 g per 1 kg of fish, the mobility of opisthorchis metacercariae was preserved for three days. By the fourth day, mobility in some metacercariae began to decrease, reducing by 50% on the fifth day, and completely ceasing on the tenth day. Before testing, fish were held in water for 20–30 minutes. Immobile metacercariae were stored in saline solution.

In conclusion, salting does not fully affect the viability of metacercariae; helminths are destroyed only in areas penetrated by salt. Under heat exposure, salt penetrates deeper layers, reducing water content, causing metacercariae to deform and disintegrate.

To reduce the incidence of opisthorchiasis, additional veterinary and sanitary measures are proposed for regions with very high and high incidence rates.

Justification of novelty and importance of the results. For the first time in the past 50 years, a large-scale parasitological monitoring of natural water bodies was conducted in opisthorchiasis-unfavorable regions of the Republic of Kazakhstan (Akmola, Pavlodar, North Kazakhstan, West Kazakhstan, Karaganda, and Kostanay regions). Based on the full parasitological dissection of 13 species of fish of the *Cyprinidae* family, trematodes from the *Opisthorchidae* family (*Opisthorchis felineus*, *Metorchis bilis* and *Pseudamphistomum truncatum*) were identified in three species (ide, tench, and silver crucian carp), indicating the potential for human infection with both opisthorchiasis and metorchiasis. PCR analysis confirmed the possibility of mixed trematode invasions of opisthorchiasis. For the first time, a comparative study of the nutritional value of ide meat, a primary source of opisthorchiasis infection, was conducted. The resistance of opisthorchiasis metacercariae to physical and chemical factors was determined. Depending on the prevalence of opisthorchiasis among the population, the territory of the country was categorized into different clusters.

Alignment with science development directions or government programs. The scientific research was conducted as part of the scientific and technical program of the Ministry of Agriculture of the Republic of Kazakhstan, titled «To Study the Epizootiological Characteristics of the Country's Territory for Fish Opisthorchiasis and Develop Veterinary-Sanitary Measures to Improve Their Effectiveness» (No. BR10764899-OT-23) for 2021–2023.

Description of the doctoral student's contribution to each publication. According to the materials of the dissertation, the co-authors published 9 scientific papers, including: 3 articles - in publications recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan; 1 article - in a journal included in the Scopus database; 2 articles - in collections of international scientific and practical conferences; 1 The article contains in the state register of rights to copyrighted objects a certificate of entry of information and 1 methodological manual, 1 recommendation.

Volume and structure of the dissertation. The dissertation follows a standard format, comprising an introduction, literature review, research materials and methods, results of independent research, summary and assessment of research results, conclusion, list of references, and appendices. The dissertation spans 117 pages, illustrated with 20 tables and 26 figures.