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TREMATODA AND CESTODA SPECIES IN CYPRINID FISH FROM SMALL LAKES OF THE KOSTANAY REGION

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Abstract

This study presents data on the epizootiological monitoring of parasitic diseases in fish from Kostanay region. It confirms the presence of previously identified permanent natural foci of opisthorchiasis in the southern districts, specifically in the Uly-Zhilanchik and Torgay rivers. Additionally, the research conducted in Zhangeldy and Amangeldy districts revealed the existence of biohelminths with both epizootiological and epidemiological significance.

This study aimed to investigate the presence of parasites dangerous to humans and carnivores in cyprinid fish species. Specifically, it focused on identifying the second intermediate hosts for opisthorchiasis, metorchiasis, and ligulosis: *ide* (*Leuciscus idus*) and *bream* (*Abramis brama*). From 2021 to 2023 seven reservoirs of the Kostanay region were the monitoring objects of the parasitological situation: Verkhnetobolskoe reservoir, rivers: Torgay, Uly-Zhilanchik, Zhaldama, Tobol, Zhelkuar, Akkol Lake, where shellfish-bitiniids, waterfowl and crustaceans live in large numbers, as the first intermediate host of pathogens *Opisthorchis felineus* (*Metorchis bilis*), *Ligula imestinalis*.

Examination of eight *ide* (*Leuciscus idus*) specimens caught in the Uly-Zhilanchik River revealed the presence of metacercariae of two parasitic trematodes: *O.felineus* and *M.bilis*, while the prevalence was 18.5% and 8.3%, and the infestation intensity was 1-3 and 2, respectively. In the Amangeldy region, metacercariae *O. Felineus* and plerocercoids *Ligula imestinalis* were found in two fish species: one individual of the *ide* species and two individuals of the *bream* species caught from the Torgai River. At the same time, the prevalence was 6.6% and 16.6%, and the infestation intensity was 2 and 1, respectively.

Parasitic diseases of fish were not detected in the waters of Denisovsky and Zhitikarinsky districts.

Based on Polymerase Chain Reaction (PCR) analyses conducted using specific primers at the National Center of Biotechnology LLP laboratory in Astana, Kazakhstan, two distinct types of opisthorchid parasites were identified in two *ide* (*Leuciscus idus*) specimens collected from Kostanay region. The species were confirmed to be *Opisthorchis felineus* and *Methorchis bilis* (PCR protocol dated April 24, 2023).

Key words: biohelminths; bitiniid mollusks; fish; invasion; metacercariae; natural focus; opisthorchiasis.

Introduction

Veterinary services play a vital role in safeguarding public health by ensuring the safety and high quality of fish products [1]. While fish is a valuable source of nutrition, it can also harbor dangerous parasites that cause serious helminthiasis in humans. Among these, opisthorchiasis ranks as

one of the leading concerns. Notably, opisthorchiasis is classified as a natural focal parasitosis, meaning it occurs in specific geographical areas with distinct ecological conditions favorable for parasite transmission [2].

Opisthorchiasis is a type of oral biohelminthiasis.

The pathogen's range extends from the Yenisei River basin to the western borders of Europe, but the spread of the disease in humans is focal [3]. The level of infection of the population with opisthorchis is driven by social and household factors: lifestyle (traditions, habits), the degree of development of fishing, the proportion of fish in the diet, methods of culinary processing of fish, the sanitary condition of the area [4].

Due to the fact that the intermediate and second intermediate hosts live in reservoirs, foci of opisthorchiasis are concentrated near rivers. The world's largest outbreak of this disease was formed in the Ob-Irtysh River basin. In the lower reaches of the Irtysh and the middle reaches of the Ob, the invasiveness of opisthorchiasis among rural populations reaches staggering levels of 90-95%. Even preschool children are frequently infected, highlighting the severity of the public health concern [5].

The highest morbidity rates of the population (up to 1000 per 100 thousand) are registered here. The reason for such an exceptional importance of this territory in the epidemiology of opisthorchiasis is the presence of an extremely developed river floodplain that provides conditions for the circulation of the pathogen of the disease. Territories with the above quantitative indicators of population infestation are hyperendemic. The development of *Opisthorchis felinus* occurs with a triple change of hosts: the first intermediate (mollusks), the second intermediate (fish) and the final (mammals) [6].

The final hosts of the parasite include humans, cats, dogs, pigs and more than 25 species of wild mammals whose diet includes fish (fox, arctic fox, sable, ferret, otter, mink, water vole, muskrat, etc.). As a result, timely diagnosis and monitoring

Materials and Methods

The work was performed at the Department of Veterinary Medicine of the Non-Profit Joint Stock Company "Akhmet Baitursynuly Kostanay Regional University" (Kostanay). For definitive diagnosis, samples were further analyzed at the laboratory of National Center of Biotechnology LLP, Astana.

The study of the epizootological situation for parasitic diseases, including fish opisthorchiasis, was carried out in the Kostanay region from 2022 to 2023. Within the framework of the budget program "To study the epizootological characteristics of the country's territory for especially dangerous

of parasitic diseases of fish such as ligulidosis, diplostomiasis are very much in demand, and liver flukes of the *Opisthorchidae* family are of the greatest epidemiological importance. The life cycle of this trematode is carried out with the participation of gastropods of the family *Bithyniidae*. The chain of the cycle is continued by fish of the *Cyprinidae* family, which play a major role in the spread of opisthorchiasis, as well as domestic and wild fish-eating animals. A person becomes infected with this parasite by eating infected individuals of the carp family. This disease is widespread in the Palearctic and Indochina [7].

Fishing is an efficient industry that generates significant income. The potential reserve of the fishing industry is to limit the spread of parasitic diseases of fish, the organization of rational preventive measures within the framework of epizootological supervision [8].

The Kostanay region boasts numerous rivers and lakes, vital resources for agriculture, industry, and recreational and commercial fishing. In this context, timely diagnosis and monitoring of diseases such as ligulidosis, diplostomiasis is of great importance for fish farming and industry, and liver flukes of the *Opisthorchidae* family are of the greatest epidemiological importance. Ensuring the helminthological safety of fish products for human consumption and addressing the impact of parasitic diseases on fish populations in the region remain pressing concerns [9].

This study investigated the prevalence of specific parasites in different fish species inhabiting water bodies across Kostanay region, aiming to assess the overall parasitic burden and its potential impact.

diseases and to develop veterinary and sanitary measures to improve their effectiveness" and the concluded agreement between the NLC "Akhmet Baitursynuly Kostanay Regional University" and LLP "Kazakh Scientific Research Veterinary Institute" No. 04/8-21-32 from 07.09.2021, as well as the topics of the master's thesis "Monitoring of the parasitological situation of helminthiasis of fish in the territory of Kostanay region".

The objects of monitoring of the parasitological situation in 7 reservoirs of Kostanay region became: Verkhnetobolskoe reservoir of the Torgai river, the Uly-Zhilanchik river, the Zhaldama river, the

Tobol River, the Zhelkuar river, the Akkol lake, where live in large numbers the bitiniid mollusks (the first intermediate host of *Opisthorchis felineus* metacercariae).

A total of 6 species of fish of the Cyprinidae family (*Cyprinidae*) in the amount of 247 specimens were studied. Namely, crucian carp (*Carassius carassius*), ide (*Leuciscus idus*), bream (*Abramis brama*), tench (*Tinca tinca*), roach (*Rutilus rutilus*), carp (*Cyprinus carpio*).

The species membership of fish was determined by the atlas “Fish Identifier” (Myagkov H.A., 1994) and the textbook “System of commercial fish” (Azizov H.A., Moiseev P.A., 1996).

The study of fish was carried out by a complete helminthological autopsy using the Scriabin method. A compressor method was also used to diagnose fish for infection with *O. Felineus* and *M.bilis* metacercariae.

The fish to be examined was determined up to the species. Then, having freed the middle part of the body from the scales, the skin was cut with scissors along the middle line of the back, and two vertical incisions from the first incision to the lateral line outlined the area of the middle third of the back. The skin was removed from it and a layer of muscles 2-3 mm thick was cut off, which was then examined in the compressor using binoculars (Beer, 1987). For each fish, a thorough external examination assessed skin and fin condition,

followed by detailed inspection of the gills. The abdominal cavity was then opened, and internal organs including the heart, liver, gallbladder, spleen, swim bladder, kidneys, genitals, and gastrointestinal tract were systematically examined for any abnormalities. Particular attention was paid to the most likely localities of metacercariae – subcutaneous connective tissue and surface layers of muscle tissue. The diagnosis of diplostomiasis was also made using the compression method of examining the eyes of fish. For this technique, the eyes were removed from the eye cavities, opened with sharp scissors and clamped between two compressor glasses. Then the obtained material was examined under a microscope.

Metacercariae of opisthorchid fish were identified in the laboratory of the Department of Veterinary Medicine, Akhmet Baitursynuly Kostanay Regional University. The identification process involved a compressor method followed by microscopic examination using a Levenhuc light microscope. Microscopic analysis of the helminth larvae was performed using an MBS binocular microscope at 16x magnification. Additionally, to confirm the infection of fish with opisthorchiasis, muscle samples of 5 fish affected by opisthorchiasis metacercariae (ide) were sent to the laboratory “National Center of Biotechnology” LLP, Astana (PCR protocol dated 04/24/2023).

Results

The study investigated the epizootiological situation for fish parasitoses in the Kostanay region, focusing on Zhangeldy, Amangeldy, Denisov, and Zhitikara districts due to their close proximity to freshwater resources, primarily smaller rivers. The functional stability of parasite foci in these areas is attributed to the presence of all necessary links in the opisthorchid life cycle: intermediate hosts (bitiniid mollusks as the first host and cyprinid fish as the second) and definitive hosts.

This study aimed to select freshwater fish, specifically those belonging to the *Cyprinidae* and *Salmonidae* families, from rivers, lakes, and reservoirs in the specified regions. Four cyprinid species (*tench*, *ide*, *bream*, and *roach*) and one salmonid species (*ripus*) were chosen for investigation, as detailed in Table 1.

Table 1 – Helminthiasis of fish in the water basins of the Kostanay region

Name of the reservoir	Types of fish	Researched	Affected	Type of parasite	The extent of the invasion%	Infestation intensity, instance
Zhangeldy district						
Uly-Zhilanchik River	<i>Ide</i>	45	5	<i>Opisthorchis felineus</i>	18,5	1-3
			1	<i>Metorchis bilis</i>	8,3	2
	<i>Roach</i>	15				
	<i>Tench</i>	12				
	<i>Bream</i>	11				

Torgai River	<i>Crucian carp</i>	17				
	<i>Roach</i>	14				
Akkol Lake	<i>Sazan</i>	12				
	<i>Roach</i>	10				
	<i>Ripus</i>	3				
Amangeldy district						
Zhaldama river	<i>Roach</i>	13				
	<i>Tench</i>	7				
Torgai River	<i>Ide</i>	15	1	<i>Opisthorchis felineus</i>	6,6	2
	<i>Roach</i>	13				
	<i>Bream</i>	12	2	<i>Ligula imestinalis</i>	16,6	1
Denisov district						
Verkhnetobolskoe reservoir	<i>Ide</i>	12				
	<i>Bream</i>	10				
	<i>Roach</i>	15				
Zhitikara district						
Zhelkuar river	<i>Ide</i>	9				
	<i>Tench</i>	2				
Total:		247	9			

From the research data reflected in Table 1, it can be seen that most of the fish studied in the reservoirs in Zhandyrdy and Amangeldy, Denisov, Zhitikara districts were free of parasites: *ide*, *roach*, *tench*, *bream*, *crucian carp* (Zhaldama river, Zhelkuar river, Verkhnetobolskoe reservoir and lake Accol), *bream* (river Uly-Zhilanchik), *carp*, *ripus* (lake Accol). However, several fish species studied in the reservoirs of Zhandyrdy and Amangeldy districts were susceptible to parasitic diseases. Metacercariae were found in eight *ide* caught in Zhandyrdy district from the Uly-Zhilanchik rivers: *O. Felineus* and *M. Bilis*, while the prevalence was 18.5% and 8.3%, and the intensity of 1-3 and 2 specimens, respectively. In Amangeldy district in the Zhaldama River, the fish is completely free of any parasites, and no helminths were found in the roach (Torgai River). And in two species of fish: one individual *ide* and two individuals *bream*, caught from the Torgai River, metacercariae *O. Felineus* and plerocercoids *Ligula imestinalis* were found, respectively. At the same time, the prevalence was 6.6% and 16.6%, and the infestation intensity was 2 and 1 specimens, respectively. Parasitic diseases of fish were not diagnosed in the waters of Denisov and

Zhitigara districts. The analysis of the infestation of fish from different reservoirs indicates that the *ide* is the species most susceptible to invasion of *O. Felineus*, *Metorchis bilis*. In the fish of the species: *bream*, pathogens of ligulosis were found in single specimens. As a result of the conducted studies, a natural focus of opisthorchiasis infestations of the Uly-Zhilanchik and Torgai rivers was established, and a significant accumulation of the intermediate host of the mollusk of the genus *Bithynia* was also found in the coastal waters.

Among the six fish species examined, only the *ide* harbored opisthorchid metacercariae, as detected by the compressor method. Out of 45 *ide* infected with opisthorchids with an invasion intensity of 2-3 copies, 8 individuals were found, as shown in Figures 2 and 3. No metacercariae of opisthorchids were found in other fish species (*ripus*, *crucian carp*, *carp*, *roach*, *bream*, *tench*).

Analysis by the National Center of Biotechnology LLP, Astana (PCR protocol, April 24, 2023), revealed two opisthorchid species among five fish with metacercariae. Two *ides* harbored both *Opisthorchis felineus* and *Methorchis bilis* (Figure 1).

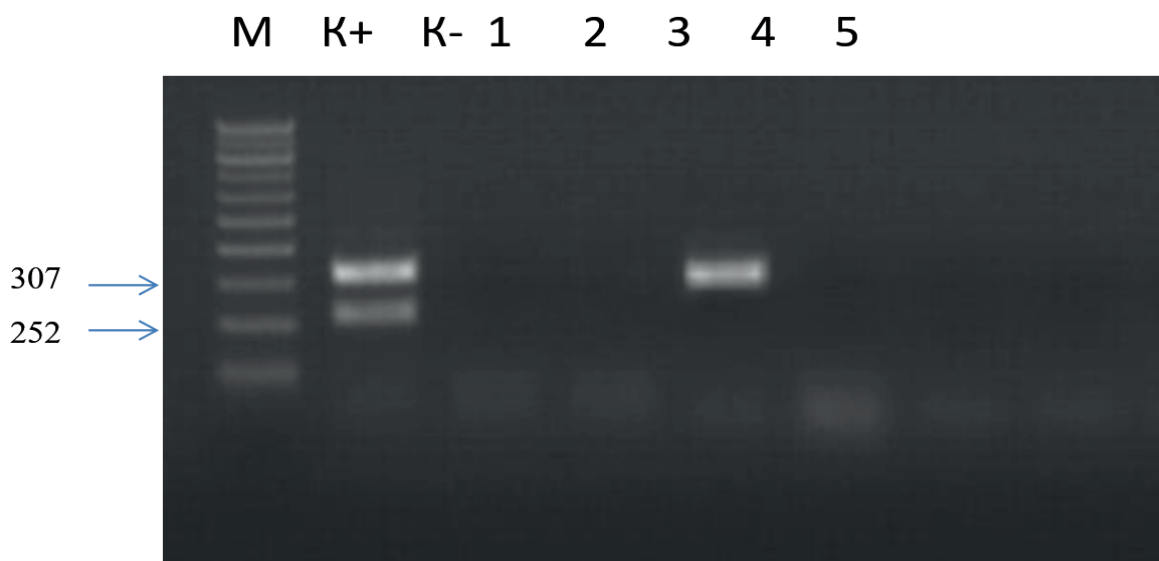


Figure 1 – Electrophoregram of PCR results with specific primers M – marker 1-5 – studied samples, K+ – positive control, K – – negative control

The pathogens *Opisthorchis felineus* and *Methorchis bilis* were confirmed in two of the five provided samples through PCR analysis, as shown in Figure 1.

Discussion

Ligulosis in bream is monoinvasia, the causative agent of which is the cestode *Ligula intestinalis*. Invasive fish are concentrated mainly in shallow water areas. The high rates of infection of fish with ligules are explained by the significant number of fish-eating birds of the *gull* family in reservoirs. Gulls and crustaceans are the main reservoir in which the population of the idler is preserved in nature. The life span of the helminth in the intestines of the bird is limited to 2 – 5 days. However, given the ability of seagulls to travel long distances and make flights from one reservoir to another, the spatial possibilities of the spread of invasion are significant [10].

It is quite difficult to diagnose metorchosis in fish as well as opisthorchiasis by classical

parasitological methods of analysis. Identifying the causative agent of metorchosis has been challenging due to the remarkable similarity between existing species of the pathogen [11]. Modern DNA-based diagnostics employing PCR with specific primers enabled the differentiation of two opisthorchid species. PCR successfully amplified fragments (amplicons) with distinct molecular weights: 307 bp for *Opisthorchis felineus* and 252 bp for *Methorchis bilis*. This confirms the presence of mixed metacercariae infections with both parasite species in the isolated specimens [12].

Opisthorchiasis, metorchiasis, and fish ligulosis persist as substantial public health concerns requiring continued focus in medical, biological, and veterinary research and practice.

Conclusion

According to the results of studies conducted in Zhangeldy and Amangeldy districts of Kostanay region, biohelminths of epizootological and epidemiological significance were found.

According to the results of monitoring of parasitic diseases in fish, it was found that the natural focus of opisthorchiasis and metorchiasis was formed within the boundaries of Zhangeldy and Amangeldy districts of the Kostanay region. The Uly-Zhilanchik and Torgai rivers are natural foci of opisthorchiasis with a high level of epidemiological risk. Favorable conditions

for shellfish habitat cause the incidence of fish (ide) with opisthorchiasis and metorchiasis. The prevalence by opisthorchiasis and metorchiasis was 18.5% and 8.3%, and the infestation intensity of 1-3 and 2 specimens, respectively. In Amangeldy district in the Torgay River, parasitization of *Ligula intestinalis* plerocercoids was detected in two ticks, the prevalence was 6.6%, and the infestation intensity was 1-2 specimens.

Parasitic diseases of fish were not diagnosed in the waters of Denisov and Zhitikara districts. The findings from the epizootological monitoring of

fish parasitic diseases highlight the crucial role of continued research on parasite system components. This research will provide an objective assessment of the region's epidemiological and epizootological state, facilitating forecasting and monitoring of the parasitological situation.

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