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VETERINARY-SANITARY EXPERTISE OF THE FISH FROM THE SHAGALALY RIVER

Fish completes the food chain of the water and is used an indicator of the water pollution. This article presents the results of biochemical, microbiological and parasitological studies of fish caught in the Shagalaly River. A total of 106 specimens of fish belonging to the Cyprinidae family were examined. As a result, it was established that there was no ammonia in the muscles of fish meat, the reaction to hydrogen sulfide was negative, the concentration of hydrogen ions in the studied samples were in the range of 6.6 – 6.7. The number of mesophilic aerobic and facultative anaerobic microorganisms was not observed at a cultivation cell of 1×10^5, which corresponds to the norm. Coliform bacteria was not found. When determining bacterial contamination for a reductase test, the samples were discolored after more than 3 hours, which indicates the absence of reductase. Metacercariae of the Opisthorchiidae family were found by the microscopy of muscles. The infection of ide by Opisthorchis felineus was 12.1%, Metorchis bilis – 6.09%, Metorchis xanthosomus – 10.9%, Pseudamphistomum truncatum – 2.4%. Pseudamphistomum truncatum was found in the muscles of the crucian carp, the infection rate was 29.1%. Molecular genetic analysis was carried out for the species differentiation of Opisthorchis felineus and Metorchis bilis metacercariae.

Key words: fish, veterinary-sanitary expertise, Shagalaly river, Opisthorchiidae, multiplex PCR.

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Шағалалы өзенінен ауланған балықтарды ветеринарлық-санитарлық бақылау

Балық су қоймасының қоректік тізбегін аяқтайды, сондықтан су объектісінің ластану индикаторына айналады. Мақалада Шағалалы өзенінен ауланған балықтардың биохимиялық, микробиологиялық және паразитологиялық зерттеу нәтижелері берілген. Тұқы тұқымдастығына жататын барлығы 106 балық зерттелген. Зерттеу барысында Шағалалы өзенінен ауланған балық етінде аммиак болмағаны анықталған. Шағалалы өзенінен ауланған балық етінде күкіртсутек реакциясы теріс нәтиже көрсетті. Зерттелген балықтардың сутектік көрсеткіші 6.6 – 6.7 шамасында болды. Микробиологиялық зерттеу нәтижесінде нормаға сәйкес, мезофилді аэробты және факультативті-анаэробты микроорганизмдер өсіндісі 1×10^5 ерітіндіде байқалмаған. Зерттеу объектісінде ішек микрофлорасы тобындағы (колиформ) бактериялар байқалмаған. Редуктаз сынамасына жүргізілген зерттеулерде, балық сынамалары 3 сағаттан артық уақытта түссізденген, бұл редуктазаның (бөгде микрофлора ферменті) жоқ екенін дәлелдейді. Шағалалы өзенінен ауланған балық етін микроскоппен зерттеу нәтижесінде Opisthorchiidae тұқымдасына жататын метацеркарилер табылған. Зерттелген язь балығының Opisthorchis felineus қоздырғышымен инвазиялануы 12,1%, Metorchis bilis – 6,09%, Metorchis xanthosomus – 10,9%, Pseudamphistomum truncatum – 2,4%. Шағалалы өзенінен ауланған Мөңке балығы Pseudamphistomum truncatum-мен инвазиясы 29,1% болды. Opisthorchis felineus пен Metorchis bilis метацеркарилерінің түрлік дифференциациясы үшін молекулалық-генетикалық талдау жасалды.

Түйін сөздер: балық, ветеринарлық-санитарлық бақылау, Шағалалы өзені, Opisthorchiidae, мультиплексті ПТР.

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Ветеринарно-санитарная экспертиза рыбы реки Шагалалы

Рыба завершает трофическую цепь водоема и является индикатором загрязнения водного объекта. В данной статье приведены результаты биохимического, микробиологического и паразитологического исследования рыбы, выловленной в реке Шагалалы. Всего было исследовано 106 экземпляров рыб, относящихся к семейству карповых. В результате установлено, что в мыщцах мяса рыбы отсутствует аммиак, реакция на сероводород была отрицательной, концентрация водородных ионов исследуемых образцов была в пределах 6.6 – 6.7. При микробиологическом исследовании на рост количества мезофильных аэробных и факультативно-анаэробных микроорганизмов рост микробных клеток не наблюдался в разведении 1×10^5, что соответствует норме. Бактерии группы кишечной палочки не обнаружены. При определении бактериальной обсеменённости на редуктазную пробу образцы обесцвечивались более чем через 3 часа, что говорит об отсутствии редуктазы. При микроскопировании мыщц найдены метацеркарии семейства Opisthorchiidae. Зараженность язя возбудителями Opisthorchis felineus составила – 12,1%, Metorchis bilis – 6,09%, Metorchis xanthosomus – 10,9%, Pseudamphistomum truncatum – 2,4%. В мышцах карася был обнаружен Pseudamphistomum truncatum, зараженость составила 29,1%. Для видовой дифференциации метацеркарий Opisthorchis felineus и Metorchis bilis был проведен молекулярно-генетический анализ.

Ключевые слова: рыба, ветеринарно-санитарная экспертиза, река Шагалалы, Opisthorchiidae, мультиплексный ПЦР.

Introduction

Worldwide consumption of fish has increased rapidly, especially with the recognition of its nutritional and therapeutic benefits. In addition to being an important source of protein, fish is enriched with essential minerals, vitamins and fatty acids. Consumption of fish meat contributes to the prevention of heart and vascular diseases [1]. The American Heart Association recommends consuming fish at least twice a week to achieve your daily intake of omega-3 [2].

World Health Organization recommends consuming at least 16 kg of fish products per person per year. People in our country consume less than 3 million tons of fish per year and prefer river fish, which often becomes a source of human food poisoning [3]. Therefore, the places where fish is caught and traded should be examinated by veterinary-sanitary expertise and in some cases, laboratory analysis [4].

When carrying out a veterinary and sanitary examination of fish, organoleptic indicators are assessed (appearance, condition of mucus, eyes, scales, fins, color of gills, consistency of muscle tissue, shape of the abdomen, etc.), and, if necessary, laboratory tests are carried out, which include determination of the concentration of hydrogen ions (pH), determination of ammonia and hydrogen sulfide, reaction to peroxidase and reductase test.

The ecological state of the aquatic environment has a significant impact on the quality of the fish [5]. The main contaminating substances of water are copper, iron, ammonium and nitrate nitrogen, sulfate ions, oil products, phenols, hexavalent chromium compounds, manganese, zinc, cadmium, fluorides, boron. Wastewater discharge by enterprises and utilities, surface runoff of agricultural lands are the main causes of water pollution [6].

Mathew J. et al. approved that fish is the main indicator of the aquatic ecosystems around the world [7].

Dirk Willem Kleingeld et al. proposed to study three groups of fish as an indicator of water purity: salmon, carp and other fish species [8].

Manjit S.A. et al. studied aquatic ecosystems and the influence of calcium hypochlorite (washing powder) on fish`s histopathological changes in gills and liver. Studies had shown that an increase concentration of washing powder leads to the damage of the liver tissue in different concentrations (2.5, 10, 30 and 50 ppm) within 96 hours [9].

Riad H. Khalil et al. concluded that the increased levels of ammonia, nitrites and organic matters contribute to the development of branchial mycosis (the causative agent is *Branchiomyces demigrans*) in Nile tilapia and common carp [10].

Piazza C.E. et al. investigated the effect of domestic wastewater on fish of the species *Poecilia*

vivipara. During the chemical analysis of fish in laboratory and in situ experiments, the authors confirmed importance of the usage of this fish species as an indicator for environmental monitoring in coastal regions [11].

The level of water pollution is estimated by the value of the complex index of water pollution and is classified into 7 classes: class 1 – very clean, class 2 – clean, class 3 – moderately polluted, class 4 – polluted, class 5 – dirty, class 6 – very dirty, 7 class is extremely dirty. An extremely high level of water pollution is recorded at three waters, these are Maybalyk Lake and the Shagalaly River, Kylshakty River [12].

The Shagalaly River is located on the territory of the Akmola and North Kazakhstan regions and belongs to the 7th class water pollution, where the concentration of copper and phenol is over 0.1 mg / l, of oil products is over 5 mg / l, of easily oxidized organic substances in terms of BOD (O2) is over 60 mg / l, of dissolved oxygen is over 2 mg / l [13]. According to the unified water quality classification system for 2021 (I quarter), the Shagalaly river is assigned to the 5th class, due to the excess of the chemical oxygen consumption standards [14].

The ichtyofauna of the Shagalaly River is rich and diverse. The river is stocked with whitefish larvae raised in the Zerenda fish hatchery and commercial fish species such as ide, pike, carp, and perch [15].

Despite the activity of catching and using commercial fish from the Shagalaly River, there is a minimal number of studies about veterinary examination of the fish products from the river. The data is received from the study of fish from the southwestern tributary of the Shagalaly River (Lake Kopa) for parasites, and as a result monogens, trematodes, cestodes, nematodes and crustaceans were found. According to the authors, the extent of infection with *Diplostomum spathaceum* on Lake Kopa was 25%, *Tetraonchus monenteron* – 34.3%, *Triaenophorus nodulosus* – 16.41%, *Tylodelphys clavata* – 31.34%, *Rhapidascaris acus* – 10.44%, *Ergasilus sieboldi* – 2.98% [16]. The presence of parasites in fish is the main obstacle to the sale of the fish product. For example, among disqualified fish in Poland, the highest number was the fish with parasitic invasion (45.97%) [17].

The aim of this study is the veterinary and sanitary examination of the fish from the Shagalaly River to the biochemical, microbiological and parasitological indicators.

The most dangerous parasite transmitted to humans through undercooked dangerous fish are trematodes of the *Opisthorchiidae* family [18]. The larvae of this parasite, metacercariae are located in the upper layer of muscle tissue (from 2 to 4 mm) and in the subcutaneous tissue in the back, are less common in the fins, gills, and intestinal walls [19, 20]. The fish becomes invasive after 6 weeks infection and capable to infecting the definitive host. The duration of the period of fish invasion depends on the fish species and water temperature [21]. Metacercariae are susceptible to drying, high concentration, high temperatures and can only die at high temperatures. Among all the stages of metamorphosis, only metacercariae are able to take root and multiply in mammals [22].

Materials and methods

The object of the study for the experimental work was fish of the Cyprinidae family (ide and crucian carp) from Shagalaly River.

Biochemical analysis of fish: the determination of the concentration of hydrogen ions, hydrogen sulfide and ammonia was carried out according to the generally accepted method [23].

Microbiological analysis of fish was carried out according to GOST 10444.15-94 to determine the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM). The identification of coliform bacteria was determined according to GOST 31747-2012. In addition, bacterial contamination was checked using the reaction to the reductase test modified by M. Kondratova [24].

Parasitological studies of fish muscles for the presence of the causative agent of opisthorchiasis were carried out according to the ST RK 2779-2015 standard. Metacercariae were identified using specialized literature [25].

Molecular studies were carried out by multiplex PCR test using primers for *Opisthorhis felineus – CO1nOf-F* and *CO1nOf-R*, for *Metorchis bilis – COInMb-F* and *COInMb-R*. Amplification was performed in a final reaction volume of 25 µl containing 1 × buffer, 2.5 mM MgCl2, 1U Phusion DNA polymerase and 2 mM dNTP, 100 pmol primer and extracted DNA. PCR was performed under the following conditions: denaturation at 95°C for 5 minutes, then 35 cycles of 15-second denaturation at 95°C, then primer annealing at 62°C for 25 seconds and extension at 72°C for 30 seconds, and final elongation for 5 min at 72°C [26].

Results and Discussion

Biochemical analysis of the fish caught from the Shagalaly River was carried out according to the following indicators – determination of the concentration of hydrogen ions (pH), hydrogen sulfide and ammonia (Table 1). A total of 106 fish were examined, and the 82 specimens were ides and the 24 specimens were crucian carps.

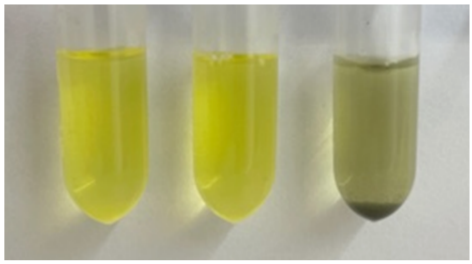
The accumulation of intermediate and final decay products in fish muscles contributes to the change of hydrogen ions to the alkaline side. The concentration of hydrogen ions in the studied samples was in the range of 6.6 – 6.7, which is in conformity with good-quality fish.

When proteins are decomposed under anaerobic conditions, hydrogen sulfide accumulates. Determination of hydrogen sulfide was carried out by heating minced meat. The ides caught from Shagalaly River had a negative reaction to hydrogen sulfide. In the studied crucians, the reaction to hydrogen sulfide was negative, and only 2 fish samples showed traces.

Table 1 – Biochemical analysis of fish

Type of fish	pH			Hydrogen sulfide			Ammonia		
	Rate	Number of fish, sch	Result	Rate	Number of fish, sch	Result	Rate	Number of fish, sch	Result
Ide	± 6,6	82	fresh	«-»	82	negative	greenish yellow, transparent	82	absent
Crucian carp	±6,7	24	fresh	«-», «±»	24	negative, traces	greenish yellow, transparent	24	absent

The appearance of ammonia in fish meat indicates the breakdown of amino acids under the influence of microflora. Determination of ammonia was carried out using Nessler's reagent. In the presence of ammonia, ammonium salts form mercurammonium iodide with Nessler's reagent, which turns the colour of the reaction to yellow-brown. The studied samples were transparent, light yellow in color, which indicates the absence of ammonia (Figure 1).



a b c

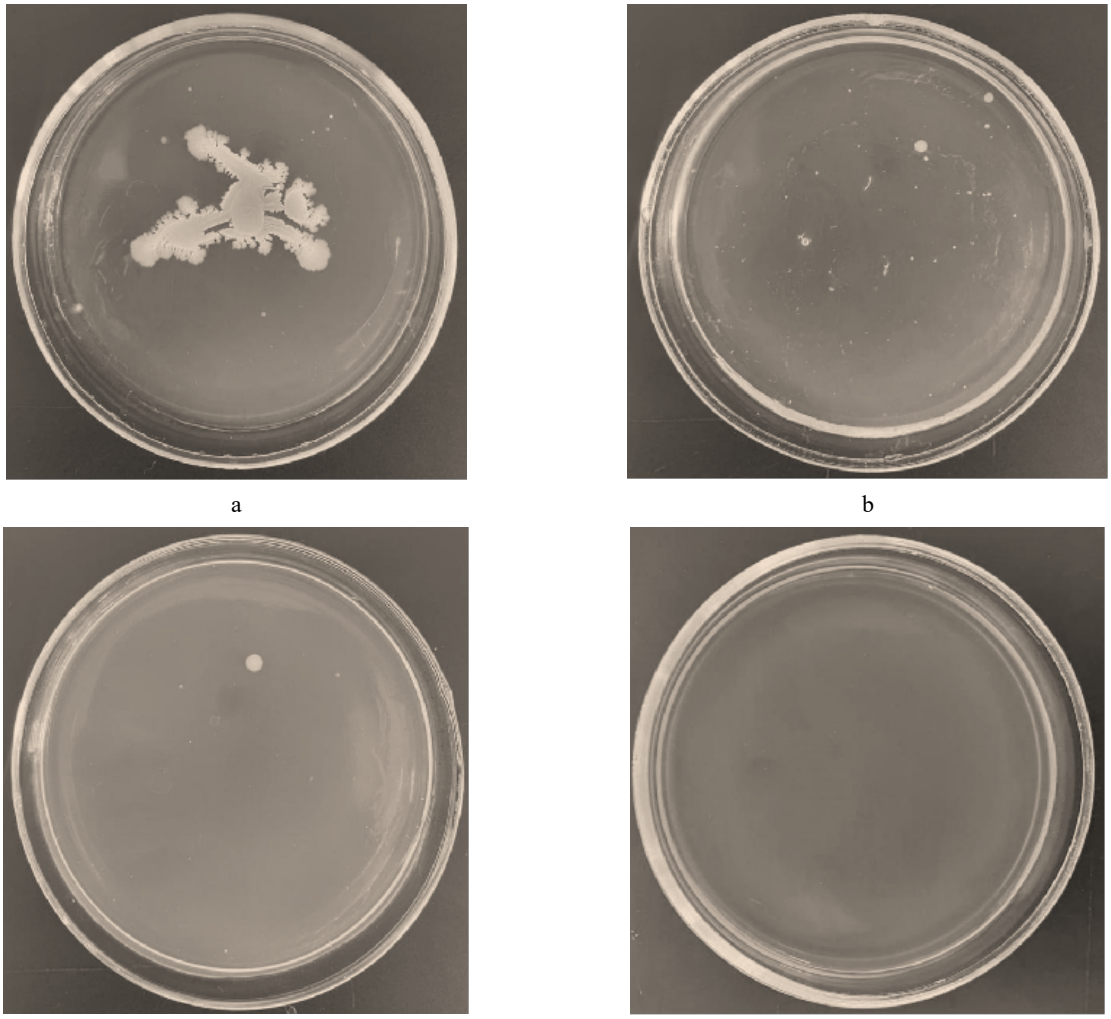
a – ide; b – Crucian carp; c – negative control

Figure 1 – Determination of ammonia with Nessler's reagent

Microbiological analysis of fish was carried out to determine the number of mesophilic aerobic and facultative anaerobic microorganisms, and to determine the coliform bacteria and reductase test modified by M. Kondratova.

To determine MAFAM, we used a nutrient medium – meat-peptone agar. As a result the large growth of microbial cells was observed at a cultivation cell of 1×10^2, in further cultivation cell (1×10^3, 1×10^4) samples, the growth of microbial cells was decreased. At a cultivation cell of 1 × 105, the growth of microbial cells was not observed, according to the required standards. An example of determining MAFAM in a fish sample (ide) is shown in the figure (Figure 2).

Coliform bacteria tend to ferment lactose. As a result of fermented lactose acids appear in fish product, which change the color of the indicator. Determination of coliform bacteria in all prepared samples (ide and crucian carp) was carried out on a nutrient medium SDS-broth. Fish is considered safe, if the color of the nutrient medium remains unchanged when diluted with 10^{-3} (0.001 g) for 48 hours (Figure 3).

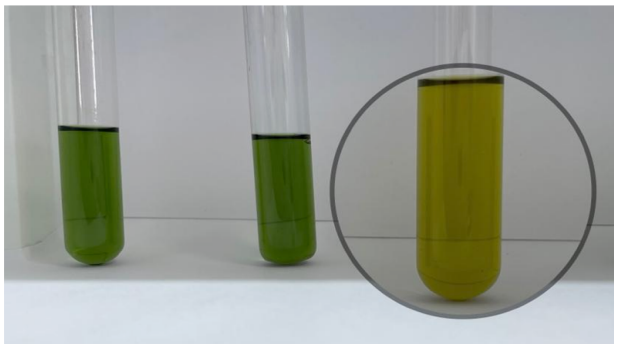


a b

c d

a – 1×10^2, b – 1×10^3, c – 1×10^4, d – 1×10^5

Figure 2 – Results of MAFAM study



a b c

a – ide; b – Crucian carp; c – negative control

Figure 3 – Examination of fish
for the presence of enterobacteria

During the examination of the fish from Shagalaly River, discoloration and turbidity was not observed. It indicates the absence of lactose-negative and lactose-positive enterobacteriaceae.

The bacterial contamination was additionally studied by reductase test modified by M. Kondratova. This method is based on the identification of reductase (a waste product of extraneous microflora) in fish meat using an indicator. Under the influence of reductase, the indicator (methylene blue) becomes discolored. Depending on the amount of microflora, the fish sample becomes discolored over a period of time, from 20 minutes to 2.5 hours. When determining the bacterial contamination of fish meat, the samples became discolored after more than 3 hours, which indicates the absence of reductase.

The compression method was applied for parasitological research to determine presence of various types of metacercariae. Various types of metacercariae were identified by microscoping of fish muscles. The identified metacercariae belong to the *Opisthorchiidae* family (*Opisthorchis felineus* or *Metorchis bilis*), *Metorchis xanthosomus,* and *Pseudamphistomum truncatum*.

It is impossible to identify *O. felineus* from *M. bilis* by morphological characteristics, for the reason that the maximum size of *O. felineus* coincide with the minimum size of the *M. bilis* (Figure 4).

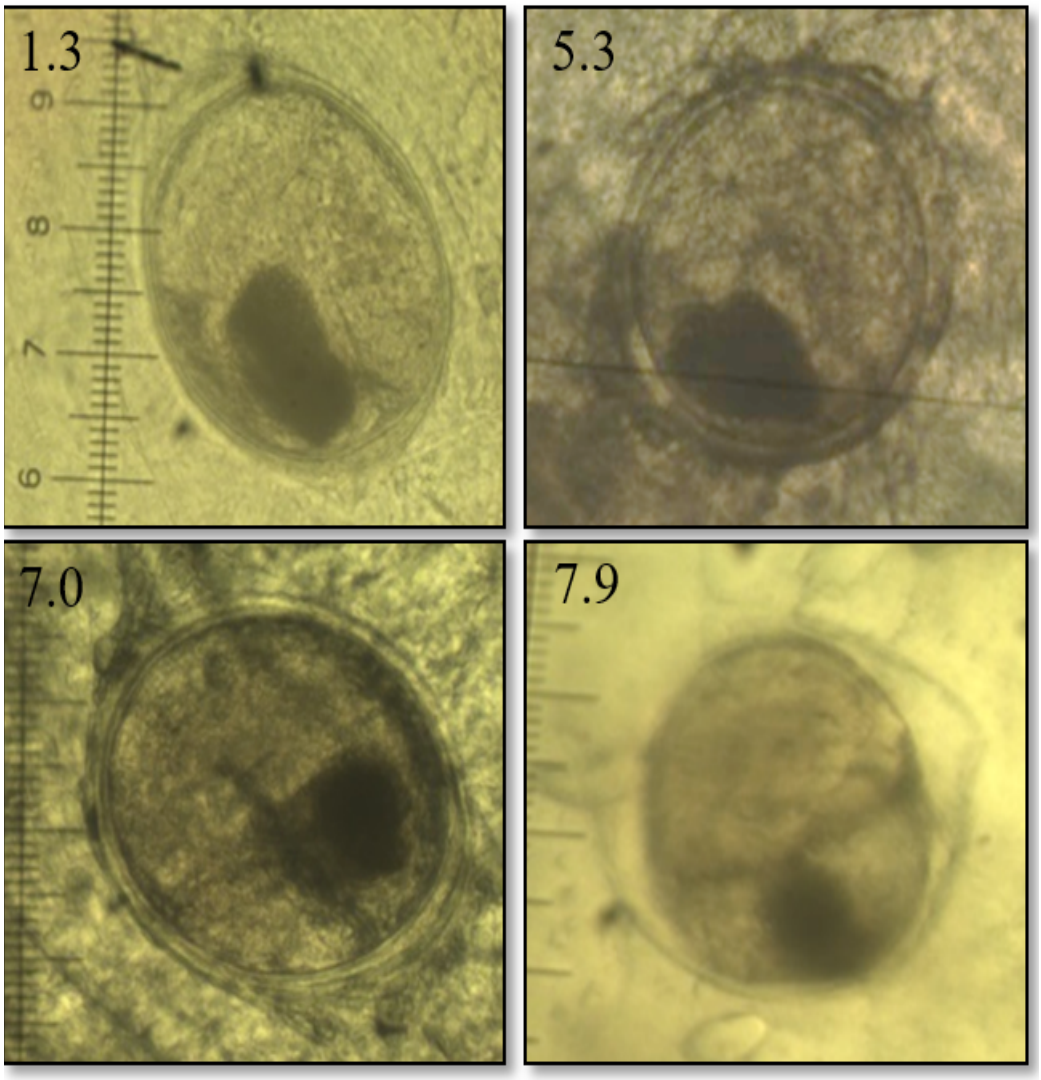
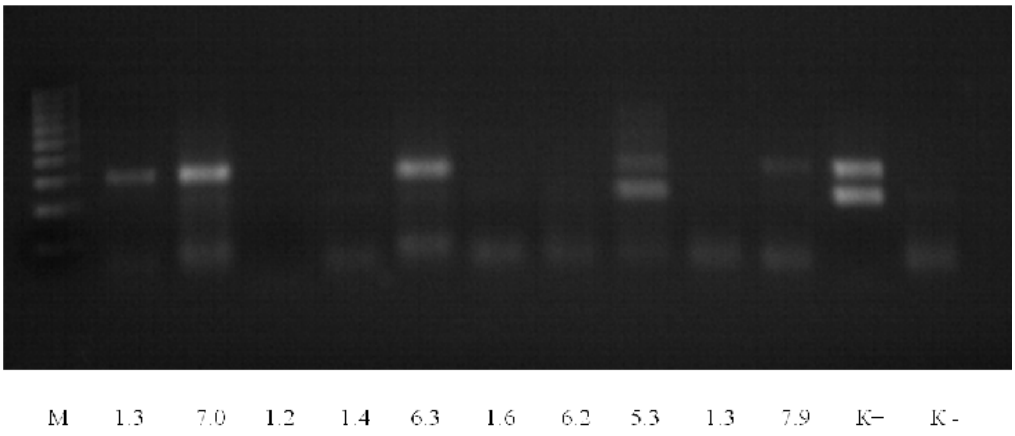


Figure 4 – Metacercariae of *Opisthorchiidae* family
(1.3, 5.3, 7.0, 7.9 – number of extracted samples)

O. felineus has a round, slightly oval shape, size varies from 0.18 × 0.21 to 0.33 × 0.42, the excretory vesicle is filled with black granules, and the shell is thin, two-layered, and transparent. *M. bilis* is ellipsoidal, the size varies from 0.13 × 0.18 to 0.15 × 0.21, excretory vesicle is filled with black granules, and thin membranes are transparent, two-layered, and thinner than *O. felineus*.

To the differentiation of the two species of, molecular analysis was carried out based on multiplex PCR using mitochondrial cluster primers. Amplification of the test samples with primers *CO1nOf-F/R* and *COInMb-F/R* resulted in PCR products of 307 and 252 bp, containing two bands (Figure 5). Samples 1.3, 6.3, 7.0, 7.9 belong to *O. felineus*, and sample 5.3 belongs to *M. bilis*.



M- DNA ladder (bp), 1.3, 6.3 , 7.0, 7.9, 5.3, 1.2, 1.4, 1.6, 6.2, 1.3 –
extracted DNA samples isolated from various types of metacercariae

Figure 5 – Electrophoretogram of multiplex PCR for differentiation of *O. felineus* and *M. bilis*

The infection of ide by causative agent *O. felineus* was 12.1%, *M. bilis* – 6.09%, *M. xanthosomus* – 10.9%, *Pseudamphistomum truncatum* – 2.4%. *P. truncatum* was found in the muscles of the crucian carp, the infection rate was 29.1%.

Conclusion

Fish of the carp family (ide and crucian carp) was used as an indicator of water pollution in the Shagalaly River in our studies. Ecological condition of the river did not affect the biochemical parameters of the fish. The concentration of hydrogen ions in the studied samples was in the range of 6.6 – 6.7, which corresponds to the norms of good-quality fish. The reaction to hydrogen sulfide and ammonia was negative.

Growth number of mesophilic aerobic and facultative anaerobic microorganisms is compliant in the studied samples. Coliform bacteria and the reductase were absent.

However, metacercariae *O. felineus, M. bilis, M. xanthosomus*, and *P. truncatum* were identified by the parasitological research of the ide. The total infection of the ide by the larvae of the *Opisthorchiidae* family was 31.7%. *P. truncatum* was found in the muscles of the crucian carp, the infection rate was 29.1%. Ecological condition of the Shagalaly River affects the decrease of the resistance of the fish, which causes the infestation of the fish by parasites.

Conflict of interest

The authors have declared no conflict of interest.

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