

## Conference Paper

# Method for Diagnostics of Carp Fish Diplostomosis During Veterinary and Sanitary Examination

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**Abstract**

Diplostomosis is an invasive disease of carp fish that can cause significant damage to the fish farm. It is widely distributed in Russia, Kazakhstan, Ukraine and Belarus. Sexually mature individuals parasitize the intestines of fish-eating birds of the genus Laridae. The intermediate host is freshwater fish and mollusks of the genus Lymnaea. The disease cycle is represented by several migrations of the parasite from the reservoir to the intermediate hosts, followed by ingestion. Fry and fish with a low immune status are most susceptible to this disease. When introduced into the body, trematodes can inoculate fish with various pathogenic and conditionally pathogenic microflora inherent in the habitat, which can complicate the production of high-quality products that are safe for both humans and animals. Therefore, the implementation of our patented “Method for assessing the quality of meat of fish infected with diplostomosis” during the veterinary and sanitary examination is important. The invention involves diagnostic measures, namely, to determine the quality of fish meat, which were carried out in this study. The method consisted of a step-by-step study of the pathogen obtained by destroying the integrity of the vitreous body and sowing exudate by collecting the material with a Pasteur pipette with transfer to the MPA. These were kept in a certain temperature regime for at least 48 hours. The researchers examined the plates with the inoculations and described the morphology of the resulting colonies, with preliminary staining of the smears according to Romanovsky-Giemsa. The colonies were counted using the Goryaev camera. The method used helped to detect the parasitic disease together with the bacteriological purity of the fish, which makes it possible to issue a correct veterinary and sanitary assessment for a given invasion. The data were processed statistically; a correlation relationship between the intensity of invasion and the level of the bacteriological flora of fish products was established. The most significant was the relationship of contamination with bacteria of the E. coli group and of the genus Pseudomonas and Staphylococcus. The obtained value of the KMAFAMnM index exceeded the indicator of healthy fish.

**Keywords:** diplostomosis, post-diplostomosis, methods, identification, parasitology, microbiology.

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## 1. Introduction

Fish farming remains a developing industry in the World market today. In 2019, more than 100 thousand tons of fish and other aquatic organisms were harvested, intended for free sale [1].

Along with the development of this industry, the issue of entering industrial circulation of high-quality products that are safe for both humans and animals remains important. Today, there are often cases of detection of pathogenic agents in fish, such as viruses, bacteria, fungi, helminths, protozoa, etc. [2].

To ensure food safety of fish products, a document that controls the main indicators of the quality of products in the territory of the Russian Federation was introduced - the Technical Regulations of the Customs Union. It presents the standards for each individual species of aquatic organisms [3].

According to the parasitological indicators of fish products, mandatory studies are provided for the presence of nematodes, trematodes, cestodes and worms, as they cause general helminthiasis in humans and animals [4, 5].

It should be noted that diseases that can affect the quality of products, while not being a source of invasion of the human body, remain poorly studied today. These include, among other things, diplostomosis [6].

## 2. Methods and Equipment

In our own research, we planned to: 1) study the methods of helminthological dissection of fish using an innovative method invented by us: "A method for assessing the quality of meat of fish infected with diplostomosis" to determine the causative agents of diplostomoses in freshwater fish; 2) to carry out identification of flukes for each disease; 3) to determine the microbial contamination of fish [7].

### 2.1. Methods

To achieve the objectives set, the developed "Method for assessing the quality of meat of fish infected with diplostomosis". The invention relates to a veterinary and sanitary examination, namely, to determining the quality of fish meat during diplostomosis.

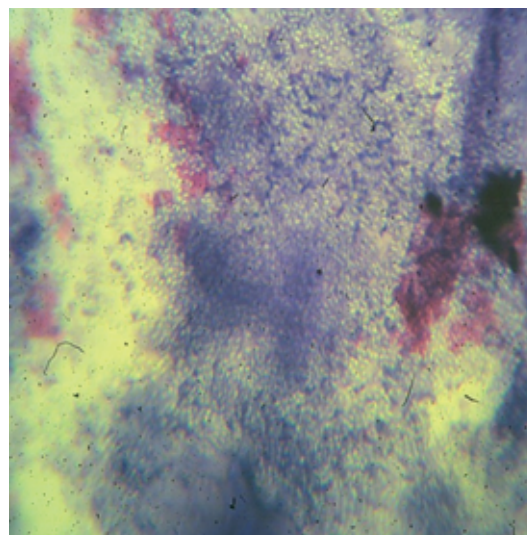
On the territory of the Russian Federation, a method for diagnosing an invasive disease of fish, such as diplostomosis, based on the removal of the lens, is applicable. It

is conducted by crushing the lens with a microscope slide, followed by obtaining larvae [8, 9].

The technical result is achieved by the fact that the method for assessing the quality of meat of fish infected with diplostomosis is carried out in the following sequence: processing of a biological object, followed by destruction of the integrity of the outer membrane of the eye, taking a sample from the affected tissue area, sowing on mesopotamia agar, with an exposure of 48 hours, analyzing the morphology of obtained colonies, determining the nature of growth, staining of smears according to Romanovsky-Giemsa and their counting [10, 11].



**Figure 1:** Examination of the lens of a fish eye



**Figure 2:** Obtained colonies of microorganisms

According to the invention, the lens of the eye of a fish is used as a biological object, which is crushed between the glasses, a sample is taken for inoculation, and the counting is carried out using the Goryaev camera, with a result equal to less than  $5 \times 10^4$  CFU / kg for conditionally pathogenic microflora; single indicators for pathogenic, fish meat is classified as benign, and with indicators over  $5 \times 10^4$  CFU / kg by conditionally pathogenic micro-flora or the presence of exceeding the norm objects in meat for: *L. monocytogenes*, *V. Parahemolyticus*, *Salmonella* spp. and others, which belong to the category of substandard [12].

An example of a specific implementation of the method was proposed: the fish entering the laboratory of veterinary and sanitary examination and suspicious of a diplostomosis disease is subject to microbiological examination. Before sowing, hands are treated with 70% ethyl alcohol (Figure 1). The lens is removed from the vitreous body by an incision, placed on a sterile glass slide, covered with another slide and crushed; the contents are collected with a sterile Pasteur pipette, then transferred to Petri dishes with MPA, prepared by adding 2-3% agar to the meat broth - agar, followed by autoclaving and adding chicken egg white with double the amount of distilled water [13].

Crops are kept at a temperature of 18-25 ° C for at least 48 hours. The inoculation dishes are examined, the morphology of the resulting colonies is described, smears are stained according to Romanovsky-Giemsa and microscopic, the result is counted using the Goryaev camera. When the result is less than  $5 \times 10^4$  CFU / kg, according to conditionally pathogenic microflora, and zero indicators for pathogenic, fish meat is classified as benign and released for free sale (Figure 2). With indicators over  $5 \times 10^4$  CFU / kg for pathogenic microflora or presence in meat: *Coliform*, *S. Aureus*, *L. monocytogenes*, *V. Parahemolyticus*, are classified as substandard and are disposed of [14, 15].

The effectiveness of the method is confirmed by the studies, for which fish, infected with diplostomosis, was processed by the method according to the prototype and the claimed method.

### 3. Results

We examined 300 specimens of carp fish with characteristic signs of diplostomosis. The data obtained was processed biometrically. The result is presented in tables.

When studying fish, the pathogen *Diplostomumspathaceum* was identified in 100% of cases.

TABLE 1: Helminthological research of fish

Type of fish	Individuals examined	Infected with diplostomes, individuals	Intensity of invasion	Extensiveness of invasion
Hypophthalmichthys molitrix (silver carp)	58	12	6.54	24.4
Cyprinus carpio (Carp)	129	39	2.05	23.9
Abramis brama (Carp bream)	75	13	2.61	14.8
Ctenopharyngodon idella (White amur)	38	7	3.0	15.6

### 4. Discussion

The highest rates of invasion extensiveness were observed in silver carp and carp - 24.4 and 23.9, respectively, while bream and grass carp were characterized by lower invasion rates.

The degree of invasion of commercial fish ranged from 1 to 7 larvae per individual.

TABLE 2: Bacterial contamination of commercial fish

Isolated microorganisms	Bacterial contamination of commercial fish, CFU / g			
	Silver carp	Carp bream	Carp	White amur
Healthy fish				
Coliform bacterias	23.4 ± 0.005	10.1 ± 0.01	15.7 ± 0.006	8 ± 0.002
QMAFanM, CFU / g × 10 <sup>4</sup>	3.14 ± 0.037	1.55 ± 0.42	1.25 ± 0.29	2.6 ± 0.39
Genus Bacillus	–	36.5 ± 0.077	–	39.2 ± 7.0
Genus Pseudomonas	47.1 ± 0.04	76.0 ± 0.041	37.09 ± 0.028	67.0 ± 0.29
Genus Staphylococcus	69.0 ± 0.07	82.0 ± 1.3	56.0 ± 0.04	78.0 ± 0.016
Fish infested with Diplostomum spathaceum larvae				
Coliform bacterias	72.86 ± 0.87***	21.9 ± 0.056***	36.4 ± 0.023***	22.3 ± 0.07***
QMAFanM, CFU / g × 10 <sup>4</sup>	24.0 ± 0.9***	2.9 ± 1.3	6.18 ± 1.19***	8.18 ± 4.27***
Genus Bacillus	148.7 ± 8.6	116.0 ± 0.67***	129.0 ± 0.31	312.5 ± 2.17***
Genus Pseudomonas	216.4 ± 8.4***	83 ± 0.4	99.0 ± 0.17***	230.1 ± 1.2***
Genus Staphylococcus	256.6 ± 8.5***	116.4 ± 0.74***	123.2 ± 0.29***	162.8 ± 0.76***
Genus Listeria	180.1 ± 7.1	–	161 ± 0.42	–
Genus Klebsiella	33.1 ± 0.06	–	–	–

Note: \*\*\* – p ≤ 0.001

According to the data presented in Table 2, it was determined that the bacterial contamination of fish infested with Diplostomum spathaceum metacercariae significantly exceeds that of healthy fish. Thus, the contamination with bacteria of the E.

coli group of fish infested with diplostomosis was significantly higher by 1.5–3.4 times (silver carp); bacteria of the genus *Pseudomonas* - 2.8–3.9 times; bacteria of the genus *Staphylococcus* - 1.4–3.3 times. The KMAFAMnM index in the meat of infested fish was significantly higher by 1.5–6.5 times. At the same time, bacteria of the genera *Bacillus*, *Listeria*, *Klebsiella* were found in the meat of silver carp and carp, which were not found in healthy fish.

## 5. Conclusion

The method used solves several problems of identifying a parasitic disease together with the bacteriological purity of fish, which makes it possible to issue a correct veterinary and sanitary assessment for a given invasion.

We also found that the diplostomosis disease is the cause of the development of pathogenic microorganisms in fish. This reduces the quality of the products obtained and is the reason for the occurrence of food poisoning in humans and animals.

A direct correlation was established between the intensity of the invasion of *Diplostomum* spp., and QMAFAMnM in fish - with an increase in the intensity of invasion, fish seed production increases.

When assessing the quality of fresh fish infested with metacercariae by diplostomoses, it is necessary to carry out additional bacteriological analysis of fish meat according to the proposed “Method for assessing the quality of fish meat infected with diplostomosis”.

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## Conflict of Interest

The authors have no conflicts of interest.

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