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Bothriocephalosis in the South of Russia: actual methods of diagnosis and treatment

Victoria Horosheltseva[1,2,*], *Tatiana* Strizhakova[1], *Anna* Poluyan[1], *Timofey* Barabashin[1], and *Efim* Kozhurin[1]

[1]Russian Federal Research Institute of Fisheries and Oceanography (FSBSI «VNIRO»), Azov-Black Sea branch of «VNIRO» («AzNIIRKH»), Rostov-on-Don, Beregovaya st. 21 «B», 344002, Russia
[2]South Federal University, Rostov-on-Don, Stachki Avenue 194/1, 344006, Russia

Abstract. The information has been collected and analyzed on the causative agent of the dangerous fish disease bothriocephalosis, which currently has more than 20 synonyms in the literature. The disease is currently reported worldwide, with the exception of Antarctica. When infecting fish, the pathogen is localized in the intestines and, first of all, has a negative mechanical effect, moreover, it affects the growth of fish. The size of the parasite adapts to the size of the host organism. Anthelmintics are used as methods of combating the disease, as well as biological control methods based on interrupting the life cycle of the parasite. The southern region of the Russian Federation is the most favorable territory for the development of bothriocephalosis. Despite this, the region as a whole has a positive status for this disease. Over the past 3 years, according to the official data of regional veterinary services, only 6 cases of the establishment of restrictive measures (quarantine) for bothriocephalosis in relation to fish farms have been noted.

1 Introduction

The frequency of occurrence of parasitic diseases in aquaculture and their impact on commercial fish farming is becoming more pronounced every year. In connection with the intensification of pond fish farming, the study of such diseases (in particular, biology and ecology of pathogens, factors of their development and distribution, etc.) in the southern regions of Russia is becoming a necessary element of sustainable development of the entire fishery industry.

Bothriocephalosis is one of the most dangerous and widespread fish diseases caused by the tapeworm *Schyzocotyle (Bothriocephalus) acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea). The causative agent of bothriocephalosis began its worldwide spread from Japan, together with the fish being acclimatized, and is currently registered worldwide, with the exception of Antarctica. Various regions of China, Mongolia, Russia (eastern Siberia and the Far East) are considered to be the native habitat of Bothriocephalosis [1]. On the territory of the Russian Federation, natural foci of

* Corresponding author: horosheltseva_v_n@azniirkh.ru

bothriocephalosis have formed in natural reservoirs of the Krasnodar, Stavropol Territories, Rostov, Moscow, Tyumen, Voronezh, Lipetsk, and Novgorod Regions [2].

The disease caused by this pathogen is usually chronic. Among the symptoms, external ones are distinguished: changes in the behavior of fish (lethargy of movements, swimming near the surface of the water, refusal to feed), swelling of the abdomen, sometimes drooping strobili of the parasite from the anus; the internal symptoms are the following: damaged mucous membrane and stretching of the intestinal walls, focal hemorrhages and inflammation, accumulation of exudate containing desquamated epithelial cells [3, 4].

Fish of older age groups are carriers of helminths, their invasion is asymptomatic. In fry and underyearlings, the disease is acute. They gather in flocks on the fresh water inflow, near the coast and quickly perish.

Infection occurs when fish feed on copepods (intermediate host of the parasite). With the transition of fish to feeding on benthos, the infection sharply decreases.

The southern region of the Russian Federation is the leader in the production of commercial aquaculture [5]. Such a position has been achieved, among other things, through the intensification of pond fish farming. Favorable climatic conditions, the presence of final and intermediate hosts of the parasite are the factors that bring about the development of the causative agent of bothriocephalosis. For this reason, fish farms must comply with all veterinary and sanitary requirements. Failure to comply with the norms of the technological process threatens the invasion of farmed fish by a tapeworm, which is especially dangerous for juveniles.

In accordance with the Order of the Ministry of Agriculture of the Russian Federation, restrictive measures are applied to the fish farm when the causative agent of bothriocephalosis is detected. *S. acheilognathi* is also listed as a Regional Pathogen (PRI) by the US Fish and Wildlife Service [6].

2 Materials and methods

The basis for this study was the analysis of literary sources, as well as the information from the state veterinary service about cases of detection of bothriocephalosis (Republic of Adygea, Astrakhan Region, Volgograd Region, Republic of Kalmykia, Krasnodar Territory, Rostov Region, Republic of Crimea, city of Sevastopol). To search for literary sources on the biology and ecology of the causative agent of bothriocephalosis, there have been used specialized scientific search systems, electronic archives, means of searching for articles and links (Scopus, Web of Science, NCBI, Scirus, Google Scholar, Science Research Portal, ResearchIndex, Elsevier, Springer and etc.).

3 Taxonomic notes

S. acheilognathi has over twenty known synonyms. It was originally described as three species. In 1934, the Japanese helminthologist Satyu Yamaguti first described the parasite found in a small carp fish *Acheilognathus rhombeus* Temmink, Schlegel, 1846 from Lake Ogura (Kyoto Prefecture, Honshu) in Japan [7]. He named it as two different species - *B. acheilognathi* and *B. opsariichthydis.* In 1955, Chinese helminthologist Liang-Sheng Ye described several more specimens obtained from grass carp (*Ctenopharyngodon idella*) in southern China as *B. gowkongensis*. The taxonomic names of these species were recognized as synonyms by later authors, and Yamaguti's original name of the species was retained in accordance with priority rules.

To date, the following synonyms for the causative agent of bothriocephalosis can be found in the literature: *B. acheilognathi* Yamaguti, 1934; *S. fluviatilis* Achmerov, 1960; *B.*

opsariichthydis Yamaguti, 1934; *B.gowkongensis* Yeh, 1955; B. *phoxini* Molnar, 1968 and others [8]. According to the information provided in the National Center for Biotechnological Information (NCBI), the scientific name *Schyzocotyle acheilognathi* is preferred, and *Bothriocephalus acheilognathi* is the homotypic synonym of the parasite. Generally accepted international common names are Asian fish tapeworm or Asian tapeworm.

4 Life cycle of the causative agent of bothriocephalosis

The emergence and spread of the disease is facilitated by non-compliance with mandatory veterinary and sanitary requirements and violations of the technological process, lack of quarantine, violation of the timing and densities of stocking, uncontrolled transport of fish, the lack of systematic prevention, drainage and freezing of pond beds, land reclamation, disinfection and disinfection in farms. All measures contributing to the prevention of the occurrence of bothriocephalosis at the fish farm are specified in the regulatory legal acts.

The development of the parasite occurs with the participation of intermediate hosts, they can be cyclops of many species: *Mesocyclops leuckarti, M. dybowski, M. crassus, Ectocyclops phaleratus, Termocyclops taihkonensis, Cyclops strenuus, C. vicinus, Microcyclops bicolor, Macrocyclops fusus, E. macrocyclops fus serrulatus, E. macrurus, Phyllodiaptomus blanci, Acantocyclops vernalis A. bicuspidatus, A. viridis*. In all likelihood, the list of cyclops can be significantly expanded.

The life cycle of the parasite lasts about a year, helminthes that have entered the body of fish in autumn ripen by April of the following year, that is, after 200–240 days.

Tapeworm eggs come out with the faeces of the fish into the water and fall to the bottom of the reservoir. Depending on the temperature, the formation of the larva, coracidium, is completed in the egg within 1.5–4 days. At a water temperature of 16-19 °C, development occurs during 3-4 days, at 25-30 °C it lasts 1.5-2 days. According to some foreign authors, at a temperature of 28–30 °C, the eggs hatch within 1–5 days, and at a temperature of about 14–15 ° C they do it in 10–28 days [9]. The other stages of the parasite development also depend directly on the water temperature.

The source of the invasion is sick fish, parasite carriers, producing bothriocephalosis eggs in the reservoir; and invaded cyclops, in which the procercoid develops - the stage of development of the parasite at which it is able to infect fish.

The lifespan of helminthes in the intestines of fish and the entire life cycle of bothriocephalosis is approximately one year [9].

The course of the disease has a pronounced seasonal character. Infestation of underyearlings increases from mid-May to mid-summer. With the transition of fish to feeding on benthos, the infection sharply decreases. The maximum infection rate occurs in late summer - early autumn. Low temperatures retard the development of eggs and larvae and slow down the completion of the life cycle.

5 Effect on the host organism

S. acheilognathi has a mechanical, toxic, trophic and inoculatory effect on the organism of sick fish. Being localized in the intestine, cestodes, first of all, have a negative mechanical effect on the fish organism. At dissection of infected fish, a thinning of the intestinal walls is found, through which the strobila of parasites are clearly visible. The causative agents of bothriocephalosis are attached to the intestinal wall with the help of the scolex, and focal hemorrhages are formed at the attachment points of the parasite. The mucous membrane becomes bumpy, covered with a small amount of yellowish-red mucus. The liver of such fish is usually reduced in volume, flabby, and clay-colored. The kidneys are somewhat enlarged and also flabby. In addition to these manifestations of the disease, there is a degradation of intestinal microvilli, a decrease in the activity of liver enzymes, as well as pancreatic enzymes and a decrease in hemoglobin levels. Inflammation can lead to hemorrhage, anemia and necrosis [10, 11].

Hematopoiesis increases in the initial stage of the disease. Later, hematopoietic activity decreases under the influence of toxins secreted by cestodes. The toxic effect of *S. acheilognathi* contributes to the defeat of a significant part of the intestinal mucosa of carp. Lysis of cells, the formation of foci of necrosis is noted [12].

The pathogenicity of cestodes also manifests itself in a decrease in intestinal filling indices, with a difference being up to 50 %.. Proteinograms of infected fish have deviations of a dystrophic nature: albumin-globulin ratio (0.9 ± 0.03) is lower than that in healthy individuals (1.11 ± 0.04) [13].

Parasitization by *S. acheilognathi* causes a number of pathological processes in fish, namely, a decrease in concentration of:

- total sugar in white muscles, swim bladder and brain;
- glycogen in hepatopancreas, white muscle;
- the total amount of pentoses (structural elements of nucleic acids and nucleotides) in the tissues of the kidneys, heart, gills, and brain;
- mucopolysaccharides and hexosamines, which is regarded as a violation of the synthesis of substances of mucopolysaccharide nature and as one of the possible reasons for an increase in the permeability of connective tissue elements and blood vessels, leading to tissue changes and a decrease in the immune responses of the invaded carp [14]. In order to normalize energy metabolism and plastic processes and increase the productivity of fish, it is necessary to balance their feeding rations in terms of carbohydrate content.

Juveniles are particularly vulnerable, their death was observed by many authors [15, 16, 17]. The mortality rate of fry from bothriocephalosis sometimes reaches 90% [18]. Fish of older age groups, as a rule, do not get sick, but are parasitic carriers. Many weedy and wild fish species can transfer the pathogen.

In the infected fish, a 30% retardation in weight was recorded, which manifested itself in the first or second year of life [19, 15]. According to V.A. Musselius [19], in the pond where the disease was observed, the yield of carp underyearlings was 23% lower (67 instead of 90%). The lethal intensity level for them was 80–100 and more parasites per fish. In addition to affecting the growth rate, nutritional status and yield of farmed fish, bothriocephalosis reduces the general immunity of the organism and aquaculture animals become more susceptible to other diseases, such as bacterial ones. Foreign authors also point to a decrease in appetite, less growth rates, and depletion of the fish body [12, 20, 21].

6 Fish susceptible to bothriocephalosis

According to the latest data, *S. acheilognathi* was recorded in more than 300 fish species [8, 13, 22]. However, all the fish feeding on the intermediate hosts of the cestode can be considered susceptible to the parasite. The parasite was also recorded in aquarium fish (mollies, guppies, and others). In the tropics, some species of cichlids and eleotrid are infected. The most susceptible fish are planktophages. Perch, pike, tropical catfish seem to be infested very rarely [8].

Despite the large number of definitive hosts, the parasite prefers cyprinids, namely the carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella*. Most of the reported cases of the parasite found usually concern carp raised in fish farms. This is due to the fact that under conditions of pond fish farming, carp, grass carp, silver carp are most often used as objects of aquaculture. In the early stages of development, these fish species feed on zooplankton, including cyclops, which are intermediate hosts of the pathogen of bothriocephalosis. Dense fish stockings provoke rapid spread of the pathogen among aquatic organisms, and that is one of the factors in the development of mass epizootics.

7 Detection and identification methods

In accordance with the Sanitary and Epidemiological Rules, pathogens of bothriocephalosis are not dangerous to humans and do not require special rules to work with them.

The microscopic method is used to detect the causative agents of bothriocephalosis. The invasion is diagnosed by examining water, faeces, and finding eggs and cestode segments, or by examining the intestines of fish.

When examining water, it is possible to establish the presence of helminth eggs in the investigated reservoir. To do this, a water sample is first passed through membrane filters, after which the precipitate is washed and centrifuged. A preparation is made from the resulting precipitate and viewed under the microscope. Depending on the study goals, the viability of the eggs can be established by staining them with acridine orange or thiazine red fluorochrome [22].

An autopsy of the fish with subsequent examination of the intestinal contents makes it possible to establish the species belonging of the helminth, to obtain quantitative characteristics of infection, as well as to record pathological changes in the organs and tissues of the fish with cestodes. The gastrointestinal tract is either dissected into a petri dish or squashed between two glass plates if the intestine is relatively thin-walled. The extracted worms are fixed with a hot solution of buffered 10% hot formalin (3.8% - 4% formaldehyde solution). As to the scolex, it is preserved in 95% or 100% ethanol for further molecular research. The fixed worms are stained and processed by standard techniques [21].

Classical identification methods use optical equipment to study native or preserved preparations [8, 20].

Modern methods are based on molecular diagnostics. The gene sequence found for *S. acheilognathi* has allowed one to determine the species of cestodes. The sequence was deposited in the GenBank (NCBI) under accession numbers KY711155-KY711166. The portal shows an isolate and partial sequence for the EMS4 gene of the small subunit of ribosomal cestode RNA; complete sequence of internal transcribed spacer 1 (5.8S ribosomal RNA gene) and internal transcribed spacer 2; partial sequence of the large subunit gene of ribosomal RNA.

8 Methods for the prevention and treatment of the disease

Among the methods of treatment and prevention of bothriocephalosis, two areas are distinguished: drug therapy and interruption of the life cycle of the parasite (biological methods). On the territory of the Russian Federation, two drugs Microsal and Phenomix are currently allowed to treat bothriocephalosis in fish farming. Both formulations are added to feed and show good efficacy in fish treatment.

Methods of disease prevention include mandatory compliance with veterinary and sanitary requirements and technological process, quarantine, timing and stocking densities, control of fish transport, systematic prevention, drainage and freezing of pond beds, land reclamation, disinfestation and disinfection.

Biological methods of control are based on the use of elimination properties of living organisms in relation to the causative agent of the disease and its intermediate hosts. Peled underyearlings who are resistant to *S. acheilognathi* infection and consume coracidia (the stage of helminth development) and copepoda (intermediate hosts of the causative agent of botrocephalosis), can act as eliminators. Mixed fish farming of carp and peled underyearlings reduces the infestation of carp with cestodes. Also, a positive effect was noted when creating optimal conditions for the development of Cladocera in ponds and thus suppressing Copepoda, which are intermediate hosts of the causative agent of bothriocephalosis [33].

9 Distribution of the causative agent of botryocephalosis among aquaculture species in the south of Russia

Bothriocephalosis is included in the List of infectious animal diseases for which the regionalization of the Russian Federation territory is carried out on the basis of the Order of the Ministry of Agriculture. The statuses of the regions are reflected in the automated system "Cerberus". Now 82 regions of the Russian Federation have the status of a "region with an undefined status" (Tambov region, Murmansk region, Lipetsk region, etc.); Volgograd, Kurgan, Novgorod and Belgorod regions are unfavorable in regard to bothriocephalosis. So, the transportation of aquatic animals from these regions will be carried out only if special veterinary rules are complied.

The Southern Federal District, consisting of 8 constituent entities (Republic of Adygea, Astrakhan Region, Volgograd Region, Republic of Kalmykia, Krasnodar Territory, Rostov Region, Republic of Crimea, city of Sevastopol) generally has a positive status regarding this disease. Over the past three years, according to the official data of regional veterinary services, the restrictive measures (quarantine) concerning bothriocephalosis in fish farms have been implemented only six times (Figure 1).

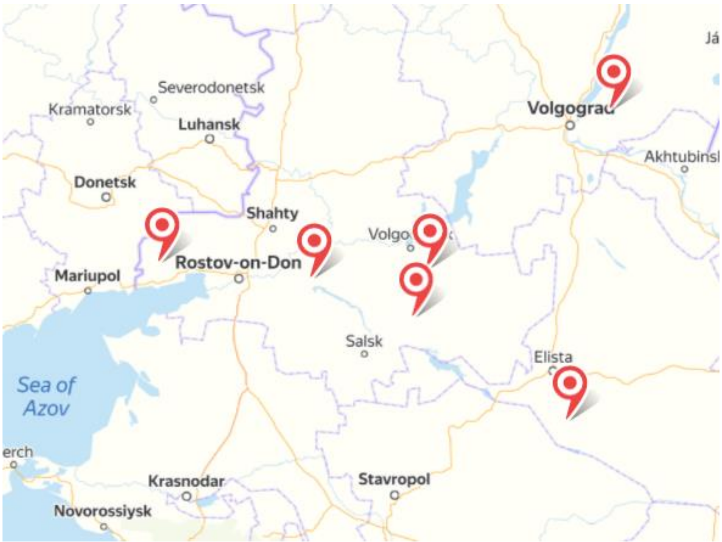

Fig. 1. Map of detected cases of bothriocephalosis in fish breeding enterprises in the south of the Russian Federation for the last 3 years (2018-2020) (open data).

10 Conclusion

Bothriocephalosis, caused by the tapeworm *Schyzocotyle (Bothriocephalus) acheilognathi* Yamaguti, 1934 (Cestoda: Bothriocephalidea), is currently recognized as a dangerous disease of fish, especially for juvenile aquaculture objects. The native area of the parasite is the Far East, but in the 21st century bothriocephalosis is recorded on all the continents, with the exception of the poles.

Bothriocephalosis has a mechanical, toxic, trophic and inoculatory effect on the organism of a diseased fish. Juveniles are especially vulnerable, mortality in this age group sometimes reaches 90%. Fish of older age groups, as a rule, do not get sick, but are parasitic carriers. Many weedy and wild fish species can transfer the pathogen. The infected fish have a poor weight gain, 30% less than in healthy fish, the yield of fingerlings of some aquaculture species is reduced by more than 20%.

Thus, bothriocephalosis is one of the main diseases in aquaculture, causing a decreased efficiency of fish rearing under artificial conditions. In the conditions of the southern region of the Russian Federation, which is a zone with favorable conditions for the emergence of foci of bothriocephalosis, constant ichthyopathological control is required when rearing fish. Lack of monitoring can entail significant economic losses both from direct death of fish, and indirectly from a decrease in feed conversion rates, condition factor, etc. The emergence and spread of the disease is facilitated by non-compliance with mandatory veterinary and sanitary requirements and violations of the technological process, lack of quarantine, violation of deadlines and stocking densities, uncontrolled fish transport, lack of systematic preventive measures, drainage and freezing of pond beds, land reclamation, disinvasion and disinfection in farms.

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