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BOTHRIOCEPHALUS SPP. INFECTION OF CYPRINIDAE: EPIZOOTOLOGY, CLINICAL FEATURES AND PATHOGENESIS, DIAGNOSTICS, THERAPEUTIC AND PROPHYLACTIC MEASURES

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ABSTRACT

Bothriocephalosis of fish is a disease caused by tape worms Bothriocephalus opsariichthydis and Bothriocephalus acheilognathi parasitizing in the anterior part of intestine. Bothriocephalus spp. infection is widely spread among fish at pond farms, cage fish farms in cooling ponds of thermal power and nuclear power stations and in natural reservoirs. One have revealed Bothriocephalus in 26 species of fish attributed to Cyprinidae family, in salmons (Arctic salmon) and in some predatory fish (catfish, pikeperch). Different species of cyclopes serve as the intermediate hosts necessary for development of helminths. Fish fry and fingerlings are the most susceptible ones to infection. Fish of older age groups are less susceptible to this parasite. The data on biology, epizootology, clinical features and pathogenesis, diagnostics, therapeutic and prophylactic measures against this infection are represented. The results of the field trials with microsal against Bothriocephalosis in carps and grass carps carried out in different regions of the Russian Federation as well as monitoring of the safe use of microsal for the study period (2007 to 2016) are described. The daily dose of medicated feed with 2% of microsal corresponds to the daily feeding for fish. The therapeutic feeding is carried out during one day without preliminary starvation period according to the current technology of fish feeding with granulated feed. The dose level according to the active substance depends on water temperature and average fish weight and ranges 12 to 40 mg/kg. Prophylactic treatment is carried out twice a year: in late April – early May and in late August – early September (at pond farms) and in late September – early October (at cage fish farms) when the water temperature is not higher 150 C. In general the data obtained for 10 year period evidence about microsal's safety for fish and at it's current application (according to the instructions) the reasonable benefit/risk ratio is maintained in all cases.

Keywords: *Bothriocephalus opsariichthydis, Bothriocephalus acheilognathi, fish farms, microsal.*

EPIZOOTOLOGY

Carps, common carps, silver and gold carps and grass carps are mainly susceptible to Bothriocephalus spp. infection. Fish fry and fingerlings are especially sensitive to this infection. Fish of older age groups are less susceptible to this parasite. Infected fish of different age groups as well as infected Cyclops function as the source of infection. At cage fish farms transmission of infection occurs with excrements containing parasite eggs and carried by water flow. First infection of young fish occurs in trays (or fry ponds) at feeding of infected zooplankton. After transfer of fish into cages the infection level increases reaching the maximum value to the end of summer and then decreases. Due to siltation of wire gauze each cage becomes a separate biotope and Bothriocephalus infection microfocus with independent circulation of the pathogen. Two-year old fish become infected with Bothriocephalus in spring and summer, but the rate of their infection usually is not high. Infection of fish fry at pond farms occurs at feeding of zooplankton and consumption of infected Cyclops. The peak of infection extensity and intensity is recorded in July-August at rich development of zooplankton and intensive feeding of fish. The infection extensity value achieves 80-100% with the intensity of infection - from several specimens to one hundred helminths per one fish. In autumn when the grown juveniles transfer to eating of forage and the number of copcpods becomes fewer the rate of infection reduces. Increase of Bothriocephalus infection level among fish depends on the temperature conditions. Temperature lowering retards the development of infection. Helminths develop from eggs to adult stages at the water temperature of 16-19°C for 33-34 days as while at 20-25°C for 19-25 days. If fish becomes infected by Bothriocephalus in autumn parasites reach mature stage only to April of the following year (post 200-240 days).

CLINICAL SIGNS AND PATHOGENESIS

Pathoanatomical changes in fish infected with Bothriocephalus depend on the infection intensity and terms of parasitizing. One can observe the most manifested changes in the anterior and middle parts of the intestine. In places of contact of helminth strobila with the intestine thinning of its wall usually is observed as a result of destruction of the mucous, muscular and partially serous membranes. Helminth parasitizing in the intestine of fish leads to deterioration of digestion processes. In infected carp fingerlings and yearlings hemoglobin level in blood reduces by 25- 30% as while polymorphonuclear leukocyte and neutrophil counts increase. Carp tiny infected by Bothriocephalus demonstrate growth and development decrease. Exhaustion, anemic gills, movement reduction are recorded in infected one-year- old fish. They cannot survive winter and die in the middle of March or early April. Chronic inflammation of the intestinal mucosa is observed in infected two-year-old carps. They have a growth slowing, poor digestion of feed and anemic condition. Intestinal obstruction and reduced growth rate take place at the infection intensity more than 12 helminths per one fish. Death of carp

fingerlings caused by Bothriocephalus occurs at parasitizing of more than 50 helminths in one fish.

DIAGNOSTICS

Bothriocephalosis is diagnosed on the basis of epizootologic data, clinical signs of disease and the results of helminthological examination of fish. The structure of parasite scolex is the most reliable diagnostic sign of Bothriocephalus species. B. opsariichthydis has a heart-shaped scolex with muscular pariental disk and deep, open bothridia as while B. acheilognathi - a spherical scolex with deep, half closed bothridia. For investigations one use not less than 25 fry, fingerlings and yearlings as well as 10-15 two-year-old carps from each pond (at pond farms); 40-50 fingerlings and 10-15 two-year-old fish from each pontoon line (at cage fish farms). Breeding herd and rearing fish are examined using coprologic methods. To reveal the latent parasite carriage two-week fry (the most susceptible ones to Bothriocephalus infection) are transferred to adult fish suspected in infection. 2-3 weeks later fry is examined and diagnosed for latent Bothriocephalus infection. Autopsy of infected fish is carried out as follows: one cut abdominal wall of fish with scissors, extract intestine with tweezers, put it into Petri dish, separate intestine from other organs, cut it along or squeeze out the contents from the intestines adding some water and count parasites according of the number of scolexes. Then the pathogen species is identified. Coprologic examinations for presence of helminth eggs include collection of fish excrements and recovery of parasite eggs. Fish excrements are collected from cage walls by net made of gauze № 50 during lifting. Samples of excrements from 3(or fewer) cages from each pontoon line are combined into one 3-4 g sample and examined by one of the following methods.

Method 1. One take a sample of 0.5 g from each combined excrement sample and examine by the method of native smear.

Method 2. 0.5 g from each combined excrement sample is added into a 100 ml glass beaker and 30 ml of water is added. The sample is stirred thoroughly using a glass rod and the obtained suspension filtered through strainer. Water is added. The volume of filtered suspension is adjusted to 60 ml. The contents is transfused into Petri dish in small portions of 10-15 ml and examined under microscope (MBS; magnification 7X8) for detection of Bothriocephalus eggs. The size of eggs - 0,05 - 0,054 ><0,03 - 0,038.

THERAPEUTIC AND PROPHYLACTIC MEASURES

Bothriocephalus infection in Cyprinidae is included in the list of quarantine and especially dangerous diseases of fish according to The Order №173 of September 29, 2005 of the Ministry of Agriculture of Russia. If Bothriocephalus infection is diagnosed in fish then the pond farm is announced as unsafe for this infection and restrictions are imposed. It is forbidden to transfer the fish stocking material to non-infectcd waters, pond and cage fish farms.

At pond farms: infected ponds are drained and subjected to disinfection using caustic lime (25 cwt/ha) or by bleaching powder (6 cwt/ha). In winter the drained ponds are deep freezed, in spring arc dried that leads to death of helminth eggs and infected intermediate hosts - crustaceans and Cyclops (Muzykovsky A.M., Skachkov D.P., et all., 1987; Skachkov D.P., Muzykovsky A.M., et all., 1990).
At cage fish farms with high level of Bothriocephalus infection: cages for juvenile fish with a mesh up to 10 mm is changed weekly and with larger mesh size – according to contamination. Cages from infected fish are cleaned from dirt, washed with water and dried at the temperature 20°C at least for 24 hours or kept in 2% formalin solution for 2 hours and then washed with water. Warm water for incubation shop, sites for rearing of juvenile fish and keeping of producers as well as lor try ponds is taken from the channel or cooling reservoir upstream to cage lines or from some other water source stationary safe for Bothriocephalus infection. Cages with juvenile fish are placed at the distance of 15-20 meters from the shore in sections of cooling reservoirs with a depth not less than 5 meters, forming sanitary zone between cage lines in range of 50-60 m. Herewith the cages with fry and fingerlings on pontoon lines should be located upstream in relation to cages with fish of older age (Skachkov D.P., Gorokhov V.V., et all., 1995).
Prophylactic treatment by anthelmintics is carried out twice a year: at the end of April-beginning of May and at the end of August- beginning of September (at pond farms) and at the end of September-beginning of October (at cage fish farms) at the water temperature not higher 15°C. Medical treatment by anthelmintics is performed at any time of the year if diagnostic indications are present. Microsal is used for such treatment of fish. The daily dose of medical feed with 2% of Microsal corresponds to the daily norm of fish feeding by mash. The therapeutic feeding is carried out for one day without preliminary starvation period using the current technology of fish feeding with granulated feed. The dose level according to the active substance depends on water temperature and average fish weight and ranges 12 to 40 mg/kg (Skachkov D.P., 2008).

Table 1.The daily dose of 2% medicated feed with Microsal, the % of fish body weight

Water temperature, °C	Fish body (weight), g				
	20-50	50-100	100-250	250-500	More than 500
12	2,0	1,6	1,3	1,0	0,8
15	3,0	2,0	1,6	1,2	1,0
18	4,0	3,0	2,0	1,6	1,3
21	5,0	4,0	3,0	2,0	1,6
24	6,0	5,0	4,0	3,0	2,0
27	7,0	6,0	5,0	4,0	2,2
30	8,0	7.0	6,5	4,5	2,5

Before general treatment every lot of the drug as a part of medical feed is tested on tish in one pond. If no complications are observed within 3 days treatment of all fish in every pond is carried out.
25 fish recovered from each pond are subjected to helminthological autopsy before and 4-5 days after medical feeding to determine the treatment effectiveness. If necessary the medical feeding can be repeated 10-20 days after the first treatment (Skachkov D.P., Arkhipov I.A., 2009).
During the period of 2007-2016 one produced and sold 37225 kg of the Microsal to fish farms of 23 Territories and Regions (Altai, Krasnodar and Stavropol I erritories; Astrakhan, Bryansk, Vladimir, Volgograd, Voronezh, Kaliningrad, Kaluga, Kursk, Lipetsk, Moscow, Orenburg, Orel, Perm, Rostov, Ryazan, Samara, Saratov, Sverdlovsk, Tambov, Tula, Chelyabinsk Regions). 1098150 kg of fish was treated [4-6]. The therapeutic effectiveness of the agent for carps appeared to be 100% as while for white Amur - 87.5% (Skachkov D.P., Pavlovich G.M., 2012).

Table 2. The data on used agent amounts and quantity of treated fish

The country	Years	Amount of the drug	Average dose according to the active substace per 1 kg of fishmass (ichthyomass)	Treatment course (days)	Treated fish (kg)
Russia	2007	3450			69000
	2008	1720			34400
	2009	2460			49200
	2010	3770			75400
	2011	3555	40 mg/kg	1	71100
	2012	6050			121000
	2013	3980			79600
	2014	6080			121600
	2015	4300			86000
	2016	1860			37200

In general all data obtained for 10 year period evidence about Microsal safety for fish and at it's correct application (according to the instructions) the reasonable benefit/risk ratio is maintained.
Microsal is packed by 20 kg in paper bags which are put in polyethylene bags. This agent amount is sufficient for preparation of one ton of medicated feed.
According to the results of our work The Federal Service for Veterinary and Phytosanitary Supervision issued the State Registration Certificate on Microsal as an agent for application against cestodoses of Cyprinidae in ponds (Skachkov D.P., 2013, 2015).

At cage fish farms to prevent reinfection with Bothriocephalus spp. fish should be transferred to other cages in a day after treatment at water temperature higher 16°C and on day 3-5 at the water temperature 14°C and lowcr. At farms with high incidence of Bothriocephalus infection a complex of other measures according to the corresponding regulation documents can be carried out along with treatment by Microsal. Restrictions are cancelled and farms are considered to be safe for Bothriocephalus inlection if no infected fish are revealed during parasitological examinations throughout a year.

CONCLUSION

It can be concluded that the obtained results of our ten-year work evidence microsal's safety for fish and at it's current application (according to the instructions) the reasonable benefit/risk ratio is maintained in all cases.

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