Т. 10. №4. 2024
https://doi.org/10.33619/2414-2948/101

UDC 619:616.993:597.552.51:639.3.09(479.24) https://doi.org/10.33619/2414-2948/101/24
AGRIS L20; L72

THE SPECIES COMPOSITION AND PREVALENCE OF THORNY-HEADED WORMS (Acanthocephala: Echinorhynchidae, Neoechinorhynchidae AND Pomphorhynchidae) IN TROUT, COMPLEX MEASURES TO CONTROL THEM IN FISH FARMS IN THE TERRITORY OF AZERBAIJAN REPUBLIC

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ВИДОВОЙ СОСТАВ И РАСПРОСТРАНЕНИЕ СКРЕБНЕЙ (Acanthocephala: Echinorhynchidae, Neoechinorhynchidae И Pomphorhynchidae) У ФОРЕЛИ, КОМПЛЕКСНЫЕ МЕРЫ БОРЬБЫ С НИМИ В РЫБНЫХ ХОЗЯЙСТВАХ АЗЕРБАЙДЖАНСКОЙ РЕСПУБЛИКИ

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Abstract. During period 2015-2020 spreading of acanthocephaliasis in the rainbow and brown trout in the fish breeding farms of Azerbaijan Republic was studied. Totally, from 668 fish examined specimens 430 (64.37%) were infected. Correlation of data on the extensiveness and intensity of invasion by thorny-headed worms (Acanthocephala) in trout farms is ventured to make an assumption about unfavorable epidemiological situation in trout breeding farms of the Republic of Azerbaijan. For the purpose of control acanthocephaliasis in trout, a number of drugs were used. The most effective drug was Tetramizole-20%, which was adopted in the trout farms.

Аннотация. В период 2015–2020 гг. изучено распространение акантоцефалеза радужной и пятнистой форели в рыборазводных хозяйствах Азербайджанской Республики. Всего из 668 исследованных экземпляров рыб 430 (64,37%) оказались зараженными. Сопоставление данных об экстенсивности и интенсивности инвазии скребнями в форелевые хозяйства позволяет сделать предположение о неблагоприятной эпидемиологической ситуации в форелевых хозяйствах Азербайджанской Республики. С целью борьбы с акантоцефалезом форели был применен ряд препаратов. Наиболее эффективным из них оказался Тетрамизол-20%, который используется в форелевых хозяйствах.

Keywords: trout, acanthocephaliasis, veterinary drugs, trout farms.

Ключевые слова: форель, акантоцефалез, ветеринарные препараты, форелевые хозяйства.

The rapid development of global aquaculture leads to the need to develop measures to protect fish from parasitic diseases. As a result of multiply invasion by various pathogens, including helminth infections, fish remain half-grown; the quality of meat deteriorates and loses marketable appearance, in some cases mass mortality is observed. Therefore, the study of fish helminth infections is considered one of the most actual problems not only in national level, but in worldwide too [1, 2].

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The study of parasitic diseases of salmonids in fish farms has scientific and practical importance. The high calorie fish meat is enriched by proteins, vitamins and microelements. It is used as a dietary product in some specific diseases of gastrointestinal tract [3]. Despite the fact that Azerbaijan Republic has favorable climatic conditions for the development of fish industry, the water basins eligible for aquaculture are gradually declining due to several environmental factors [4].

The control of thorny-headed worms has some specific moments in the system of parasitosis control. Acanthocephala is a phylum of parasitic flatworms, characterized by the presence of an eversible proboscis, armed with spines. This organ is used for nutrient absorption and attachment to the intestinal wall of the host. The parasite has a naked neck and body. The species composition of the spiny-headed worms is defined by the number and appearance of the spines on proboscis. The representatives of above-mentioned phylum can infect a terrestrial and water vertebrates as definitive hosts [5].

So, the life cycle of Acanthocephala usually requires only one crustacean intermediate host. Adult worms were located on the abdominal cavity of freshwater and marine fish species [6].

Adult members of this phylum were located and feed on the intestinal walls of fresh and marine water fishes. Adult members of Acanthocephalan parasites are highly specialized dioecious parasites that are located in the intestines and take up their nutrition through their teguments. Adult Acanthocephalans are all gut parasites which develop through one or more intermediate hosts which lay their eggs into the intestinal lumen moved with feces [5].

The epizootic situation in the republic in trout breeding farms was studied by N. L. Nechaev (1962), Mikayilov T. K. (1969), Heydarov A. A. (1970), Pashayev N. A. (1970, 1991), Suleymanova A. V. (2011, 2013), Suleymanova A. V. and Nasirov A. M. (2015), Suleymanova A. V. (2020) [7-16]. However, it should be recognized that investigations in veterinary epidemiology have declined in recent decades due to objective economic and social reasons.

Taking into account the above-mentioned facts, we set a goal to obtain the species composition and some epizootological peculiarities of acanthocephaliasis in fish farms of the Azerbaijan Republic.

Material and methods

In the period 2015-2020 investigations were carried out in the territory of the Chaykend, Chukhur, Gabala, Zagatala-Sheki, Goranboy, and Khachmaz trout breeding factories. In this case, 668 fish specimens were examined (Table). The studies were carried out depending on the epizootological situation. Also, the bioecology, age and sex of studied fishes were taken into account.

Specimens were examined by method of full parasitological dissection [17]. Field works were implemented out in the branch veterinary laboratories of the Azerbaijan Scientific Research Institute of Veterinary. All routine histological investigations were carried out at the Department of Diseases of Fish and Bees of the above-mentioned organization.

The larvae of spiny-headed worms in the tissues of the eyes, brain, muscles and internal organs were obtained using the compressor method and examined by using a Phenix XTL-165-VT stereo microscope. Adult worms were fixed in dehydrated ethyl alcohol and 4% formaldehyde. Species of thorny-headed worms was defined by the number and structure of spines located on the proboscis.

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Table

SPECIES COMPOSITION AND NUMBER OF STUDIED FISH

Fish	*Chaykend trout hatchery*	*Zagatala trout hatchery*	*Sheki trout hatchery*	*Goranboy trout hatchery*	*Gabala trout hatchery*	*Khachmaz trout hatchery*	*Total*
Rainbow trout, *Salmo gairdnerii* Richardson, 1836	—	120	85	90	80	40	415
Brown trout, *Salmo fario* Linnaeus, 1758	70	—	—	115	68	—	253
Total:	70	120	85	205	148	40	668

The effectiveness of anthelminthic granulated drug Tetramizole 20% was tested at first in laboratory, and then in field conditions in the above-mentioned trout breeding factories. Thus, the weight of experimental fishes was measured in accordance with the existing methodology. For experimental investigation 25 specimens of rainbow trout were selected. These specimens infected by species *Metechinorhynchus truttae* and *Pomphorhynchus laevis* were kept in a pool with flowing water. The drug was added to food at the rate of 1.0 g per 1 kg of fish live weight 3 times a day for 14 days and used after 30 minutes. On the 15th day, the therapeutic effect of the drug was defined by holding full parasitological dissection of selected specimens. During the field epidemiological study in the trout farms this course of treatment was repeated.

Measurements and photomicrographs were taken using a Phmias ver 3.0 software for the digital camera Phenix HD MCD300U(C)/TP mounted on stereo microscope. All data were biometrically processed. Extensiveness of invasion (IE) is defined as the ratio of fish invased by parasites to the total number of examined specimens (%). The intensity of invasion (II) is the number of parasites of in host specimen [18]. Thereby, the extensiveness of invasion (EI) was calculated in accordance with the following formula:

$$IE = \frac{X_{inf}}{X} \times 100\%$$

X_{inf} — number of infected fishes, X — total number of dissected fishes.

Results and Discussion

Totally 430 fish specimens from the 668 examined in the spring and summer in the above-mentioned trout breeding factories were infected by spiny-headed worms. The re-description of the registered acanthocephalan species (*Metechinorhynchus baeri*, *M. salmonis*, *M. truttae, Neoechinorhynchus rutili*, *Pomphorhynchus laevis,*) is given.

The causative agents of acute metechinorhynchosis of trouts in Azerbaijan are two species (*M. salmonis*, *M. truttae*). Two other species (*M. baeri* and *Neoechinorhynchus rutili*) are not so significant. Development of all representatives of genus *Metechinorhynchus* occurs with the participation of an intermediate host (amphipods). Intermediate hosts for *M. salmonis* are species *Pontoporeia affinis* and *Gammarus pulex*, respectively. The definitive host for this species is trouts. Infection occurs in the summer, during the season of intensive feeding of fish and the development of intermediate hosts. *M. salmonis* has a short pear-shaped body with a cylindrical proboscis bent at an angle to the body axis (Figure 1, B). The proboscis has 14 longitudinal rows of hooks, 10-11 hooks in each row. The length of males is 3.0-6.3 mm, females 6-8 mm.

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Figure 1. Spiny worms of trout, registered in Azerbaijan. A — *Metechinorhynchus baeri*; B — *M. salmonis*; C — *Neoechinorhynchus rutili*; D — *Pomphorhynchus laevis*; E — *M. truttae*; F — *N. rutili*, anterior part with proboscis; Magnification: ×0.7 (A, D), ×1 (B, C, E), and 1.5 (F)

Fish can be infected by another species (*M. truttae*) in feeding by benthic organisms, including amphipod crustaceans from the family Gammaridae. Adult helminths live in the intestines of trout for 4-5 months. *M. truttae* has a narrow cylindrical body, with a cylindrical proboscis, ventrally curved and armed with 20-22 longitudinal rows of hooks, 13-16 hooks in each row (Figure 1, E). The length of the male is 8-10 mm.

Thorny-headed worms cause inflammation of the mucous membrane by penetrating the intestinal wall with their proboscis. A proliferation of connective tissue is noted around the proboscis. In some cases, lime deposits are observed in the affected area (petrification), and the intestines lose the ability to absorb digested food. In some cases, during mass infection, a decrease in the weight of fish was noted. The high level of the intensity of invasion by *M. truttae* (more than 100 worms per one fish specimen) can provoked the mass mortality of trouts.

The female of *M. baeri* is slightly large (11-12 mm) in comparison with male (7.5 × 0.6-0.7 mm). The proboscis is cylindrical, 0.8-1.0 × 0.3-0.4 mm, slightly bent. The length of the anterior hooks is 0.08-0.10, middle 0.09-0.11, posterior 0.04-0.07 mm. The length of the proboscis sheath is 1.4 mm. The lemnisci reach the posterior edge of the sheath. The testes are shifted to the back of the body and lie closely one after the other. There are 6 cement glands, arranged regularly, sometimes almost in pairs. The dimensions of eggs are equal 0.10-0.13 × 0.022-0.024 mm (Figure 1, A).

The body of the species *Neoechinorhynchus rutili* is fusiform, bent on the ventral side (Figure 1, C). Lemnisci contain 1-2 nuclei. The proboscis is small, round, sometimes slightly oval (Figure 1, F). Male 1.5-6.5 × 0.25-1.6 mm. Proboscis sheath 0.15-0.38 × 0.09-0.17 mm, lemnisci of unequal

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length. Testes oval. The size of female 2.50-12.00 × 0.30-1.68 mm. The length of the terminal hooks is 0.06-0.08, median 0.03-0.04, basal 0.02-0.04 mm. The eggs are 0.016-0.043 mm long, oval, with three shells. Intermediate hosts: larvae of alderflies, ostracods *Candona angulata*, *C. candida*, *Cypria turneri*, *C. ophtalmica*, *Cyclocypris laevis*. In case of mass infection, it can cause damage to the intestinal mucosa. Cases of death of trout and carp fingerlings have been described. The size of males and females of the species *Pomphorhynchus laevis* are 6-8 mm and 10-30 mm, respectively (Figure 1, D). The neck part is 5-6 mm, the bulb is situated anteriorly with a diameter of 3 mm. The proboscis has 18-20 longitudinal rows of hooks. The hooks in 7 rows, located in the front of the body, are relatively large. Testes oval (0.6-0.9 × 0.64 mm), located in the middle part of the body. The development of the parasite occurs with the participation of an intermediate host. The definitive host for this parasite can be a wide range of hosts, and amphipods can be the intermediate host. Cyprinid fish are considered to be the reservoir hosts of this species [19].

For the first time this parasitic worm is recorded by V. A. Dogel and B. Ya. Bikhovsky (1939) not far from the island Sara in Southern Caspian Sea in Aral barbel (*Luciobarbus brachycephalus*), Wels catfish (*Silurus glanis*), Vimba bream (*Vimba vimba persa*), European perch (*Perca fluviatilis*), in the representatives of the family Gobiidae. In recent years *Pomphorhynchus laevis* is recorded in the rivers Kura and Araz in Khramulya (*Varicorhinus capoeta gracilis*), Aral barbel (*Luciobarbus brachycephalus*), Schneider (*Alburnoides bipunctatus*) [8]. Also, this species of spiny-headed worm is described from the intestines of carp (*Cyprinus carpio*), Silver carp (*Hypophthalmichthys molitrix*), Aral barbel (*Luciobarbus brachycephalus*) in fish farms of Shirvan, Neftchala, Kura and Araz rivers, in Devechi gulf. For the first time acanthocephalans in trouts in the territory of Azerbaijan Republic was recorded by N. A. Pashayev (1991) [11].

The severity of the disease can be minor or extreme. High level of mortality can be recorded in fry and young fish. Three months after infection, the first obvious symptoms of the disease appear. Pathological changes are determined by damaging of intestines. The fish die due anemia and weight loss. As can be seen in diagram (Figure 2), acanthocephaliasis was recorded in all trout breeding factories.

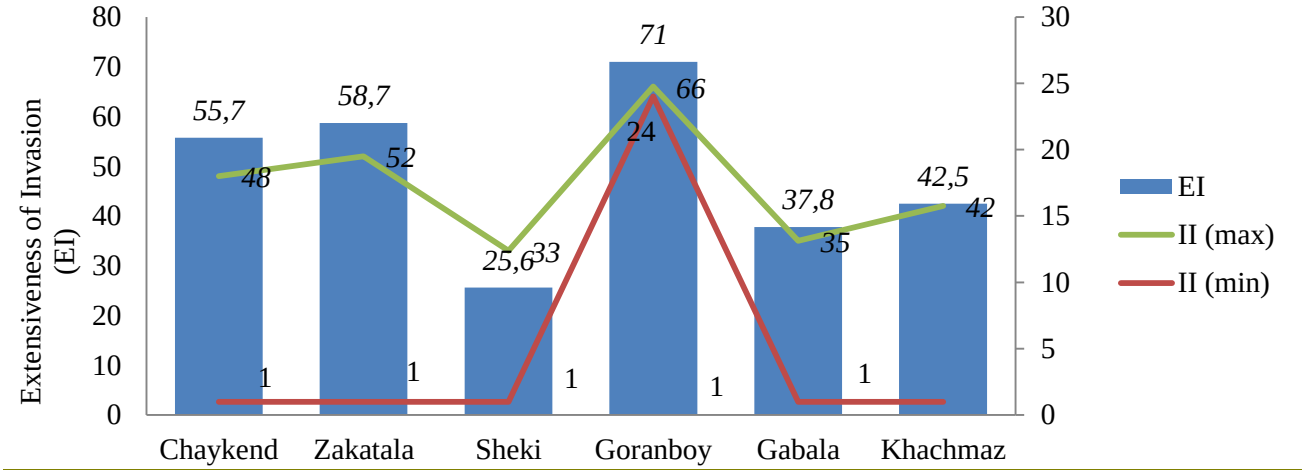

Figure 2. Dynamics of extensiveness and intensity of invasion in trout breeding farms in the territory of Azerbaijan Republic

Studying the graph shows that the highest extensiveness of invasion was recorded in the Goranboy trout breeding farm (71%). At the same time, the data from the two another farm (Zagatala and Chaykend) were not differed from the Goranboy farm. Therefore, they can be combined into one group. The average extent of EI was recorded in Khachmaz (42.5%) and Gabala

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(37.8%) trout farms. And only in the Sheki (25.6%) trout farm was a relatively low EI level recorded. The maximum values of invasion intensity approximately corresponded to those of extensiveness. However, the highest minimum values were observed only in the Goranboy region. This fact indicates the presence of a serious outbreak of this disease in the country. So, the urgent implementation of the treatment and preventive measures described in this study is required.

Recommendations

1. For prevention of new outbreaks of acanthocephaliasis prohibiting of the fish capture from trout farms is recommended.

2. Close-meshed water pipes should be installed for prevention infiltration of crustaceans infected by thorny-head worms to fishponds and canals.

3. The internal organs of fish must be utilized in accordance with biosecurity standards adopted in aquaculture.

4. In fry feeding nutritional supplements, enhancing the immunity can be used.

5. Fish farmed for reproductive and commercial purposes can be a main source of invasion. Thereby, attention should be paid to preventive and sanitary measures in trout farms.

6. Drug is used early in the morning for 14 days (before the first feeding). Pieces of spleen, liver and flour are added to food. The mixture is sprayed in equal quantities in different parts of the ponds or canals or placed in specially prepared containers. The evaluation of the drug efficiency is determined after ten days by repeating tests.

7. The disinfection of the bottom of pond before filling it with 0.25% sodium hypochlorite solution (containing 8% chlorine) for 50 minutes at the rate of 1 liter per m^2 of area is recommended.

Conclusions

During the period of investigation, an epizootic situation in trout farms was studied. The highest level of extensiveness and intensity of invasion was recorded in the Goranboy trout breeding farm (71% and 24-66 parasite specimens/fish). In other farms, level of above-mentioned rates was feebly marked. Analysis of correlations between the extensiveness and intensity of invasion by acanthocephaliasis allows to conclude: the epidemiological situation in trout breeding farms of the Republic of Azerbaijan is unfavorable.

A test of the anthelmintic drug Tetramizole 20% showed its high efficiency in the treatment of acanthocephalosis, giving 100% therapeutic potency. The drug was adopted in trout farms in Azerbaijan. Now it is currently widely used by farmers.

Acknowledgments. The author thanks Dr. Mehdi Ali (Azerbaijan Scientific Research Institute of Veterinary, Azerbaijan) for helps during some laboratory studies. Besides, by farmers and owners of trout farms were established all conditions for successfully implementation of this investigation.

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Работа поступила
в редакцию 07.03.2024 г.

Принята к публикации
15.03.2024 г.

Ссылка для цитирования:

Suleymanova A. The Species Composition and Prevalence of Thorny-Headed Worms (Acanthocephala: Echinorhynchidae, Neoechinorhynchidae and Pomphorhynchidae) in Trouts, Complex Measures to Control Them in Fish Farms in the Territory of Azerbaijan Republic // Бюллетень науки и практики. 2024. Т. 10. №4. С. 163-171. https://doi.org/10.33619/2414-2948/101/24

Cite as (APA):

Suleymanova, A. (2024). The Species Composition and Prevalence of Thorny-Headed Worms (Acanthocephala: Echinorhynchidae, Neoechinorhynchidae and Pomphorhynchidae) in Trouts, Complex Measures to Control Them in Fish Farms in the Territory of Azerbaijan Republic. *Bulletin of Science and Practice, 10*(4), 163-171. https://doi.org/10.33619/2414-2948/101/24