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THE ROLE OF AMPHIBIANS IN MAINTAINING PARASITIC ZOONOSES (TREMATODOSIS) IN FISH IN THE REPUBLIC OF MOLDOVA

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Abstract

The paper presents data on the identification of the helminth fauna structure of ecaudata amphibians from Pelophylax and Bufo genera, and the determination of its role in maintaining parasitic zoonoses (trematodosis) in fish in the Republic of Moldova. As result of helminthological investigations 4 helminths species was established: Opisthioglyphe ranae Froelich, 1791; Tylodelphys excavata Rudolphi, 1803; Isthmiophora melis Shranch, 1788 and Neodiplostomum major Dubinina, 1950. This trematode species from a taxonomic point of view fall into a class (Trematoda), 3 orders (Plagiorchiida, Echisnostomida, Diplostomida), 3 families (Omphalometridae, Echinostomatidae, Diplostomidae) and 4 genera (Opisthioglyphe, Tylodelphys, Isthmiophora, Neodiplostomum). All this species of helminths comune in amphibians and fish, species of trematodes, for the fish are a negative impact, because causing various zoonosis. The need to write such a paper is due to the fact that the study of ichthyoparasites in the Republic of Moldova was carried for a long period of time (since 1963), but at the same time there was no discussion about the groups of organisms that contribute to the maintenance of the causative parasitic agents of various trematodes.

Key words*: amphibians, fish, Moldova, parasitic zoonosis.*

INTRODUCTION

Several causative agents of infectious diseases, including bacteria, viruses, parasites, and fungi, can be transmitted from animal to to another through different routes, including penetration through wounded or abrasive skin, ingestion, animal bites, vectors (insects), and animal-to-human contact (inhalation of respiratory particles or skin/mucous membrane contact) (Gauthier, 2015; Rahman et al., 2020). The pathogens that usually exist in animals can infect another animals, or humans either directly or via a vector (Wolfe et al., 2007).

Within aquatics, the general perception is that there are few zoonotic diseases considered as important (Shamsi, 2019). For those that are detected, the number of cases per year is small compared to other zoonotic diseases in animals or humans. While this might be correct, there is a possibility that this is an underestimate due to poor awareness and lack of monitoring and surveillance. However, for those that are diagnosed, the consequences can be severe, including death of host (Zorriehzahra & Talebi, 2021).

Emergence of zoonotic agents is a serious threat to global health and causes great damage worldwide (World Health Organization (WHO), 2021)

Amphibians can serve as a source of exposure to zoonotic agents for fishes. Although little published information is available regarding transmission of diseases or of parasitic agents from amphibians to fishes, several organisms have been identified as potential concerns, less amphibians. Awareness of the hazards of the formation and maintenance of a parasitic zoonosis in an ecosystem, require a sustainable helminthological management of all animal species, especially in amphibians, which can reduce sure the risk of infection fish from amphibians.

MATERIALS AND METHODS

Observation, collecting and obtaining data on the anurans from *Pelophylax* (*Pelophylax ridibundus* Pallas, 1771; *Pelophylax lessonae* Camerano, 1882; *Pelophylax esculentus* Linnaeus, 1758) and *Bufo* (*Bufo bufo* Linnaeus, 1758; *Bufo viridis* Laurenti, 1768) genera was

performed in the area of the Republic of Moldova.

The helminthological analysis of biological samples was performed according to the standard method proposed by K.I. Skrjabin, which involves the examination of all the internal organs of the animal (Moravec & Skorıkova, 1998). Helminthological research of the parenchymal organs was performed with the help of compressors, and the digestive tract - by successive washes. The collection, fixing, determination and processing of the helminthological material was carried after the methods proposed by various authors (Koprivnikar et al., 2006; Koprivnikar & Poulin, 2009; Krone & Streich, 2000; May & Anderson, 1983; Moravec & Kaiser, 1994; Nickol, 1985; Okulewicz, 2008).

The determination of the helminthological material was performed according to standard methods (Ryzhikov et al., 1980).

In order to quantify the characteristic of helminthes contamination, the intensity indexes (II, specimens) was calculated - the minimum and maximum number of parasites of a species and the extent of invasion (EI, %) - the percentage of host contamination by a parasite species.

RESULTS AND DISCUSSIONS

Amphibians are one of the main components of the biocenosis, which directly contributes to the formation of parasitic zoonoses in fish. This important role attributed to amphibians in the formation and maintenance of various zoonotic agents specific to hydrobionts (fish) at least for a single developmental stage of helminths, egg, and larvae, adult is thanks to the fact that amphibians are a group of animals with an amphibiont way of life, thus populating both terrestrial and aquatic habitats. Due to this way of life of amphibians, for the entire annual life cycle (*Pelophylax* genus) or for part of the life cycle, the stage of lava development, until maturity, and the reproduction and laying of eggs every year must be in the aquatic environment (*Bufo* genus) are veridical transmitters of various groups of parasitic agents to various groups of hydrobionts, especially fish. As established by Aho (1990), because amphibians have invaded a great variety of habitats and exhibit numerous patterns of life histories, reproductive modes, body size, and trophic relationships, they are excellent systems for exploring patterns and processes that influence the organization of helminth communities. By acting as generalist predators and as preys, amphibians play a role as intermediate and definitive hosts of a great variety of parasites in the aquatic and terrestrial food chains (Koprivnikar et al., 2012).

According to the helminthological investigations performed on amphibians from *Rana*, *Pelophylax* and *Bufo* genera, in area of the Republic of Moldova, the presence of 4 trematodes species specific to fishes was established: *Opisthioglyphe ranae* Froelich, 1791; *Tylodelphys excavata* Rudolphi, 1803; *Isthmiophora melis* Shranch, 1788 and *Neodiplostomum major* Dubinina, 1950. This trematode species from a taxonomic point of view fall into a class (Trematoda), 3 orders (Plagiorchiida, Echisnostomida, Diplostomida), 3 families (Omphalometridae, Echinostomatidae, Diplostomidae) and 4 genera (*Opisthioglyphe, Tylodelphys, Isthmiophora, Neodiplostomum*).

Tylodelphys excavata is a species, wich is characterized by the trixenic life cycle, with the obligatory participation of 3 hosts: 1 intermediate host, 2 intermediate host and the definitive host. Marita of *Tylodelphys excavata* parasitizes the intestines of storks of the genus *Ciconia*, which are also their obligate definitive hosts. *Planorbarius corneus* mollusk species, in the life cycle of this trematode, are first intermediate hosts, in whose body cavities the stage of cercar develops (Erhan & Gherasim, 2022; Szidat, 1935).

The metacercar of *Tylodelphys* sp. is a specific parasite freshwater fish (second intermediate host) and parasitizes in the vitreous body of the eyes.

On the territory of the Republic of Moldova, trematodes of the *Tylodephys* genus were detected in the following fish species: *Tinca tinca, Scardinius erythrophthalmus* and *Silurus glanis*. In these fish species, from 5 to 11 trematodes were recorded in one specimen.

According to the helminthological investigations carried out in amphibians, the intensity of invasion with this trematode species was established from 49 to 97 specimens in an individ (Table 1).

According to the helminthological research carried in fish, infestation was recorded in 27.3% of cases, and in amphibians, infestation was recorded in 52.8% of cases (Figures 1, 2).

Table 1. The intensity of invasion with trematodosis common to amphibians and fish

No.	Invazion	Amphibians	Fish	Amphibians	Fish	Amphibians	Fish
		Minimum		Maximum		Medium	
1.	*Tylodelphys* spp.	49	5	97	11	75	8
2.	*O. rane*	2	2	98	10	50	6
3.	*I. melis*	6	1	42	13	24	7
4.	*N. major*	1	6	6	57	3.5	31.5

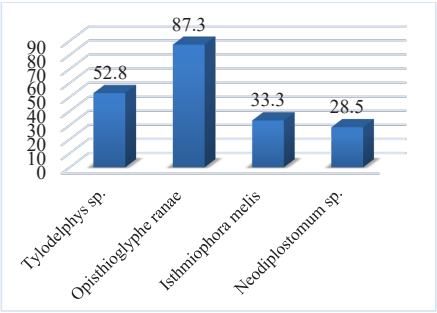

Figure 1. Parasitological indices of amphibians

Larvae of *Tylodelphys* Diesing, 1950 are major digenean pathogens of fish and amphibians. *Tylodelphy*s spp. may induce mass mortality of fish and increase their susceptibility to predation. Even though *Tylodelphys* spp. cause substantial damage to aquaculture systems, surprisingly little is known regarding the taxonomy of this commercially important genus with a limited number of visible autapomorphic identification features.

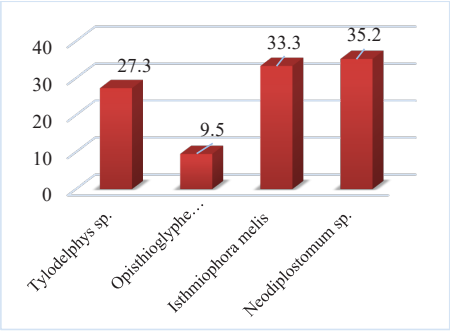

Figure 2. Parasitological indices of fish (after Maritz, 1964; Moshu, 2014)

Opisthioglyphe ranae Fröhlich, 1791 trematode is widespread in amphibians. The first intermediate host are molluscs: *Lymnaea stagnalis, L. limosa, Galba palustris*, less often - *Radix ovata* and *R. auricularia*. Their infestation occurs by ingesting eggs containing miracidia. Sporocysts are formed in the snails, which produce cercariae. They are removed from the molluscs mainly in the morning hours. The second intermediate host is the larvae of amphibians, dragonflies, coleopterans and aquatic snails (mainly from the family Limnaeidae) in whose bodies, in the period of 6-10 days, metacercariae are formed.

Infestation of amphibians occurs through ingestion of molluscs, but also in cases of cannibalism. The infestation of fish with this trematode species is due to the fact that amphibians eliminate the eggs of this trematode in the aquatic environment - an environment strictly specific to fish, and the fish use intermediate hosts infected with this trematode species in their diet.

In our country, the trematode *Opisthioglyphe ranae* was determined by A. Moșu and Sokolov S. (2013) in the *Percuttus gleni* Dybowschi, 1877 fish species, in Cahul Lake and the affluents of the Prut River - the Draghiște River and the Racovați River, where a prevalence of invasion was in 9.5% of cases (Figure 2).

In amphibians, the trematode species *Opisthioglyphe rane* is a species frequently encountered both in juveniles and in adults. When evaluating the obtained data, an intensity of invasion with this trematode species was established from 2 specimens in young amphibian forms to 98 specimens in an adult individual (Table 1), and the prevalence of invasion was recorded in 87.3% of cases (Figure 1).

Isthmiophora melis Schrank, 1788 is a species, wich is characterized too by the trigenic life cycle, with the obligatory participation of 3 hosts: 1 intermediate host, 2 intermediate host and the definitive host.

The first intermediate host is the freshwater mollusc *Lymnaea stagnalis*.

The second intermediate hosts are fish (*Carassius auratus*) and amphibians (*Pelophylax esculentus*).

The list of definitive hosts includes over 30 species of mammals including dogs, cats,

hedgehogs, otters, martens, rats, pigs, badgers, foxes and humans.

According to the helminthological data carried on fish on the territory of our country, it was found that fish are infected with *Isthmiophora melis* species in 33.3% of cases, and the number of specimens in an individual varies from 1 to 13 specimens (Maritz, 1963; Maritz, 1964; Moshu, 2014) (Table 1, Figure 2).

In the investigated amphibians, a prevalence of invasion was recorded in 33.3% of the cases, and the intensity of the invasion was recorded from 6 to 42 specimens in the specimen (Table 1, Figure 1).

Another trematodosis specific to both amphibians and fish is *Neodiplostomum major* Dubinina, 1950.

Neodiplostomum major species is a specific parasite of diurnal birds of prey. The intermediate hosts are the gastropod species *Planorbis planorbis* and *Planorbcirius comeus*.

Amphibians and fish serve as reservoir or paratenic hosts. Be that as it may, however, the fact remains that amphibians, as intermediate hosts, play a very clear role in the circulation of helminths and serve as a source of invasion of this group of fish parasites, but of the definitive hosts with this species of trematodes.

Moreover, the infection of amphibians occurs more frequently in their larval stage. The trematode has a wide range of reservoir hosts, which includes reptiles, birds and mammals.

This trematode parasitizes in the eyeball, often in the lens, less often in the vitreous body of fish. In fish, their infection can reach up to 35.2% of cases, and in one fish they can be found from 6 to 57 specimens (Moshu, 2014) (Table 1, Figure 2).

The losses caused by neodiplostomosis occur not only as a result of the death of fry, the deterioration of the body mass of sick fish and the quality of the meat, but also of their consumption by ichthyophage birds, which easily capture malnourished, cataract-affected fish (Mishanin, 2012; Novak, 2010).

In amphibians, this trematodosis was found only during the summer, with a prevalence of 28.5% of cases, and in one host can be found from 1 to 6 copies (Table 1, Figure 1).

These hazardous amphibians parasitic agents outbreaks reported (Table 1) indicate the importance of monitoring amphibians-derived zoonotic diseases.

All this species of helminths comune in amphibians and fish, species of trematodes (*Tylodelphys excavata, Opisthioglyphe rane, Isthmiophora melis, Neodiplostomum major*), for the fish are an negative impact, because causing various zoonosis.

Thanks to the determination of the presence of these species of trematodes in amphibians, their role in the formation and maintenance of outbreaks of fish parasitic zoonoses is demonstrated, and the massive losses caused by helminthosis are determined by their mass spread and affecting a large number of fish.

These losses can be expressed through the following causes: the mass death of fish, especially the fry; in many helminthiasis the fish is cachexic, remains undeveloped, stagnating the process of obtaining fish production.

Fish acquired these parasites while feeding on benthic invertebrates (mollusks, aquatic insect larvae, leeches) and free-swimming cercariae.

Therefore, as can be concluded from the above data, the circulation of helminths from a structural and functional point of view is a complex dynamic process, based especially on intermediate hosts (cercari host, metacercari host). With their participation, additional channels are formed, through which a large part or even the entire flow of invasive larvae from intermediate hosts and unique duplicate systems is created (Sharpilo, 1979). Because of them, a number of helminth species could apparently survive significant historical periods and reach our time from other geological epochs, despite the elimination of the primary definitive hosts by natural selection.

The high degree of infestation with these trematodosis specific to fish, demonstrates the role of amphibians as eliminators of the invasive stages of helminths with a dangerous impact both for fish and for human health. The probability of reducing the number of amphibians certainly extends to the increase the degree of infestation with various trematodosis in fish, but other animal species (eggs, larvae, adults).

CONCLUSIONS

It has been studied the helminth fauna of amphibians in the *Pelophylax* and *Bufo* genera and the determination of its role in maintaining parasitic zoonoses (trematodosis) in fish in the Republic of Moldova.

As result of helminthological investigations 4 helminths species was established: *Opisthioglyphe ranae* Froelich, 1791; *Tylodelphys excavata* Rudolphi, 1803; *Isthmiophora melis* Shranch, 1788 and *Neodiplostomum major* Dubinina, 1950. This trematode species from a taxonomic point of view fall into a class (Trematoda), 3 orders (Plagiorchiida, Echisnostomida, Diplostomida), 3 families (Omphalometridae, Echinostomatidae, Diplostomidae) and 4 genera (*Opisthioglyphe, Tylodelphys, Isthmiophora, Neodiplostomum*).

All this species of helminths comune in amphibians and fish, species of trematodes, for the fish are a negative impact, because causing various zoonosis.

In this sense, the role of amphibians in the formation and maintenance of outbreaks of fish parasitic zoonoses was demonstrated, and the massive losses caused by helminthosis are determined by their mass spread and affecting a large number of fish species.

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