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Anurans (*Amphibia*) - VECTORS OF THE PARASITIC AGENTS TO WILD AND DOMESTIC ANIMALS IN MOLDOVA

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Abstract

In this scientific work are exposed data with reference to the description of the diversity of the helminthic fauna of ecaudata amphibians from Ranidae and Bufonidae families, and the determination of its role as vectors for various groups of helminths to wild and domestic animals. As result of helminthological investigations during 2020-2022 years, 17 helminths species (secernentea, trematoda, palaeacanthocephala) was established: H. variegatus Rudolphi, 1819; G. varsoviensis Sinitzin, 1905;C.urniger Rudolphi, 1819; P. robusta Szidat, 1928; P. brumpti Buttner, 1951; P. confusus Looss, 1894; T. excavata Rudolphi, 1803; D. subclavatus Pallas, 1760; O. ranae Froelich, 1791 S. falconis Szidat, 1928; H. cylindracea Zeder, 1800; P. medians Olsson, 1876, C. ornata Dujardin, 1845; O. filiformis Goeze, 1782; I. neglecta Diesing, 1851; S. lupi Rudolphi, 1809; A. ranae Schrank, 1788). Of the 17 helminth species detected in amphibians, a special importance is attributed to the presence of 4 species of medical-veterinary importance helminths (Spirocerca lupi, Codonocephalus urniger, Parastrigea robusta, Strigea falconis), which can cause sterility, parastrigeosis and strigeosis in birds, as well as spirocercosis in dogs, foxes, wolves and occasionally in goat, horse, cattle and other.

Key words*: anurans, Moldova, parasitic agents, vectors, wild and domestic animals.*

INTRODUCTION

In recent decades, the effect of anthropogenic factors on natural ecosystems has been significantly highlighted. One of the consequences of this process was certain changes in the parasitic cenosis of amphibians. During the anthropogenic transformations of the environment, the biological diversity of communities is reduced, their structure is simplified, and a number of ecological niches are released or destroyed. At the same time, invasive species appear, carrying with them parasitic complexes unusual for these ecosystems. This creates a potential risks of zoonotic invasions, such as cercariosis, alaryosis, spirocercosis, etc.

Thus, the helminhstudy of the parasitic fauna and the structure of invasive species is very relevant, because the amphibians can be serve as intermediate hosts (Bolt et al., 1993; Gonz´alez & Hamann 2007; Jackson & Tinsley, 1998; King et al., 2007; Moravec & Kaiser, 1994) or as paratenic hosts (Iacob, 2019; Eberhard & Brandt, 1995; Gonz´alez & Hamann, 2007; Jackson & Tinsley, 1998; Moravec & Skorıkova, 1998; Nickol, 1995), for a wide variety of helminths specific to the vertebrates.

The helminthological and ecological study of amphibians from various types of ecosystems, depending on the degree of synanthropization and contact with other species of invertebrate and vertebrate animals allow the establishment of the parasitological condition, some characteristics in the pathogenesis of the formation of outbreaks of parasitic agents and the elaboration of biological measures to control and prevent the parasitic diseases in productive animals, because in the structure of the helminth fauna of amphibians are some helminths species that are antagonistic to other helminths species specific to the animals (Gherasim, 2016).

The importance of such research is very high, developing a conceptual framework about particularities in helminthology populations of anurans throughout the annual life cycle that will lead to the possibility to identification of new helminths species in the fauna of Moldova, but also, elaboration of new effective and

harmless methods of biological control of some helminths specific to productive animals.

MATERIALS AND METHODS

The study of anurans from Ranidae (*Rana dalmatina* Fotzinger in Bonaparte, 1839; *Pelophylax lessonae* Camerano, 1882; *Pelophylax ridibundus* Pallas, 1771; *Pelophylax esculentus* Linnaeus, 1758) and Bufonidae (*Bufo bufo* Linnaeus, 1758; *Bufo viridis* Laurenti, 1768) families was performed in aquatic and terestrial ecosystems from Moldova.

Helminthological research in amphibians was carried out according to the specific research methodology, which involves the examination of all the internal organs of the amphibians (Moravec & Skorıkova, 1998). The obtaining, collection, processing, determination and fixing 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, 1995; Okulewicz, 2008).

After collection, fixing and processing, the assembly of the helminthological material is carried out using paraffin rings according to the method proposed by Seinhorst (1959) and described by Erhan & Gherasim (2022).

In order to assess the risk of vectorization of dangerous helminth species for other groups of animals, the main parasitological indices (II, specimens; EI, %) of helminth species were evaluated depending on the host species.

RESULTS AND DISCUSSIONS

Amphibians as definitive, intermediate, complimentary and reservoir hosts, for various species of parasites, are "vector" organisms which, being obligatory in the development of parasites, constitute the favorable environment for the penetration, development and conservation of evolutionary forms of parasitic agents.

Amphibians are a category of biological vectors that, moving from the aquatic environment to the terrestrial one, ensure development and multiplication, at least for one biological stage, which gives them the role of source of a pathogen and, at the same time, transmits a variety of parasitic forms. They have a special role in the contamination of areas favorable to certain parasites and participate directly in the formation of parasitic zoonoses.

According to the investigations of amphibians from Ranidae (*Pelophylax ridibundus, P. lessonae* , *P. esculentus, Rana dalmatina*) and *Bufonidae* (*Bufo bufo*, *Bufo viridis*) families in Moldova, 17 species of helminths were identified: *Haematoloechus variegatus, Codonocephalus urniger*, *Opisthioglyphe ranae, Paralepoderma brumpti, Prosotocus confuses, Tylodelphys excavate, Diplodiscus subclavatus, Parastrigea robusta, Strigea falconis, Gorgodera varsoviensis*, *Haplometra cylindracea, Pleurogenoides medians, Cosmocerca ornate, Oswaldocruzia filiformis, Icosiella neglecta, Spirocerca lupi,* and *Acanthocephalus ranae*, which from a taxonomic point of view fall into 3 classes, 7 orders, 16 families and 17 genera.

4 of the 17 species of helminths determined in amphibians species previously mentioned, cause various parasitosis common to wild, domestic and pet animals.

In the context of the development of science and the increase of the diversity of animals helminthologically investigated in our country, it is certain that the number of species that are registered in wild, domestic animals and to humans has increased in the last 10 years.

The parasitic diseases of animals (domestic, wild) include spirocercosis, which is caused by the *Spirocerca lupi* Rudolphi, 1809 nematode. This disease is spread all over the world, but in Moldova this nematode, which causes the disease, was detected for the first in 2019 in the southern area.

This species of nematode forms spirocercosis in carnivores (dog, fox, wolf), and accidentally in goats, horses, cattle, pigs, etc., it is located in the esophagus, clinically characterized by digestive, cardiovascular and general disorders (Iacob, 2019).

Another species of helminths with veterinary medical importance found in the investigated amphibians is *Codonocephalus urniger* Rudolphi, 1819 - a trematode with trixene life cycle.

The species of aquatic snails *Lymnaea stagnalis* and *L. palustris* are the intermediate hosts, but the amphibians for this trematode are the complementary hosts. In their body parasitize the larval forms, in the stage of metacercariae of the trematode *Codonocephalus urniger*. The species of birds as *Botaurus slellaris*, *Ixobrychus minutus*, *Ardea purpurea*, *Egretta garzetta* et al, are the definitive hosts. The infection of amphibians occurs from the tadpole stage and ends with adult specimens. The metacercariae has a pathogenic action on the hosts in the sense that in the strong infestations of the genital glands of amphibians with this parasite, total castration is found.

Amphibians stop laying eggs and does not show a reproductive instinct any more. The infested ovary has a dirty yellowish colour and is very small in volume.

Parastrigea robusta Szidat, 1928, from the Strigeidae family is another species of trematode with veterinary medical importance that was detected in amphibians in the muscles and less often on the mesentery. The larval form of this species (metacercarie) is also found in fish (*Abramis brama*, *Atherina mochon pontica*, *Alburnus alburnus* and other). The adult forms are parasitic in the intestines of herons and of day predators, especially of the Ardeiformes order - *Ardea cinerea, A. purpurea*. The infection of birds with trematode of *Parastrigea robusta* species, this is causes parastrigeosis.

The *Strigea falconis* Szidat, 1928 is a trematode species similar to family Strigeidae that was found in amphibians under the muscular fascias around the neck, chest, legs, under the serosa of the esophagus and goiter, in the connective tissue between the trachea and esophagus, under the skin of the neck, chest and legs.

Adult forms parasitize in the bird intestines of different orders: Falconiformes, less often Strigiformes and accidentally Passeriformes (*Oriolus melanocephalus*), Galliformes (*Meleagris gallopavo*), Gharadriiformes (*Charadrius dubius*) and Columbiformes (*Streptopelia chinensis*) causing Strigeosis. The larval forms of meso- and metacercariae are found in birds of the Ardeiformes, Columbiformes, Ralliformes, Steganopodes, Anseriformes, Charadriiformes, Lariformes, Falconiformes, Coraciiformes, Galliformes, Strigiformes, Piciformes, Passeriformes orders, as well in amphibians *P. lessonae* species.

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

The metacercar of *Tylodelphys excavata* is a specific parasite freshwater fish (2intermediate host) and parasitizes in the vitreous body of the eyes.

Of all the investigated amphibian specimens from the Ranidae family, the *P. ridibundus* species (n = 45) was established to be the most common, and assessing the diversity of their helminth fauna, the helminth infestation in 75.0% of cases was established, among which in the aspect of mono invasion in 25.0% of cases, and in aspect of poly invasion in 50.0% of cases (Figure 1).

In the investigated *P. lessonae* specimens (n = 19), it was found that 69.3% of cases were infested, all of which were in the aspect of polyinvasion, while in their hybrid - *P. esculentus* (n = 16), the infestation in 68.0% of cases was established, of which in the aspect of monoinvasion - 18.7% of cases, and in the aspect of polyinvasions the infestation in 49.3% of cases was established, and in the specimens *R. dalmatina* (n = 12), the infestation was registered in 52.0% of cases, of which monoinvasions in 15.8% of cases and respectively 36.2% of cases in terms of polyinvasions (Figure 1).

At the investigated amphibians of the Bufonidae family (*Bufo bufo* n = 30, *B. viridis* n = 11) was established that the infestation predominates in the same way in aspect of polyinvasions, thus in the *Bufo bufo* species the infestation in 88.0% of cases was established, of which in aspect of monoinvasions in 24.0% of cases and polyinvasions in 64.0% of cases, and in *B. viridis* the species the infestation in

85.0% of cases was established, of which in aspect of monoinvasions in 35.0% of cases and respectively 50.0% of cases in terms of polyinvasions (Figure 1).

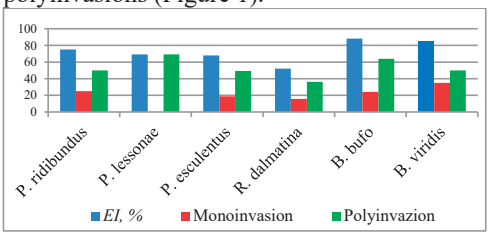

Figure 1. Parasitological indices of amphibians in mono - and polyinvasion aspect

The analysis of the helminthological data obtained during the spring-summer-autumn seasonal period allowed us to establish the nature of the distribution of helminths depending on the host and the research area, with a particularly important value, which contributes to the determination of the seasonal dynamics of the formation of parasitic zoonoses. Of all species of helminths detected in amphibians, 4 species of trematodes (*Tylodelphys excavata, Codonocephalus urniger, Parastrigea robusta, Strigea falconis*) and one species of nematode (*Spirocerca lupi*) are of medical-veterinary importance, causing tylodelphyosis (blindness) in fish, codonocephalosis, parastrigeosis, strigeosis in birds and spirocercosis of insectivorous mammals and canids, respectively.

When assessing the degree of helminth infestation of amphibians, taking into account the area and time of detection of helminths, the age of the host and the parasite, it is possible to assess the period, stations and places of infection of the definitive hosts (fish, reptiles, birds, mammals), which is particularly important for determining the role of amphibians as vectors in the formation and maintenance of outbreaks of parasitic zoonoses. Thus, for the trematode species *Tylodelphys excavata*, *Parastrigea robusta* and *Strigea falconis*, which were determined only in the southern part of the country, the most favorable vectorization period is autumn, for the nematode species *Spirocerca lupi* - summer, but for the trematode species *Codonocephalus urniger* - spring and summer, respectively, which were determined in the center and southern part of the country. Although these data are remarkable for the high abundance of the invasion, only in the spring period were all 5 species of helminths of medical-veterinary importance established (Table 1).

The causes of differences in the degree of infestation of amphibians with helminthes in different biotopes are complex. Thus, the faunal diversity in biotopes populated by amphibians depends of the high abundance of definitive, intermediate, paratenic hosts (mollusks and insects), which determine the degree of infestation with helminthes transmitted through the food chain. The high density of amphibians themselves, as a result, batrachophage predators, leads to their intensive infestation with helminthes in the larval stage.

Table 1. The degree of infestation with helminthes of medical veterinary importance depending on the seasonal period

No.	Invasion	Seasonal period		
		Spring	Summer	Autumn
1.	*Tylodelphis excavata*	40.00%	-	59.50%
2.	*Parastrigea robusta*	5.00%	-	33.30%
3.	*Strigea falconis*	10.00%	42.40%	88.10%
4.	*Codonocephalus urniger*	23.80%	73.90%	-
5.	*Spirocerca lupi*	25.00%	87.90%	-

In addition to the fact that amphibians vector various species of helminths to vertebrate animals (domestic, pet, wild), amphibians also vector species of helminths to invertebrates. In this study, we established that in the case of vectorization of helminthic elements to invertebrate by amphibians, we found that the presence of the trematode species *H. cylindracea* is antagonistic to the Fasciola hepatica species, which causes fasciolosis in farm animals.

In the Republic of Moldova, this disease is quite widespread, and veterinary specialists apply medicinal methods to combat this species of trematodes that cause considerable damage.

It should be noted that these methods have a series of shortcoming: drug treatment involves expenses and consists of the toxic and immunosuppressive action of the antiparasitic preparations on the animal organism treated. At the same time, the way of using these preparations orally presents a difficulty for administration to a large number of animals. As a result of deworming through the

administration of anthelmintics, after a short period of time, the parasitic forms (eggs) are eliminated again in the external environment (water, vegetable mass), which then contribute to the infestation and re-infestation of the animals.

The role of amphibians in the biological control of ruminant fasciolosis is explained by the fact that they are definitive hosts of the trematode species *Haplometra cylindracea* which in the cercariae stage parasitizes in the snail species *Lymnaea truncatula* - intermediate hosts for the *species Fasciola hepatica*.

These antagonistic relationships between trematodes *Fasciola hepatica* and *Haplometra cylindracea* trematodes we have realized both in field conditions and in the laboratory conditions. Initially, *Fasciola hepatica* and *Haplometra cylindracea* miracidia were obtained. To achieve the proposed goal, adult fascioles were collected from animals slaughtered at the slaughterhouse, from which eggs were obtained. In the thermostat at a temperature of 24-26^{0}C, over 10 days, from the eggs *Fasciola hepatica* miracidia were obtained.

The miracidia of *Haplometra cylindracea* were obtained by examining all the internal organs of amphibians (*Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis*) applying the standard method proposed by the academician Ryzhikov et al. (1928). *Haplometra cylindracea* specimens were kept alive in a thermostat, at a constant temperature of 37^{0}C, in physiological solution.

In order to achieve the method of combating fasciolosis in the laboratory conditions, the experiments were carried out in 3 glass vessels of identical sizes and according to the same scheme.

In the first glass vessel, with a volume of 0.5 liters of water, 50 miracidia of *Fasciola hepatica* and 50 miracidia of *Haplometra cylindracea* were introduced, in a ratio of 1:1.

In the second glass vessel, with a volume of 0.5 liters of water, 50 miracidia of *Fasciola hepatica* and 25 miracidia of *Haplometra cylindracea* were introduced, in a ratio of 2:1.

In the third glass vessel, with a volume of 0.5 liters of water, only miracidia of *Fasciola hepatica* (control batch) were introduced.

During 14 days, at regular time intervals (T = 24 h), the number of *Fasciola hepatica* and *Haplometra cylindracea* miracidia was calculated.

During the experiments, in vessels no. 1 and no. 2 significant results were obtained starting from the second day of contact between *Fasciola hepatica* and *Haplometra cylindracea* species.

As a result, in vessel no. 1, in which the species were introduced in a 1:1 ratio, *Fasciola hepatica* miracidia were absent at the 8th day of research. In vessel no. 2, in which the species were introduced in a ratio of 2:1, *Fasciola hepatica* miracidia were absent at the 9th day of research. In vessel no. 3 (control), there was practically no numerical change in the *Fasciola hepatica* miracidia, a fact that demonstrates its presence in the absence of the antagonistic species *Haplometra cylindracea*.

The results of the experiments are presented in Table 2.

Table 2. The result of the antagonistic action between *Haplometra cylindracea* on *Fasciola hepatica* miracidia

No. day	Vessel no. 1		Vessel no. 2		Vessel no. 3	
	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*	No. of miracidia of *F.hepatica*	No. of miracidia of *H.cylindracea*
1	50	50	50	25	50	-
2	49	50	48	25	50	-
3	32	50	40	25	50	-
4	24	50	35	25	50	-
5	16	50	22	25	50	-
6	7	50	14	25	50	-
7	3	50	8	25	50	-
8	0	50	2	25	50	-
9	0	50	0	25	50	-
10	0	49	0	25	50	-
11	0	49	0	25	50	-
12	0	49	0	25	49	-
13	0	49	0	24	49	-
14	0	49	0	24	49	-

The experiences in the field were carried in the private cattle breeding farm of SRL "STRAPID", Călăraşi district.

In order to achieve the proposed goal, 100 heads of cattle, aged 3-5 years, were helminthologically investigated by the coprological method - Darling. As a result of these investigations, it was established that cattle were infected with *Fasciola hepatica* in 35.0% of cases.

In the spring, in the household favorable to fasciolosis, the land where the cattle were to graze, parasitological investigations were carried out and the presence of snails of *Lymnaea* genus was established. This land was divided into two lots.

The first lot - experimental, in which 1000 specimens of miracidia of the *Haplometra cylindracea* species were introduced into the environment. The second lot - control, in which *Haplometra cylindracea* miracidia were not introduced.

In the same time, the cattle were divided into two lots of 50 heads each. The research was carried out on cattle over the course of the entire year 2016: in the spring - until the start of the grazing period and in the autumn - before their transition to the stable. Coprological samples were taken, in order to establish the level of fasciola infestation. The results of the experiments are presented in Table 3.

Table 3. The result of using *Haplometra cylindracea* miracidia in combating fasciolosis in ruminants

The experimental lot	**Number of cattle, n**	***Haplometra cylindracea* introduced, n**	**Level of infestation by *Fasciola hepatica* (EI - %)**	
			The fifth month	**The tenth month**
The first experimental lot	50	1000	17.0%	19.0%
The second control lot	50	-	18.0%	84.0%

During the research period of the level of infestation with Fasciola hepatica in the cattle that grazed on first Lot and second Lot, different values were established.

Therefore, during the 5th month, the cattle that grazed on Lot no. 1 and Lot no. 2 with *Fasciola hepatica* in 17.0% and 18.0% of the cases, respectively were infected. Contrary to the research carried out during the 5th month, in the 10th month, in the cattle that grazed on Lot no. 1, in which of the *Haplometra cylindracea* miracidia were introduced, a maintenance of the level of infestation with *Fasciola hepatica* was established (19.0 % of cases), while, in the cattle that grazed on Lot no. 2 (the control lot), in which *Haplometra cylindracea* miracidia were not introduced, the level of infestation of the cattle with *Fasciola hepatica* increased considerably and their infestation was established in 84.0% of cases.

Thus, in order to reduce the risk of contamination of domestic and wild animals with fasciola, it is effective to implement this previously described method, which consists in the biological elimination of fascioliasis in ruminants by introducing amphibians (Amphibia: Anura) which are infested with the trematode *Haplometra cylindracea* in a biotope that is favorable to the development of the trematode *Fasciola hepatica*. This is a low difficulty method, without the toxicity and immunosuppressive action of chemicals, and with minimal expenses.

The advantages of the biological strategies for the dehelmintization of ruminants attributes a new qualitative effect, which allows to increase the productivity and viability of cattle. At the same time, the method can be applied for combating fascioliosis in all affected biotopes.

CONCLUSIONS

It has been studied the helminth fauna of amphibians in the Ranidae and Bufonidae families and has been established by 17 species of helminths: *Haematoloechus variegatus*; *Codonocephalus urniger*; *Opisthioglyphe ranae*; *Paralepoderma brumpti*; *Prosotocus confusus*; *Tylodelphys excavata*; *Diplodiscus subclavatus*; *Parastrigea robusta*, *Strigea falconis*; *Gorgodera varsoviensis*; *Haplometra cylindracea*; *Pleurogenoides medians*, *Cosmocerca ornata*; *Oswaldocruzia filiformis*; *Icosiella neglecta*; *Spirocerca lupi*; *Acanthocephalus ranae* from 3 classes, 7 orders, 16 families and 17 genera.

The presence of 5 species of medical-veterinary importance helminths, (*Spirocerca lupi, Codonocephalus urniger, Parastrigea robusta, Strigea falconis, Tylodelphis clavata*) has been established, which can cause sterility, parastrigeosis, strigeosis in birds, as well as spirocercosis in dogs, foxes, wolves and occasionally in goat, horse, cattle and other and tylodephiosis in fish.

The degree of helminth infestation in amphibians in the aspect of mono- and polyinvasions was evaluated and it was found that in all investigated species the polyinvasion infestation predominates as a result of the

coexistence of several helminth species in the same host.

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