Pelophylax ridibundus (Amphibia: ranidae) as paratenic host of *Spirocerca lupi* species (Secernentea: spirocercidae) in the Republic of Moldova

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Abstract
The paper presents data on the identification of the helminth fauna structure of Pelophylax ridibundus species and the determination of its role as intermediate and paratenic host for various groups of vertebrate helminths. 84 specimens of Pelophylax ridibundus (26 - males, 31 - females, 27 - juveniles) from the Dniester River, border area of Talmaza village, Ştefan Vodă district, were helminthologically investigated. As result of helminthological investigations, it was established that the structure of the helminth fauna of Pelophylax ridibundus species is characterized by 13 species of helminths (Haematoloechus variegatus Rudolphi, 1819; Codonocephalus urniger Rudolphi, 1819; Opisthioglyphe ranae Froelich, 1791; Paralepoderma brumpti Buttner, 1951; Prosotocus confusus Looss, 1894; Tylodelphys excavata Rudolphi, 1803; Diplodiscus subclavatus Pallas, 1760; Parastrigea robusta Szidat, 1928, Strigea falconis Szidat, 1928; Cosmocerca ornata Dujardin, 1845; Oswaldocruzia filiformis Goeze, 1782; Icosiella neglecta Diesing, 1851; Spirocerca lupi Rudolphi, 1809), from 13 genera, 11 families (Omphalometridae, Haematoloechidae, Plagiorchiidae, Lecithodendriidae, Diplodiscidae, Diplostomatidae, Strigeidae, Cosmocercidae, Molineidae, Onchocercidae, Spirocercidae), 6 orders (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Spirurida), 2 classes (Trematoda, Secernentea) and 2 phylums (Plathelminthes, Nematoda). Of the 13 helminth species detected, a special importance is attributed to the nematode *Spirocerca lupi Rudolphi, 1809, for which the taxonomic classification, synonims, hosts, organic specificity and biological cycle are described, for which the Pelophylax ridibundus species is as paratenic host. The diversity and intensity of helminth infestation of Pelophylax ridibundus has been established*
Keywords: *Pelophylax ridibundus, Spirocerca lupi, paratenic host.*

Introduction

Helminthological research is more frequently focused on the study of the degree of helminth infection in domestic, wild, pets' animals and human. Currently, in different regions of the world several taxa of animals species are insufficiently studied helminthologically, including amphibians.

Amphibians are the most primitive terrestrial vertebrate animals, which has preserved the aquatic and terrestrial way of life, being vectors of various parasitic agents. In some cases, amphibians participate not only at the contamination of domestic and wild animals, but are an important factor in maintaining their circulation in the nature and actively participate in the formation of parasitic zoonoses [1].

In the foreground, the study of helminth fauna in amphibians is also interesting in terms of knowledge of fauna.

The study of amphibian helminth fauna, the specificity of the circulation in the natural and anthropized biotopes and their contact with the host, allow the establishment of the parasitological situation, some characteristics in the pathogenesis of the formation of outbreaks of parasitic agents and the elaboration of measures with epizootic and epidemiological impact.

In addition to the faunal importance of research, anurans are definitive hosts for several classes of helminths, including Cestoda, Monogenea, Trematoda, Secernentea and Palaeacanthocephala [1, 3-6, 8-11, 13-16, 18-20]. They also serve as intermediate hosts [7, 11, 12, 17, 19, 20] or as paratenic hosts [12, 8-10, 13, 14, 16] for a wide variety of helminths specific to the vertebrates.

It is known that the wild animals are an important source of parasites for humans and domestic animals. According to researchers from the Republic of Moldova, it was found that out

of 178 parasitic agents established in wild animals, 20 species were recorded in humans and domestic animals [21, 29].

The parasitic diseases of domestic and wild vertebrates include spirocercosis, which is caused by the nematode *Spirocerca lupi* Rudolphi, 1809. This disease is spread all over the world and in the Republic of Moldova the nematode, which causes the disease, was detected for the first time.

In the context of determining the sources of the spread of parasitosis in domestic animals, pets and humans in the Republic of Moldova, it is necessary to conduct an in-depth study of helminth fauna, especially in amphibians *Pelophylax ridibundus*, and identify its role as a paratenic host for various groups of parasitic agents.

Materials and methods

The investigations regarding the study of the helminth fauna, the determination of the degree infestation by helminth of *Pelophylax ridibundus* species were carried in the laboratory of Parasitology and Helminthology of the Institute of Zoology.

A total of 84 amphibian specimens (males - 26, females - 31, juveniles - 27) collected from the Dniester River, Talmaza village area in the Stefan-Voda district during the years 2019-2020 were helminthologically investigated.

The amphibians were determined by external characters [23].

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 [27]. 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 [22, 23, 24, 25, 26, 28]. The determination of the helminthological material was performed after standard methods [25].

To quantify the contamination characteristic by helminthes, the Intensity indice was calculated (*II, exemplars*) – the minimum and maximum number of parasites of a species and the extensivity of invasion (*EI, %*) – the percentage of host contamination by a species of parasite.

Laboratory helminthological investigations of biological samples of Peloplylax ridibundus to the presence of helminths or helminthic elements (eggs, larvae), allowed to obtain data of special value in order to determine the importance of amphibians in the formation and maintenance of outbreaks of common parasitic organisms in wild animals, pets and human.

Results and discussions

Unlike other species of green and brown frogs, *Pelophilax ridibundus*, due to its ecological plasticity is able to populate a wide range of aquatic habitats, from natural (permanent or temporary, with standing or flowing water) to moderately-polluted in lowland areas [23].

The study of helminth fauna in amphibians, the specificity of circulation in natural and anthropized biotopes and their contact with the host, allow establishing the parasitological situation, some characteristics in the pathogenesis of parasitic agent outbreaks and development of measures with epizootic and epidemiological impact.

According to the helminthological investigations performed on *Pelophylax ridibundus* from Talmaza village, Ștefan-Vodă district, the presence of 13 helminths species was established: *Haematoloechus variegatus* Rudolphi, 1819; *Codonocephalus urniger* Rudolphi, 1819; *Opisthioglyphe ranae* Froelich, 1791; *Paralepoderma brumpti* Buttner, 1951; *Prosotocus confusus* Looss, 1894; *Tylodelphys excavata* Rudolphi, 1803; *Diplodiscus subclavatus* Pallas, 1760;

Parastrigea robusta Szidat, 1928, *Strigea falconis* Szidat, 1928; *Cosmocerca ornata* Dujardin, 1845; *Oswaldocruzia filiformis* Goeze, 1782; *Icosiella neglecta* Diesing, 1851; *Spirocerca lupi* Rudolphi, 1809, which from a taxonomic point of view fall into 2 classes (Trematoda, Secernentea), 6 orders (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Spirurida), 11 families (Omphalometridae, Haematoloechidae, Plagiorchiidae, Lecithodendriidae, Diplodiscidae, Diplostomatidae, Strigeidae, Cosmocercidae, Molineidae, Onchocercidae, Spirocercidae) and 13 genera (*Haematoloechus,Codonocephalus,Opisthioglyphe, Paralepoderma, Prosotocus, Tylodelphys, Diplodiscus, Parastrigea, Strigea, Cosmocerca, Oswaldocruzia, Icosiella,Spirocerca*).

Unlike previous helminthological research conducted on the amphibians in the Central and Northern areas of the Republic of Moldova, in the southern area a differentiated helminth fauna structure was established. Thus, it has been found the presence of a new species of nematodes *Spirocerca lupi* Rudolphi, 1809 with faunal, bioindicative and veterinary medical importance Fig. 1.

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 [2].

The development of *Spirocerca lupi* species is heteroxenous. The coprophage beetles (Geotrupes, Scarabaeus) serve as intermediate hosts. They become infected through the ingestion of parasite eggs, and in their body the L_1 larvae hatch, which suffer two moults and become L_3 infestants after they are encapsulated. Infested beetles are ingested by paratenic hosts - amphibians, reptiles, birds, in whose body the nematode encapsulates again, and the infestation of the definitive hosts occurs through the consumption of paratenic hosts [2].

Fig. 1. *Spirocerca lupi* Rudolphi, 1809. Original

Spirocercosis evolves in countries with warm climates, and in our country the nematode that causes this disease - *Spirocerca lupi* was detected for the first time. For this disease the amphibians serve as a sure source of transmission, representing true reservoirs of infesting larvae.

One of the main factors that determine the infection with parasitic agents of animals is the type of biotope. Thus, the succession of climatic and anthropogenic transformations in biotopes can lead to the interruption of the biological cycles of parasitic agents and respectively to the disappearance of historically formed parasitic systems, or (rarely) can influence to the increase of helminth diversity.

Quantitative analysis of parasitological indices in *Pelophylax ridibundus* demonstrate that infestation with the trematode species *Opisthioglyphe ranae* was recorded in 69.0% of cases (*II* – 8-86 exemplars), with *Haematoloechus variegatus* in 38.1% of cases (*II* -1-10 exemplars), with *Codonocephalus urniger* in 47.6% of cases (*II* -1-30 exemplars), with *Prosotocus confusus* in 22.6% of cases (*II* -1-32 exemplars), *Diplodiscus subclavatus*în 10.9% of cases (*II* - 4-9 exemplars), with *Paralepoderma brumpti* in 39.3% of cases (*II* -1-9 exemplars), with *Tylodelphys excavata* in 36.9% of cases (*II* – 8-96 exemplars), with *Parastrigea robusta* in 7.1% of cases (*II* – 3 exemplars), with *Strigea falconis* in 3.6% of cases (*II* – 150 exemplars), with *Oswaldocruzia filiformis* in 1.2%

of cases (*II* – 1 exemplar), with *Cosmocerca ornata* in 19,0% of cases (*II* – 4-40 exemplars), with *Icosiella neglecta* in 26.2% of cases (*II* – 1-14 exemplars), but with *Spirocerca lupi* species the infestation was recorded in 21.4% of cases (*II* – 2-98 exemplars) (*Table 1*).

When evaluating the parasitological indices obtained, it has been established that the highest degree of infestation was with the species of trematode *Opisthioglyphe ranae,* and among nematodes - with *Icosiella neglecta* and *Spirocerca lupi* species.

Helminthological investigations depending of the host sex performed on 57 mature individuals (males - 26, females - 31) of *Pelophylax ridibundus* s demonstrated that the infestation degree by helminthes depends on the helminth species and the host sex. Although, the males of the species are the first individuals to come out of hibernation and have a longer contact with the environment, they were characterized by a simpler helminth structure being infested with only 7 species of helminths, of which 6 species of trematodes (*Haematoloechus variegatus*,*Codonocephalus urniger*, *Opisthioglyphe ranae, Paralepoderma brumpti, Prosotocus confusus*, *Tylodelphys excavata*) and one species of nematode (*Icosiella neglecta*) *Table 2*.

Table 1.

Parasitological indices of *Pelophylax ridibundus* species (n = 84)from the studied ecosystems

No	Invasion	*EI - %*	*II, ex.* min-max
TREMATODA			
1	*Opisthioglyphe ranae*	69.0	8-86
2	*Haematoloechus variegatus*	38.1	1-10
3	*Codonocephalus urniger*	47.6	1-30
4	*Prosotocus confusus*	22.6	1-32
5	*Diplodiscus subclavatus*	10.9	4-9
6	*Paralepoderma brumpti*	39.3	1-9
7	*Tylodelphys excavata*	36.9	8-96
8	*Parastrigea robusta*	7.1	3
9	*Strigea falconis*	3.6	150
SECERNENTEA			
10	*Oswaldocruzia filiformis*	1.2	1
11	*Cosmocerca ornata*	19.0	4-40
12	*Icosiella neglecta*	26.2	1-14
13	*Spirocerca lupi*	21.4	2-98

Unlike males, the females of *Pelophylax ridibundus* were infected by 13 species of helminthes, of which 9 species of trematodes (*Haematoloechus variegatus, Codonocephalus urniger, Opisthioglyphe ranae, Paralepoderma brumpti, Prosotocus confusus, Tylodelphys excavata,Diplodiscus subclavatus, Parastrigea robusta, Strigea falconis,*) and 4 species of nematode (*Cosmocerca ornata, Oswaldocruzia filiformis, Icosiella neglecta, Spirocerca lupi*), whose degree of infestation differs from one species to another (*Table 2*).

Another question frequently addressed in the literature is the study of helminth fauna depending on the age of host. Various authors (69, 79, 98, 128 - teza) affirm that the degree of helminth infestation increases with the age of host, but according to our investigations in the juveniles of *P. ridibundus* collected from the southern part of the republic, their infestation with 5 species of trematodes was established: (*Haematoloechus variegatus, Codonocephalus urniger,*

Opisthioglyphe ranae, Paralepoderma brumpti, Tylodelphys excavata,) whose extensivity overrun that of adults (*Table 2*).

Table 2.

Parasitological indices depending of the sex and age of host *Pelophylax ridibundus* from the studied ecosystems

No	Invasion	Sex				Age			
		♂, n=26		♀, n=31		Adults, n=57		Juveniles, n= 27	
		EI -%	*II, ex.*	*EI - %*	*II, ex.*	*EI - %*	*II, ex.*	*EI - %*	*II, ex.*
TREMATODA									
1	*O. ranae*	76.9	4-180	45.2	38-86	59.6	4-180	88.9	8-41
2	*H. variegatus*	50.0	1-30	38.7	1-4	43.9	1-30	29.6	3
3	*C. urniger*	38.5	1-30	83.9	2-11	63.2	1-30	14.8	1
4	*P. confusus*	38.5	1-14	29.0	1-32	33.3	1-32	-	-
5	*D.subclavatus*	-	-	29.0	4-9	15.8	4-9	-	-
6	*P. brumpti*	61.5	1-8	29.0	10-26	43.9	1-26	29.6	3-9
7	*T. excavata*	57.9	26-49	38.7	9-96	47.4	9-96	14.8	8
8	*P. robusta*	-	-	19.4	3	10.5	3	-	-
9	*S. falconis*	-	-	9.7	150	5.3	150	-	-
SECERNENTEA									
10	*O. filiformis*	-	-	19.4	1	10.5	1	-	-
11	*C. ornata*	-	-	38.7	6-10	21.1	6-10	-	-
12	*I. neglecta*	19.2	1-3	29.0	1-14	24.6	1-14	-	-
13	*S. lupi*	-	-	58.1	2-98	31.6	2-98	-	-

Therefore, the results of helminthological research revealed that the amphibians species *Pelophylax ridibundus* from the Ranidae family in the natural ecosystems of lower Dniester River is infested with 13 species of helminthes (trematodes - 9, nematodes - 4), as well as the identification of the nematode species *Spirocerca lupi* show the role of amphibians in maintaining and forming the foci of parasitic agents specific to vertebrates.

Conclusions

1. It has been studied the helminth fauna of 84 specimens (males - 26, females - 31, juveniles - 27) of Pelophylax ridibundus from the natural ecosystem - the Dniester River, from the village of Talmaza from the Stefan-Voda district.
2. The structure of the helminth fauna of the host species was established - Pelophylax ridibundus has been represented by 13 species of helminthes: *Haematoloechus variegatus* Rudolphi, 1819; *Codonocephalus urniger* Rudolphi, 1819; *Opisthioglyphe ranae* Froelich, 1791; *Paralepoderma brumpti* Buttner, 1951; *Prosotocus confusus* Looss, 1894; *Tylodelphys excavata* Rudolphi, 1803; *Diplodiscus subclavatus* Pallas, 1760; *Parastrigea robusta* Szidat, 1928, *Strigea falconis* Szidat, 1928; *Cosmocerca ornata* Dujardin, 1845; *Oswaldocruzia filiformis* Goeze, 1782; *Icosiella neglecta* Diesing, 1851; *Spirocerca lupi* Rudolphi, 1809, from 2 classes, 6 orders, 11 families and 13 genera.
3. The degree of helminth infestation of the species *Pelophylax ridibundus* increases with age, because in juveniles the infestation has been established with 5 from 13 species of helminths detected.

4. It was estimated that the structure of the helminth fauna in *Pelophylax ridibundus* depends on the helminth species and of the host sex, thus in males the presence of 7 from 13 species of helminths was established the, and in females - all 13 species of helminths were found.
5. It was determined the role of amphibian species *Pelophylax ridibundus* in maintaining and forming parasitic foci of *Spirocerva lupi*, which causes Spirocercosis in vertebrates, and for which the amphibians serve as a paratene hosts.

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