

Determination of the degree of helminth infestation of the ecaudate amphibians (the Anura: Ranidae, Bufonidae) depending on the biotic factors in conditions of the Republic of Moldova

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Abstract. In this study is made an ecological analysis of the species composition, structure of the helminth community and the invasion degree of Ranidae and Bufonidae families in different habitats in Moldova. As a result, it has been established the presence of 17 helminths species. It was found that the diversity of helminth fauna of the studied amphibians is structured depending on the host species, biotop, biotic factors, gender and depending of the host ontogenesis. Thus, the greatest diversity of helminthes in amphibians in the family Ranidae was established in *Rana ridibunda* and *Rana lessonae* with 17 species, and for the species from the Bufonidae family, no different values were established, so each host species being infested with 3 species of helminthes.

Keywords: helminthological investigation, helminths, Ranidae, Bufonidae, Moldova.

Determinarea gradului de infestare cu helminți a amfibienilor ecaudați (Anura: Ranidae, Bufonidae) în dependență de factorii biotici în condițiile Republicii Moldova

Rezumat. În acest studiu se face o analiză ecologică a compoziției speciilor, structurii comunității de helminți și a gradului de infestare cu helminți a amfibienilor din familiile Ranidae și Bufonidae în diferite habitate din Moldova. Ca rezultat, sa stabilit prezența a 17 specii de helminți. S-a constatat că diversitatea faunei helmintice a amfibienilor studiați este structurată în funcție de specia gazdă, biotop, factorii biotici, gen, dar și în funcție de ontogeneza gazdei. Astfel, cea mai mare diversitate de helminți la amfibienii din familia Ranidae s-a stabilit la speciile *Rana ridibunda* și *Rana lessonae* cu 17 specii, iar pentru speciile din familia Bufonidae nu s-au stabilit valori distincte, astfel fiecare specie gazdă fiind infestată cu câte 3 specii de helminți.

Cuvinte cheie: investigații helmintologice, helminți, Ranidae, Bufonidae, Moldova.

1. INTRODUCTION

It is known that the wild animals are an important source of parasites for humans and domestic animals.

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 the research, anurans are definitive hosts for several classes of helminthes, including Cestoda, Monogenea, Trematoda, Secernentea and Palaeacanthocephala [3-5, 7, 9-11, 13, 15, 16].

They also serve as intermediate hosts [2, 7, 8, 14-16] or as paratenic hosts [2, 3, 5,6, 9, 10, 13] for a wide variety of helminthes specific to the vertebrates. Therefore, helminth infracommunities (i.e., communities of parasite infrapopulations in a single host) are ideal systems for investigations of host responses to simultaneous parasitic infections and within-host interactions between co-infecting parasites.

Species of parasites in all of these groups may infect the host in different stages of the life cycles. Any specimen may be infected by more than one parasite simultaneously or at different times.

Therefore, several factors contribute to the dynamics and structure of parasite communities, such as seasonality, environmental heterogeneity or factors associated with the host, such as spatial distribution, population density, body size, age and host gender [1].

Obtaining and evaluating scientific data as a result of helminthological investigations on the species of ecaudata amphibians will elucidate their role as vectors of different groups of parasitic agents, but also the importance in the prophylaxis of helminthes specific to domestic, wild and company animals in conditions of the Republic of Moldova.

2. MATERIALS AND METHODS

Observing, collecting and obtaining data on the complex of anurans from *Ranidae* (*Rana ridibunda*, *R. lessonae*, *R. esculenta*, *R. temporaria*, *R. dalmatina*) and *Bufo* (*Bufo bufo*, *B. viridis*) families was performed in the center and south of Moldova.

All investigations regarding the study of the helminth fauna, the determination of the infestation degree by helminth of amphibian species was carried out in the laboratory of Parasitology and Helminthology of the Institute of Zoology.

Helminthological analysis was performed according to the standard method, which involves examination of all internal organs of the animal [22]. Helminthological investigations of the parenchymal organs were carried out with the help of compressors, and of the digestive tract - by successive washing.

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The collection, fixation, determination and processing of the helminthological material was carried out according to the methods proposed by different authors [17-21, 23, 24]. After collecting, fixing and processing, the helminthological material was mounted using paraffin rings according to the method proposed by Seinhorst (1959). The helminthological material was determined according to contemporary methodology [22].

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.

Laboratory helminthological investigations of amphibian samples on the presence of helminthes or helminth elements (eggs, larvae) will contribute to obtaining data of great value in order to determine the importance of amphibians in the formation and maintenance of outbreaks of parasitic agents common to pets, domestic animals and wildlife.

3. RESULTS AND DISCUSSIONS

According to the helminthological investigations performed on amphibians from Ranidae and Bufonidae families in the center and southern area of the Republic of Moldova, the presence of 17 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; *Gorgodera varsoviensis* Sinitzin, 1905; *Haplometra cylindracea* Zeder, 1800; *Pleurogenoides medians* Olsson, 1876, *Cosmocerca ornata* Dujardin, 1845; *Oswaldocruzia filiformis* Goeze, 1782; *Icosiella neglecta* Diesing, 1851; *Spirocerca lupi* Rudolphi, 1809 *Acanthocephalus ranae* Schrank, 1788, which from a taxonomic point of view fall into 3 classes (Trematoda, Secernentea) (Trematoda, Secernentea, Palaeacanthocephala), 7 orders (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Spirurida, Echinorhynchida), 16 families (Omphalometridae, Haematoloechidae, Plagiorchiidae, Lecithodendriidae, Gorgoderidae, Diplodiscidae, Diplostomatidae, Pleurogenidae, Diplodiscidae, Strigeidae, Macroderoididae, Cosmocercidae, Molineidae, Onchocercidae, Spirocercidae, Echinorhynchidae) and 17 genera (*Haematoloechus*, *Codonocephalus*, *Opisthioglyphe*, *Paralepoderma*, *Prosotocus*, *Gorgodera*, *Tylodelphys*, *Diplodiscus*, *Parastrigea*, *Strigea*, *Haplometra*, *Pleurogenoides*, *Cosmocerca*, *Oswaldocruzia*, *Icosiella*, *Spirocerca*, *Acanthocephalus*).

According to the analysis of the investigation performed, *Rana ridibunda* Pallas, 1771 was the species with the most helminth specimens investigated (n=45) and as a result, their infestation with 17 helminthes species was established, of which 12 species of trematods (*Opisthioglyphe ranae* Fröhlich, 1791, *Haematoloechus variegatus* Rudolphi, 1819, *Prosotocus confusus* Looss, 1894, *Diplodiscus subclavatus* Pallas, 1760, *Codonocephalus urniger* Rudolphi, 1819, *Paralepoderma brumpti* Buttner, 1951, *Pleurogenoides medians* Olsson, 1876, *Parastrigea robusta* Szidat, 1928, *Haplometra cylindracea* Zeder, 1800, *Gorgodera varsoviensis* Sinitzin, 1905, *Strigea falconis* Szidat, 1928, *Tylodelphys excavata* Rudolphi, 1803), 4 species of secernentea (*Oswaldocruzia filiformis* Goeze, 1782, *Cosmocerca ornata* Dujardin, 1845, *Icosiella neglecta* Diesing, 1851, *Spirocerca lupi* Rudolphi, 1809) and a species of the class Palaeacanthocephala (*Acanthocephalus ranae* Schrank, 1788).

For 13 of the 17 helminths species detected *Rana ridibunda* is the definitive host, and for 4 helminths species it is the paratenic host.

At helminthological investigation of the *Rana lessonae* species (n = 19), the presence of 17 helminths species was established too, of which 12 trematodes species (*Opisthioglyphe ranae* Fröhlich, 1791, *Haematoloechus variegatus* Rudolphi, 1819, *Prosotocus confusus* Looss, 1894, *Diplodiscus subclavatus* Pallas, 1760, *Codonocephalus urniger* Rudolphi, 1819, *Paralepoderma brumpti* Buttner, 1951, *Pleurogenoides medians* Olsson, 1876, *Parastrigea robusta* Szidat, 1928, *Haplometra cylindracea* Zeder, 1800, *Gorgodera varsoviensis* Sinitzin, 1905, *Strigea falconis* Szidat, 1928, *Tylodelphys excavata* Rudolphi, 1803), 4 species of secernentea (*Oswaldocruzia filiformis* Goeze, 1782, *Cosmocerca ornata* Dujardin, 1845, *Icosiella neglecta* Diesing, 1851, *Spirocerca lupi* Rudolphi, 1809) and a species of the class Palaeacanthocephala (*Acanthocephalus ranae* Schrank, 1788).

For 13 of the 17 helminthes species detected *Rana lessonae* is the definitive host, and for 4 helminthes species it is the paratenic host.

In *Rana esculenta* (n=16) the presence of 12 species of helminths was determined, of which 8 species of trematodes (*Opisthioglyphe ranae* Fröhlich, 1791, *Haematoloechus variegatus* Rudolphi, 1819, *Gorgodera varsoviensis* Sinitzin, 1905, *Prosotocus confusus* Looss, 1894, *Diplodiscus subclavatus* Pallas, 1760, *Codonocephalus urniger* Rudolphi, 1819, *Pleurogenoides medians* Olsson, 1876, *Tylodelphys excavata* Rudolphi, 1803), 3 species of secernentea (*Oswaldocruzia filiformis* Goeze, 1782, *Cosmocerca ornata* Dujardin, 1845, *Icosiella neglecta* Diesing, 1851) and a species of the class Palaeacanthocephala (*Acanthocephalus ranae* Schrank, 1788)

For 9 of the 10 helminthes species detected *Rana esculenta* is the definitive host, and for one helminthes species it is the paratenic host.

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According to the helminthological investigations carried out at the *Rana dalmatina* species (n = 12) the presence of 5 species of helminthes was established, of which 3 species of trematodes (*Opisthioglyphe ranae* Fröhlich 1791, *Haematoloechus variegatus* Rudolphi 1819, *Parastrigea robusta* Szidat, 1928) and 2 species of secernentea (*Oswaldocruzia filiformis* Goeze, 1782, *Cosmocerca ornata* Dujardin, 1845). At 4 of the 5 species of helminthes detected, *Rana dalmatina* is the definitive host, and for one species it is the paratenic host.

The amphibian species from the family Bufonidae - *Bufo bufo* (n = 30) and *Bufo viridis* (n = 11) the presence of 3 species of helminthes was determined each, of which 2 species of nematodes (*Oswaldocruzia filiformis* Goeze, 1782, *Cosmocerca ornata* Dujardin, 1845) and a species of acanthocephalus (*Acanthocephalus ranae* Schrank 1788), for which these serve as definitive hosts.

In order to quantify the characteristic of helminth contamination detected in amphibian species of the families Ranidae and Bufonidae, the main parasitological indices were calculated which include the minimum and maximum number of parasites of one species (II, specimens) and the percentage of contamination for each host species with each helminth species (EI, %) Table 1.

At the same time, the helminthological researches, depending on the host ontogenesis were performed, and in order to carry out this research, the amphibians from two age categories were helminthologically investigated: adults and juveniles.

Thus, 17 juveniles of *Rana ridibunda* species were helminthologically investigated, in which the presence of 4 species of helminthes was established, of which 3 species of trematodes (*Opisthioglyphe ranae* Fröhlich, 1791, *Haematoloechus variegatus* Rudolphi, 1819, *Diplodiscus subclavatus* Pallas, 17) and a species of secernentea (*Cosmocerca ornata* Dujardin, 1845).

The results of the researches allow us to conclude that with the increase of the size and age of the amphibians, the degree of helminthes infestation also increases. This peculiarity demonstrates the intensification of the diet of adult amphibians and the accumulation of parasitic agents in their body, as well as increasing the size and diversity of foods that promote the simultaneous penetration of more parasitic agents into the host body, leading to increased infestation.

Therefore, according to the results of the helminthological research obtained, it was established that the diversity of helminthes and their infectivity is higher in amphibians in floodplain lakes with relatively constant environmental conditions. Amphibians from anthropized aquatic habitats with dynamic environmental conditions are less infected

Table 1. Infection level of ecaudata amphibians from Ranidae and Bufonidae families

Nr.	Host	<i>Rana ridibunda</i> n=45		<i>Rana lessonae</i> n=19		<i>Rana esculenta</i> n=16		<i>Rana dalmatina</i> n=12		<i>Bufo bufo</i> n=30		<i>Bufo viridis</i> n=11	
	Invasion	II, ex.	EI, %	II, ex.	EI, %	II, ex.	EI, %	II, ex.	EI, %	II, ex.	EI, %	II, ex.	EI, %
TREMATODA													
1.	<i>O. ranae</i>	1-114	88.9	1-94	73.7	1-64	87.5	1-23	66.7	-	-	-	-
2.	<i>H. variegatus</i>	1-5	75.6	1-3	10.5	1-4	18.8	1-3	50.0	-	-	-	-
3.	<i>G. varsoviensis</i>	1-3	22.2	1-2	15.8	1	18.8	-	-	-	-	-	-
4.	<i>P. confusus</i>	1-98	80.0	1-111	63.2	1-46	56.3	-	-	-	-	-	-
5.	<i>D. subclavatus</i>	1-15	86.7	1-15	89.5	1-11	25.0	-	-	-	-	-	-
6.	<i>C. s. urniger</i>	1-63	22.2	1-6	36.8	1-11	25.0	-	-	-	-	-	-
7.	<i>P. brumpti</i>	1-21	6.8	1-26	57.9	-	-	-	-	-	-	-	-
8.	<i>P. medians</i>	1-43	17.8	1-32	31.8	1-6	56.3	-	-	-	-	-	-
9.	<i>S. falconis</i>	1-14	22.2	1-31	42.1	-	-	-	-	-	-	-	-
10.	<i>P. robusta</i>	1-55	22.2	1-8	42.1	-	-	1-11	83.3	-	-	-	-
11.	<i>T. excavata</i>	1-228	88.9	1-187	57.9	1-114	87.5	-	-	-	-	-	-
12.	<i>H. cylindracea</i>	1-28	75.6	1-21	36.8	-	-	-	-	-	-	-	-
SECERNENTEA													
13.	<i>O. filiformis</i>	1-4	24.4	1-3	15.8	1-7	87.5	1-3	41.7	1-28	83.3	1-27	54.5
14.	<i>C. ornata</i>	1-10	86.7	1-3	52.6	1-19	87.5	1-6	66.7	1-27	60.0	1-133	81.8
15.	<i>I. neglecta</i>	1-5	26.7	1-9	26.3	1-13	37.5	-	-	-	-	-	-
16.	<i>S. lupi</i>	1-98	53.3	1-70	57.9	-	-	-	-	-	-	-	-
PALAEACANTHOCEPHALA													
17.	<i>A. ranae</i>	1-4	11.1	1-2	26.3	1-4	37.5	-	-	1	26.7	1-2	27.3

with the same diversity of species; they are characterized by a relatively small number of helminthes species and a low level of helminth invasion.

The causes of differences in amphibian infestation by helminthes in different biotopes are complex. Diversity, high abundance of mollusks and arthropods (intermediate, definitive, paratenic hosts) cause a high level of infestation of amphibians with transmission through food chains. The high density of the amphibians themselves, and, accordingly,

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of the predators batrachophages, leads to intensive infestation of frogs by larval stages of helminthes.

4. CONCLUSIONS

- (1) The helminth fauna of 133 specimens of ecaudata amphibians (Ranidae, Bufonidae) was studied from Central Forest (Codrii Reservation, Ciuciuleni village) and in the natural ecosystem - the Dniester River, from the village of Talmază from the Stefan-Voda district.
- (2) It has been established the presence of 17 helminths species: *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; *Gorgoderă varsoviensis* Sinitzin, 1905; *Haplometra cylindracea* Zeder, 1800; *Pleurogenoides medians* Olsson, 1876, *Cosmocerca ornata* Dujardin, 1845; *Oswaldocruzia filiformis* Goeze, 1782; *Icosiella neglecta* Diesing, 1851; *Spirocercă lupi* Rudolphi, 1809; *Acanthocephalus ranae* Schrank, 1788, which from a taxonomic point of view fall into 3 classes (Trematoda, Secernentea, Palaeacanthocephala), 7 orders (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Spirurida, Echinorhynchida), 16 families (Omphalome-tridae, Haematoloechidae, Plagiorchiidae, Lecithodendriidae, Gorgoderidae, Diplodiscidae, Diplostomatidae, Pleurogenidae, Diplodiscidae, Strigeidae, Macroderoididae, Cosmocercidae, Molineidae, Onchocercidae, Spirocercidae, Echinorhynchidae) and 17 genera (*Haematoloechus*, *Codonocephalus*, *Opisthioglyphe*, *Paralepoderma*, *Prosotocus*, *Gorgoderă*, *Tylodelphys*, *Diplodiscus*, *Parastrigea*, *Strigea*, *Haplometra*, *Pleurogenoides*, *Cosmocerca*, *Oswaldocruzia*, *Icosiella*, *Spirocercă*, *Acanthocephalus*).
- (3) It was found that the diversity of helminth fauna of the studied amphibians is structured depending on the host species, so the greatest diversity of helminthes in amphibians in the family Ranidae was established in *Rana ridibunda* and *Rana lessonae* with 17 species, and the lowest diversity in *Rana dalmatina* species - with 5 species of helminths, and for the species from the Bufonidae family, no different values were established, so each species being infested with 3 species of helminthes.
- (4) It was determined that the helminth fauna of the amphibians investigated according to the age of the host shows divergent values, so that in the juveniles of the

Rana ridibunda species there was established the infestation with only 4 species of helminthes.

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