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LARVAL STAGES OF TREMATODES IN FRESHWATER SNAILS OF THE CHORNOBYL ZONE OF RADIOACTIVE CONTAMINATION

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Abstract. *In 1986, at the Chornobyl Nuclear Power Plant in the Exclusion Zone after an industrial accident, ecosystems began to have several features that distinguish them from the objects of the natural reserve fund. Parasitic systems are an informative indicator of the state of the ecosystem, which is now sporadically applied in the Exclusion Zone. Any change in the parasite population can lead to changes in the host population. The degree of imbalance in the "parasite-host" system depends on the strength and nature of the influence of external factors. At the same time, the presence of mutual adaptations of parasitic organisms and snails gives grounds to consider the "parasite-host" system comprehensively.*

Freshwater gastropod snails of various systematic groups, which can be intermediate and secondary hosts for trematode agents, were selected as an object of the study.

In order to study freshwater gastropod snails for the presence of larval stages of helminths, snails were collected from such locations as Krasne Lake, Ilya River, Chernobyl Nuclear Power Plant cooling pond, bypass channel of the Chernobyl Nuclear Power Plant cooling pond, left bank of the Prypiat floodplain, Koshevka oxbow lake, Hrubchanskyi canal in the Meshevo village.

Based on the results of the research, the presence of larval stages of trematode agents at different stages of their development (redia and metacercaria) parasitizing in the freshwater snails Lymnae stagnalis and Radix auricularia was established.

Keywords: *freshwater snails, trematodes, larval stages, Chornobyl Exclusion Zone*

Introduction

Trematodes are flatworms that belong to phylum Platyhelminthes with a leaf-like unsegmented body and flattened dorsal-ventrally. They have a complicated life cycle, which comprises adult and several developing stages, such as egg, miracidium, sporocyst, redia, cercaria, and metacercaria (cyst). Trematodes are an important component of natural ecosystems (Curtis, 2002; Curtis, 2009; Rastyazhenko et al., 2015; Brian & Aldridge, 2020; Mouritsen & Elkjær, 2020; Wiroonpan et al., 2021).

The life cycle of these internal parasites constantly includes parthenogenetic and hermaphroditic generations. The effect of the diversity is formed by a wide set of numerous biological and morphological adaptations, existing at different stages of the development of these parasites, i.e., a great variety of animal hosts (both vertebrates and invertebrates) and a broad range of eco-systems and biotopes (Galaktionov et al., 2003; Kołodziejczyk et al., 2016). Trematodes are heterogeneous with a multiple host life cycle and require snails as their first or/and second intermediate host (Zbikowska & Nowak, 2009; Zhytova et al., 2017).

In rapport to modern taxonomy, there are known more than 18000 species of trematodes (Bray et al., 2008). In the Ukrainian Polissia forest zone, more than 166 species of trematodes of animals are known (Iskova et al., 1995). Furthermore, in Ukraine, trematode circulation ways from reservoirs of forestries of Polissia Nature Reserve (Rivne, Volyn, and Zhytomyr Regions). In this research, 26 species of trematodes from 14 families were found (Zhytova et al., 2019). On the previous stage of our snail investigation from the Chornobyl Exclusion Zone, the intensity of invasion by larvae of trematodes in *Lymnaea stagnalis* was 83%, in *Lymnaea (Radix) auricularis* – 50% and *Viviparus viviparus* – 75% (Semenko et al., 2020).

Like other parasitic organisms, trematodes negatively affect organisms, populations, animal groups, and food chains in general (Kuris et al., 2008; Magalhães et al., 2015; Żbikowska & Żbikowski, 2015; Moema et al., 2019; Paviotti-Fischer et al., 2019).

Studying trematodes and their circulation ways is necessary for the successful management of hunting farms, in particular, for breeding wild animals in enclosures.

The present study was carried out for identification and determination of the prevalence and intensity of parasite stages in freshwater snails collected from several water bodies of the Chornobyl Exclusion Zone.

Analysis of recent researches and publications

Freshwater snails are known to host a wide variety of parasites. They have received considerable attention because of their involvement with some species as intermediate hosts for trematodes. This has become an issue of significant medical and/or veterinary importance with regard to the cause of parasitic diseases in people and animals, and is especially true in diseases associated with various food-borne trematodes (Faltýnková & Haas, 2006; Wang et al., 2017; Dellagnola et al., 2019; Gilardoni et al., 2019; Chantima & Rika, 2020). Many trematode species, especially from the Echinostomatidae family, require 1 or 2 snail hosts to finish their life cycles. The Echinostomatidae is the largest family within the class Trematoda. Over 60 species of echinostomatid flukes are spread worldwide (Sorensen et al., 1998; Moraes et al., 2009). Members of this family, in their adult stage, can infect a far-ranging dimension of vertebrates (predominantly birds) such as poultries, migratory birds, and sometimes mammals, including humans. The zoonotic potential of this family of parasites is associated with the ingestion of raw fishes, amphibians, and snails, which can harbor the infective stage, metacercaria (Chantima et al., 2013; Nguyen et al., 2021).

Echinostomes are morphologically distinct from other trematodes by the presence of spines around the oral sucker, forming collar spines. The number and arrangement of these spines are the basis of genus identification. Their complex life cycle involves three categories of hosts. Among them definitive, first intermediate, and second intermediate hosts in which develop seven different stages (adult specie/species, eggs, miracidia, sporocysts, redia, cercaria, and metacercaria) (Toledo et al., 2014; Fernández et al., 2016; Horák et al., 2019). Also, in several trematode species that have the rediae stage, the latter comes in two distinct morphs (Poulin et al., 2019). The experimental study showed the possibility of native metacercariae (*Echinostoma revolutum, Echinoparyphium aconiatum*, and *Hypoderaeum conoideum*) settlement in non-specific snail species (*Potamopyrgus antipodarum*) (Zbikowski & Zbikowska, 2009).

Furthermore, there are cases in which trematoda, developing in gastropod snails, exploits first host for metacercarial encystment (Van den Broek & de Jong, 1979; Chai et al., 2011; Chantima et al., 2013; Sohn et al., 2017).

The shape of metacercarial cysts is variable and may be spherical (e.g. *Echinostoma trivolvis*), hemispherical (e.g. *Fasciola hepatica* or *Zygocotyle lunata*), flask-shaped (e.g. *Philophthalmus hegeneri*), ovoidal (e.g. *Parorchis acanthus*). Ventral lid or mucus plug may be present in a specific area of the cyst wall (e.g. *Fasciola hepatica*). During excystation, the ventral lid opens or the mucus plug is dissolved. Mechanisms of these processes are not fully explored. The structure of encysted metacercaria is differ from one species to another. Numerous histochemical, histological, and ultrastructural studies show that appreciable specific variation exists in both the chemical composition and their number of cyst layers (Fried et al., 1994).

Echinostome cercaria and furcocercous cercaria commonly develop in the body of viviparid and plumonate snails (*Lymnaea spp., Filopaludina spp.*) (Chontananarth & Wongsawad, 2013). *Echinostoma revolutum* and other similar species (*Ec. trivolvis* and *Ec. robus-*

tum) are possible to reveal via molecular genetic analyses (Pantoja et al., 2021). Cercarial numbers are known to vary both spatially and temporarily in aquatic ecosystems influenced by a range of factors, such as wind, water currents, and the specific geomorphology and biota of a habitat (Morley & Lewis, 2013).

Materials and methods of research

Collection of snails was carried out in the period from August 2021 till September 2021 using a simple random sampling approach and with the help of a strainer or net. Snails were collected in the following waterbodies as Krasne Lake, Ilya River, Chornobyl NPP cooling pond, bypass channel of the Chornobyl NPP cooling pond, left bank of the Pripyat floodplain, Koshevka oxbow lake, and Hrubchansky canal in the Meshevo village.

Then the research was conducted at the laboratory of the Department of Pharmacology, Parasitology and Tropical Veterinary Medicine of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine.

In the laboratory, snails were placed in glass jars with tap water until they were identified. Identification was performed according to conchological characteristics and also based on morphological features (Brandt, 1974 (Fig. 1); Stadnichenko, 1990, 2006; Kruglov, 2005).

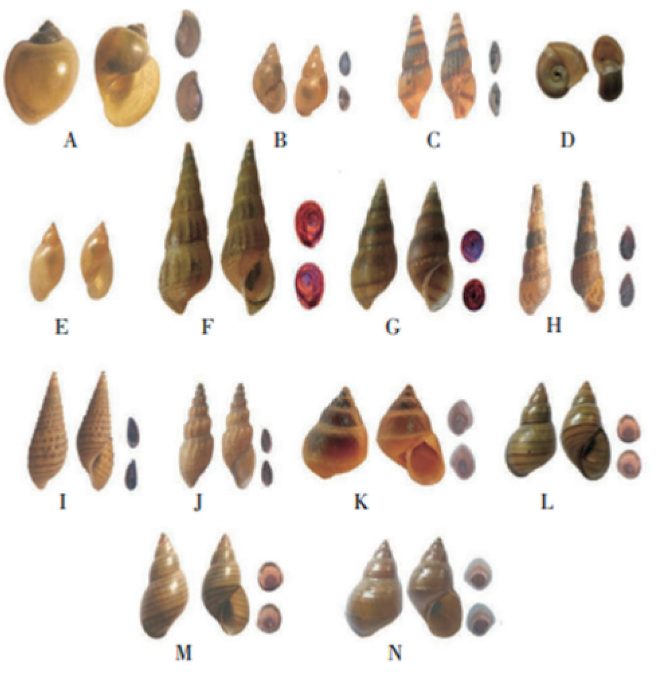

Fig. 1. Shell and operculate characteristics of snails collected from the collecting sites. A: *Pila polita*; B: *B. siamensis*; C: *Clea helena*; D: *I. exustus*; E: *Lymnaea auricularia rubiginosa*; F: *B. crostula crostula*; G: *Brotia wycoffi*; H: *M. tuberculata*; I: *T. granifera*; J: *T. scabra*; K: *E. eyriesi*; L: *Filopaludina dorliaris*; M: *Filopaludina sumatrensis polygramma*; N: *Filopaludina martensi* (Chontananarth & Wongsawad, 2013)

Subsequently, snails were examined using the compressorium technique for the detection of different stages of the development of trematoda species.

Results of the research and their discussion

Altogether 30 freshwater snails were collected, transported, and then identified and examined in the laboratory. On the basis of the shell morphology, the snail species were classified into 3 species. They included *Planorbis corneus, Lymnea stagnalis,* and *Radix auricularis* (Fig. 2–4).

In order to detect parasitic organisms, the liver and pancreas of the snails were examined under a microscope (Fig. 5–6).

Among 8 snails of the specie *Lymnea stagnalis*, 1 of them had larval stages of trematodes. The stage of the development was redia. Among the 9 studied snails of the specie *Radix auricularia*, all had a significant infestation with metacercariae. Metacercariae were morphologically classified echinostomatid species.

Among 13 snails of the species *Planorbis corneus*, no parasitic organisms were found.

So, the overall prevalence of trematodes infection in investigated snails was 33.3%, which is higher than in Chiang Mai province, Thailand where the prevalence of cercarial infection was 17.27% (428/2457) in 8 snail species collected (Chontananarth & Wongsawad, 2013). In another research, in total, *Bithynia tentaculata* showed a trematode prevalence of 12.9% and 14%, respectively (Schwelm et al., 2020). Thirteen percent of snail specimens were infected with Digenea in the Kenyan part of Lake Victoria. The highest prevalence was recorded in *Melanoides tuberculata* (64.5%), followed

Fig. 2. ***Planorbis corneus***

Fig. 3. ***Lymnea stagnalis***

Fig. 4. *Radix auricularia*

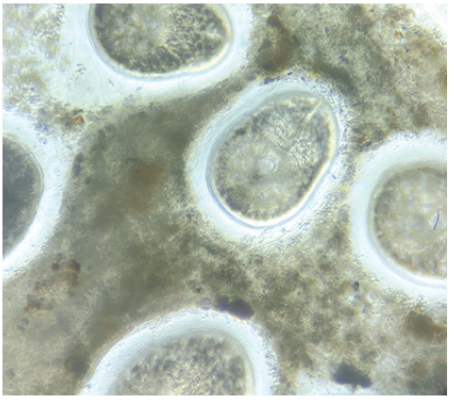

Fig. 5. Encysted metacercaria of several trematode species

by *Pila ovate* (15.4%), *Radix natalensis* (9.5%), *Bulinus ugandae* (9.1%), *Bellamya unicolor* (8.9%), *Biomphalaria pfeifferi* (7.3%), and *Biomphalaria sudanica* (4.4%) (Outa et al., 2020).

To the most frequently recorded species belong *Diplostomum spathaceum* (Rudolphi, 1819) (8% of the records), D. *pseudospathaceum Niewiadomska*, 1984 (6% of records), and *Echinoparyphium recurvatum* (von Linstow, 1873) (6% of the records) (Faltýnková et al., 2016).

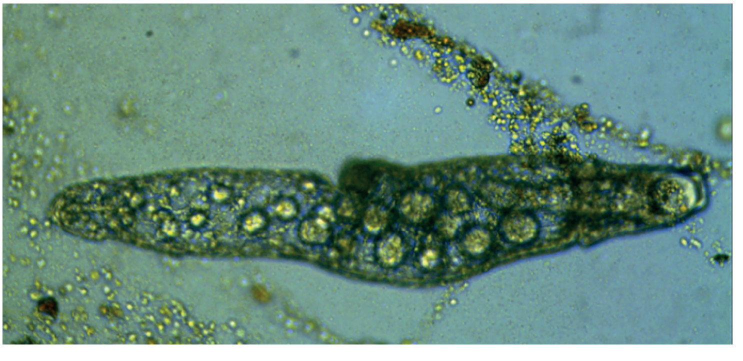

Fig. 6. Redia in hepatopancreas

Consequently, the results of the studies established the presence of larval stages of trematodes agents at different stages of the development, such as redia and metacercaria, which parasitize in the body of freshwater snails of water bodies of the Chornobyl Exclusion Zone.

Conclusions and future prospects

Studies have shown a significant infestation of snails of the species *Radix auricularia* by larval stages (metacercaria) of echinostamatid trematodes. Definitive hosts for these agents could be different species of waterfowl.

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Анотація. *В 1986 році на Чорнобильській атомній електростанції в зоні відчуження після аварії, екосистеми почали мати низку особливостей, що відрізняють їх від об'єктів природно-заповідного фонду. Інформативним індикатором стану екосистеми, який наразі спорадично застосовується в зоні відчуження, є паразитарні системи. До змін популяції паразита може призвести будь-яка зміна в популяції хазяїна. Ступінь дисбалансу системи «паразит-хазяїн» залежить від сили та характеру впливу зовнішніх факторів. У цей же час, наявність взаємних адаптацій паразитичних організмів і молюсків, дає підстави розглядати комплексно систему «паразит-хазяїн».*

Об'єктом дослідження було відібрано прісноводних черевоногих молюсків різних систематичних груп, які можуть бути проміжними та додатковими хазяями збудників трематодозів.

Для дослідження паразитичних стадій гельмінтів, прісноводних черевоногих молюсків було відібрано з таких водних об'єктів: озеро Красне, річка Ілля, Водойма-охолоджувач Чорнобильської АЕС, обвідний канал водойоми-охолоджувача Чорнобильської АЕС, лівий берег пойма Прип'яті, стариця Кошевка, Грубчанський канал с. Мешево.

За результатами проведених досліджень встановлено наявність личинкових стадій збудників трематодозів на різних стадіях їхнього розвитку (редія, метацеркарій), що паразитують в організмі прісноводних молюсків Lymnaea stagnalis і Radix auricularia.

Ключові слова: *прісноводні молюски, трематоди, личинкові стадії, Чорнобильська зона відчуження*
