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ECOLOGICAL AND PARASITOLOGICAL CHARACTERISTICS OF THE “*FASCIOLA HEPATICA* L.–*LYMNAEA (GALBA) SUBANGULATA*” SYSTEM

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Background. Fasciolosis is one of the most common zoonoses in the world, caused by the trematode *Fasciola hepatica* L. This helminthiasis leads to significant economic damage in animal husbandry and can pose a direct threat to human health. Today, in the context of climate change, detailed knowledge of the biological characteristics of the trematode *F. hepatica* circulation will make it possible to predict outbreaks of fasciolosis on farms in detail and plan effective measures to combat this dangerous disease. The purpose of this work is to clarify some aspects of *F. hepatica* biology within Berdychiv district belonging to the forest-steppe zone of Zhytomyr region.

Material and Methods. The work was carried out during 2020–2021. Collections of mollusks from water bodies on the territory of Berdichevsky district of Zhytomyr region served as the material for the research. Their identification was carried out by conchological features. Conventional methods were used for the parasitological study of mollusks. In total, more than 2000 mollusks *Lymnaea (Galba)* spp. were examined. Sampling of water and mollusks was carried out from four pasture reservoirs.

Results. The main intermediate host of *F. hepatica* is the mollusk *L. (G.) subangulata* in the territory of Berdychiv region; *L. (G.) truncatula* plays a secondary role in the distribution of this trematode. It was noted that the carriers of *F. hepatica* partenites are mainly mollusks with a shell height of 4.0 to 8.2 mm.

New data on the dynamics of infection of *L. (G.) subangulata* with the trematode *F. hepatica* was obtained. The dynamics of infestation of these mollusks with *F. hepatica*



cercariae shows a stable uniform character in the form of a two-peak curve, with peaks in May (2.51 ± 0.88 %) and August (9.19 ± 1.78 %). The overall prevalence of *L. (G.) subangulata* invasion by fasciola cercariae in the studied water bodies in 2020 was significantly lower than in 2021.

Conclusions. In modern climatic conditions, in the territory of the region, permanent water bodies have become fasciolosis foci and common places for the settlement of *L. (G.) subangulata*, infected with *F. hepatica* cercariae. The most favorable environmental conditions for fasciola and its intermediate hosts develop in spring; accordingly; for the definitive hosts, this period is quite dangerous in terms of infection of animals.

Keywords: *Fasciola hepatica*, molluscs, *Lymnaea (Galba) subangulata*, *Lymnaea (Galba) truncatula*, intermediate hosts, definitive hosts

INTRODUCTION

The trematode *Fasciola hepatica* is the causative agent of one of the world's most common zoonoses – fasciolosis. This parasite is widespread in 81 countries, it causes significant economic damage to livestock, leads to the death of animals and reduces their productivity. In addition, *F. hepatica* poses a direct threat to human health and well-being of the population in general (Vázquez *et al.*, 2018; Alba *et al.*, 2019; Beesley *et al.*, 2019; Leka, 2019; Malatji & Mukaratirwa, 2019; Zhang *et al.*, 2019; Caravedo, & Cabada, 2020; Huang *et al.*, 2020; Coelho *et al.*, 2021; Cwiklinski, Robinson, Donnelly, & Dalton, 2021; Lalor *et al.*, 2021). According to the literature (Caravedo, & Cabada, 2020), there is an expansion of endemic zones of *F. hepatica*. An estimated number of people infected by fasciola worldwide is over 17 million and the number of infected humans is constantly growing.

F. hepatica has a complex life cycle that includes intermediate and final hosts. Freshwater mollusks are intermediate hosts; the final ones are wild and domestic ruminants, as well as humans. 30 species of freshwater mollusks from the genera *Lymnaea*, *Omphiscola*, *Stagnicola*, *Pseudosuccinea* and *Radix* are intermediate hosts (Caron, Martens, Lempereur, Saegerman, & Losson, 2014; Alba *et al.*, 2019; Caravedo, & Cabada, 2020; Ngcamphalala, Malatji, & Mukaratirwa, 2022). *Lymnaea peregra* (O. F. Muller, 1774) can be an intermediate host for *F. hepatica* under natural conditions (Relf, Good, McCarthy, & de Waal, 2009). However, mollusks of the genus *Galba*, in particular *Galba truncatula*, play the dominant role in the infection transmission.

The ability of fasciola to infect and reproduce in their intermediate mollusk hosts, as well as their adaptation to a wide range of final mammalian hosts, contribute to their high transmission and spread.

Researchers note (Zhang *et al.*, 2019) that today fasciolosis, which is one of the most important and dangerous trematodoses, does not attract enough scholarly attention in connection with climate change towards warming, which will possibly affect the spread of *F. hepatica*.

In recent years in Ukraine, the intensity of research on the infection of freshwater mollusks by larvae of the trematode *F. hepatica* has decreased. Currently, there are isolated data concerning parasitocenoses of cattle in the Central region of Ukraine, in particular a study of the epizootiology of fasciolosis of these animals in Zhytomyr region (Hud & Dovhiy, 2021). Therefore, despite the existing publications on the spread of fasciolosis in Ukraine, studies of some aspects of the biology of *F. hepatica* under modern environmental conditions are rather topical, particularly because they can be useful for the

prediction of fasciolosis outbreaks in certain areas. Given the lack of data in recent years on the infection of freshwater mollusks by paratenites and larvae of *F. hepatica* in Zhytomyr region and its forest-steppe zone, the aim of our research was to clarify some aspects of *F. hepatica* biology in Berdychiv district.

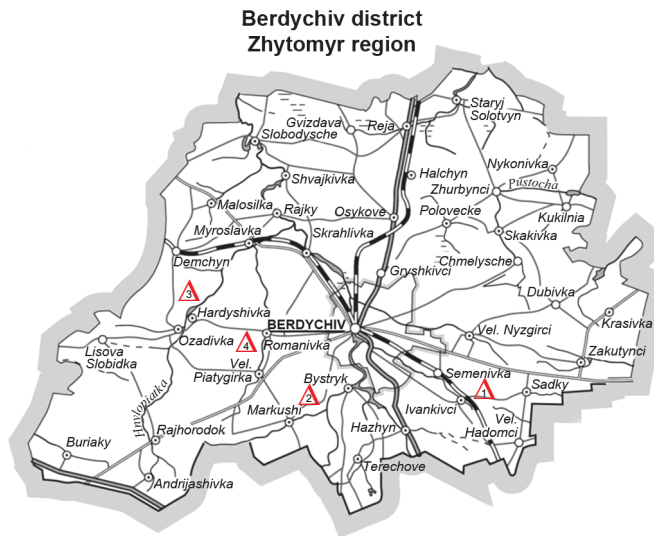


Fig. 1. Map showing the sites of data sampling from water bodies of Berdychiv district: Δ – sites of material sampling

During the collection of mollusks, their population density was determined. For this purpose, standard wooden frames with a surface area of 1 m² were used (Stadnichenko, 2006).

Water sampling was performed at mollusk collection sites. The analysis was performed on the same day in the laboratory of the Polissia National University using standard methods (Yatsyk, Denisova, Chernyavskaya, & Zimina, 1995). Identification of mollusks was carried out by conchological features, taking into account their anatomical data (Stadnichenko, 2004).

Parasitological studies of mollusks were performed by conventional methods (Chernogorenko, 1983). The morphology of cercariae was studied using vital dyes: neutral red and sulphate Nile blue. Live and fixed cercariae were measured. In the course of parasitological study of the mollusks, bioethical norms were not violated.

The advanced analytics software package Statistica 6.0 was used for statistical processing of the primary data.

RESULTS AND DISCUSSION

Conditions necessary for the successful existence of intermediate hosts – mollusks of the subgenus *Galba* – are vital for the circulation of fasciola in a particular area. Today, due to climate change towards warming, it is the permanent water bodies that ensure the successful overwintering of pond snails (*lymnaea*), which is necessary for the completion of the development cycle of *F. hepatica* in early spring. At present, temporary reservoirs do not play an important role in the spread of fasciolosis due to their drying up.

The exploration of the area under study revealed four water bodies inhabited by pondsnails of the subgenus *Galba*, namely *Lymnaea (G.) subangulata* and *L. (G.) truncatula*. The typical habitats of these pondsnails in Berdychiv district are ponds and canals that occur on pastures. Mollusks *L. (G.) subangulata* and *L. (G.) truncatula* were not found in permanently dark and poorly heated water bodies.

The population density of mollusks depends on the water body type, the season of the year, the composition of dissolved minerals and oxygen content in water. Thus, in spring, the population density of *L. (G.) subangulata* in the studied reservoirs ranged from 2 to 18 specimens/m², while at the end of summer it increased to 26 specimens/m². For *L. (G.) truncatula*, the values of this indicator were 1–3 specimens/m² in spring and 15 specimens/m² in summer (**Table 1**).

It was found that *L. (G.) subangulata* and *L. (G.) truncatula* play the main role in the spread of fasciolosis, the incidence of the former species is 88 %, that of the latter – only 12 %.

Table 1. Mean population density of *Lymnaea (Galba) subangulata*

Habitat	Mollusk species	Population density, specimens/m ²			
		2020		2021	
		Spring (May)	Summer (August)	Spring (May)	Summer (August)
Habitat I	<i>L. subangulata</i>	12 ± 0.41	16 ± 0.41	14 ± 0.28	21 ± 0.41
Habitat II	<i>L. subangulata</i>	10 ± 0.57	15 ± 0.41	11 ± 0.41	11 ± 0.40
Habitat III	<i>L. subangulata</i>	9 ± 0.89	14 ± 0.28	11 ± 0.41	19 ± 0.41
Habitat IV	<i>L. subangulata</i>	14 ± 0.44	21 ± 0.27	15 ± 0.41	23 ± 0.41

Pond snails, infected by the trematode *F. hepatica*, inhabit reservoirs with a similar chemical composition of water and rather different quantitative ratios of substances dissolved in water (**Table 2**). In the water of the examined reservoirs inhabited by *L. (G.) subangulata*, as well as the pastures of the district, an increased content of nitrogen nitrates (2.8–70.80 mg/L) was detected. This is probably due to the pollution of water bodies with agricultural and household waste as well as decomposition of organic residues.

Mass emergence of the trematode *F. hepatica* intermediate hosts – mollusks *L. (G.) subangulata* and *L. (G.) truncatula* – was observed in the second half of April and in early May. The active life of these mollusks begins at water temperature of about 10–14 °C.

Collected in May 2020–2021, *L. (G.) subangulata* and *L. (G.) truncatula*, which successfully overwintered, belonged mainly to two previous year's generations – mature (spring) and young (autumn) ones. With the onset of spring, young mollusks of the autumn generation continue their development. After reaching sexual maturity, these mollusks begin to reproduce. In one of the studied reservoirs, some individuals with a shell height of more than 8.2 mm were found. This indicates the ability of *L. (G.) subangulata* to survive two winters.

Temperature is the main factor influencing the growth and development of fasciola partenites and larvae in mollusks. In winter, the process of hatching larvae of *F. hepatica* is suspended and resumes only in spring, with the release of mollusks from overwintering. In recent years (2020–2021), there has been an increase in average daily temperature in spring and summer, which accelerated the development of *F. hepatica* from the beginning of embryogenesis in eggs released into the environment to the formation of adolescents after cercariae emerge from mollusks.

Table 2. Chemical composition of water in the examined habitats of galba

Indices	Habitats	Villages			
		Semenivka	Bystryk	Hardyshivka	Romanivka
		Waterbodies			
	I	II	III	IV	
pH		6.85	6.45	7.12	7.27
Oxidation (mg/dm ³)		6.24	6.64	5.68	6.08
General hardness (mmol/dm ³)		8.4	6.0	5.8	3.6
Clorides (mg/dm ³)		93	58	105	36
Sulphates (mg/dm ³)		15.06	7.14	16.01	9.53
Ammonia nitrogen (mg/dm ³)		5.133	0.514	0.113	0.197
Nitrogen nitrates (mg/dm ³)		0.427	0.031	0.033	0.044
Nitrogen nitrites (mg/dm ³)		70.80	4.96	2.87	2.71

During the study period (2020–2021), the infestation of mollusks by fasciola partenites and cercariae in water bodies of Berdychiv district pastures reached 2.08–18.75 % (4.98 ± 0.47 % on average). The low level of infection 0.8–4.0 % (2.28 ± 0.68 % on average) of *L. (G.) subangulata* by partenites and cercariae of *F. hepatica* was recorded in May and reached 0.8–4.0 % (2.28 ± 0.68 % on average), while the maximum, recorded in August, was 4.44–18.75 % (8.07 ± 1.99 % on average) (Table 3).

The generalized results indicate that the dynamics of the prevalence of *L. (G.) subangulata* invasion has a stable one-type nature represented by a two-vertex curve, with peaks in May (2.51 ± 0.88 %) and August (9.19 ± 1.78 %) (Fig. 2). Compared with the results of previous studies [3] on the dynamics of infection of *(G.) subangulata* by the trematode *F. hepatica* in Zhytomyr region, the peak of fasciola invasion of this species of mollusks shifted from September to August. In our opinion, this is due to the increase in average daily temperatures in recent years.

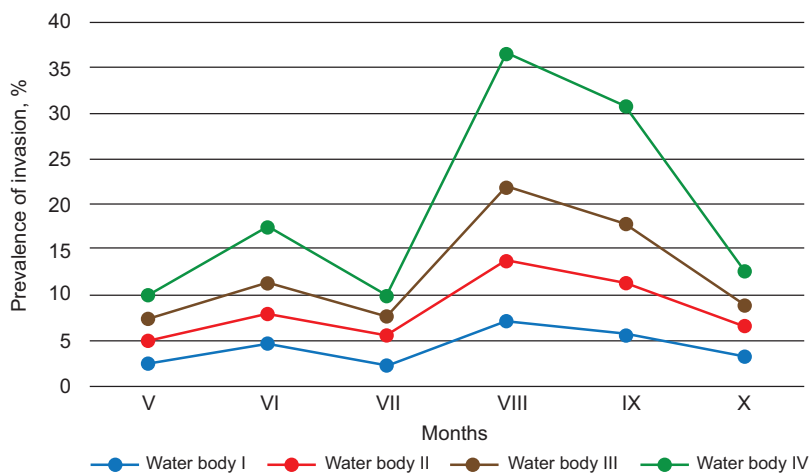

Fig. 2. Seasonal dynamics of prevalence of *L. (G.) subangulata* invasion by fasciola partenites and cercariae in water bodies of Berdychiv district

Table 3. Seasonal variation in prevalence of *Lymnaea (Galba) subangulata* by *F. hepatica* cercariae on the pastures of Berdychiv district, Zhytomyr region in 2020–2021

Habitat	Examination time	2020			2021			Total, %
		number of examined mollusks	number of infected mollusks	PI, %	number of examined mollusks	number of infected mollusks	PI, %	
I	May	37	1	2.71	42	1	2.38	2.53±1.77
	June	40	2	4.35	41	2	4.88	4.60±2.24
	July	41	2	2.44	44	1	2.27	2.35±1.64
	August	48	3	6.25	49	4	8.16	7.22±2.62
	September	42	4	4.76	46	3	6.52	5.68±2.47
	October	46	2	4.35	48	1	2.08	3.2±1.82
Total for the year		260	11	4.23±1.24	270	12	4.44±1.20	4.34±0.89
II	May	41	1	2.43	40	1	2.5	2.47±1.7
	June	42	1	2.38	47	2	4.26	3.37±1.91
	July	44	1	2.27	48	2	4.17	3.26±1.85
	August	45	2	4.44	45	4	8.89	6.67±2.63
	September	42	2	4.76	46	3	6.52	5.68±2.47
	October	46	1	2.17	43	2	4.65	3.37±1.91
Total for the year		260	8	3.08±1.07	269	14	5.21±1.36	4.16±0.87
III	May	40	1	2.5	41	1	2.44	2.47±1.72
	June	47	1	2.12	42	2	4.76	3.37±1.91
	July	48	1	2.08	44	1	2.27	2.17±1.51
	August	45	3	6.67	42	4	9.52	8.05±2.91
	September	46	2	4.34	45	4	8.89	6.59±2.60
	October	41	1	2.43	46	1	2.17	2.30±1.61
Total for the year		267	9	3.37±1.10	260	13	5.0±1.35	4.18±0.87
IV	May	38	1	2.63	40	1	2.5	2.56±1.79
	June	39	2	5.12	43	3	6.98	6.10±2.63
	July	48	1	2.08	45	1	2.22	2.15±1.50
	August	48	5	10.42	48	9	18.75	14.58±3.66
	September	46	4	8.69	48	8	16.67	12.77±3.44
	October	42	1	2.38	40	2	5.0	3.66±2.06
Total for the year		261	14	5.36±1.40	264	24	9.09±1.77	7.24±1.13

Comment: PI – Prevalence of invasion

It should be noted that fewer *L. (G.) subangulata* were infected by *F. hepatica* after winter season than in autumn due to the death of some of the fasciola-infested lymnaeid snails in winter under the influence of low temperatures.

Part of the mollusks that overwintered is made up of individuals that were infected in late autumn; thus, the development of parthenogenetic generations was not completed before winter.

Further development of partenites occurs in mollusks after overwintering.

In *L. (G.) subangulata*, which were infected in spring, the release of *F. hepatica* cercariae occurs no later than June. In mid-summer (July) there was a decline in mollusk infection (2.49 ± 0.82 % on average), which in our opinion is due to a decrease in their population density caused by the death of old mollusks, a large proportion of which were infected by *F. hepatica*. From July, a rapid increase in invasion begins. In August and early September, cercariae of *F. hepatica* were detected mainly in mollusks of the spring generation of the current year, their infestation level being rather high. Due to the death of old individuals that overwintered, the number of the latter in the population was insignificant; thus, the cases of invasion among them were rare.

According to the results of the study, the overall extent of invasion of *L. (G.) subangulata* by fasciola cercariae in the examined water bodies in 2020 was significantly lower than in 2021 ($P \leq 0.05$), which is probably due to weather conditions (**Table 4**).

Table 4. The results of statistical comparisons of the general prevalence of *L. (G.) subangulata* invasion in water bodies of Berdychiv district in 2020–2021

Year	Q ± m _x	t	P, %
2020	4.01 ± 0.61	2.04	95.86
2021	5.93 ± 0.72		

In the spring and summer period of 2020 in Berdychiv district, the average daily air temperature was 11 °C, the average precipitation – 56.6 mm, the average relative humidity – 70 %, while in 2021 the values of these indicators reached 12 °C, 72 mm and 74 %, respectively.

In the autumn period, late October – early November, when water temperature drops to +5–8 °C, the mollusks of the autumn and partly of the spring generations of the current year prepare for overwintering by immersing in muddy soil and becoming sedentary.

The study has proved the dependence of the seasonal dynamics of *L. (G.) subangulata* invasion on both the season of the year and the proportion of the number of individuals of different ages in the mollusk population. Infection of individuals with a shell height of 4.0 to 8.2 mm by *F. hepatica* cercariae was detected. At the same time, fasciola cercariae were not found in mollusks with a shell height of 0.8–3.8 mm.

The analysis of the size and age (2020–2021) of individuals of the studied sample of *L. (G.) subangulata* showed that populations of these mollusks included individuals of several generations. The main size and age groups were as follows: 1) mollusks of the previous year's spring generation with a shell height of 4.3–6.3 mm in spring, and 5.7–7.0 mm in autumn; 2) mollusks of the previous year's autumn generation with a shell height of 3.6 to 5.6 mm; 3) mollusks of the spring generation of the current year, which reached a shell height of 2.9–4.9 mm before overwintering; 4) mollusks of the autumn generation of the current year whose shell did not exceed 3 mm in height before overwintering (the range of shell height 0.8–2.8 mm).

The general sample under study was dominated by individuals with a shell height of 0.8–4.9 mm (68 % of the total number), which means that mollusks of the current year's generations made up the larger part of the population (**Fig. 3**).

This is evidenced by the distribution of mollusks infected by cercariae of *F. hepatica* by size and age groups (**Fig. 4**).

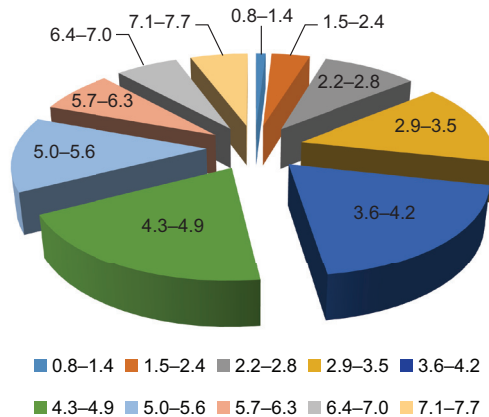


Fig. 3. Size and age structure of *L. (G.) subangulata* populations based on the samples collected in 2020–2021

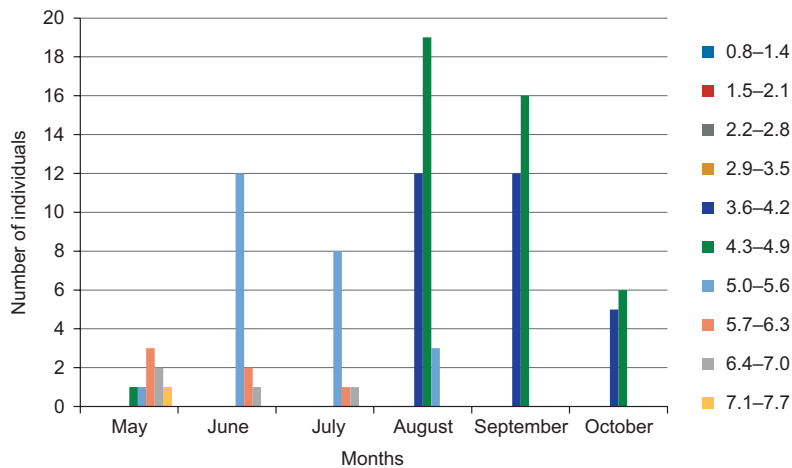


Fig. 4. Seasonal distribution of infected *L. (G.) subangulata* by age groups (2020–2021)

After overwintering, in May, individuals with a shell height of 5.7–7.7 mm accounted for 75 % of all the infected mollusks, although their share in the *L. (G.) subangulata*-population structure reached only 19.42 % of the total sample.

In summer (June–July), individuals with a shell height of 5.0–5.6 mm make up the most infected group, which accounts for 80 % of all the infected mollusks. In August, the overall picture changes significantly. Among the infected *L. (G.) subangulata*, the dominant group is mollusks with a shell height of 4.3–4.9 mm (61 % infected, their share reaches 19.7 % of the total sample of mollusks). In September and October, it is overtaken by a group with a shell height of 3.6–4.2 mm (44 % infected, 19.4 % of the total sample of mollusks). Both groups are represented by individuals of the spring generation of the current year, which are dominant in the population. These mollusks overwinter and remain infected until spring. However, in spring the extent of their invasion is significantly lower, due to the mortality of the infected *L. (G.) subangulata* in winter.

The generalized results of our study show that the potential intermediate hosts for *F. hepatica* in Berdychiv district are *L. (G.) subangulata* and *L. (G.) truncatula*, which

is consistent with previous research data (Dovhiy & Hud, 2020; Hud & Dovhiy, 2021; Zhytova, 2021) that indicate the same species of mollusks as intermediate hosts for fasciola in the territory of Ukrainian Polissia. It should be noted that the *L. (G.) goupili* and *L. (G.) oblonga* mollusks of the *Galba* subgenus can also act as intermediate hosts for *F. hepatica*, but they were not found in the water bodies under study.

It is known (Bennema *et al.*, 2011; Beesley *et al.*, 2017) that the life cycle of *F. hepatica* depends on environmental and climatic factors. In particular, the presence of vegetation, wetlands, water bodies, high animal density, as well as the lack of prevention and treatment of fasciolosis contribute significantly to the spread of fasciolosis. On the pastures of Berdychiv district, there are permanent water bodies with stagnant water. These habitats with relatively stable water levels, shores overgrown with grassy vegetation, and a large number of cattle from individual farms provide favorable conditions for the successful circulation of *F. hepatica*.

CONCLUSIONS

Based on the obtained results, we can conclude that under modern climatic conditions, in the territory of Berdychiv district of Zhytomyr region, permanent water bodies have become foci of fasciolosis and common habitats for *L. (G.) subangulata* and *L. (G.) truncatula*. The most common and numerous species *L. (G.) subangulata* (occurrence 88 %) plays the leading role in the spread of fasciolosis. In these mollusks, infection by *F. hepatica* was registered at a shell height of 4.0 mm or more. Therefore, during the epizootological assessment of water bodies for fasciolosis, small *L. (G.) subangulata* do not need to be examined, which significantly improves and facilitates the effectiveness of work. The most favorable environmental conditions for *F. hepatica* and its intermediate hosts develop in the spring, accordingly, for the definitive hosts, this period is quite dangerous in terms of the possibility of their infection by fasciola.

Thus, elucidation of some issues in the biology of the trematodes *F. hepatica* in Berdychiv district will enable scientists to predict the infection of animals with fasciolosis and stop outbreaks of this disease in farms by preventing contact between fascioles and their definitive hosts.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal studies: All international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [O.Z.]; methodology, [O.Z.]; validation [O. Z., Yu.S.]; formal analysis, [O. Z., Yu.S., O.A.]; investigation, [O.P., Yu.S., A.L.]; resources [O.Z.; Yu.S.; O.A.]; data curation, [O.P., A.L.]; writing – original draft preparation [O.Z.]; writing – review and editing, [O.Z.]; visualization, [O.Z.]; supervision, [O.Z.].

All authors have read and agreed to the published version of the manuscript.

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ЕКОЛОГО-ПАРАЗИТОЛОГІЧНА ХАРАКТЕРИСТИКА СИСТЕМИ “*FASCIOLA HEPATICAL.–LYMNAEA (GALBA) SUBANGULATA*”

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Вступ. Фасціольоз є одним із поширених у світі зоонозів, спричинений трематодою *Fasciola hepatica* L. Цей гельмінтоз призводить до значних економічних збитків у тваринництві та може являти загрозу безпосередньо для здоров'я людини. На сьогодні, за умов кліматичних змін, докладні знання біологічних особливостей циркуляції трематоди *F. hepatica* дадуть можливість детально спрогнозувати спалахи фасціольозу в господарствах і спланувати дієві заходи боротьби з цим небезпечним захворюванням. Мета роботи – з'ясувати деякі аспекти біології *F. hepatica* в межах Бердичівського району, що належить до лісостепової зони Житомирщини.

Матеріали та методи. Роботу виконували протягом 2020–2021 рр. Матеріалом для досліджень слугували збори молюсків із водойм на території Бердичівського району Житомирської області. Їхню ідентифікацію проводили за конхологічними ознаками. Для паразитологічного дослідження молюсків застосовували загальноприйняті методи. Загалом обстежено понад 2000 екз. *Lymnaea (Galba) spp.* Відбір проб води, молюсків здійснювали з чотирьох пасовищних водойм.

Результати. Основним проміжним хазяїном *F. hepatica* на території Бердичівського району є молюски *L. (G.) subangulata*, другорядна роль у поширенні цієї трематоди належить *L. (G.) truncatula*. Відмічено, що носіями партеніт *F. hepatica* є переважно молюски з висотою черепашки від 4,0 до 8,2 мм. Отримано нові дані щодо динаміки зараження *L. (G.) subangulata* трематодою *F. hepatica*. Динаміка зараженості цих молюсків церкаріями *F. hepatica* показує стійкій однотиповий характер у вигляді двовершинної кривої, з піками у травні ($2,51 \pm 0,88\%$) і серпні ($9,19 \pm 1,78\%$). Загальна екстенсивність інвазії *L. (G.) subangulata* церкаріями фасціол у досліджених водоймах у 2020 р. вірогідно менша порівняно з 2021 р.

Висновки. За сучасних кліматичних умов на території регіону постійні водойми стали осередками фасціольозу та звичайними місцями оселення *L. (G.) subangulata*, заражених церкаріями *F. hepatica*. Найсприятливіші умови зовнішнього середовища для фасціоли і її проміжних хазяїв утворюються навесні, відповідно для дефінітивних хазяїв цей період є досить небезпечним із точки зору зараження тварин.

Ключові слова: *Fasciola hepatica*, молюски, *Lymnaea (Galba) subangulata*, *Lymnaea (Galba) truncatula*, проміжні хазяї, дефінітивні хазяї