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Article

Morphological and Biological Development of Fasciola Hepatica Larvae in a Mollusk

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Abstract: The article presents an analysis of literary data on the gastropod mollusk family Lymnaedae, which is an intermediate host of fasciolae, as well as the results of studies conducted in 2010-2023 on the stages of development of fasciolae parthenites in the body of mollusks.

Keywords: mollusk, sporacyst, radia, cercariae, adolescariae

1. Introduction

Nowadays, to meet the demand of the world population for high-quality products, it is necessary to develop livestock breeding at an innovative level, protecting animals from various infectious, non-infectious and invasive diseases, which is one of the most important and urgent tasks. The aim is to study the developmental stages of parthenite fascioles in pulmonary gastropod molluscs, which act as intermediate hosts in the spread of fascioliasis, widespread among cattle, sheep and goats in some districts of Samarkand region. Extent of study of the problem. The causative agents of fascioliasis, *F. gigantica* and *F. hepatica*, are found in many countries of the world and cause dangerous helminthic diseases in animals and humans. In particular, there is sufficient information on the prevalence of these trematodes in the regions of Central Asia, Armenia, Georgia, Azerbaijan, the African continent, Vietnam, Arab countries and Hawaii [1].

F. gigantica is the most dangerous and highly pathogenic species among the causative agents of fascioliasis and, due to its morphological and biological characteristics, is widespread in countries with warm climates, including Central Asia and Transcaucasia. *F.gigantica* is widely distributed in all regions of Uzbekistan and on the territory of the Republic of Karakalpakstan [2].

The reason for this is that from the biological point of view, *F. gigantica* sharply differs from *F. hepatica* in that all developmental periods are long and parthenogenetic development occurs in a completely different type of aquatic molluscs (phytophilic - herbivorous). In the territory of Uzbekistan, our scientists have established that intermediate hosts of *F. gigantica* are 4 species of large limnaids *Lymnaea aurcularia*, *L. bactriana*, *L. subdisjuncta*, *L. impura* [3].

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The available literature describes that only one species of *Fasciolopsis buski* parasitises the human gastrointestinal tract, while species of *Fasciola halli*, *Fasciola indica*, *Fasciola Californica*, *Fasciola indes magna*, *Parafasciolopsis fasciola morpha*, cause fasciolosis in mammals and are found in the USA, Russia, Belarus and India [4].

As a result of our long-term observations, the participation of *Lymnaea truncatula* and *L. thiessea* in the larval development of *F. hepatica* in the climatic conditions of Uzbekistan was once again established [5]. After getting fertilised eggs of *F. hepatica* with faeces of the main host into water, or in wet conditions when the ambient temperature reaches 15-25°C, within 3 days 10-25 days the larva of the parasite of the first generation - ciliated miracidium comes out. Intermediate hosts of this trematode are pulmonary gastropod mollusks living in various freshwater bodies [6]. *F. hepatica* has been found to parasitise 15 species of mammals in Uzbekistan [7]. To maintain its viability and to continue its further development, Myracidium actively penetrates the organism of the respective intermediate hosts, the molluscs *L. truncatula* and *L. thiesseae*. In the liver of intermediate hosts, it metamorphoses and transforms into a second-generation larva, a sac-shaped sporocyst. From the germ cells, sporocysts develop into third-generation fasciola larvae - rediae, which emerge parthenogenetically, i.e. without hatching.

Miracidia, which have entered the mollusk organism by different ways, undergo regressive metamorphosis (cilia, nerve nodes, eyes, and protonephridia disappear), as a result of which embryos of different shapes turn into second-stage sporocyst larvae. Such sporocysts receive their development in the liver of the mollusk, and it continues until the formation of redia, the third-generation larva, by parthenogenesis.

Redia are sac-like clusters of parthenogenic cells having a mouth, muscular pharynx and sac-like gut. The redia are divided into two groups. Fourth generation larvae, caudal cercariae, emerge from the cells of one group, and daughter rediae emerge from the cells of the second group.

Parthenogenetic reproduction of *Fasciola larvae* continues indefinitely, until the death of the mollusk. The exit of cercariae from the mollusc's body is divided into dark and light phases. Molluscs are of theoretical and practical importance, given that they participate as intermediate hosts in the spread of trematode diseases in Uzbekistan, it is important to study their systematics, species composition, ecology, ethology and biogeography.

In order to become infectious, cercariae excreted from the mollusk body must undergo a developmental stage, cystogonia, which for *F. hepatica* occurs in the external environment. During this process, cercariae released into the water (according to our special observations) cling to the substrate (mainly to aquatic plants) and attach their body with suction cups, and then quickly covered with cystogenic glands, which quickly harden in the water. The tail part is compressed by the outer shell, after some time due to intense movement, the tail is torn off, and the larva with the help of the juice of cystogenic glands forms an inner shell and turns into a cyst (adolescarium). Adolescarium is the last stage of the fifth generation *F. hepatica* larva, which becomes infectious to the definitive hosts.

The necessity of passing the stage of development of cystogonium (adolescaria) is that cercariae, after leaving the mollusk organism, are unstable to exogenous and endogenous factors. It is for this reason that such a larva can retain its invasiveness in the external environment for a long time. The shell of the *F. hepatica* cyst consists of four layers: the first is the outer, incompletely developed layer; the second is a thin, fibrous layer; it contains mucoprotein and polysaccharide acid; the third layer is densely structured and has a mucoprotein, mucopolysaccharide and neutral polysaccharide sublayer; the fourth layer consists of protein-lipid plates [8]. For this reason, our studies have shown that it takes at least 2-3 days for the last three layers of Fasciola (*F. hepatica*) cysts to fully form, and only then do they become infectious to their definitive hosts.

The maritagonia process takes place in the organism of the main host, where adult maritagonia emerge from the invasive larvae infected by various pathways. The adult *F.*

hepatica adopts the path of sexual reproduction, and can lay several tens of thousands, even more than a hundred thousand eggs in a single day. For example, it was found that *F. hepatica* releases about 1 million eggs in one week [9].

It should be noted that adults of *F. hepatica* reproduce sexually, and in the larval stage - parthenogenetically. Sexual reproduction occurs in the organism of the main host, the embryogony period - in the external environment, parthenogenetic reproduction - in the intermediate host - in the mollusk organism. The period of cystogonia development proceeds in exogenous conditions [10].

The developmental period of Maritogonia proceeds in the internal environment, i.e., in the body of vertebrates; an adult individual of *F. hepatica* lives in the host body for up to several years.

2. Materials and Methods

Gastropod molluscs of the family Lymnaedae collected from biotopes of Okdarya, Payaryk, Ishtikhon districts of Samarkand region served as the material of the study. Epizootological, clinical and malacological methods were used in the study.

3. Results and Discussion

Studies were conducted on the molluscs *Lymnaea truncatula* and *Lymnaea thiesseae*, belonging to the family *Lymnaedae*. In the world fauna there are more than 150 species of the family *Lymnaedae*, which are divided into genera *Lymnaea* and *Aenigmomphiscola*. In Central Asian regions 34 species of the genus *Lymnaea* are found, which are divided into 8 subgenera.

As for the molluscs considered as the object of the present study, the shell of the mollusk *Lymnae truncatula* is brown, high cone-shaped, with 4-5 equally growing whorls, the tangent line is straight, the last whorl is not swollen. Shell whorls are separated by strongly raised, stepped, deep, almost non-angled sutures, mouth flat [11]. The clam *L.truncatula* can be active for 8-10 months and sometimes longer, in different biogeocenoses of Uzbekistan throughout the year [12]. The mollusk *Lymnaea thiesseae* has a brown or bright brown shell, cone-shaped egg, thin wall, 4-5 irregularly growing whorls, the tangent line is straight, the last whorl is more swollen and higher. Shell spirals are characterised by strongly convex, stepped, but not many deeper and higher whorls than in the first type, and also by gentle sutures. The mouth is ovoid, elongated and flat, the upper part is straight or partially angular [13].

Ecological parameters of the habitat of molluscs of the family Lymnaedae are largely similar and do not differ in any way, the mollusc *L.truncatula* is found together with other species of molluscs of this family in springs, spring waters, small ponds, ditches and canals. Depending on the regional location of this species, numbers increase in March-June, giving 2-3 generations per year. Adults lay eggs in the soil, and after 1.5-2 weeks, young molluscs hatch from the eggs. The life cycle lasts about a year. The mollusk *L.thiesseae* inhabits rapidly drying and shallow springs, as well as on the edges of ditches and in silt, amphibiont-telmatophile, polygamous species. Often occurs together with *L.truncatula* and species belonging to this systematic group. Numbers increase in April, May, June. Also, like many representatives of this family life expectancy is up to one year [14].

The degree of infestation of molluscs *L.truncatula* and *L.thiesseae* by Fasciola hepatica parthenites collected from biotopes of some districts of Samarkand region was studied (Figure 1-2).

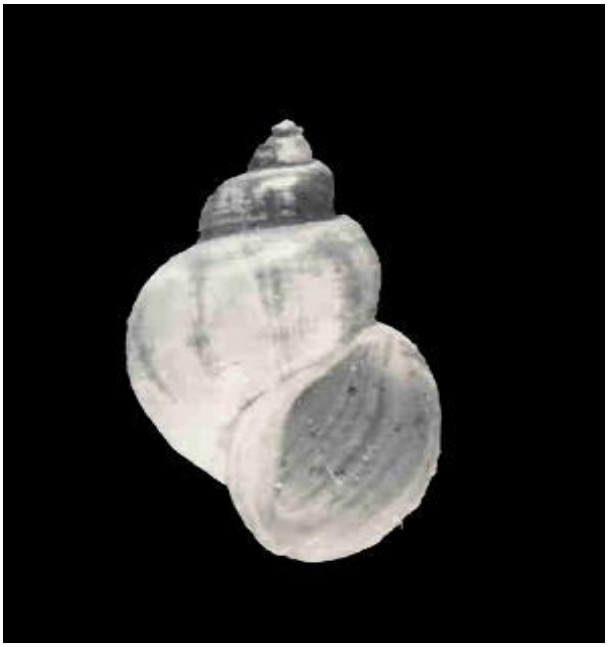

Figure 1. *Lymnaea truncatula*

Figure 2. *Lymnaea thiesseae*

The collection of molluscs of species *L.truncatula* and *L.thiesseae* collected from biotopes of some districts of Samarkand region is presented. Molluscs were studied according to generally accepted methods [15]. For this purpose, the molluscs, which appeared in the biotopes selected as an experimental site, were collected on Sundays weekly during the year in order to study the level of infection by parthenites of fascioles, kept in laboratory conditions, dissected and examined.

Firstly, the collected molluscs were placed in 10 beakers filled with clean water and introduced green plant leaves under laboratory conditions. At a certain interval of time, the leaves were examined with a magnifying glass for the presence of adolescariae. The liver of dead molluscs was crushed and examined on a slide with two or three drops of clean water using the 8th objective and 7th eyepiece of the microscope. If parthenites were not detected in the clam liver sample, clams from this beaker were taken into separate beakers and the remaining leaves of the plants on the 2nd, 3rd, 4th and 5th day were

examined. Adolescaria collected from these leaves were injected into experimental animals kept in the vivarium and their pathogenic properties were studied.

Analysing the results of our long-term studies of mollusks *L.thiesseae* (Table 1) we revealed that individuals of 4-4.5 mm size mainly appear in biotopes from March, and in April and May sporocysts of young parthenites of fascioles are not detected in laboratory conditions.

Table 1. Results of a laboratory study on the mollusc *Lymnaea thiesseae* (2010 - 2023)

Years and months of experimentation	Number of molluscs examined (total)	Number of infested molluscs (total)		Number of detected partenites, including					
				Sporocysts		Rhedia		cercariae	
		Number	%	Number	%	Number	%	Number	%
2010 July	40	3	7,5	3	100,0				
2010 August	65	6	9,2	5	83,3	1	16,7		
2010 September	95	13	13,7	10	76,9	2	15,4	1	7,7
2010 October	45	6	13,3	4	66,7	1	16,7	1	16,7
2011 July	40	3	7,5	3	100,0				
2011 August	65	7	10,8	6	85,7	1	14,3		
2011 September	95	14	14,7	10	71,4	2	14,3	2	14,3
2011 October	45	7	15,6	3	42,9	2	28,6	2	28,6
2012 July	50	4	8,0	4	100,0				
2012 August	70	6	8,6	5	83,3	1	16,7		
2012 September	95	13	13,7	9	69,2	2	15,4	2	15,4
2012 October	40	5	12,5	3	60,0	1	20,0	1	20,0
2013 July	45	3	6,7	3	100,0				
2013 August	60	6	10,0	5	83,3	1	16,7		
2013 September	95	12	12,6	8	66,7	3	25,0	1	8,3
2013 October	40	5	12,5	3	60,0	1	20,0	1	20,0
2014 July	45	3	6,7	3	100,0				
2014 August	70	6	8,6	6	100,0				
2014 September	90	9	10,0	6	66,7	2	22,2	1	11,1
2014 October	40	6	15,0	4	66,7	1	16,7	1	16,7
2015 July	45	3	6,7	3	100,0				
2015 August	70	7	10,0	6	85,7	1	14,3		
2015 September	95	11	11,6	7	63,6	3	27,3	1	9,1
2015 October	40	5	12,5	2	40,0	2	40,0	1	20,0
2016 July	45	3	6,7	3	100,0				
2016 August	75	6	8,0	5	83,3	1	16,7		

2016 September	90	9	10,0	6	66,7	2	22,2	1	11,1
2016 October	40	6	15,0	4	66,7	1	16,7	1	16,7
2017 July	50	4	8,0	4	100,0				
2017 August	65	5	7,7	4	80,0	1	20,0		
2017 September	90	11	12,2	7	63,6	2	18,2	2	18,2
2017 October	45	7	15,6	3	42,9	2	28,6	2	28,6
2018 July	55	5	9,1	5	100,0				
2018 August	80	6	7,5	5	83,3	1	16,7		
2018 September	90	10	11,1	7	70,0	2	20,0	1	10,0
2018 October	45	6	13,3	3	50,0	2	33,3	1	16,7
2019 July	60	6	10,0	6	100,0				
2019 August	70	7	10,0	6	85,7	1	14,3		
2019 September	100	11	11,0	8	72,7	2	18,2	1	9,1
2019 October	100	12	12,0	8	66,7	2	16,7	2	16,7
2020 July	80	6	7,5	6	100,0				
2020 August	90	6	6,7	5	83,3	1	16,7		
2020 September	110	13	11,8	10	76,9	2	15,4	1	7,7
2020 October	120	15	12,5	11	73,3	2	13,3	2	13,3
2021 July	60	5	8,3	5	100,0		0,0		
2021 August	100	9	9,0	7	77,8	2	22,2		
2021 September	120	16	13,3	12	75,0	2	12,5	2	12,5
2021 October	110	14	12,7	10	71,4	3	21,4	1	7,1
2022 June	65	5	7,7	5	100,0				
2022 July	75	6	8,0	6	100,0				
2022 August	310	27	8,7	23	85,2	4	14,8		
2022 September	150	16	10,7	11	68,8	3	18,8	2	12,5
2022 October	170	18	10,6	12	66,7	4	22,2	2	11,1
2023 June	120	4	3,3	4	100,0				
2023 July	110	9	8,2	8	88,9	1	11,1		
2023 August	115	10	8,7	8	80,0	2	20,0		
2023 September	195	13	6,7	9	69,2	2	15,4	2	15,4
2023 October	180	23	12,8	12	52,2	7	30,4	4	17,4
2023 November	140	21	15,0	10	47,6	6	28,6	5	23,8
2023 December	95	16	16,8	4	25,0	8	50,0	4	25,0
Total	**5095**	**529**	10,4	**383**	72,4	**95**	18,0	**51**	9,6

In August, on average, 8.7% of all molluscs examined were infested, of which rediae were found in 15.8% of molluscs, and cercariae were not found in molluscs in this month, while 11.7% were infested in September and 16.3% of all molluscs in October.

Several changes have occurred in *L.thiesseae* based on the results of surveys conducted in the last 2 years, for example, in our observations in 2022, only in the first June of the summer season, sporocysts were detected in 5 clams out of 65 clams in biotopes (7.7%), while rediae and cercariae were not detected. Of the 120 clams collected in 2023, sporocysts were found to have formed in 4 specimens or 3.3 per cent. In contrast, in mollusks that are the result of our multi-year studies, our 2023 observations in November found cercariae in 5 of 140, or 23.8 per cent of 21 infected mollusks, and in 4 specimens examined in the laboratory in December of 95 mollusks, or out of a total of 16 mollusks infected, cercariae were found in 25.0 per cent of the samples.

Out of 5095 examined specimens of molluscs *L.thiesseae*, Fasciola parthenitis at different stages was found in 529 or 10.4%, of which sporocysts - 383 or 72.4%, rediae - 95 or 18.0%, cercariae - 51 or 9.6%.

4. Conclusion

1) It has been established that 2 species of gastropod molluscs *L. truncatula* and *L. thiesseae* are involved in fascioliasis caused by *F. hepatica*. Hepatica, 2 species of gastropod molluscs *L. truncatula* and *L. thiesseae* are involved in fasciolosis caused by *F. hepatica* in conditions of Samarkand region.
2) According to the results of our research, 10.4% of the studied molluscs were infected with Fasciola parthenitis, of them sporocysts - 72.4%, rediae - 18.0%, cercariae - 9.6%.
3) It was established that these territories of Samarkand region, where our studies were conducted, are extremely unhealthy for fasciolosis, and also the conducted studies showed that mollusk *L. thiesseae* is infected by young parthenites of *F. hepatica* only after winter anabiosis and participates in aggravation of epizootic condition of fasciolosis up to cold days of winter.

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