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ИССЛЕДОВАНИЕ ВОСПРИИМЧИВОСТИ *HELIX POMATIA* (MOLLUSCA, HELICIDAE) К ПРОТОСТРОНГИЛИДАМ (NEMATODA: PROTOSTRONGYLIDAE) ПРИ МНОГОКРАТНОМ ЗАРАЖЕНИИ

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Реферат

Цель исследования – изучение восприимчивости моллюсков вида *Helix pomatia* к протостронгилидам при многократном заражении.

Материалы и методы. Экспериментальное заражение моллюсков вида *H. pomatia* (Mollusca, Helicidae) протостронгилидами (Nematoda: Protostrongylidae) осуществляли последовательно 4 раза через каждые 14–20 сут. Через 2 недели после каждого очередного заражения часть моллюсков вскрывали с целью определения экстенсивности и интенсивности инвазии (ИИ), коэффициента внедрения личинок в моллюсков, стадии развития в моллюсках. Личинок протостронгилид получали из ноги моллюска, которую отсекали и исследовали под микроскопом компрессорным методом. Полученные результаты обработали статистически, используя программу Statistica 7.

Результаты и обсуждение. Выявлено, что после каждого нового заражения ИИ и коэффициент внедрения повышаются. Данные исследований показали, что моллюски остаются восприимчивыми к инвазии протостронгилидами в условиях многократного заражения. После 3 и 4-го заражений относительное число внедрившихся в моллюсков личинок меньше, по сравнению с 1 и 2-м инвазированием. Это означает, что у хозяина существуют механизмы, ограничивающие увеличение интенсивности инвазии протостронгилидами в условиях повторяющихся заражений.

Ключевые слова: Helix pomatia, протостронгилиды, экспериментальное заражение, чувствительность к повторному заражению.

Introduction

Protostrongylidae (Nematoda) are widely distributed parasites infecting ruminants, lagomorphs and carnivores and causing high economic damage. Various species from this family have been found at Europe, Asia, Africa and Australia [5]. They are biohelminths having mollusks, mainly land snails (Gastropoda: Pulmonata) as intermediate hosts. Infection of these intermediate hosts is performed by stage 1 larvae from feces of a definitive host. During development in a mollusk organism, larvae transform after 2 molting into infective stage 3 larvae. After being swallowed by definitive hosts they develop into adult forms.

There are a lot literature data of studies of mollusks' susceptibility to infections and formation of immunity in these invertebrates which play highly important part in various helminths' life cycles. Particularly interesting are studies of protective reactions' mechanisms of gastropods in cases of trematode infections where they play part of intermediate hosts. This aspect is analyzed in depth in fundamental works of Ataev, Polevschikov [1], Ataev, Dyachkov, Polevschikov [2], Be'er [3], Be'er, Voronin [4]. These works present results of comparative immunological analysis of protective reactions of gastropods, e. g. *Biomphalaria glabrata*, *Lymnaea stagnalis* to infection with trematodes *Schistosoma mansoni*, *Trichobilharzia ocellata*, *Opisthorchis felineus*, etc.

Main factors of cell immunity of mollusks according to most authors are: a) phagocytosis removing remains of degrading parasites and taking part in encapsulation; b) leukocytosis, i. e. fast increase in leukocytes and amoebocytes numbers in response to helminths' presence; c) nacrezation in response to parasites' irritating effect, such as with pearl formation; d) incapsulation when cell elements concentrate and form capsule around alien body. It was a common opinion for a long time that protective reactions of invertebrates have antigene-nonspecific character, which was based on a point of view of absence of molecules of immunoglobulin suprafamily in invertebrates. However, studies of latter years have shown that haemocytes of mollusks playing the leading part in their immunity have a full complex of features characteristic for any phagocyte taking part in reactions of inborn immunity in multicellular animals regardless of their organization levels [2]. Also a presence of immunoglobulin-like molecules and mRNA receptor to adrenocorticotropic vertebrate hormone have been demonstrated, showing a long history and high evolutionary conservatism of immunity mechanisms.

Resistance of mollusks to infection with these parasites is as a rule specific at population level and may be characterized with a specific (non-zero) level of infection with parasites from local populations and with low level of mortality of the infected mollusks. It should be also noted that compatibility between various dems of mollusks and trematodes developed in the course of their coevolution may be broken under effects of changing environment factors. In such cases there is a possibility of development of temporary or pseudoresistance [3] to trematode infection which should be taken into consideration in studies of mollusk immunity.

So, in mechanisms of formation of gastropode response to parasite infections both cell and humoral factors play an important part.

Recently, several experimental studies of complex relationships inside «parasite–host» systems, including «Protostrongylidae–mollusks» system were performed [7–10, 13]. Our team had also performed some studies in this area [6, 11, 12]. However, there are still no such studies or precise data on establishment of immunity in intermediate hosts after being infected with Protostrongylidae. So, this work had been performed to study a character of susceptibility of *Helix pomatia* mollusks to Protostrongylidae in repetitive infections conditions. A choice of intermediate host was based on preliminary data about its susceptibility to infections with small lung nematodes. Also, it had proved itself the most appropriate object for work in experimental conditions [11].

Materials and methods

The experiment used juvenile mollusks from species *Helix pomatia* (Mollusca, Helicidae). To exclude mollusks naturally infected with Protostrongylidae they were collected from locations free from ruminants' grazing. Larvae of stage 1 were taken from sheep and goat feces. For active stage 1 larvae feces probes were studied using Vaida method. Eighty specimens of mollusks collected have been infected. Four consecutive infections were performed after each 14–20 days. After each 2 weeks after each consecutive infection 20 mollusk specimens were dissected and infection parameters analyzed. The mollusks' experimental infections were performed as follows: feces with highly active larvae were placed into a glass jar. Mollusks remained in the feces for 1,5 hr. Then they were kept in glass aquariums on soil at 20–25 ^{o}C. High air humidity in aquariums was provided for through regular water spraying; mollusks were fed with grass and leaves. Before dissection, their length and weight were measured. Protostrongylidae larvae were obtained from mollusk foot which was diced and studied under microscope using compression method. Numbers of larvae in each mollusk foot were counted and their stage of development noted. For higher reliability of the results obtained the so-called infestation coefficient (numbers of larvae per 1 g of muscle mass) was used. Statistical processing of the results was performed using Statistica 7 (Star Soft, Inc).

Results and discussion

Total weight of mollusks from the 1st group (single infection) before dissection was 26,94 g, total parasite load was 5927 larvae and infestation coefficient – 220 larvae per 1 g. The same parameters for 2nd, 3rd and 4th groups were, respectively: weight – 34,03 g, parasite load – 8680 larvae, infestation coefficient – 255 larvae/g; 30,3 g, 9692 larvae, 320 larvae/g; 32,93 g, 10667 larvae and 324 larvae/g. Graphical representation of these results can be seen at Fig. 1. It shows that weights of mollusks from all groups have no significant differences. However, parasite load and infestation coefficient increase after each subsequent infection. Basing on the results obtained we may reach a conclusion that the mollusks are sensitive to subsequent infection and, so, do not gain full immunity.

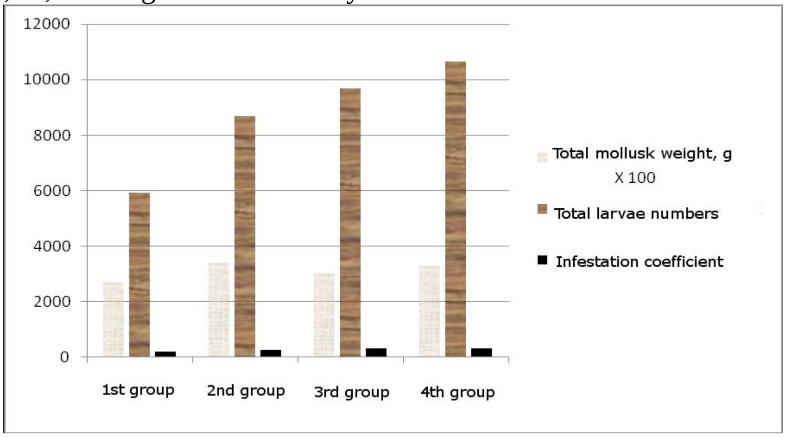

Fig. 1. Results of experimental infections of *Helix pomatia* mollusks with Protostrongylidae

However, there are some nuances here. Development of the larvae inside mollusks from the 1st group (single infection) have led to formation of stage 2 larvae (Fig. 2). The development inside mollusks of the 2nd group produced helminths of stage 2 and stage 3 (Fig. 3) in about equal numbers. After 3rd and 4th infections we have found mostly stage 3 larvae (Fig. 4).

Decrease of numbers of larvae at «fresh» stages after subsequent infections means that after 3rd and 4th infections relative numbers of the larvae infesting the mollusks are less than that after 1st and 2nd ones. These results prove that the mollusk host studied has mechanisms able to partially restrict intensity of Protostrongylidae infestation in conditions of subsequent infections. These data are in accord with those in works of Ataev [2, 3] and Be'er [4] and literature data given in these for other mollusk species and trematode parasites.

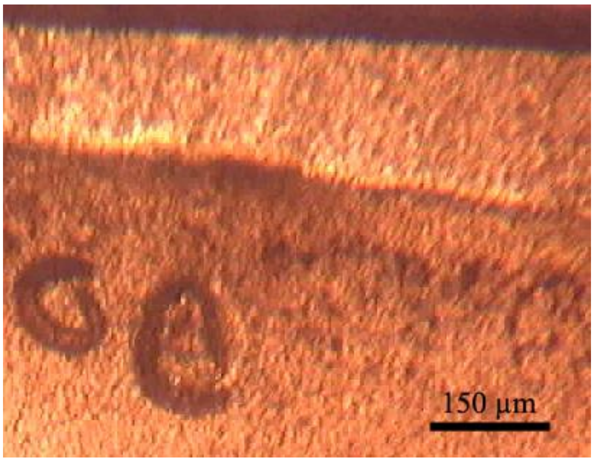

Fig. 2. The foot of *Helix pomatia* experimentally infected with Protostrongylidae (compression; stage 2 larvae)

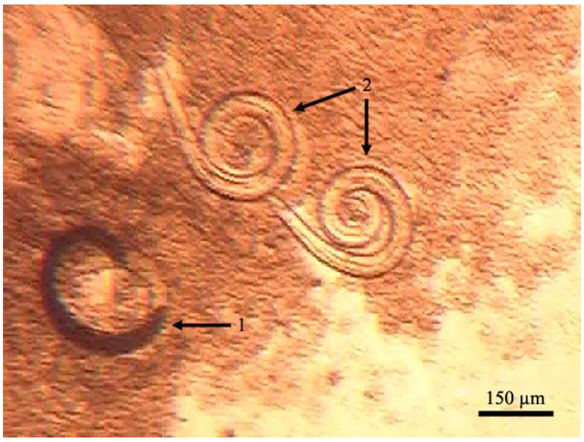

Fig. 3. The foot of *Helix pomatia* experimentally infected with Protostrongylidae (compression; 1 – stage 2 larvae; 2 – stage 3 larvae)

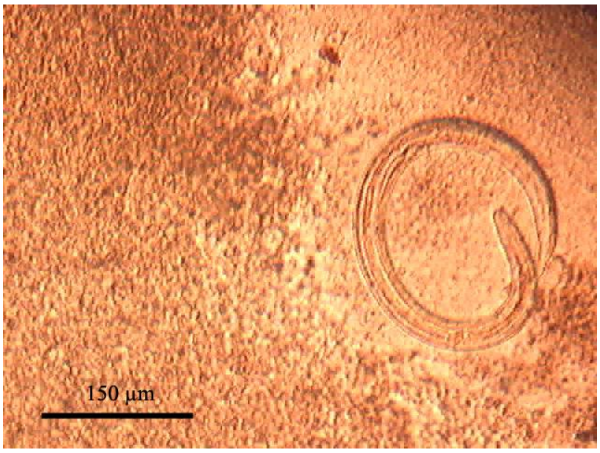

Fig. 4. The foot of *Helix pomatia* experimentally infected with Protostrongylidae (compression; stage 3 larvae)

An interesting fact should be noted here: after compression of significant part of mollusks from 3rd and 4th groups we have found inside them an aggregation of specific darkly colored cells around stage 3 larvae (Fig. 5). Such host reaction may be explained as a start of encapsulation, restriction and elimination of the parasites. It was seen 1,5 months post infection.

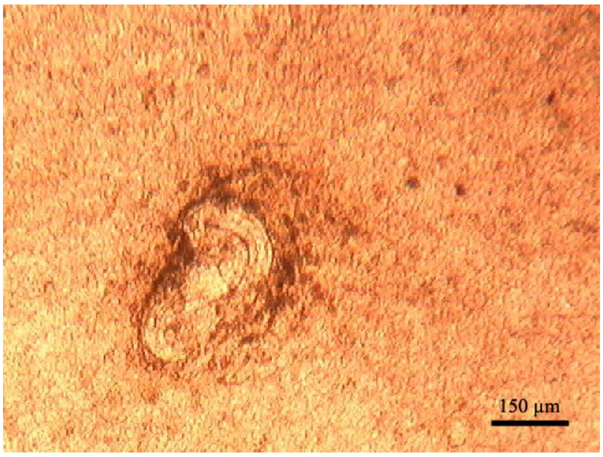

Fig. 5. The foot of *Helix pomatia* experimentally infected with Protostrongylidae (compression; stage 3 larva and an aggregation of host cells around it)

Conclusions

An analysis of the data obtained allows us to propose that experiments performed show a complexity of relationships inside «parasite–host» system, for further clarification of which subsequent studies are necessary.

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