Research Article

Angiostrongylus vasorum: Experimental Infection and Larval Development in Omalonyx matheroni

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The susceptibility and suitability of Omalonyx matheroni as an intermediate host of Angiostrongylus vasorum and the characteristics of larval recovery and development were investigated. Mollusks were infected, and from the 3rd to the 25th day after infection, larvae were recovered from groups of 50 individuals. The first observation of L2 was on the 5th day, and the first observation of L3 was on the 10th day. From the 22nd day on, all larvae were at the L3 stadium. Larval recovery varied from 78.2% to 95.2%. We found larval development to be faster in O. matheroni than in Biomphalaria glabrata. Our findings indicate that this mollusk is highly susceptible to A. vasorum. Infective L3 were orally inoculated into a dog, and the prepatent period was 39 days. This is the first study to focus on O. matheroni as an intermediate host of A. vasorum.

1. Introduction

The nematode Angiostrongylus vasorum is a parasite of wild and domestic canids. Adult worms are found in the right ventricle, pulmonary artery, and its branches, where sexual reproduction and oviposition take place. The first-stage larvae (L1) hatch in the alveoli, migrate up the bronchial tree, and are swallowed and then excreted into the environment along with the host feces. Infection frequently leads to pneumonia, loss of racing performance, coughing, and anemia [1]. Severely infected dogs may develop cardiac insufficiency and pulmonary fibrosis, followed by weight loss, hemorrhagic diatheses, and death [2, 3]. Several terrestrial and aquatic mollusks may act as intermediate hosts [4–7]. The genus Omalonyx (Pulmonata: Stylommatophora) belongs to the family Succineidae, which is composed of hermaphroditic terrestrial pulmonates that are morphologically diverse. Omalonyx sp. have a reduced flat shell and slug-like body, and they can be found in humid soil and in macrophytes [8–10]. They have a broad geographical distribution east of the Andes in South America and in the Lesser Antilles Islands [9], including localities where A. vasorum is known to occur [11, 12]. These mollusks are important intermediate hosts of the trematode Leucocloridium [13–15] and are able to support the life cycle of Angiostrongylus costaricensis in the laboratory [12]. There is no record of Angiostrongylus naturally infecting Omalonyx. This investigation aimed to evaluate the susceptibility and suitability of Omalonyx matheroni as an intermediate host of A. vasorum and to analyze the parasite’s larval development from L1 to L3. Studies on the development of A. vasorum in different hosts contribute to the understanding of the parasite’s biology and of the host-parasite relationship.

2. Methods

2.1. Mollusks. Young individuals (from 25 to 30 days old) of O. matheroni (n = 1150) measuring from 9 to 14 mm in length, raised under laboratory conditions, and from parental specimens from Pampulha Lake in Belo Horizonte, Minas Gerais State, Brazil were employed in this trial.
Table 1: Larval recovery from groups of 50 Omalonyx matheroni experimentally infected with 12500 L1 of Angiostrongylus vasorum (250 L1 per mollusk).

| DPI | Number of larvae that entered the hosts | Percentage of larvae that entered the hosts (%) | Number of larvae recovered | Percentage of larvae recovered (%) | Number of L1 recovered | Number of L2 recovered | Number of L3 recovered |
|-----|----------------------------------------|-----------------------------------------------|---------------------------|-----------------------------------|------------------------|-----------------------|------------------------|
| 3   | 11997                                   | 95.9                                         | 10009                     | 83.4                              | 10009                  | 0                     | 0                      |
| 4   | 11900                                   | 95.2                                         | 9300                      | 78.2                              | 9300                   | 0                     | 0                      |
| 5   | 12038                                   | 96.3                                         | 10113                     | 84.0                              | 9364                   | 749                   | 0                      |
| 6   | 11937                                   | 95.5                                         | 9750                      | 81.7                              | 5675                   | 4075                  | 0                      |
| 7   | 12149                                   | 97.2                                         | 10154                     | 83.6                              | 6417                   | 3737                  | 0                      |
| 8   | 12225                                   | 97.8                                         | 11635                     | 95.2                              | 7446                   | 4189                  | 0                      |
| 9   | 11987                                   | 95.9                                         | 9942                      | 82.9                              | 5349                   | 4593                  | 0                      |
| 10  | 12187                                   | 97.5                                         | 10207                     | 83.8                              | 4001                   | 5675                  | 531                    |
| 11  | 12006                                   | 96.1                                         | 10012                     | 83.4                              | 1782                   | 7028                  | 1202                   |
| 12  | 12033                                   | 96.3                                         | 10024                     | 83.3                              | 1484                   | 6435                  | 2105                   |
| 13  | 12076                                   | 96.6                                         | 10131                     | 83.9                              | 932                    | 6768                  | 2431                   |
| 14  | 11993                                   | 95.9                                         | 9974                      | 83.2                              | 0                      | 6413                  | 3561                   |
| 15  | 12207                                   | 97.7                                         | 11018                     | 90.3                              | 0                      | 5246                  | 5772                   |
| 16  | 11972                                   | 95.8                                         | 9891                      | 82.6                              | 0                      | 3858                  | 6033                   |
| 17  | 11984                                   | 95.9                                         | 9923                      | 82.8                              | 0                      | 2322                  | 7601                   |
| 18  | 11905                                   | 95.2                                         | 9539                      | 80.1                              | 0                      | 1784                  | 7755                   |
| 19  | 11954                                   | 95.6                                         | 9840                      | 82.3                              | 0                      | 1081                  | 8759                   |
| 20  | 12075                                   | 96.6                                         | 10116                     | 83.8                              | 0                      | 364                   | 9752                   |
| 21  | 11979                                   | 95.8                                         | 9902                      | 82.7                              | 0                      | 246                   | 9656                   |
| 22  | 11910                                   | 95.3                                         | 9573                      | 80.4                              | 0                      | 0                     | 9573                   |
| 23  | 11918                                   | 95.3                                         | 9727                      | 81.6                              | 0                      | 0                     | 9727                   |
| 24  | 11995                                   | 95.9                                         | 10008                     | 83.4                              | 0                      | 0                     | 10008                  |
| 25  | 11911                                   | 95.3                                         | 9620                      | 80.8                              | 0                      | 0                     | 9620                   |

2.2. Parasites. A. vasorum L1 were obtained from the cycle maintained in the laboratory using successive passages in snails (Biomphalaria glabrata) and dogs (Canis familiaris). This strain was isolated from a dog in Caratinga, Minas Gerais State [11].

2.3. Mollusk Infection. The feces of infected dogs was collected, and L1 were recovered by the modified Baermann apparatus [16]. After 24 hours of fasting, mollusks were individually placed in polystyrene culture test plates with 24 wells of 15 mm diameter (TPP—Techno Plastic Products, Switzerland) and fed with 250 L1 on a fragment of lettuce (approximately 15 mm diameter). After 8 hours, they were transferred to a plastic container (20 × 12 cm) with 250 mL of dechlorinated tap water and wood pieces. Groups of 10 individuals were kept in these containers during this trial. They were maintained at room temperature (25 to 27°C) and were fed on lettuce. Larvae that stayed in each test plate were counted and subtracted from the total amount offered to each individual. It is assumed that this is the number of larvae that entered each individual and calculate, for groups of 50 individuals: the number of larvae that entered the hosts, the percentage of larvae that entered the hosts, and the percentage of larvae recovered (Table 1).

2.4. Larval Development. From the 3rd to the 25th day after infection, larvae (L1, L2 and L3) were recovered from groups of 50 mollusks in a Baermann apparatus and fixed in Railliet-Henry at 60°C for quantification and identification of the larval stage [7]. Larval stage was identified based on published descriptions [7, 11, 17].

2.5. Dog Infection. To verify whether L3 from O. matheroni (21 days after infection) were infective, 1000 larvae were orally inoculated into a male mongrel dog weighing 10 kg born in the breeding facilities of the Universidade Federal de Minas Gerais, under the management systems on animal well-being and according to the ethics committee of the university (CETEA/UFMG). After the 20th day of infection, feces was collected daily for parasitological investigation of the presence of larvae.

3. Results

3.1. Mollusk Infection. O. matheroni was susceptible to the infection. Larvae (L1, L2 and L3) were recovered from the 3rd to the 25th day after infection. After 8 hours of contact, 95.2 to 97.8% of the larvae had penetrated the mollusks (Table 1).
3.2. Larval Development. The amount of larvae recovered each day is presented in Table 1. Mean L3 recovery is reported as the number of L3 recovered divided by the number of L1 that penetrated the host. These proportions were between 78.2% and 95.2%.

3.3. Dog Infection. Larvae were first detected in the feces in the 39th day (512 per g of feces) and increased until the 60th day (3320 per g of feces). This increase was followed by a gradual decrease that reached 1120 larvae per gram of feces on the 100th day (Figure 1). During this 100-day period, the amount of larval release varied, but larvae were never absent.

4. Discussion

Nematodes of the genus Angiostrongylus, including the species A. vasorum, can infect a wide spectrum of intermediate hosts of the class Gastropoda [18]. This system thus represents an interesting experimental model for the study of the host-parasite relationship. The susceptibility of a mollusk to a protostrongylid has been defined in terms of L1 penetration capability, the possibility of L3 development and time required to complete larval development [19, 20]. The present investigation demonstrates the susceptibility and suitability of O. matheroni as an intermediate host of A. vasorum. The percentage of L3 recovery in O. matheroni varied from 78.2% to 95.2%. The high percentage of larval recovery confirms our findings and indicates that this mollusk is highly susceptible to A. vasorum. Infective L3 recovered from these mollusks developed into fertile adults. L1 were observed in the feces of the infected dog.

Several factors influence the larval development of protostrongylids in the intermediate host such as environmental conditions (i.e., temperature) and biological conditions (i.e., hosts species and age) [21–24]. Geritcher [21] emphasized that among the environmental factors affecting the development of protostrongylid larvae in snails, the most important is temperature [21]. Low temperature (18 to 20°C) increases the time of development of the larvae, whereas high temperatures accelerate their development (25 to 28°C), as observed for the genus Angiostrongylus [17, 25, 26]. In this work, we observed that larval development of A. vasorum is faster in O. matheroni than in other known intermediate hosts [17, 27]. This conclusion is based on comparisons with data that is available in the literature. Experimental infection of several species of terrestrial mollusks (maintained at 18 to 23°C) allowed the first observations of L3 on the 16th and 17th day after infection [27]. Such low temperatures increase the time of larval development, and we are focusing our discussion on works that were performed at higher temperatures (25 to 28°C). In a trial where B. glabrata was maintained at 25 to 27°C, L2 were recovered between the 7th and 8th day after infection and L3 on the 14th and 15th [17]. Our results for O. matheroni demonstrated that L2 can be observed for the first time on the 5th day after infection and L3 can be observed for the first time on the 10th day. Furthermore, after 21 days, almost all larvae recovered were L3. The exploitation of hosts’ immune response by the parasite was discussed by Damian [28], and the encapsulation of A. costaricensis in veronicellidae slugs has been considered an example of such a process [29].

Larvae were observed in the feces of the experimentally infected dog 39 days after infection. These results corroborate those of Bessa et al. [7], Oliveira-Júnior et al. [30], and Barçante et al. [16], who observed a prepatent period varying from 28 to 108 days after infection.

In view of the high reproductive rates of O. matheroni and the feasibility of laboratory rearing (accelerated larval development, efficient larval recovery, and larval viability), we consider such mollusks very useful for the maintenance of the A. vasorum cycle in the laboratory. Moreover, this mollusk is also an interesting experimental model for studies on the host-parasite relationship of A. vasorum and its intermediate hosts.

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