STUDIES ON GROWTH AND DISTRIBUTION OF *ANGIOSTRONGYLUS CANTONENSIS* LARVAE IN *AMPULLARIUM CANALICULATUS*

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INTRODUCTION

Angiostrongylus cantonensis, the nematode parasite which inhabits in the pulmonary arteries and heart of rodents, has been recognized as the primary causative agent of human eosinophilic meningoencephalitis in Taiwan since 1944 (Nomura and Lin, 1945). The life cycle of *A. cantonensis* involves a molluscan intermediate host (Mackerras and Sandars, 1955), usually a terrestrial snail or a slug. However, some freshwater mollusks were also found to be infected in nature (Lim *et al.*, 1965) or experimentally (Alicata and Brown, 1962; Richards and Merritt, 1967). *Ampullarium canaliculatus*, a freshwater species recently introduced into Taiwan, is now widely distributed in the paddy fields and ditchwaters all over the island. The immigrant snail is cooked by some inhabitants to eat or to feed the poultry. *A. canaliculatus* has been proved to be involved in a human infection recently (unpublished data). Epidemiological investigation carried out at the village where the patient lived has revealed a heavy infection of *A. canaliculatus* with the parasite larvae. This study reports the findings of an experimental infection of *A. canaliculatus* with *A. cantonensis* with special emphasis on the development and distribution pattern of the larvae in the snail.

MATERIALS AND METHODS

Parasite : A long-term laboratory-reared strain of *Angiostrongylus cantonensis*, originally isolated from *Achatina fulica* collected from Nei-hu, Taipei, was used in this investigation. It was maintained in this laboratory by cycling through the planorbid snails, *Biomphalaria glabrata*, and adult Sprague-Dowley rats.

Snail: Egg clutches of *Ampullarium canaliculatus* were collected from two areas at Pei-tou, Taipei and hatched in the laboratory. Sibling snails were grown to 15-25 mm in a large beaker with a constant change of aerated water and cabbage.

Experiment: The first-stage larvae of *A. cantonensis* were obtained from the feces of infected rats. The feces was dissolved in aerated water and filtered through two layers of gauze. The filtrate was collected, sedimented and examined under a dissecting microscope to pick up larvae with active movement. The larvae were washed twice with aerated water and placed in a 140 × 20 mm Petri dish. Healthy snails were put in a covered Petri dish overnight. After exposure to the first-stage larvae, snails were washed with water and raised in large beakers.

Groups of three snails were killed daily since the first day of infection. The shell was crashed and removed carefully. The body was separated into four parts, head, foot, mantle, digestive tract, and other visceral organs. All parts were homogenized and digested in a pepsin-HCl solution at 37°C for 2 hr. The solution was examined carefully under a dissecting microscope for the juvenile worms.

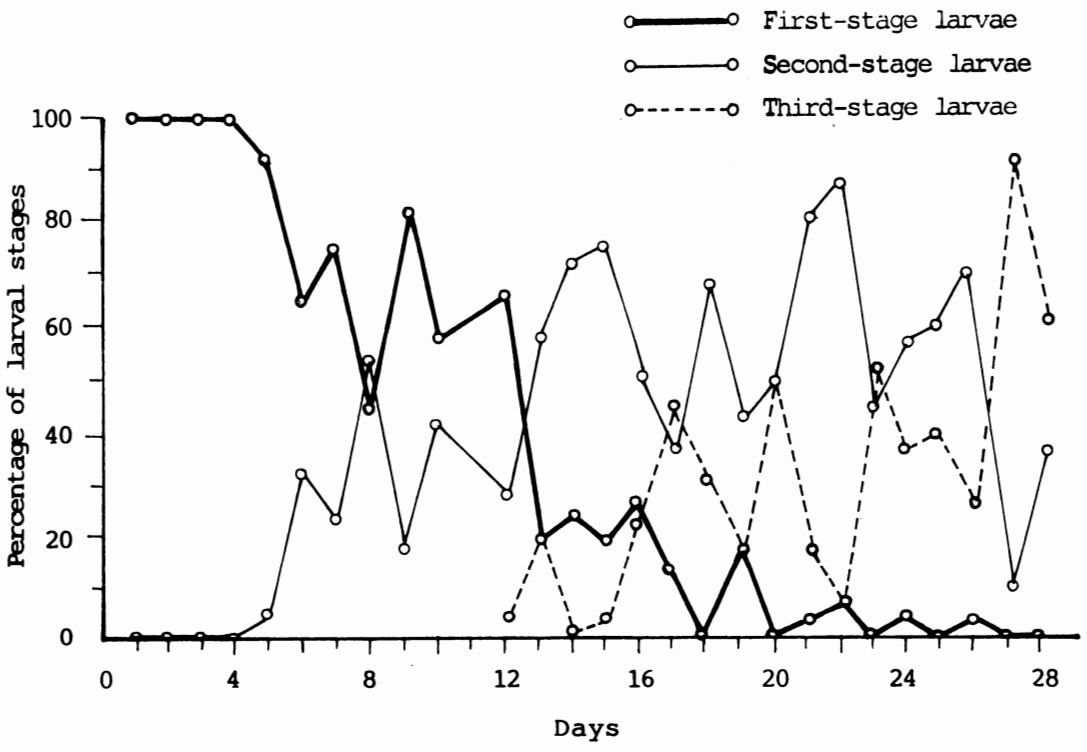

Fig. 1—Chronological development of *Angiostrongylus cantonensis* larvae in *Ampullarium canaliculatus*.

RESULTS

All the snails examined were found to be positive of *A. cantonensis* infection. Different stages of larvae were observed in various parts of the snail and the distribution pattern altered with the infection course.

The percentages of each stage of larvae are shown in Fig. 1. The first molt was observed on the fifth day of infection and the second molt on the 12th day. First-stage larvae decreased during the course of infection while third-stage larvae increased. The second-stage larvae first appeared on the fifth day, reached the peak on the 22nd day, and decreased subsequently.

Fig. 2 shows the total percentages of larval stages found in each part of the snail. In the first four days of infection, juvenile worms were found only in the digestive tract. This suggested that mouth is the primary route of *A. cantonensis* transmission in this freshwater snail. As the percentage of larvae found in the digestive tract decreased during the course of infection the percentage in headfoot region increased. The paucity of larvae existed in the visceral organs, including liver, heart, ovary, except for a very short period in the beginning of the first molt which indicated that these organs might be only a temporary place during the juvenile migration. The greatest density of larvae was found in the headfoot region in the later phase.

At the end of examination period, most parasites (62.1%) recovered were third-stage larvae (Fig. 1).

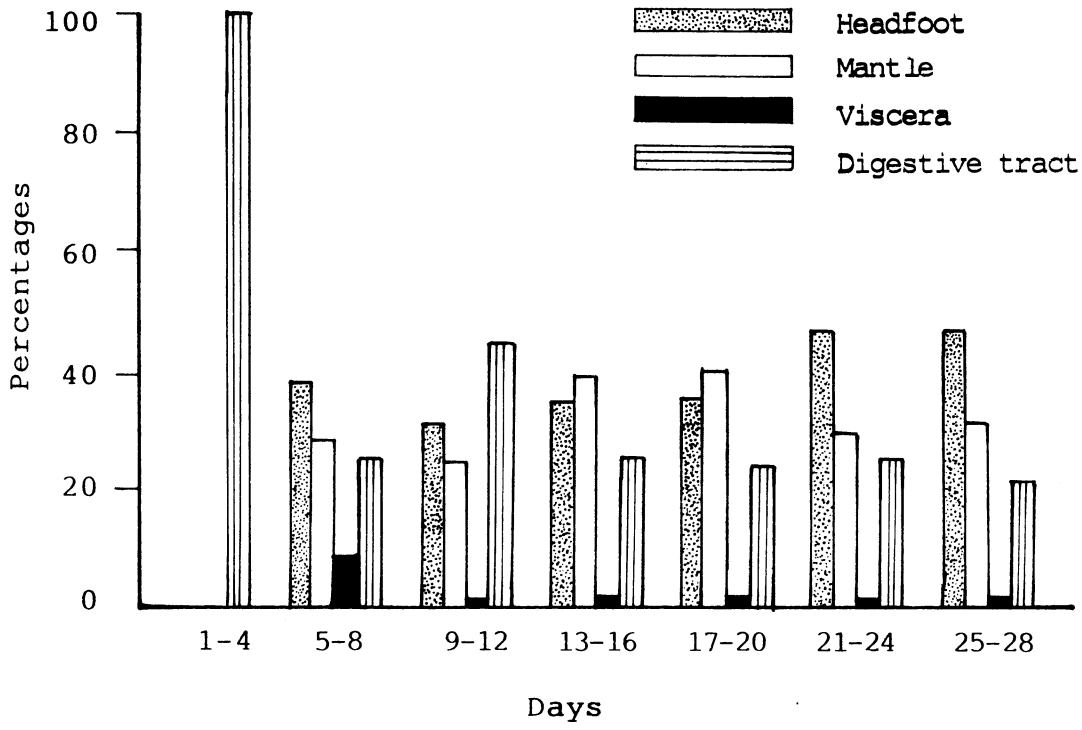

Fig. 2—Distribution patterns of the intramolluscan stages of *Angiostrongylus cantonensis* in *Ampullarium canaliculatus*.

DISCUSSION

The development of *A. cantonensis* in molluscan host have been studied by various investigators (Mackerras and Sandars, 1955; Alicata, 1965; Wen, 1977). A number of studies have pointed out that *A. canaliculatus* may also serve as an intermediate host for transmission of this parasite (Yen *et al.*, 1985; Chen *et al.*, 1986). However, chronological distribution of the intramolluscan stages of the parasite has not been followed in this freshwater snail species.

Alicata (1965) demonstrated that first-stage larvae of *A. cantonensis* may enter the body of the mollusk equally effective either through the digestive tract or by active penetration of its cuticle. Wen (1973) demonstrated that the invasion of *Achatina fulica* by *A. cantonensis* was effected primarily through the respiratory pore. In the present study, we found that the possible invasion route for the parasite larvae to *A. canaliculatus* takes place through digestive tract since first-stage larvae were found only in the digestive tract during the first four days. The snail is usually found in the water in natural condition, but it is possible to be infected by the parasite through a respiratory route experimentally.

Yen *et al.* (1985) suggested that infection of aquatics nails by *A. cantonensis* is through the penetration of foot. However, no consecutive examination data can support this suggestion. When distribution pattern was monitored one month after infection as indicated in their study, most parasites might have migrated from the site of invasion to the site of predilection.

Within the mollusk, first-stage larvae undergo two molts and become third-stage larvae. It seems that molting can occur in any part of *A. canaliculatus*, since all three stages of larvae could be found in all parts examined except the density varied. The highest density was in headfoot region and the lowest in visceral organs. This distribution has its significance in epidemiology since viscera is usually removed during the preparation for human consumption while headfoot is the main edible part. The development of *A. cantonensis* larvae in *A. fulica* was followed for 24 consecutive days by Wen (1977). High densities of infection were found in the lung, collar, and foot. First molt occurred on the 7th day of infection while second molt on the 17th day. The differences in distribution pattern and development duration in these two snail species indicated that *A. canaliculatus* is even more dangerous than *A. fulica* when eaten by people. Unfortunately, the hazards due to the widespread distribution of this freshwater snail is increasing in Taiwan. Apparently, it has taught a lesson to the people in Taiwan about the introduction of foreign snails like *A. fulica*. Due to the absence of its predator, the new species has become populated in all habitats within a comparatively short time. The undesirable results include the damage of farm products, orchards, gardens, and other agriculture lands, transmitting parasites (Yen *et al.*, 1985; Chen *et al.*, 1986), and other effects which cause an ecological unbalanced state.

SUMMARY

The intramolluscan development of *Angiostrongylus cantonensis* larvae in *Ampullarium canaliculatus*, a fresh-water migrant snail which multiplies in tremendous rate in Taiwan, was followed for 28 consecutive days in this study. The snails were infected with first stage larvae of *A. cantonensis* and groups of three snails were sacrificed daily to study various larval stages in different organs. *A. cantonensis* larvae were found in all snails examined. During the first four days, they were obtained only from the digestive tract. Mouth is thus suggested as the primary route of natural infection. The first molt was observed on the fifth day of infection, and the second molt, the 12th day. Molting occurred in almost all kinds of organs examined. Studies on tissue tropism showed that the predilection site of *A. cantonensis* in this freshwater snail was headfoot region. Relatively few larvae were found in visceral organs including liver, heart, and ovary. Most parasites, 62.1%, recovered on the 28th day of infection were third-stage larvae.

ACKNOWLEDGEMENTS

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