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POSSIBLE ROLE OF TADPOLE OF *RANA CHENSINENSIS* AS AN INTERMEDIATE HOST OF *ANGIOSTRONGYLUS CANTONENSIS*

Yuzaburo OKU, Ken KATAKURA, Jun-ichi NAGATSUKA and Masao KAMIYA

Department of Parasitology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

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Tadpoles of *Rana chensinensis* became infected by exposure to the 1st stage larvae of *Angiostrongylus cantonensis*, and the larvae developed into the 2nd stage 12 to 16 days after the infection. It was suggested that the tadpole might act as an intermediate host of *A. cantonensis*.

INTRODUCTION

Angiostrongylus cantonensis is a causative agent of human eosinophilic meningoencephalitis. Its natural final hosts are limited to the rodents belonging to the genera *Rattus*, *Melomys* and *Bandicota*, and the intermediate hosts are various snails and slugs.[1] In addition, frogs and toads are known to be paratenic hosts of *A. cantonensis*.[2,3] Recently, two cases of eosinophilic meningoencephalitis after eating toads were reported in Japan.[5] The present investigation was carried out to determine the role of tadpole as an intermediate host rather than a paratenic host of *A. cantonensis*.

MATERIALS AND METHODS

Eggs of *Rana chensinensis* were collected at the foot of Mt. Soranuma, Hokkaido, Japan, and hatched in our laboratory. Tadpoles of 4 weeks after hatcking were exposed to the 1st stage larvae of *A. cantonensis* obtained from the lung of an infected albino rat and kept at room temperature. Each pair of tadpoles was sacrificed 4, 8, 12 and 16 days after the exposure. One of the two was fixed with 10% formalin solution, and the paraffin sections for microscopy were stained with hematoxyline-eosin. The distribution of larvae in the tadpole body and the histologic reactions at the host side were examined in these sections. The other specimen was used to determine the developmental stage of the larvae. The liver, pancreas, intestines and mesentery were pressed under cover slips.

RESULTS

The nematode larvae were found on or in the liver, pancreas, stomach, upper intestine, mesentery, abdominal wall, subcutis and gills, but not in the muscle or in other organs (fig. 1). Host tissue reactions of the tadpole 4 days after the infection were very slight, e. g., few small and spherical cells with scanty cytoplasm surrounded the larvae. In the 8 to 16 day cases, most of the larvae were enveloped in oval capsules (fig. 2). Most of the capsules were small, 80 to 120 μ in diameter, and contained 1 or 2 larvae. The large capsules, 160 to 300 μ, contained 3 to 7 larvae. The large ones were found only on the surface of the visceral organs and abdominal wall, and the larvae were surrounded by many reticulocyte-like cells and a few fibroblast-like cells (fig. 3). The fibroblast-like cells increased in number with the progress of the infection. The first stage larvae were found mainly 4 and 8 days after the infection. And the 2nd stage larvae were found 12 and 16 days after the infection (fig. 4).

DISCUSSION

It has been reported that various snails and slugs are possible intermediate hosts. CHENG & BRUTON (1965) reported that the oyster, *Crassostrea virginica*, and clam, *Mercenaria mercenaria*, are experimental intermediate host of *A. cantonensis*. As yet, no vertebrate has been regarded as the intermediate host; and some vertebrates, such as cattle, pigs, fishes, sea snakes, frogs and toads have been reported as possible paratenic hosts.[1] It was found that twenty-three out of 43 frogs, *Hyla aurea*, from a market garden in Noumea, New Caledonia, harbored infective larvae in their tissues.[3] In Okinawa Prefecture *Bufo asiaticus*, *Rana catesbeiana*, *Rana limnocharis* and *Rhacophorus leucomystax* were naturally harbored the 3rd stage larvae.[2] Recently, two cases of eosinophilic meningoencephalitis in Okinawa Prefecture were reported in persons who had taken raw liver of toad, *Bufo asiaticus*, as medicine.[5] It was reported that the 3rd stage larvae were found most frequently in the stomach and intestinal wall with the mesentery, and some were in the liver and muscle of frogs and toads.[2] This finding corresponds with the present results. The encapsulated larvae were surrounded by many reticulocyte-like cells and a few fibroblast-like cells. This result was not the same in the frogs and toads; however, almost all of cells in the capsules may become fibroblast-like cells because the fibroblast-like cells increased in number progressively.

Frogs and toads are known to be paratenic hosts because the 3rd stage larvae can infect them and survive. In the present study the tadpole could be infected with the 1st stage larvae, which could develop into the 2nd stage larvae; therefore, it can be concluded that the tadpole of *Rana chensinensis* possibly acts as an intermediate host of *A. cantonensis*. Further investigations, however, are necessary.

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Explanation of plate

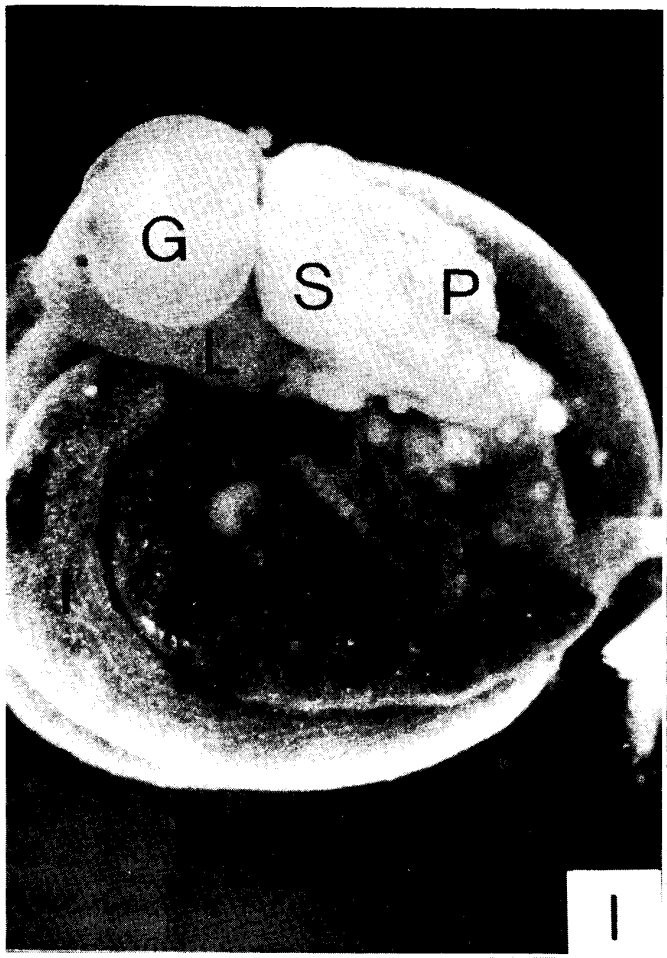
Plate

Fig. 1 Dorsal view of the digestive organs of the tadpole, *Rana chensinensis*, infected with *A. cantonensis*; liver (L), stomach (S), gall bladder (G), pancreas (P) and intestine (I) with encapsulated larvae ×18

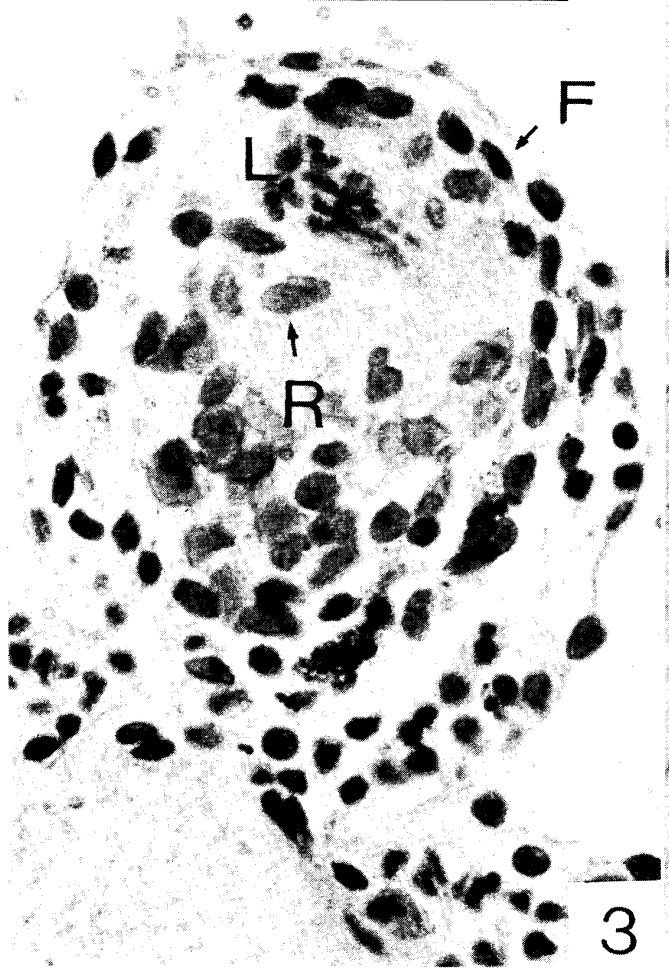
Fig. 2 Section of stomach (S), pancreas (P) and mesentery (M) showing encapsulated larvae ×105

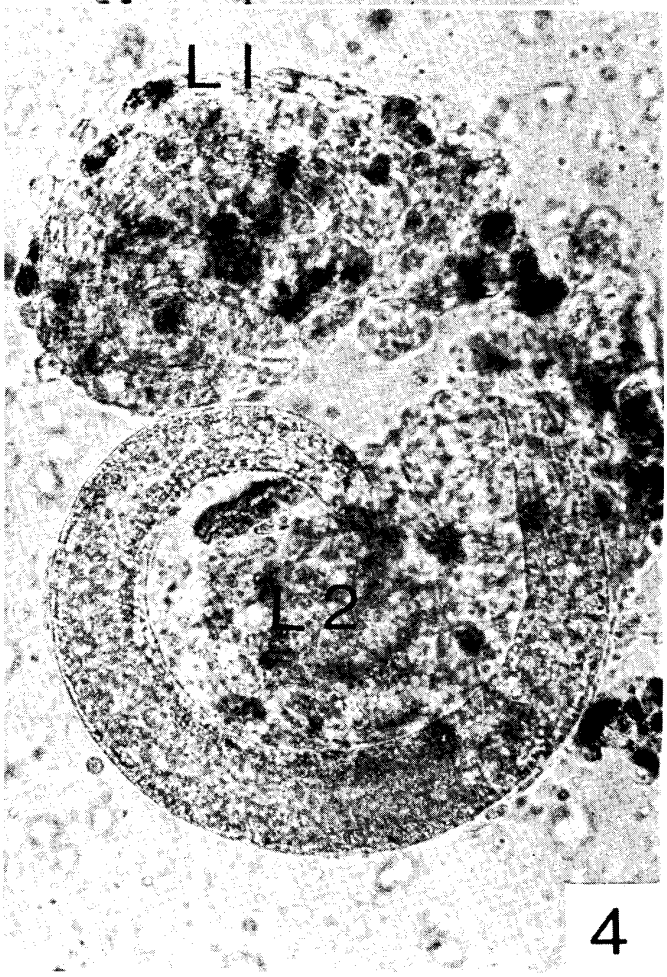
Fig. 3 A larva (L) on serosa of stomach surrounded by many reticulocyte-like cells (R) and a few fibroblast-like cells (F) ×740

Fig. 4 A 1st stage larva (L 1) and a 2nd stage larva (L 2) obtained from a tadpole 12 days after the infection ×360


G
S
P
1


M
S
2


F
L
R
3


L1
L2
4