[Jpn. J. Parasitol., Vol. 42, No. 3, 193–198, June, 1993]

# Susceptibility of Yellow Tree Monitor (*Varanus bengalensis*) as Experimental Host for *Angiostrongylus cantonensis* Infection

PRAYONG RADOMYOS[1], ANCHALEE TUNGTRONGCHITR[2] AND RANGSUN PRAEWANICH[2]

(Accepted for publication; January 29, 1993)

**Abstract**

Thirty yellow tree monitors (*Varanus bengalensis*) were studied for susceptibility of *Angiostrongylus cantonensis* infection. These monitor lizards can be infected only with third stage larvae and the parasite still persisted and survived. The organ found most infected with larvae was the liver. More than 90% of third stage larvae were recovered from the yellow three monitors. The third stage larvae were encysted in the liver and after digestion, were still infective to the rats. The high percentage of *Angiostrongylus cantonensis* infection in yellow tree monitors may have a significant role in the distribution of this worm because the people in north and northeast Thailand like to eat this raw yellow tree monitors as well as raw snails.

**Key words:** *Angiostrongylus cantonensis,* Yellow tree monitors, monitor lizards, *Varanus bengalensis,* paratenic hosts

## Introduction

*Angiostrongylus cantonensis* is a neurotropic and pulmonary parasite of the rat. It is a recognized cause of eosinophilic meningitis or meningoencephalitis in humans who have eaten molluscan intermediate hosts harbouring the 3rd-stage larvae (Rosen *et al.*, 1967). Hundreds of cases of eosinophilic meningitis caused by this parasites have been reported in Thailand (Punyagupta *et al.*, 1970). Most of the patients are raw freshwater snails especially *Pila* spp., which act as intermediate hosts of this parasite. An infection rate of about 3% has been reported in rats and 28% of third stage larvae in snails were found in the natural environment (Harinasuta *et al.*, 1965). This prevalence is quite high and should be an important focus of epidemiological study.

In 1990, 5 patients with eosinophilic meningitis were admitted at Siriraj Hospital, Bangkok. One died, another was paralyzed and the rest recovered later. Autopsy revealed many fifth stage larvae of *Angiostrongylus cantonensis* in the brain of the fatal case. All patients gave a history of eating raw or partially cooked yellow tree monitors (*Vananus bengalensis*) (Fig. 1) prior to experiencing symptoms. None of them mentioned consuming raw *Pila* snails. The cases mentioned above led to the suspicion that yellow tree monitors might have an important role in the epidemiology of this parasite. Therefore, this study was carried out to clarify the susceptibility of the yellow tree monitors to experimental *Angiostrongylus cantonensis* infection.

## Materials and Methods

### *Animals*

Thirty yellow tree monitors (*Varanus bengalensis*), weighing 300–400 gram were purchased from a commercial farm. They were pretested by dissection of some animals in the whole experimental groups. The stool examination of the animals were also performed. The result found to be negative for infection with

[1]*Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok, 10400, Thailand.*
[2]*Bangkok School of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.*

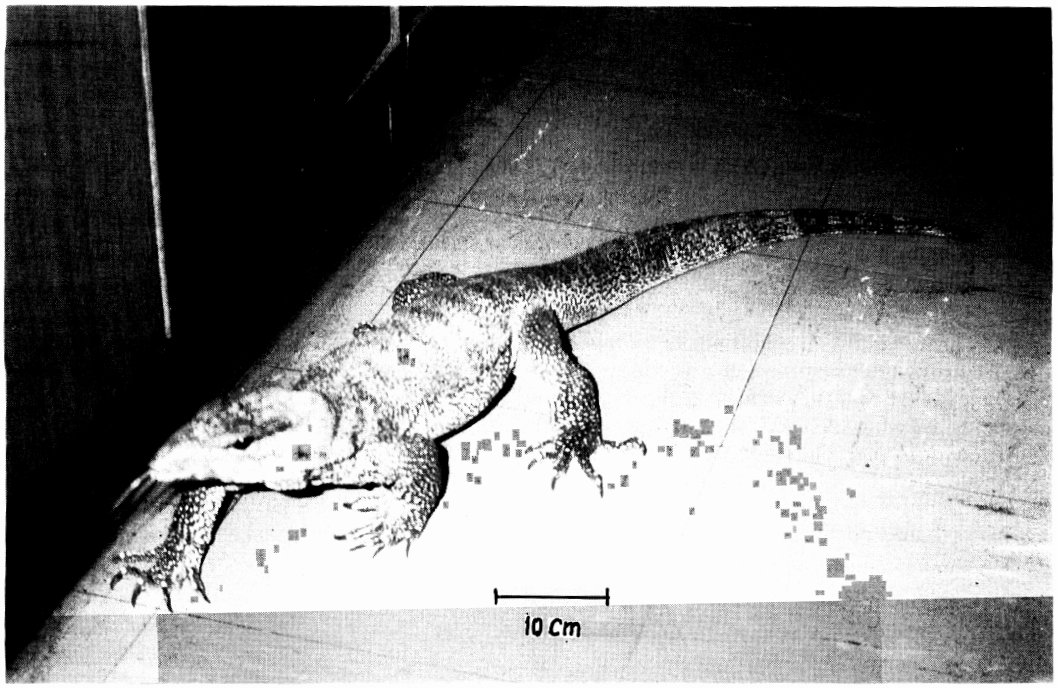


Fig. 1 Yellow tree monitor *Varanus bengalensis.*

*Angiostrongylus cantonensis* before the experiment. The animals were divided in three groups as follows: Group 1) Ten yellow tree monitor were infected with first stage larvae of *Angiostrongylus cantonensis* collected by sedimentation from the feces of naturally infected wild rats caught from the ricefields. The adult worms from postmortem examination of these naturally infected wild rats was also confirmed to be *Angiostrongylus cantonensis*; Group 2) Ten yellow tree monitors were infected with third stage larvae from infected *Biomphalaria glabrata* snails in which the parasite is well developed (Richards and Merritt, 1967). Inocula for the yellow tree monitors in this group contained 175–225 larvae which were prepared by micro-isolating individual organisms with a fine-bore pipette into small dishes, which were then carefully examined to check the total number of larvae present (Weinstein *et al.*, 1963); Group 3) Ten yellow tree monitors without any treatment served as the controls.

All yellow tree monitors were infected by introducing the inoculum into the mouth with a syringe and blunt-tipped needle (Weinstein *et al.*, 1963). As the infections progressed, animals were observed for any signs of abnormal behavior which might be attributed to the infection.

*Parasitological studies*

All of the yellow tree monitors were maintained in the laboratory animals house. Raw chicken was the food given to these monitor lizards until 28–45 days post-infection which is the time needed for development into the mature parasite in rats. They were then sacrificed and all organs were carefully examined for developmental stages of *Angiostrongylys cantonensis.* The heart, lung, pulmonary artery and its branches were carefully dissected and examined for worms. The brain was examined under a dissecting microscope for lesions and parasites, and fixed for further histologic study. The liver was also examined for the presence of the worm.

*Statistical analysis*

Difference in the number of larvae found in each group of tree yellow monitor were analyzed using the Chi square test.

## Results

Negative findings were obtained in all ten yellow tree monitors from group 1 which were infected with first stage larvae of *Angiostrongylus cantonensis* (Table 1). The result was also negative for the control group (group 3).

In group 2, the mature parasite was not found in any organ although the parasite still persisted and survived as third stage larvae. The organ found most infected with larvae was the liver. More than 90% of the third stage larvae were recovered from yellow tree monitors (Table 2) and all were still alive. The third stage larvae were encysted in the liver (Fig. 2 and 3). After digestion, they were still infective to the definitive host, rats. The adult worms from infected rats were reexamined again as *Angiostrongylus cantonensis*.

## Discussion

*Angiostrongylus cantonensis*, the rat lung worm was first found in rats in south China in 1935 and two years later in rats in Taiwan. The

Table 1 The infectivity of *Angiostrongylus cantonensis* in the yellow tree monitors (*Varanus bengalensis*) in 3 groups

| | Experimental animals | | |
|---|---|---|---|
| | Group 1 | Group 2 | Group 3 |
| Stage of infected lrva | first | third | – |
| Number of animals used | 10 | 10 | 10 |
| Number of animals infected | – | 10* | – |
| Organ found infected larva | | | |
| –liver | – | 10* | – |
| –heart | – | – | – |
| –brain | – | – | – |
| –lung | – | – | – |
| –pulmonary artery | – | – | – |
| –muscle | – | – | – |

*Significant $p<0.05$

Table 2 Recovery of parasite from yellow tree-monitors in Group 2 after infection with third stage larva of *Angiostrongylus cantonensis*

| No. | No. of parasite inoculated | No. of parasite recovered | Percent recovery |
|---|---|---|---|
| 1 | 225 | 216 | 96.0 |
| 2 | 225 | 214 | 95.1 |
| 3 | 200 | 182 | 91.0 |
| 4 | 200 | 167 | 83.5 |
| 5 | 200 | 184 | 92.0 |
| 6 | 175 | 20 | 11.4 |
| 7 | 200 | 191 | 95.5 |
| 8 | 200 | 186 | 93.0 |
| 9 | 200 | 188 | 94.0 |
| 10 | 200 | 163 | 81.5 |

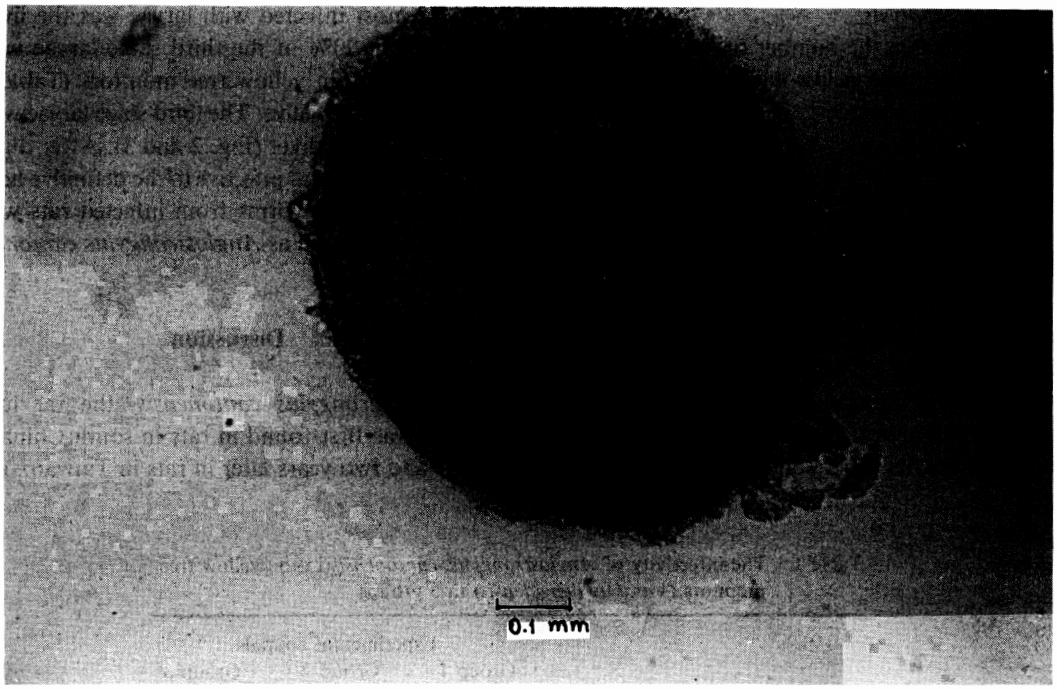


Fig. 2 Encysted third stage larva of *Angiostrongylus cantonensis* in the liver of yellow tree monitor.

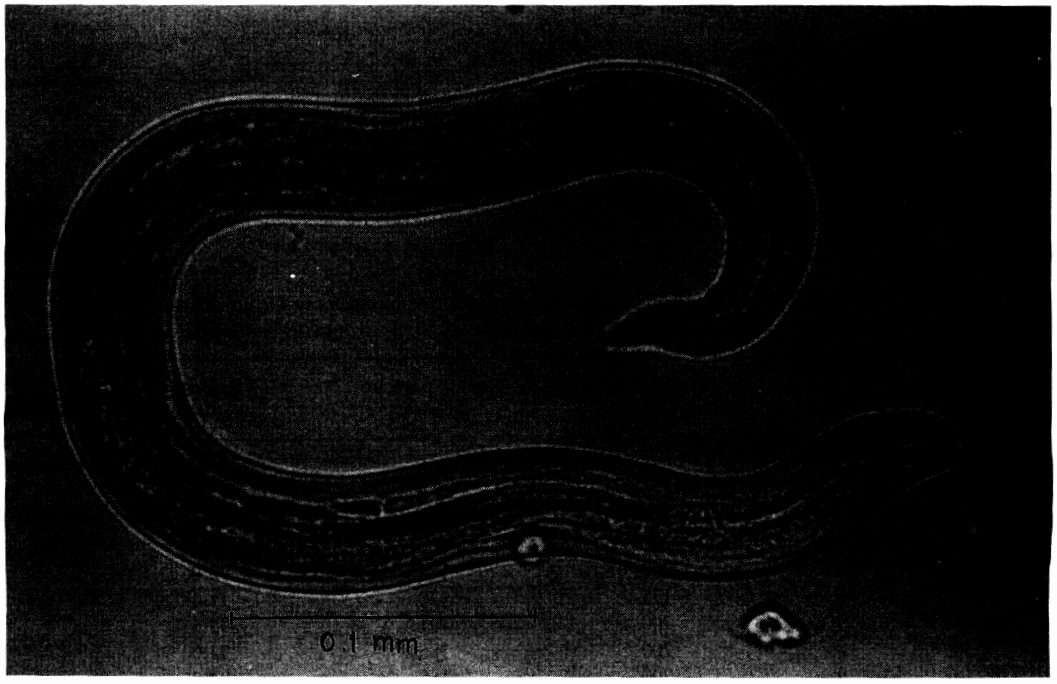


Fig. 3 Excysted third stage larva of *Angiostrongylus cantonensis* from the liver of yellow tree monitor.

first parasitologically confirmed human infection was from Taiwan in 1945. The public health importance of cerebral angiostrongyliasis was not recognized however, until 1962 when Rosen and his associates reported the parasite in the brain of a Filipino who died in Hawaii (Cross, 1987). The occurrence of this worm has been reported in several localities (Wallace and Rosen, 1969; Agular *et al.*, 1981; Campbell and Little, 1988; Witoonpanich *et al.*, 1991). The typical form of eosinophilic meningitis is prevalent and widely distributed in Thailand especially in the northeastern and central parts of the country (Punyagupta *et al.*, 1970). Among 484 patients with eosinophilic meningitis, most gave a history of consuming raw snails or rare *Pila* snails within 30 days before the onset of symptoms. Other patients had eaten other raw or rare animal food (Punyagupta *et al.*, 1970). Only 20% of 257 *Pila* snails, collected from the endemic areas which served as a source of the *Pila* snails consumed by patients, were infected with third-stage larvae of *Angiostrongylus cantonensis*. The number of larvae per infected snail was low (Harinasuta *et al.*, 1965). Therefore, animals other than gastropods may have a significant role in the distribution of this worm.

There are several reports of naturally infected frog and toads harboring third stage larvae such as *Hyla aurea* in New Caledonia (Ash, 1968), *Bufo asiaticus, Rana catesbeiana, Rana limmnucharis* and *Rhacopholus leucomystax* in Okinawa Prefecture, Japan (Asato *et al.*, 1978). It is known that no other reports of vertebrates as complete intermediate host of *Angiostrongylus cantonensis*. However, it has been shown that the first stage larvae of *Angiostrongylus cantonensis* can develop into infective third stage larvae in tadpole of the clawed frog, *Xenopus laevis* in the experimental laboratory (Oku *et al.*, 1980). The current study clearly confirms that *Angiostrongylus cantonensis* can infect yellow tree monitors and maintain the infective third stage larvae; however, they could not develop further beyond this stage. The results also show that infective stage larvae can survive for as long as 1–1½ month in the yellow tree monitors. However this experiment could not show the maximum survival period in this host. It has already been proven that rats are the definitive host of *Angiostrongylus cantonensis* and the parasite can develop into the mature stage after infection with third stage larvae after about 1–1½ months (Harinasuta *et al.*, 1965). Although the parasite is not capable of completing its life cycle in yellow three monitors, it is very important in connection with the epidemiological distribution of the parasite in its natural environments.

At the present time, the natural food eating habbit of yellow tree monitors is not known, it should be like that of other reptiles that eat all kinds of small animals including snails. Its eating habbit possibly infect it larvae.

It must be stressed that the recovery rate of infective third stage larvae was high. Therefore, the susceptibility of yellow tree monitors to infection with this worm is also very high and is important in the dispersal of worm. There is no report concerning the natural prevalence of *Angiostrongylus cantonensis* in yellow tree monitors but there was also a high percentage of *Angiostrongylus cantonensis* infection from our preliminary survey. This is of epidemiological importance because the people in northeast Thailand like to eat raw yellow tree monitors as well as raw snails.

**References**


1) Agular, P. H., Morera, P. and Pascual, J. (1981): First record of *Angiostrongylus cantonensis* in Cuba. Am. J. Trop. Med. Hyg., 30, 963–965.
2) Asato, R., Sato, Y. and Otsuru, M. (1978): The occurrence of *Angiostrongylus cantonensis* in toads and frog in Okinawa Prefecture, Japan. Jpn. J. Parasitol., 27, 1–7.
3) Ash, L. R. (1968): The occurrence of *Angiostrongylus cantonensis* in frogs of New Caledonia with observation on paratenic hosts of metastrongyles. J. Parasitol., 54, 432–436.
4) Campbell, B. G. and Little, M. D. (1988): The finding of *Angiostrongylus cantonensis* in rats in New Orleans. Am. J. Trop. Med. Hyg., 38, 568–573.
5) Cross, J. H. (1987): Public health importance of *Angiostrongylus cantonensis* and its relatives. Parasitology Today, 3, 367–368.
6) Harinasuta, C., Setasubun, P. and Radomyos, P.

(1965): Observations on *Angiostrongylus cantonensis* in rats and mollusks in Thailand. J. Med. Ass. Thailand, 48, 159–171.

7) Oku, Y., Katakura, K. and Kamiya, M. (1980): Tadpole of the clawed frog, *Xenopus laevis*, as an experimental intermediate host of *Angiostrongylus cantonensis*. Am. J. Trop. Med. Hyg., 29, 316–318.
8) Punyagupta, S., Bunnag, T., Juttijudata, P. and Rosen, L. (1970): Eosinophilic meningitis in Thailand: epidemiologic studies of 484 typical cases and the etiologic role of *Angiostrongylus cantonensis*. Am. J. Trop. Med. Hyg., 19, 950–958.
9) Richards, C. S. and Merritt, J. W. (1967): Studies on *Angiostrongylus cantonensis* in molluscan intermediate hosts. J. Parasitol., 53, 382–388.
10) Rosen, L., Loison, G., Laigret, J. and Wallence, G. D. (1967): Studies on eosinophilic meningitis. 3 epidemiologic and clinical observations on Pacific islands and the possible etiologic role of *Angiostrongylus cantonensis*. Am. J. Epidemiol., 85, 17–44.
11) Wallace, G. D. and Rosen, L. (1969): Studies on eosinophilic meningitis. V. Molluscan hosts of *Angiostrongylus cantonensis* on Pacific Islands. Am. J. Trop. Med. Hyg., 2, 206–216.
12) Weinstein, P. P., Rosen, L., Laqueur, G. L. and Sawyer, T. D. (1963): *Angiostrongylus cantonensis* infection in rats and rhesus monkeys, and observations on the survival of the parasite *in vitro*. Am. J. Trop. Med., 12, 358–365.
13) Witoonpanich, R., Chuahirum, S., Soranastaporn, S. and Rojanasunan, P. (1991): Eosinophilic myelomeningo-encephalitis caused by *Angiostrongylus cantonensis*: A report of three cases. Southeast Asian J. Trop. Med. Public Health, 22, 262–267.