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Survey of *Angiostrongylus cantonensis* in rats and giant African land snails in Phitsanulok province, Thailand

Apichat Vitta^{1*}, Raxsina polseela¹, Seangchai Nateworanart², Muncharee Tattiyapong³

¹Department of Microbiology & Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand

²Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand

³National Institute of Animal Health, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Chatuchak, Bangkok 10900, Thailand

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ABSTRACT

Objective: To survey the *Angiostrongylus cantonensis* (*A. cantonensis*) or the rat lungworm in a rat, definitive host, and in a giant African land snail (*Achatina fulica*), the intermediate host, in Phitsanulok, Thailand. **Methods:** Rats and giant African land snails were captured from Tha Pho sub-district, Phitsanulok, Thailand. Rats were killed and examined for adult *A. cantonensis*. The artificial digestion method following Baermann technique were used for isolation third stage larvae of *A. cantonensis*. **Results:** Sixty-two rats were captured and they were identified as *Rattus argentiventer*, *Rattus rattus* (*R. rattus*), *Bandicota savilei*, and *Bandicota indica* but only one animal (*R. rattus*) of 62 rats (1.61%) was positive with adult worm of *A. cantonensis*. The third stage larvae of *A. cantonensis* were examined on 307 *Angiostrongylus fulica* snails. It was found that the overall infection rate was 12.38% (38 infected out of 307 *Achatina* snails). **Conclusions:** This study demonstrates that *A. cantonensis* is available in the natural hosts of Phitsanulok. This suggests that the transmissions of this parasite to human may occur in this region.

1. Introduction

Human angiostrongyliasis is a food-borne parasitic zoonosis caused by infection with the larval stage of *Angiostrongylus cantonensis* (*A. cantonensis*) or the rat lungworm. This parasite has been known to be the primary cause of eosinophilic meningitis or meningoencephalitis[1–5]. It has been reported from several parts of the world, particularly from Southeast Asia to the Pacific Islands[4,5]. Recently, at least 2 827 cases of human angiostrongyliasis have been recorded worldwide and over half of these cases (1 337) are reported from Thailand[5]. Although four species of Metastrongyloidea are found in Thailand, namely, *A. cantonensis*[6], *Angiostrongylus siamensis*[7], *Angiostrongylus malaysiensis*[8] and *Thaistrongylus harinasuti*[7], *A. cantonensis* is the only known cause of the disease in the country. Moreover, this parasite can occasionally be the cause of ocular angiostrongyliasis[9,10].

To complete the life cycle, *A. cantonensis* requires rodents as a definitive host and mollusks as intermediate hosts[11]. Several species of rodents from many parts of the world have been reported as the natural definitive hosts, but the most

common genera were *Rattus* and *Bandicota*[12–14]. Various species of terrestrial and land snails are reported to serve as natural intermediate hosts for *A. cantonensis*[15–18]. A giant African land snail, *Achatina fulica*, is responsible for wide spread infection of this parasite. Recently, however, in Phitsanulok there were few reports of natural infection of the worms in rats and giant African land snails. The present work is to make a preliminary assessment of *A. cantonensis* infestation of the natural hosts captured around Naresuan University, Phitsanulok province.

2. Materials and methods

2.1. Collection of adult *A. cantonensis* from captive rats

Rats were collected from Tha Pho sub-district, Phitsanulok during May 2010 to February 2011. Traps were baited with banana and the trapped rats were brought every morning to laboratory for examination. They were identified according to 'Mammals of Thailand'[19]. The rats were euthanized by placing the trap into the plastic box containing a cotton wool soaked with diethyl ether. The thoraxes were opened to remove the lungs and hearts which were placed in Petri dishes containing normal saline. Female *A. cantonensis* worms were identified by their Barber pole pattern and the long vagina with no projection

*Corresponding author: Apichat Vitta, Department of Microbiology & Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.
E-mail: apichatv@nu.ac.th

at tip of the tail. The worms were collected and fixed in 10% formalin.

2.2. Collection of 3rd stage larvae of *A. cantonensis* from giant African land snails

Giant African land snails were collected from Phitsanulok during rainy season (May–August 2010) temporarily maintained in a plastic box and fed lettuce leaf. The hard shell of each land snails was removed. The snails were chopped into small pieces, blended and then incubated with 1% pepsin solution, at 37 °C for 1 hour. The 3rd stage larvae were isolated by the Baermann techniques. The apparatus was constructed a glass funnel connected to a short piece of rubber tubing at the outlet. Several layers of gauze were laid over a wire mesh at the bottom surface of the funnel. With the outlet closed, the digested solution was poured into the funnel until the fluid level touched the wire mesh. The apparatus was left standing for 30 minutes to allow larvae migrate into the rubber tube. At the end of this period, the fluid containing mainly enriched L3 larvae was released into a Petri dish. They were morphologically identified using the following criteria: (1) the body is long, thin and delicate; (2) It has two chitinous rods at the anterior end and the cuticle possesses delicate transverse striations; (3) the tail is cone-shaped, slightly curved and pointed^[20].

3. Results

A total of 62 rats, comprising 6 of *Rattus rattus* (*R. rattus*), 12 of *Bandicota savilei*, 17 of *Rattus argentiventer*, and 27 of *Bandicota indica* (*B. indica*) were captured and examined. The overall prevalence of *A. cantonensis* was 1.61%. Only one *Rattus rattus* was found to be infected with adult worms (Figure 1). 307 giant African land snails were collected of which 37 (12.37%) were infected with 3rd stage larvae of *A. cantonensis* (Figure 2).



Figure 1. The gross appearance of adult *A. cantonensis* collected from *R. rattus*.



Figure 2. The 3rd larva of *A. cantonensis* characterized by two chitinous rods (arrow) at the anterior end.

4. Discussion

Human angiostrongyliasis related to eosinophilic meningitis or meningoencephalitis is a parasitic zoonosis caused by *Angiostrongylus cantonensis*. The present studies demonstrated that *A. cantonensis* could be found in *Rattus rattus* (definitive host) and *Achatina fulica* (intermediate host) collected from Phitsanulok. These findings agreed with the study from Deng et al (2010), Chikweto et al (2009), Foronda et al (2010), Zhang et al (2008), Zhang et al (2008), Lv et al (2009), Thiengo et al (2010), Harinasuta (1965), Namue and Wongsawad (1997) and Pipitgool et al (1997)^[12–18, 21–23]. They reported that *R. rattus* and *Angiostrongylus fulica* is the definitive host and intermediate host for *A. cantonensis*, respectively. In Phitsanulok, Impand et al (1983) reported that *B. indica* and *R. rattus* were naturally infected with *A. cantonensis*^[24]. It could be concluded that *R. rattus* in Phitsanulok province serves as the naturally definitive hosts for *A. cantonensis*.

The results obtained from the present study revealed high prevalence of 3rd stage larvae in the *Achatina snails*. This finding agreed with the study of Radomyos et al (1994). They reported a high prevalence of 3rd stage larvae in yellow tree monitors collected from Phitsanulok^[25–27]. However, the infections of land snails have not been examined before in Phitsanulok. The finding shows that *A. cantonensis* in the giant land snails can serve as a source of human infection in the studied area. For this reason, eating of land snails was reported as one of the route of infection^[28,29]. Although human angiostrongyliasis has not been reported in Phitsanulok, there should be awareness of infection by this parasite in this area. In addition, the high prevalence of the worms in the land snails, intermediate host, indicates that the snails are often infected so the host and parasite relationship should be studied further.

Conflict of interest statement

We declare that we have no conflict of interest.

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