



# Houseflies: a Possible Transport Host of *Angiostrongylus* sp

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## Abstract

**A**ngiostrongylus, one of the metastrongyloid worms, is known to be pathogenic to humans. Human angiostrongyliasis can be caused by eating raw or improperly cooked land snails containing infective larvae, eg, *Pila* sp, *Pomacea* sp, *Achatina* sp, which may all serve as intermediate hosts. *Angiostrongylus* infection has also been reported among travelers returning from the Caribbean; it was concluded that infection occurred after they had consumed a vegetable salad contaminated with infective-stage *Angiostrongylus* embedded in mucus. In June 2010, we visited a field station in Kanbauk area, Yebhyu Township, Dawei District, Tanintharyi Division, in the Union of Myanmar. At the field station, many houseflies were seen around the canteen, auditorium, living room, and work areas. Thence, 468 houseflies were caught manually using clean plastic bags. After collection, they were stored in formalin detergent and transported to the laboratory in Bangkok, Thailand, for parasite examination. After washing with an ultrasonic cleaner, mites, unknown nematode larvae, and 12 larvae with prominent chitinous rods at the anterior end and constricted-tails – the morphological features resembling infective *Angiostrongylus* larvae – were detected from the wash sediments. DNA identification showed the larva was a metastrongyloid, with a 16.86% genetic differentiation from *A. cantonensis*. This finding indicated houseflies are able to carry the infective stage of the worm, and act as transport hosts for metastrongyloid larvae. It is possible that houseflies can also be a transport host of *Angiostrongylus*, the lung worm, with known medical significance to man.

**Keywords:** housefly, transport host, metastrongyloid, *Angiostrongylus*

*Angiostrongylus*, worms in the superfamily Metastrongyloidea, are parasites found in the lungs of rats. Humans are a paratenic host of the worm, and infection can occur after the ingestion of mollusks or slugs containing infective larvae.

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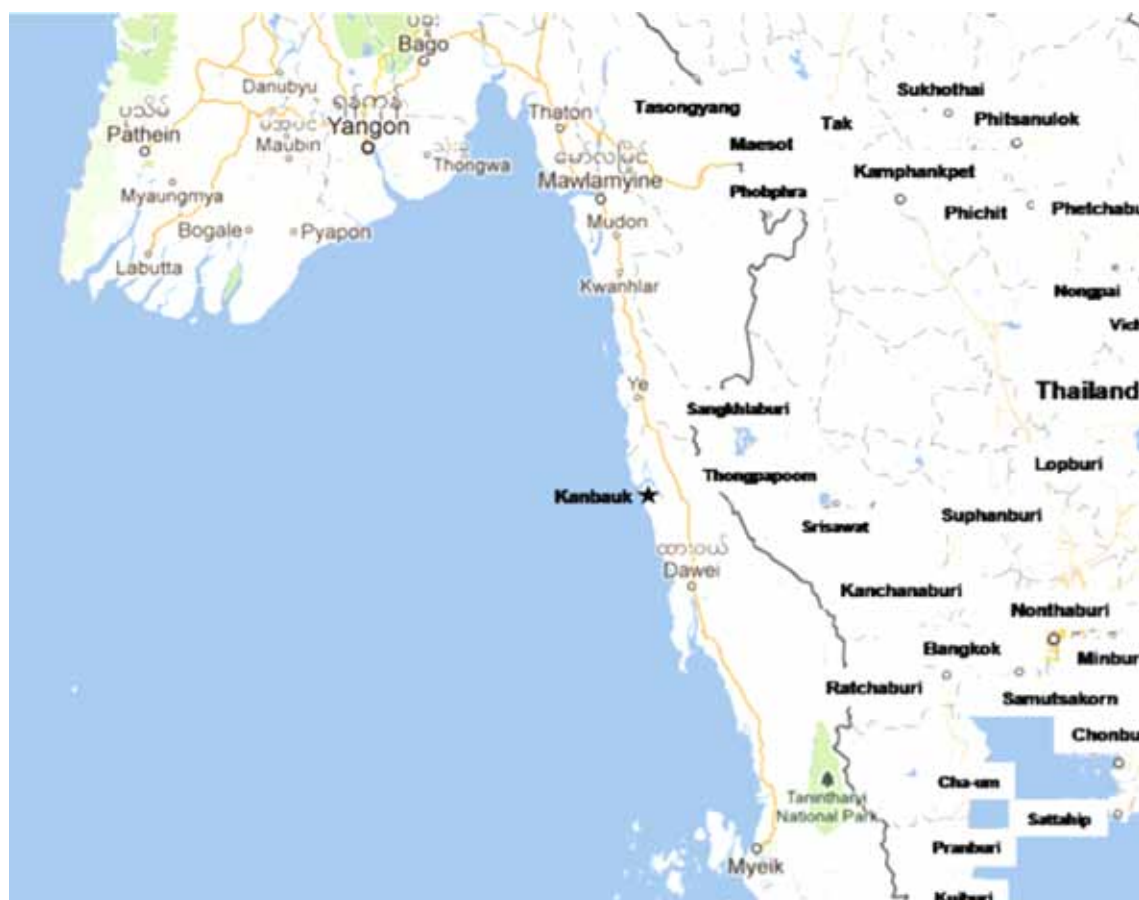
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In humans, *A. cantonensis* larvae accumulate in the central nervous system (CNS), causing eosinophilic meningitis, and may undergo partial development in the brain to become immature adults, where they then die or remain alive without further development [1]. Most incidents of *A. cantonensis* meningitis have been reported in Southeast Asia and the Pacific Basin [2,3], but sporadic cases have been reported in other

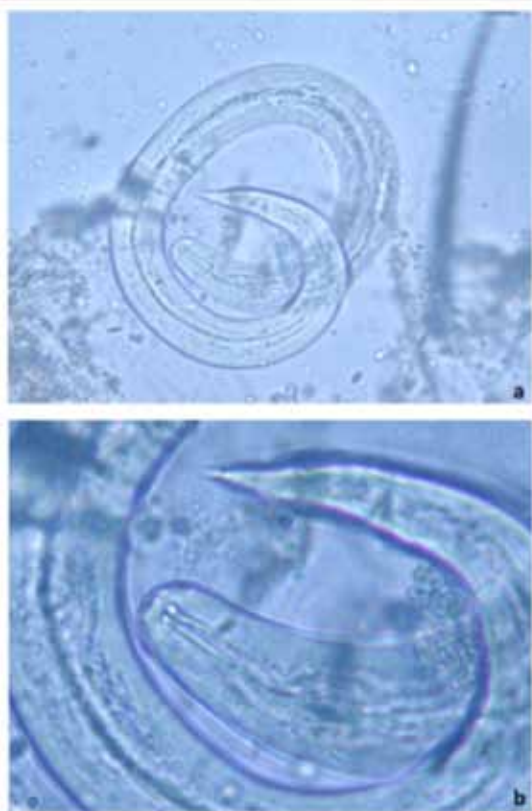
regions [4-8]. In Thailand, improperly cooked food prepared from snails (*Pila* sp or *Pomacea* sp) is the main source of *Angiostrongylus* infection. Dishes may comprise semi-cooked snails in herbs and spices, from whence live infective larvae migrate to the musculature. Vegetables and water can also be contaminated with infective larvae and serve as a source of infection [9,10]. In 2000, a group of travelers returning from the Caribbean displayed symptoms of eosinophilic meningitis. The Caesar salad consumed during the meal was suspected to contain third-stage larvae embedded in mucus [11]. If infective larvae in the environment can contaminate vegetables and water, it is possible that insects, especially houseflies, can serve as mechanical vectors of angiostrongyliasis.

PTTEP International Limited (Yangon Branch), or PTTEPI, intends to develop and

produce gas from the offshore field Block M9, owned by Myanmar Oil & Gas Enterprise (MOGE). The M9 block is located in the gulf of Moattama, off the shores of Myanmar. PTTEPI started to implement a socio-economic program in the Kanbauk area (Fig 1) in 2009, via the “Zawtika Socio-Economic Project”. It consisted of three main areas of development: 1) the health sector; 2) the education sector; and 3) the community development sector. The Faculty of Tropical Medicine, Mahidol University, was in charge of the health sector aspect. In June 2010, a team from the Department of Helminthology visited Kanbauk area, in Yebhyu Township, Dawei District, Tanintharyi Division, in the Union of Myanmar, during the Burmese rainy season. Many houseflies were observed around the field station. The flies fed and rested on the tables, walls and



**Fig 1 Kanbauk study site.**



**Fig 2 Third-stage larva of *Angiostrongylus* sp (a and b), isolated from a housefly.**

floors of the canteen, as well as in the auditorium, living room, and corridors. The workers' camp was very close to the auditorium. In the kitchen, fish remains were discarded into uncovered garbage cans, with numerous houseflies feeding on them. Based on this observation, houseflies in the area were collected manually and examined for parasites. Individual resting flies were caught with a clean plastic bag then transferred to a larger plastic bag. After placement in an ice box to slow metabolism, they were placed in a clean bottle and preserved using formalin detergent solution (10 ml formalin and 50 ml detergent dissolved in 440 ml water) [12], then transferred to the laboratory in Bangkok, Thailand.

In the laboratory, the 468 houseflies collected were separated into smaller containers; 50 houseflies per container. They were then transferred to an ultrasonic cleaner (Elma Transsonic Digital D-7700) for washing at level 5 for 15 minutes,

with the temperature off. After removing the insects, the supernatant was transferred to a centrifuge tube, and centrifuged at 2,000 rpm for 2 minutes [13]. All sediments were observed under a microscope. Microscopic examination revealed that the sediments contained a number of mites, larvae of unknown nematodes, and 12 larvae with prominent chitinous rods at the anterior end and constricted tails (Fig 2). Larvae in the latter group were suspected to be live *Angiostrongylus* third-stage larvae, since the identification features were clearly visible and no shrinkage of the larvae was exhibited. One was kept in 70% ethanol for DNA extraction. DNA identification, based on the partial nad1 mitochondrial gene (599 bp), showed 16.86% genetic differentiation from *A. cantonensis*. Although it was not an *A. cantonensis* larva, the result indicated it was a metastrongyloid larva. All houseflies caught were *Musca domestica* (Fig 3).

The field station in Kanbauk area is located near mountain forest, and has a small stream in the backyard – this represents a good habitat for definitive and intermediate hosts of *Angiostrongylus* nematodes. In this area, a full life cycle may be completed. The evidence of houseflies carrying metastrongyloid larvae on their surface can be explained by the flies feeding on dead, infected snails/slugs (intermediate hosts), as well as other paratenic hosts, such as crabs/prawns, or the slime from snails and slugs.

Infection with *Angiostrongylus*, a human metastrongyloid, is mostly caused by eating improperly cooked land snails (usually the giant African land snail, *Achatina fulica*), which contain infective larvae; the main causative agent is *A. cantonensis* [14]. This study showed that the housefly, *M. domestica*, is a transport host of metastrongyloid larvae. Although the larva discovered was not *A. cantonensis*, other species of *Angiostrongylus* have been reported to cause disease in humans, such as *A. malaysiensis*, *A. costaricensis*, and unidentified species of *Angiostrongylus* (*Parastrongylus*) [14-21]. Slom *et al* [11] concluded that a salad contaminated with infective third-stage larvae was the cause of eosinophilic meningitis among travelers. This report, with our



**Fig 3 Housefly, *Musca domestica*.**

findings, indicates humans may become infected with angiostrongyliasis without consuming infected, second intermediate hosts. Community health education should emphasize appropriate sanitation, hygienic food-preparation practices, and the prevention of food contamination by flies and other insects. In addition, to prevent zoonotic angiostrongyliasis, the consumption of raw or undercooked foods should be avoided.

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