OCCURRENCE OF A RARE DIGENETIC TREMATODE (*ECHINOSTOMA* SP.) FOUND IN A NORWAY RAT FROM PENANG, MALAYSIA

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Abstract. Trematodes commonly known as flukes are parasitic flatworms with complex life cycles, typically involving both aquatic and terrestrial hosts including rodents. The biological behaviour of these opportunistic rodents' aids in facilitating the acquisition and development of helminth parasites including trematodes. Among the family of trematodes, the Echinostomatidae has a unique distinguishing feature between members with the presence of a distinctive head collar spined around the oral sucker. A necropsy was conducted on the wild rodent's carcasses, and the gastrointestinal sections were removed and inspected. Morphometric analyses of the cleared specimens were performed using an Olympus BX41 microscope connected to an Olympus digital camera, calibrated with an eyepiece micrometer. This study discovered a trematode species known as *Echinostoma* sp. from one out of 150 wild rodents examined from Penang, Malaysia. The adult of this digenetic trematode was found with 43 collar-spined was found in the small intestine of the brown Norway rat (*Rattus norvegicus*) collected from a wet market in Penang, Malaysia. The Echinostomatidae is also well-known to cause intestinal foodborne parasitic disease, known as echinostomiasis infecting both animals and humans. This updated report on the detection of *Echinostoma* sp. will provide valuable insight into the occurrence and the possibility of *R. norvegicus* transmitting *Echinostoma* sp. to the local environment and aid in future prevention and reduce the risk of infection locally.

Keywords: Digenea, *Echinostoma* sp., Penang, rodents, trematode

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1. INTRODUCTION

The subclass Digenea is a large and impactful group within the realm of parasitic organisms, holding notable significance in medicine, veterinary science, and economics. Often referred to as digenetic trematodes or flukes, this extensive group comprises more than 18,000 species [1]. Meanwhile, the families of Echinostomatidae, Himasthlidae, and Echinochasmidae are a large group of digenea trematodes parasitizing the small intestines of fish, reptiles, birds, and mammals including rodents [2].

The biological behaviour of these opportunistic rodents has facilitated the acquisition and development of several zoonotic helminth parasites including trematodes [3]. As the definitive hosts, this mammal acquires infection through the ingestion of the second intermediate host such as snails, mussels, tadpoles, frogs, and fishes, which serve as a reservoir for encysted metacercariae. Following ingestion, the metacercariae undergo excystation in the duodenum. Subsequently, the juvenile parasites embark on a migration to the small intestine, where they attach themselves to the mucosa utilizing the ventral sucker and progress through maturation to reach the adult stage [4-5].

Among the family of trematode, the Echinostomatidae has a unique distinguishing feature between members with the presence of a distinctive head collar adorned with collar spines encircling the oral sucker. Notably, the configuration of the spines on the cephalic collar can exhibit either a unicyclic or a bicyclic arrangement, adding to the characteristic morphological traits that define this group of organisms [6].

The Echinostomatidae is also well-known to cause intestinal foodborne parasitic disease, known as echinostomiasis, which infects both animals and humans. Human echinostomiasis is caused by at least 24 species globally [2,7]. In the Asian tropics, there were human infection cases reported in Southeast Asian countries such as Malaysia, Indonesia, Thailand, and the Philippines [7].

In Malaysia, three species specifically known to cause infection in humans are *Echinostoma malayanum (E. malayanum), E. ilocanum,* and *E. revolutum* [4-7]. Furthermore, the occurrence of co-infection involving both *E. revolutum* and *E. malayanum* has been documented in both humans and rodents [8]. The case of *Echinostoma* sp. infecting rodents is very rare and has only been documented once in Malaysia by Lee in 1965 [9]. *E. malayanum* was found to infect *Rattus argentiver* in Kedah and Malacca, and there has been no reported infection of *Echinostoma* sp. in rodents recently.

Due to its complex lifecycle, it is very rare to find *Echinostoma* sp. infecting wild rodents in Malaysia. On the other hand, the close interaction between rodents and humans caused public health concerns as it increases the risk of infection. This study aims to describe the occurrence of a digenean trematode found in the wild brown Norway rodent caught at a public market and update the report on the detection of *Echinostoma* sp. in Penang, Malaysia. This will, therefore, provide valuable insight for the authorities into the occurrence and the possibility of *Echinostoma* sp. infection involving rodents for control measures to prevent human echinostomiasis cases.

2. MATERIALS AND METHODS

Rodent sampling and sample collections were done with the collaboration of Seberang Perai City Council (MBSP) and Penang Island City Council (MBPP). A simple random sampling method was conducted, and a conventional wire trap was used to trap the rodents. To attract the rodents, various baits were used, namely peanut butter and bread, artificial crabstick, and fried chicken. The cage traps were placed by the city councils where rodents' signs could be seen as burrowed signs, areas with droppings, and footprints.

A total of 150 rodents' carcasses were received from the city councils and the wild rodents were collected from several commercial and residential areas in Penang, Malaysia. Subsequently, the examined rodent carcasses were handled according to the protocols [10]. The necroscopy was performed on the carcasses and the gastrointestinal parts were extracted and separated. The separated gastrointestinal contents were tediously inspected. Distilled water was used to facilitate the dissection process. The contents were filtered through a fine-hole mesh strainer. Then, the deposited contents were transferred into a petri dish and tediously inspected under a dissecting microscope. The collected trematode was stored in a universal bottle containing 70% ethanol solution [10].

The trematode was stained with Semichon's acetocarmine, cleared with lactophenol, and viewed under a light microscope for its classical anatomy. Morphometric analyses of the cleared specimen were conducted using an Olympus BX41 microscope connected to an Olympus Digital camera, which was calibrated with an eyepiece micrometer. The morphology was identified according to Soulsby [11]. The identification based on features such as the general morphological characteristics of the family Echinostomatidae and the collar spine, which is a distinctive feature of echinostomes, were confirmed by Dr Jong-Yil Chai, an expert from Seoul National University.

3. RESULTS AND DISCUSSION

A total of 150 wild rodents comprising of five species were collected from several residential areas and wet markets around Penang, Malaysia. The dominant rodent collected was *Rattus norvegicus (R. norvegicus)* (Norway rat) with a prevalence of 58% (87/150), followed by *Bandicota indica (B. indica)* with 24% (36/150), *R. rattus* with 15.33% (23/150), and *B. bengalensis* with 2.67% (4/150).

Only one rodent host (*R. norvegicus*) was infected (1.37%, 1/150) with one identified genus (*Echinostoma*) of trematode. The infected host and other similar species were collected from Chai Leng Park wet markets in Seberang Perai, Penang, which had a high infestation of rodents showing holes chewed through its floors. It is noteworthy that rodents in the wet market were having close interactivity with the community.

Around 30 adult trematodes were collected, and the adult *Echinostoma* sp. (Figure 1) was found inhabiting the small intestine of *R. norvegicus.* The morphology of the trematode was identified as *Echinostoma* sp., and it was later confirmed through morphological features including the general characteristics of the family Echinostomatidae and the collar spine, which is a distinctive feature of echinostomes by the expert. The general characteristic of the family Echinostomatidae is the flat and elongated body. The average length for the adult *Echinostoma* sp. found in this study is 5 mm to 10 mm. Meanwhile the eggs are visible,

yellowish-brown in colour, and ellipsoidal in shape (Figure 1). As in Figure 2, the unique anterior part is the ‘head collar’, which bears a single double row of spines surrounding the oral sucker and the ventral sucker is located not far from the oral sucker similarly reported in previous studies [1,4,11].

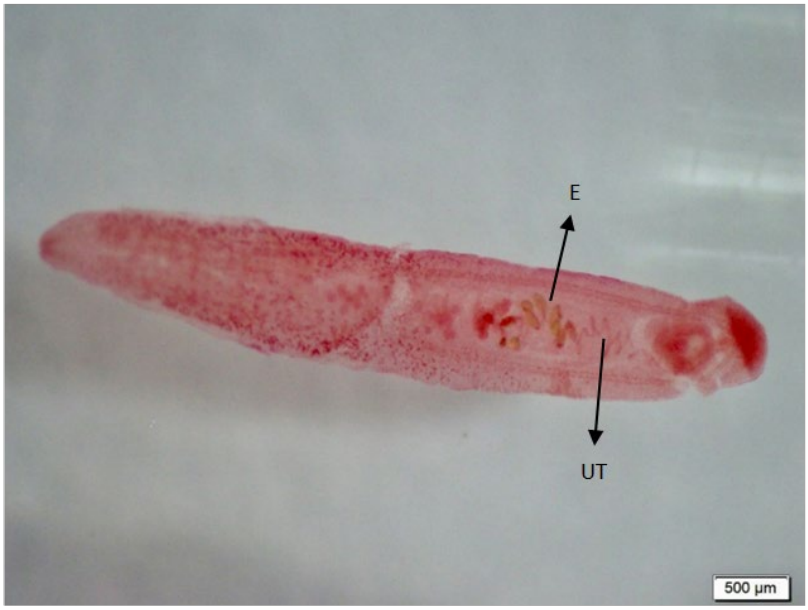

Figure 1: Whole body of adult *Echinostoma* sp. found in small intestine of the Norway rat (E: Egg; UT: Uterus)

Unlike other intestinal flukes, it has been reported that the distinctive feature of echinostomes is the collar spine. The number and arrangement of these collar spines play a crucial role as a morphological criterion for the classification of this family [12-13]. The collar spines in these configurations vary in total number, ranging from 31 to 51 [12]. The latter is encircled dorsally and laterally by a 'head-collar' with a single or double row of massive spines.

Figure 2 shows anterior part of adult Echinostoma sp. with 43-collar-spined. As in Figure 2, the specimens collected in this study exhibited 43-collar-spined around the oral sucker. The number of collar spines suggested that the trematode found in this study could most probably be *E. malayanum.* Established studies reported that *E. malayanum* is characterized by 43-44 collar spines, organized with five corner spines on each side of the shoe-shaped circumoral disc (3 oral and 2 aboral). In addition, it exhibits two groups of lateral spines, with each group consisting of 11 spines arranged in a single row, and dorsal spines comprising 11 spines (6 oral and 5 aboral) (13). However, details of these features could not be specifically ascertained in the specimens collected in this study.

Figure 2: Anterior part of adult *Echinostoma* sp. with 43-collar-spined (OS: Oral Sucker; CS: Collar Spined; VS: Uterus)

As in Figure 3(A), the vitellarium is follicular and is arranged in two different areas extending to the posterior end. Meanwhile, the digestive system comprises the pharynx that nearly reaches the ventral sucker. Simple intestinal caeca that extend to the posterior extremities is also visible in Figure 3(B) in agreement with previous report [11]. *Echinostoma* sp. is hermaphroditic; there is the presence of both male and female sexual organs in one individual. In Figure 3(C), two large lobed testes were found in the posterior half of the body and the uterus can be seen in Figure 1 as described before [4,11].

This genus possesses a complex life cycle and needs two intermediate hosts to complete its life cycle. Figure 4 shows the probable life cycle of *Echinostoma* sp. and the source of infection in the wet market environment. The first intermediate host would be the freshwater snails from the families of Planorbidae and Lymnaeidae, where the miracidia undergo development and transform into cercariae and will be released. Snails, fish, or crabs on the other hand would be the potential second intermediate hosts that carry the encysted metacercaria of the trematode [5-6].

The life cycle of *Echinostoma* sp. is continued when the rodent from the wet market is expected to consume the infected second intermediate, which could be fish, crabs, snails, or the mussels that are widely sold in the market. The metacercaria excyst in the rodent and becomes an adult trematode and is found in the small intestine of the rodent. Finally, the life cycle will be continued when the rodents defecate faeces containing the eggs into the environment. The complex life cycle and the need to use two biological intermediary hosts support the low prevalence and intricacy of *Echinostoma* sp. in rodents.

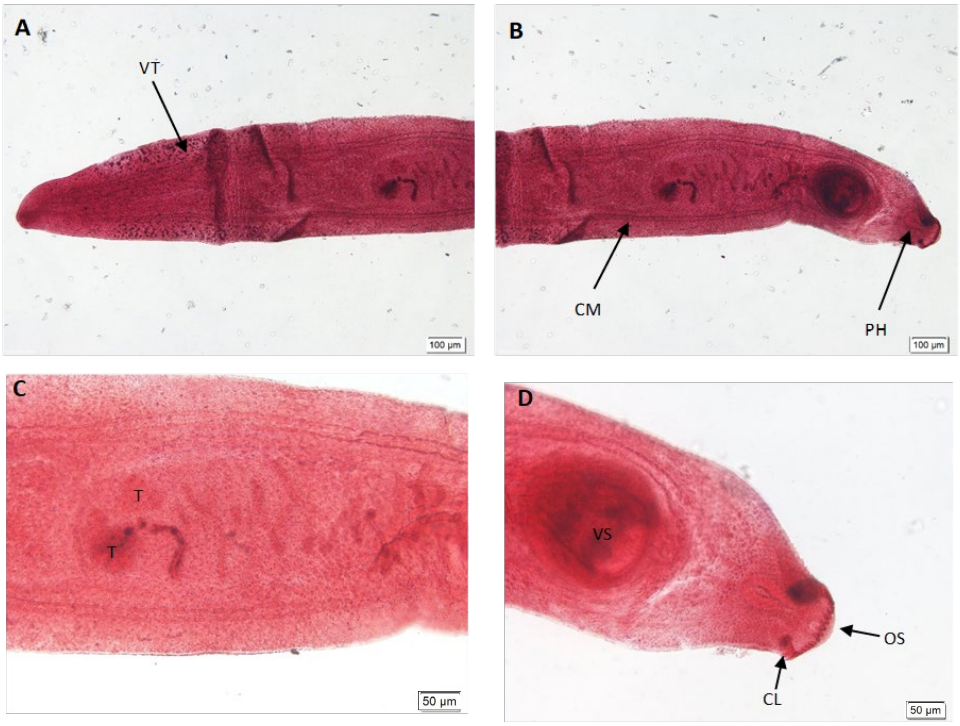

Figure 3: Adult Echinostoma sp. (A) posterior end, (B) anterior end, (C) reproductive organ, and (D) suckers (VT: Vitellarium; CM: Caecum; PH: Pharynx; T: testes; VS: Ventral Sucker; OS: Oral Sucker; CL: Collar)

On the other hand, humans may also act as *Echinostoma* sp. definitive host that will cause echinostomiasis, which is known as an intestinal foodborne parasitic disease [1,6,12,13]. Presently, at least 24 species indigenous to Southeast Asia and the Far East are responsible for echinostomiasis in humans. There are at least 56 officially recognized species in this genus. Meanwhile, in Asia, at least eight species including *E. revolutum, E. lindoense,* and *E. robustum* were identified [2].

China has reported the highest number of echinostome species with a total of 13 recorded in the country, particularly in the provinces of southeast China. The province in China recorded the most human echinostomiasis with more than 2500 cases recorded [14-15]. Along the Mekong River in Lao PDR, a total of six species of *Echinostoma* had been identified to infect humans. Meanwhile, in Khammouane province, echinostome eggs of *E. revolutum, E. malayanum,* and *E. japonicus* were identified in the faeces of residents. Meanwhile schoolchildren show gastrointestinal symptoms which are later identified due to *E. revolutum* [6,15].

In Malaysia, *E. malayanum,* and *E. ilocanum* are the two endemic species of *Echinostoma* spp. known to infect human [6]. In 1911, the first human case of *E. malayanum* was first found from labourer [16]. The second case reported was in 1963, it was discovered from an Indian child aged 5 in Kuala Lumpur was diagnosed with amoebic dysentery with only a few *Echinostoma* eggs present. Upon investigation, it was known that the children had consumed snails [17]. Additionally, to our knowledge, after two of the reported case there have been no new reported cases of echinostomiasis in Malaysia until today.

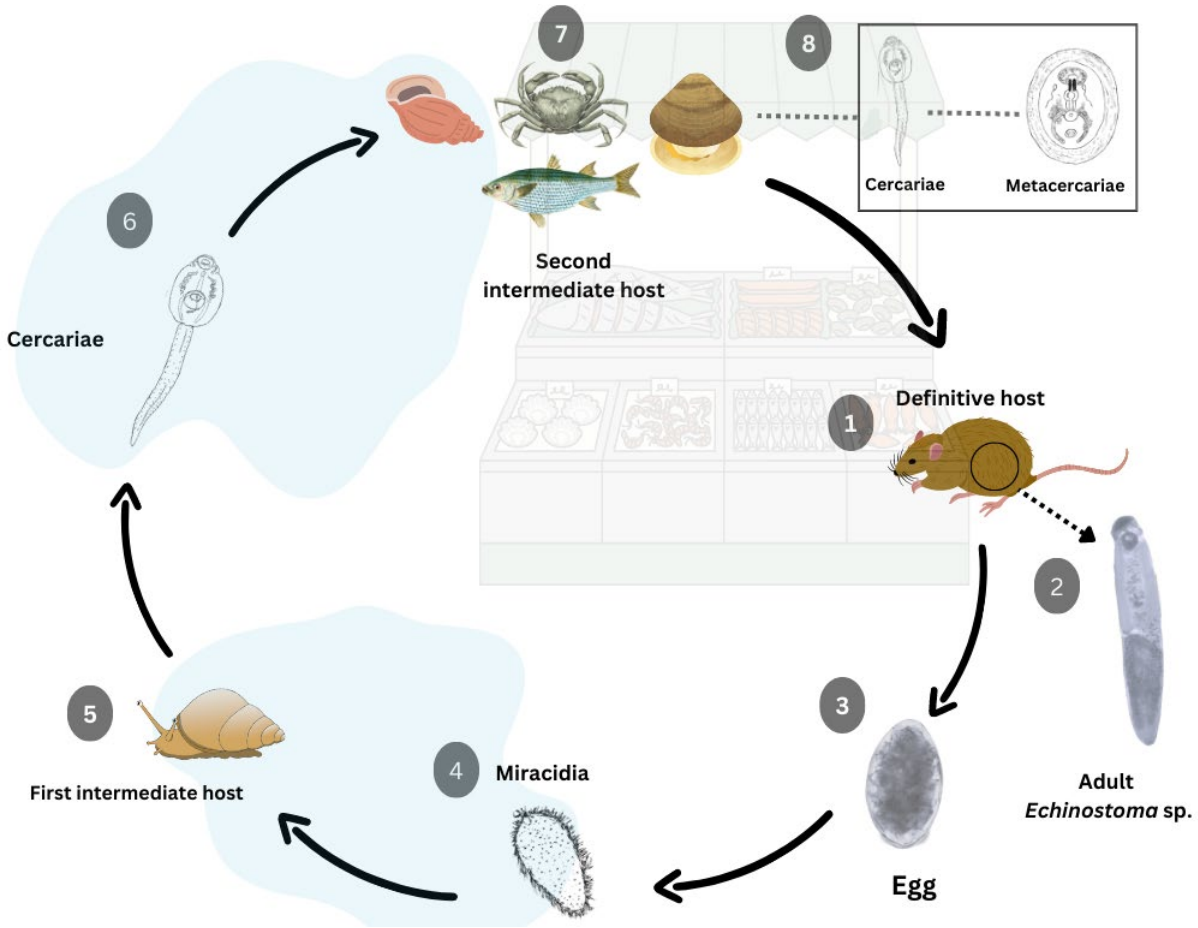

Figure 4: The probable life cycle of *Echinostoma* sp. infection in the wet market environment. (1) Wild rodents found in the wet market, (2) Adult worms in the small intestine of vertebrate hosts (rodents), (3) Eggs are excreted from host faeces, (4) Miracidia hatch in freshwater and infect the first intermediate host, (4) Intramolluscan stages develop within the snails, (5) Cercariae are released by the first intermediate host and swim and penetrate the second intermediate host (snails, crabs, fishes, clams), (6) Cercariae encyst within the second intermediate host become metacercariae and (7) Ingested by the definitive host and excyst to become adults

Human echinostomiasis signs and symptoms associated with mild-to-moderate infections, including anemia, headache, dizziness, slight stomachache, gastric pain, and loose stools [8]. Endoscopic observations have revealed that echinostomes inflict damage to the small intestinal mucosa, leading to extensive lesions in the intestinal and duodenal regions [2] as well as inducing inflammation [2,5].

In contrast to echinostomiasis infection in humans, it is a rare occurrence of rodents being infected with *Echinostoma* sp. The first study was first reported by Lee [9] in which *E. malayanum* was found to infect *R. argentiver* in Kedah and Malacca. As far as we know, there has been no reported infection of *Echinostoma* sp. in rodents recently. Echinostomiasis in rodents is often asymptomatic, however for higher parasitic load will cause inflammation at the small intestine caused by the local tissue damage which heightened inflammatory responses, goblet cell hyperplasia, and disruption of villi [5].

Co-infection of *E. malayanum* and *E. revolutum* has been found in rat and human host [9]. Rodents may act as one of the definitive hosts for echinostomiasis, and this is concerning to nearby communities. In an urban area, rodents can be found inhabiting the same space as the community and is the primary worry today. Being the definitive host, rodents can transmit the infection to the surrounding community from the voided infected faeces.

4. CONCLUSIONS

Although there are no reported cases at present, the infection can be prevented by elimination and eradication of the rodent host to lower the risk of infection in the future. Echinostomiasis can also be avoided by preventing eating raw and inadequately prepared freshwater fish, raw brackish water snails, and raw crabs. The updated report on the detection of *Echinostoma* sp. in Penang, Malaysia will provide valuable insight for the city council and other authorities for future prevention planning and strategy. Future studies involving molecular characterization of this parasite will support the morphological characterization of *Echinostoma* sp. as reported and to identify up to the species level.

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Author Contributions

All authors contributed toward data analysis, drafting, and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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