OCCURRENCE AND MORPHOLOGICAL CHARACTERISTICS OF *CYSTICERCUS FASCIOLARIS* IN WILD RODENTS FROM PENANG, MALAYSIA

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Abstract. *Taenia taeniaeformis* is a zoonotic tapeworm that causes a reduction in weight, vomiting, malnutrition, and intestinal obstruction to felines and canines. Murid and cricetid rodents are the intermediate hosts, cats and dogs are definitive hosts for this parasite whereas humans are the accidental hosts. These animals may contribute to the transmission of infectious diseases specifically in the urban environment which can be a potential threat to public health. Thus, this study aims to determine the occurrence of *T. taeniaeformis* in rodents in several selected locations in Penang, Malaysia. Wild rodent sampling activities were conducted from October 2022 until March 2023 using bait traps in Seberang Perai and Penang Island, Malaysia. The wild rodents were then dissected, and the livers were examined for the presence of cysts. The extracted parasites were examined in detail under the stereomicroscope and scanning electron microscope (SEM). The SEM images show detailed morphological characteristics of *T. taeniaeformis* (*Cysticercus fasciolaris*) at the larval stage. The larvae were found in 44 out of 146 wild rodents (30.1%). *Rattus rattus* was found to have the highest infection rate (11/20; 55%), while *Rattus norvegicus* had the lowest rate (18/86; 20.9%). This study provided a reliable reference for the parasite *C. fasciolaris*, with distinctive morphological characteristics. The study concluded that development in urban areas of Penang may influence the prevalence of *T. taeniaeformis* in both definitive and intermediate hosts by altering both hosts' behaviour, and habitat. With a high prevalence of *C. fasciolaris* among rodents, this parasite poses increased zoonotic potential to the public.

Keywords: Electron microscopy, morphology, prevalence, *Taenia*, wild rodent

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1. INTRODUCTION

Taenia taeniaeformis is known as a helminth that has been classified in the family of Taeniidae, the flatworm family. *Cysticercus fasciolaris* is known as the earliest larval stage of cat tapeworm [1]. The characteristics of this parasite represent by the large scolex with four suckers, one or two row(s) of hooks (depending on the development level of parasites), and a terminal bladder at the posterior end [2]. The size of *C. fasciolaris* seems to vary in the samples. The total number of hooks will be at least 19 up to 42 [2-3]. The body length greatly varies from 0.9 up to 32cm and depends on the matureness of the parasites [2-4].

Murid and cricetid rodents serve as intermediate hosts for this parasite species. They are infected by ingesting contaminated water or food with infected cat faeces [5]. Felines or canines are the definitive hosts of this parasite species, and the transmission happens when they consume a rodent with *C. fasciolaris* on the liver [1]. There are several cases of *T. taeniaeformis* that have been reported in humans [6-9].

In Malaysia, a lot of studies have been done related to endoparasites including *C. fasciolaris* from wild rodents and it provided prevalence rates [1,10-16]. Most of these studies were conducted in urban areas, especially in southern regions of Peninsular Malaysia such as Kuala Lumpur and Selangor. Another study showed a prevalence rate of 35% was found in wild rodents among other endoparasites found in southern regions [16]. This prevalence rate was the highest in Malaysia as of 2023.

This study also suggested the medical and zoonotic importance of *C. fasciolaris* since dogs and cats were also present in this area, there is a chance that these animals may pick up zoonotic parasites and transmit them to humans in the nearby villages. *C. fasciolaris* has been recovered from the liver part of wild rodents from the Endau Rompin National Park, Johor [13]. In their study, *C. fasciolaris* was found in 14 out of 128 wild rodents as forest and commensal hosts and the prevalence rate of *C. fasciolaris* was 7.81 %.

However, there is only one study available in Malaysia that presented a morphological study on *T. taeniaeformis* in recent dates [17]. An adult *T. taeniaeformis* was recorded with detailed morphological features in micrographs but with the cost of shrinkage and distortion due to hexamethyldisilane drying. There is also a lack of study of endoparasites including *C. fasciolaris* from wild rodents in Penang State, especially morphological study of the larval stage of *T. taeniaeformis.* As for the prevalence of *C. fasciolaris* in Penang, only one study has been done in Georgetown to determine the endoparasite infection of wild rodents from islands (Carey Island and Penang) and coastal (Kuantan and Malacca) habitat in Peninsular Malaysia related to prevalence of infections, and, to determine the role of intrinsic and extrinsic factors in the endoparasite community from the wild rat population [11]. The most recent study of *C. fasciolaris* prevalence in the Penang region was done at least 10 years ago [11].

It is important to provide its status as the zoonotic potential it possesses because of rapid urbanisation. Sampling was conducted to obtain fresh liver samples from wild rodents to extract *C. fasciolaris*. Its prevalence in wild rodents of Penang state was updated. Statistical analyses were carried out to determine the association of the prevalence of the parasite with the rat species, age group, and sex. Therefore, this study aims to provide the morphological characteristics and related measurements of *T. taeniaeformis* at the larval stage

(*Cysticercus fasciolaris*) using microscopic techniques, as well as to provide updates on the prevalence of *C. fasciolaris* in wild rodents in Penang, Malaysia.

2. MATERIALS AND METHODS

2.1 Animal Ethics Approval

All animals in this study were handled according to the Institutional Care and Use Committee (IACUC), Universiti Sains Malaysia (USM/IACUC/2023/(Exemption) (0007)). Permission was sought from the Penang Island City Council (MBPP) and Seberang Perai City Council (MBSP) after obtaining approval from the IACUC, Universiti Sains Malaysia.

2.2 Sampling Sites

Sampling activities were conducted at several selected areas namely, the commercial area, wet market, residential areas, and dump sites of Penang Island and Seberang Perai **Latitude:** 5.4141° N and **Longitude:** 100.3288° E) (Figure 1). In this study, two types of urban settings have been chosen namely the commercial areas which consist of 12 selected sampling sites, and the residential areas which consist of 13 sampling sites. Traps were placed in commercial areas which nearby shops, markets, and hawker stalls. As for residential areas, traps were placed near dumpsters at the apartment or flatlet.

Figure 1: Sampling sites as indicated in red dots.

2.3 Sampling Activities

The sampling was conducted from October 2022 until March 2023. The sampling lasted from 9 pm until midnight. Sampling activities were conducted by using collapsible cages with several baits such as crab meat, chocolate, fish cake, cheese, fried chicken, and salted fish [18]. The traps were placed near their burrows, dumpsters, and areas with infestation signs where there is the presence of their droppings. Once the rat was caught, the trap was replaced with a new one. The rats were then brought by the Penang Island City Council (MBPP) and Seberang Perai City Council (MBSP) and transported to the laboratory.

2.4 Identification of Wild Rodents

The species of rodents were identified based on [19-20]. The weight, sex, development stage, and morphometric measurement (ears, head, body, tail, and hindfoot) were measured and recorded. The sex of wild rodents was identified by the presence of testes for adults or the presence of teats. As for juveniles, the bodies of wild rodents were opened to check the presence of seminal vesicles.

2.5 Dissection of Wild Rodents

The rodent body was opened with a dissection scissor and a tweezer. The end of their intestine was cut first and then, rib bones were cut with the scissors. Their trachea was cut to be separated. All the organs were removed from the back of the body and placed on a metal tray. The gastrointestinal tracts (GITs) and livers were separated and placed on a metal tray. The cysts along with liver lobes were separated. While opening the cyst, a viable larva was then stored inside a universal bottle containing 70% ethanol and labelled date and sampling ID.

2.6 Screening for Taenia Taeniaeformis Larvae

The liver was carefully examined for searching *T. taeniaeformis*. The larvae of *Taenia taeniaeformis* were screened by finding the visible white cysts attached to the liver with the naked eye. The detailed morphological features and characteristics, including body measurements and morphology, were checked with a CellSens dimension microscope. The cysts along with liver lobes were separated and washed in 1% phosphate-buffered saline (PBS).

2.7 Microscopy Examination

a) Light Microscope (CellSens Dimension Microscope)

Identification of these larvae was carried out by submerging them in lactophenol first and then examining them under a CellSens dimension microscope. Body measurements were taken and recorded such as body length, scolex, terminal bladder (tail) and the number of suckers and hooks (the outer larger hooks and the smaller inner hooks). The whole body and tail were observed with 20X magnification. Scolex, suckers and hooks were observed with 80X magnification. A clear image of each body part of *C. fasciolaris* was obtained under the stereo microscope in the Electron Microscope Unit at the School of Biological Science,

USM. The morphology of *C. fasciolaris* was observed under a CellSens dimension microscope, Olympus SZX9 (Olympus Optical Co. Ltd. Tokyo, Japan). The larvae were identified by morphological characters and the organ which they parasitize on [21-22].

b) Scanning Electron Microscope (SEM)

A whole body of one *C. fasciolaris* was collected from one of the cysts in the liver part of a wild rodent and examined under a Leo Supra 50 VP Field Emission Scanning Electron Microscope (Carl-Ziess SMT, Oberkochen, Germany) at the School of Biological Science, Universiti Sains Malaysia. The protocol was provided by the USM Electron Microscopy (EM) Unit. The specimen was fixed in McDowell-Trump fixative prepare in 0.1 M phosphate buffer, dehydrated through a graded ethanol series (50-100%), immersed in 1-2 ml of hexamethyldisilane (HMDS) for 10 minutes, and air dried. The HDMS was decanted from the specimen vial and allowed the specimen to air-dry at room temperature. The dried sample was mounted onto an SEM specimen stub with a double-sided sticky tape or silver point and coated the specimen with gold for viewing under scanning electron microscopy.

2.8 Prevalence of C. fasciolaris from Wild Rodents

The prevalence of *C. fasciolaris* from the rats was calculated by the calculation below:

$$\text{Prevalence (\%)} = \frac{\text{No. of rats positive for } C.\ fasciolaris}{\text{No. of total rats sampled.}} \times 100$$

2.9 Statistical Analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) software package version 26. Chi-square and Independent T-test were carried out to determine whether the prevalence of *C. fasciolaris* infection was associated with the rat species, age group, and sex. A probability value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Out of 146 rodents, *C. fasciolaris* was found in 44 (30.1%) wild rats namely, *Bandicota bengalensis* (1/4; 25%), *Bandicota indica* (14/36; 38.8%), *Rattus norvegicus* (18/86; 20.9%) and *Rattus rattus* (11/20; 55%). It was found that there is a significant difference between the prevalence of *C. fasciolaris* and the rat species ($\chi^2 = 5.864$, $p = 0.015$). It indicates that *R. rattus* was found to be infected more than other species. The habitat and their behaviour might have contributed to the infection of *C. fasciolaris* in *R. rattus*. *T. taeniaeformis* infection occurs if rodents consume contaminated food or water with infected cat faeces by *T. taeniaeformis* [23]. *R. rattus* is known to be one of the dominant species in urban environments and rely on food source from humans [10,24-26]. The presence of cats in some areas overlaps with the places where rodents were captured [4,10,27-28]. *T. taeniaeformis* eggs were recovered from cat faeces in the overlapped area [14]. As to *B. indica* and *R. norvegicus*, these species of rats tend to burrow their nests near food sources such as dumpsters while *R. rattus* usually nests on trees [20,23]. Therefore, *R.*

rattus could have foraged more frequently and explored further. As a result, *R. rattus* is more likely to be exposed to *C. fasciolaris* eggs.

Besides, it was also found that the prevalence of *C. fasciolaris* infection was statistically significant in the host age group (p-value = 0.026, $p < 0.05$). It is suggested that the infection occurs more frequently in adult rats than the juvenile in which adult rats have more chance to be exposed to contaminated food or water outside the nest as they forage more frequently [29-32]. There is a higher probability of infection in adult individuals as compared to juveniles.

The number of white cysts (Figure 2) present on an infected liver varied from one to seven [2,33]. In extreme cases, *C. fasciolaris* could migrate into other organs through a central vein and systematic circulation [33]. As a result, the intermediate hosts could develop multiple cysts by continuous exposure to *T. taeniaeformis* eggs. The cysts that formed in less than one month could be found in a diameter of less than 5 mm with an undeveloped scolex. After one month, the larvae would demonstrate features of a developed scolex, which include four lateral distinct suckers and two collar rings of hooks [34].

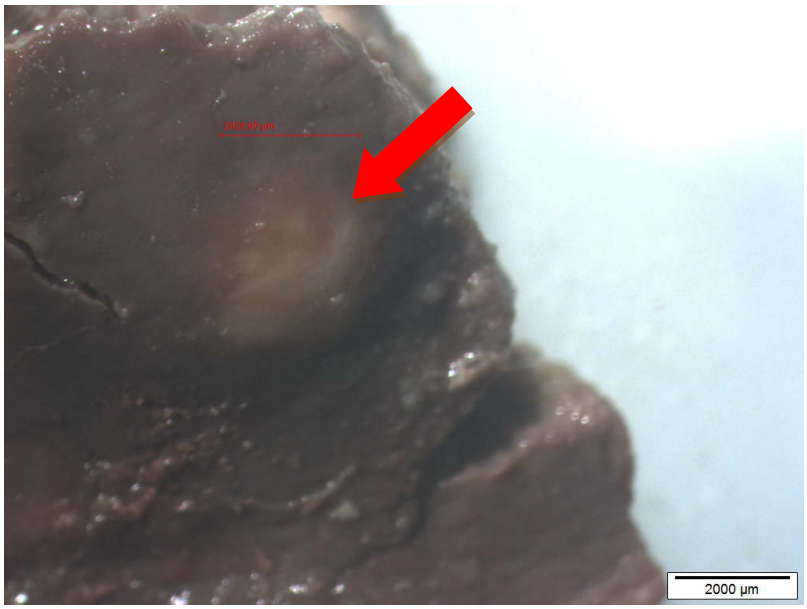

Figure 2: A white cyst (pointed with a red arrow) was embedded in the liver of a wild rodent under a CellSens dimension microscope.

Apart from that, the body length of *C. fasciolaris* found in this study is approximately 1.7 cm (Figure 3) similarly reported by [4,33]. The larvae's body length could be up to 15 to 60 cm in length [5]. The details of hooks on the scolex are one of the important keys to the identification of the cestode species. The scolex of *C. fasciolaris* consist of large, four suckers. The rostellum consists of anterior (large) and posterior (small) hooks which in total is 33 hooks and their size 248 µm (outer) and 121 µm (inner), respectively. The four suckers are visible at the scolex (Figure 4). The total number of hooks in the poorly developed posterior crown is 19 while the matured one is 34-42 hooks in total [2,4]. Whereas the total number of hooks is 34 which fits the range of total hooks as reported by previous studies [2-4]. In the liver, the immature larva develops the scolex in which one row of hooks is developed at each time. It may only possess the first row of the crown (hooks) at the scolex, and some with a varied number of hooks [2,33].

Figure 3: The whole body of *C. fasciolaris* under a CellSens dimension microscope.

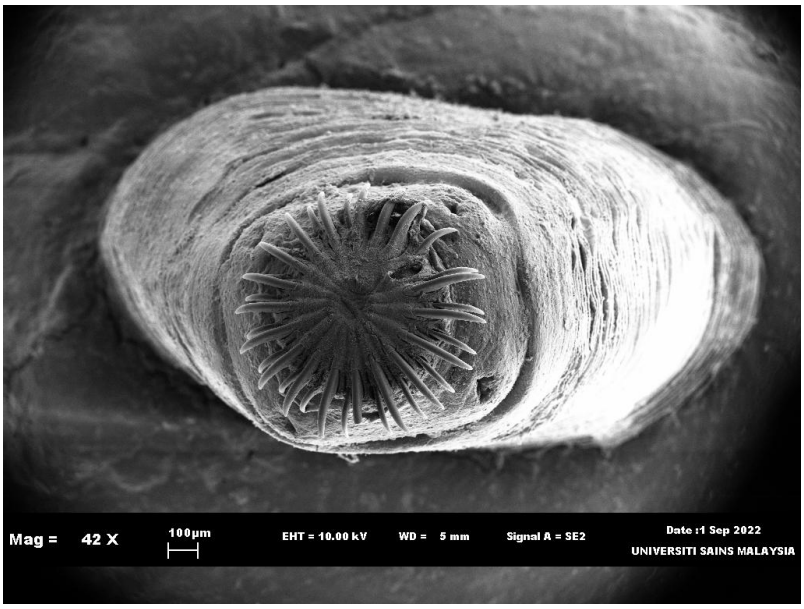

Figure 4: Electron micrograph of the scolex of *C. fasciolaris.*

The segments (Figures 5(A) and (B)) of *C. fasciolaris* appear with no genital pores. The body segments of *C. fasciolaris* provide flexibility to the parasite during attachment. The surface of the body wall is surrounded by a tegument. It works as the host-parasite interface and functions as protection, absorption, and secretion [2]. The posterior end of the parasite shows round round-shaped tail (terminal bladder) (Figure 6). The terminal bladder and the segmented strobila without the genital organs. It is one of the main characteristics of the identification of the larval stages [2].

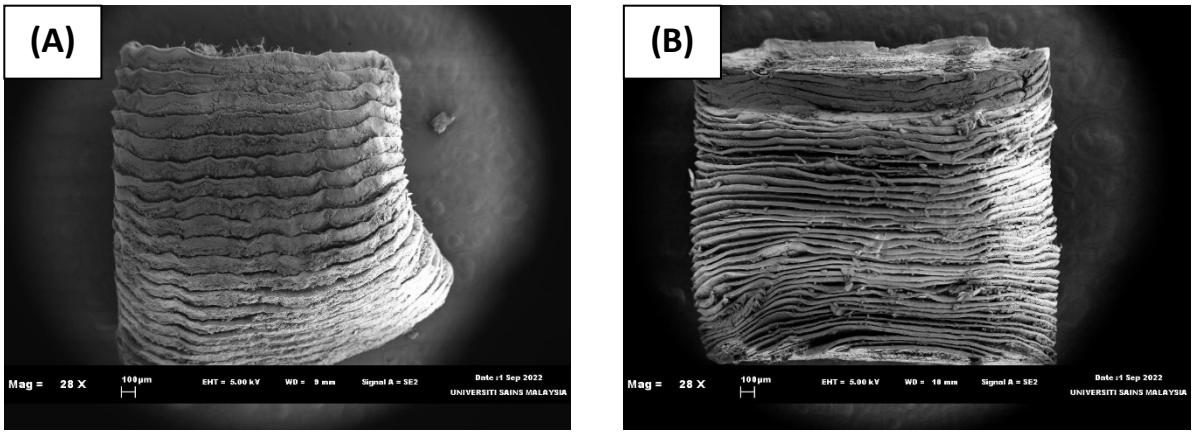

Figure 5: Electron micrograph of the mid-body segments of *C. fasciolaris.* (A) Dorsal segments and (B) Ventral segments.

Figure 6: Electron micrograph of the round bulb tail (terminal bladder) at the posterior end of *C. fasciolaris.*

4. CONCLUSIONS

The electron micrographs provide details on the morphological characteristics of *C. fasciolaris* compared to the stereomicroscope such as the exact number of hooks, the presence of suckers at the scolex, and the presence of genital pores on each body segment. It is a useful tool that provides us with some clues to understand the function of each body part.

This study concluded that development in urban areas of Penang may influence the prevalence of *T. taeniaeformis* in both definitive and intermediate hosts by altering both hosts' behaviour, and habitat. With a high presence among rodents, this parasite poses increased potential for zoonotic implications to the public. Apart from that, micrographs with morphometric parameters could be a reliable reference for species identification, specifically *C. fasciolaris*.

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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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