Ultrastructural Morphology Comparison of *Raillietina echinobothrida* and *Raillietina tetragona* In Native Chickens at Sidoarjo Regency Using Scanning Electron Microscope (SEM)

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**Abstract**

This study aimed to compare the ultrastructural morphology of *Raillietina echinobothrida* and *Raillietina tetragona* that infected the small intestine of native chickens (*Gallus domesticus*) obtained from various traditional markets in Sidoarjo Regency. The samples used in this research were 57 small intestines of native chickens (*Gallus domesticus*) sold at Tulangan, Wonoayu, Larangan, Krian, and Suko Market. The samples were macroscopic and microscopically examined using Scanning Electron Microscope (SEM). The result showed that *Raillietina echinobothrida* with prevalence of 57.89% has a yellowish string-like body (length 16-23 cm) with a round scolex (0.27-0.3 mm in diameter), round sucker (0.07x0.08-0.08x0.09 mm in size), rostellum armed with hooks has size of 0.06x0.07-0.08x0.1 mm, and unsegmented, thick, and short neck. Meanwhile *Raillietina tetragona* showed prevalence of 29.05%, also has a yellowish string-like body (length 20-28 cm) with an oval scolex (0.16-0.2mm diameter), oval sucker (0.04x0.09-0.05x0.1mm in size), rostellum armed with hooks has size of 0.03x0.06-0.04x0.66 mm, and unsegmented, thin, and long neck. The surface body of both *Raillietina echinobothrida* and *Raillietina tetragona* has a soft and velvety texture.

**Keywords:** Scanning Electron Microscope, *Raillietina echinobothrida*, *Raillietina tetragona*, native chicken

**Introduction**

Helminths or worms are the cause of various diseases that are known globally as helminthiasis (Ryabokon et al., 2013). Worms can be distinguished and identified based on their morphology, such as: body shape, body cavity, body cover, digestive tract, reproductive organs, and their organs that can attach to their predilection sites (Boundless, 2021). A lot of research has been conducted around the world to distinguish various species of worms with many methods and tools, but in Indonesia, there has never been researched to see the morphological comparison of *Raillietina echinobothrida* and *Raillietina tetragona* by using Scanning Electron Microscope (SEM).

Over et al. (1992) explained that worm infection in livestock could cause significant economic losses. This has to do with the effects of this disease that can cause the death of livestock or cause chronic symptoms such as decreased growth rate, weight loss, reduced fertility, or other symptoms that may be difficult to see. Helminthiasis in native chickens have been widely reported to have a high prevalence in several developing countries (Mukaratirwa and Khumalo, 2011).

Worms that attack poultry generally divided into three main classes, namely cestodes, trematodes, and nematodes (Ola-fadunsin et al., 2019). Infection due to cestode worms has a wider spread rate in poultry than infections caused by nematodes and trematodes. Raillietina species included in cestodes class that usually attack poultry are *Raillietina echinobothrida* and *Raillietina tetragona* (Rahayu, 2008). Raillietinasis often attacks native chickens because they live in dirty environmental conditions, so that the infection rate is higher due to the definitive host consuming paratenic and intermediate hosts containing the raillietina infective stage (Bolfa et al., 2019). Raillietina also had a high record of infection in area with high humidity and rich biodiversity and availability of
intermediate hosts, such as ants and beetles (Ilyes and Ahmed, 2013). *R. echinobothrida* is one of the most pathogenic cestode worms and it can cause nodules in the intestine and lead to degeneration and necrosis of the intestinal villi if there is heavy infestation (McDougald, 2003). Meanwhile, *R. tetragona* can cause weight loss and decreased egg production (Macklin and Hauck, 2019).

Sidoarjo is one of the regency located in East Java province. It has 18 districts and each district has a traditional market, such as Larangan Market at Candi District, Krian Market at Krian District, Suko Market at Sidoarjo District, Tulangan Market at Tulangan district, and Wonoayu Market at Wonoayu District. At these markets, the chicken sellers brought the chickens from their own farms and then sell them together with other sellers at traditional market at cheap prices. Slaughterhouse for chickens also found in traditional market. However, the environmental conditions in traditional markets are dirty and unhygienic so the spread rate of infectious diseases in chickens sold at traditional market is very high.

In reality, *R. echinobothrida* and *R. tetragona* are difficult to distinguish by only using a light microscope because the differences in their sizes are too small and have a lot of resemblances, therefore other identification and characterization methods needed to provide a higher resolution to obtain a more detailed picture of different morphological structures. Scanning Electron Microscopy (SEM) can be used as an alternative method that is suitable and has been accepted and recognized by the world material research community. Scanning Electron Microscope itself is a tool that uses electron emission to get an overview of the structure and composition of a sample (Kannan, 2018). When compared to a light microscope, which has a magnification of about 400-1000 times its original size and a resolution of 200nm, the Scanning Electron Microscope has a much greater magnification, reaching 300.000-1.000.000 times its original size and a resolution of 0.5nm with a very detailed and smooth image (Mohammed and Abdullah, 2018). Based on this background, need further research to understand the ultrastructural differences in the morphology of *R. echinobothrida* and *R. tetragona* by using a Scanning Electron Microscope (SEM) so it can help the identification process in the field later.

### Methods

#### Sample Collection

The type of this research is a laboratory exploration with a cross-sectional study. The study population is the intestines of native chickens slaughtered at Tulangan, Wonoayu, Larangan, Krian, and Suko Market. The numbers of chicken intestines that taken were 57 in total with 10 samples from Tulangan, Wonoayu, and Suko Market, 12 samples from Larangan Market, and 15 samples from Krian Market. Surgery and sample examination conducted at the Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga and Bioscience Laboratory, Universitas Brawijaya.

#### Dissection of the Small Intestine (Worm Collection)

The small intestine cut at one end and the contents slowly removed and filtered, then observe if there are worms. The worms that found placed on petridishes that already given with physiological NaCl solution. To make it easier to collect worms, the contents of the small intestine are filtered again using running water. After the contents of the small intestine filtered, open the intestine using surgical scissors and scalpels. With running water, the small intestine is washed and rinsed with a gentle massage so the worms that stick to the mucosa can be released. The washing water collected on the tray in anticipation of the worms being rinsed out. After all the worms have been taken, the number of worms is calculated and separated by the genus. Macroscopically, Raillietina worms characterized by a thin body shape, consisting of ribbon-like segments, and their size increases from anterior to posterior. After counting, some of the worms fixed with glutaraldehyde solution for SEM examination, and some were stored in 5% alcohol glycerin solution for carmine staining where the worms previously killed in hot water with a temperature of ±700C.

#### Carmine Staining

The worms obtained from the dissection of small intestine are temporarily soaked in physiological NaCl solution. The Semichen-Acetic Carmine method was used, which refers to Khulman (2006). First, the clearing stage need further research to understand the ultrastructural differences in the morphology of *R. echinobothrida* and *R. tetragona* by using a Scanning Electron Microscope (SEM) so it can help the identification process in the field later.
organ/tissue diffraction by soaking them in a solution of acidic alcohol (alcohol 70% + HCl) for 2 minutes and followed by soaking them in a solution of alkaline alcohol (alcohol + NaHCO3) for 20 minutes. The next step is the dehydration stage to remove water content by using alcohol levels of 70%, 85%, and 95%, for 5 minutes each. Then the clearing stage where the worms is soaked in Hung's I solution for 20 minutes, then mounting the worms on a glass object with a drop of Hung's II solution and covered with a glass cover. The final stage of the preparation is observation under the light microscope at 100x magnification.

**Scanning Electron Microscope (SEM)**

The SEM examination used the standard of Bioscience Laboratory, Universitas Brawijaya. The samples of adult worms of *R. echinobothrida* and *R. tetragona* to be examined are washed first with physiological NaCl solution, and then continued with the first fixation stage where the samples were soaked in 1% glutaraldehyde solution for 3 hours, then washed again using PBS (phosphate buffer saline) solution pH 7.4 for 3 times. The second fixation stage carried out using a solution of osmium tetroxide for 1 hour. Then the dehydration carried out using graded alcohol levels (30%, 50%, 70%, 80%, 90%, and 100%). The next stage is the drying stage or CPD (Critical Point Drying) and the last is coating the sample with conductive materials in the form of carbon. The sample then examined and photographed using a SEM Model JOEL type JSM-T200.

**Data Analysis**

The data obtained was an analysis of the morphology of *R. echinobothrida* and *R. tetragona* worms using a Scanning Electron Microscope (SEM) was presented descriptively.

**Results and Discussion**

**Examination and Identification of Raillietina echinobothrida and Raillietina tetragona**

The results of laboratory examinations of chicken intestines obtained from markets in Sidoarjo Regency showed that 33 of the 57 samples tested infected with *R. echinobothrida* with a total prevalence rate of 57.89% and 12 of 57 samples infected with *R. tetragona* with a total prevalence rate of 21.05%. This number is not much different from the research conducted by Kusumadewi et al. (2020) on native chickens taken from various traditional markets in DKI Jakarta and Bogor City, which showed the prevalence of *R. echinobothrida* of 59% and *R. tetragona* with 24%. Macroscopically, the adult of *R. echinobothrida* and *R. tetragona* are yellowish white in color and thin like ribbon. The worms are about 16-18 cm long. The prevalence comparison between *R. echinobothrida* and *R. tetragona* can be seen in Table 1.

<table>
<thead>
<tr>
<th>Market</th>
<th>R. echinobothrida</th>
<th>R. tetragona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulangan</td>
<td>60%</td>
<td>20%</td>
</tr>
<tr>
<td>Wonoayu</td>
<td>40%</td>
<td>10%</td>
</tr>
<tr>
<td>Larangan</td>
<td>58.33%</td>
<td>25%</td>
</tr>
<tr>
<td>Krian</td>
<td>66.67%</td>
<td>26.67%</td>
</tr>
<tr>
<td>Suko</td>
<td>60%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>57.89%</strong></td>
<td><strong>21.05%</strong></td>
</tr>
</tbody>
</table>

The highest prevalence of *R. echinobothrida* and *R. tetragona* in Sidoarjo Regency found at Krian Market, the result showed 66.67% and 26.67% respectively. It was presume that native chickens sold at Krian Market kept and reared in a dirty environment. Moreover, the density of native chicken in Krian Market is higher than other markets at Sidoarjo Regency so the transmission of *Raillietina spp.* between each individual happened faster. This is in line with statement of Rismawati et al. (2013) which stated that in general the frequency of endoparasites presence in native chickens affected by contact between infected and uninfected animals. The environmental condition in Krian Market was also dirtier than other markets at Sidoarjo Regency, with *Musca domestica* as one of the intermediate hosts of Raillietina found at excessive amount.

Identification of the anterior part of *R. echinobothrida* and *R. tetragona*, the scolex, four suckers, rostellum, and neck in different shapes and sizes between each species found. The sharp hooks surrounding the rostellum and sucker could not be seen clearly. *R. echinobothrida* has a round head, four round suckers, also a thick and short neck. Meanwhile, *R. tetragona* had an oval head, four oval suckers, and a thinner and longer neck than *R. echinobothrida*. The comparison of the morphological characteristics of *R. echinobothrida* and *R. tetragona* can be seen in Table 2.
Table 2. Comparison of the morphological characteristics of *R. echinobothrida* and *R. tetragona*.

<table>
<thead>
<tr>
<th>Morphological Characteristics</th>
<th><em>R. echinobothrida</em></th>
<th><em>R. tetragona</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Scolex</td>
<td>Scolex</td>
</tr>
<tr>
<td>Shape</td>
<td>Round</td>
<td>Oval</td>
</tr>
<tr>
<td>Scolex diameter (mm)</td>
<td>0.27-0.3</td>
<td>0.16-0.2</td>
</tr>
<tr>
<td>Sucker Shape</td>
<td>Round</td>
<td>Oval</td>
</tr>
<tr>
<td>Sucker Size (mm)</td>
<td>0.07x0.08</td>
<td>0.04x0.09</td>
</tr>
<tr>
<td>Rostellum Size (mm)</td>
<td>0.06x0.07</td>
<td>0.03x0.05</td>
</tr>
<tr>
<td>Neck Shape</td>
<td>Unsegmented, thick and short</td>
<td>Unsegmented, thin and long</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>16-23</td>
<td>20-28</td>
</tr>
</tbody>
</table>

Microscopically, *R. echinobothrida* and *R. tetragona* which were observed using a binocular microscope with a 100x magnification could be identified based on the shape and size of the scolex, sucker, rostellum, neck, and body segments. However, the sharp hooks surrounding the sucker and rostellum could not be saw clearly. The surface structure and topography of the worms could not be observe by using this microscope.

**Scanning Electron Microscope (SEM)**

The Scanning Electron Microscope (SEM) can be used to observe the surface of the ultrastructural morphology of *R. echinobothrida* and *R. tetragona* in more detail and 3-dimensional image. In the anterior part of *R. echinobothrida* and *R. tetragona*, ultrastructural forms found in the form of scolex, sucker, rostellum and neck can be seen in detail. The morphological comparison of the scolex and neck parts between *R. echinobothrida* and *R. tetragona* using a Scanning Electron Microscope (SEM) at 500x magnification can be seen in Figure 1.

Figure 1. Morphological Comparison of Scolex and Neck Parts between *R. echinobothrida* and *R. tetragona* using A Scanning Electron Microscope (SEM). A) Scolex of *R. echinobothrida* using a Scanning Electron Microscope (SEM) at 500x magnification B) Scolex of *R. tetragona* using a Scanning Electron Microscope (SEM) at 500x magnification C) Neck of *R. echinobothrida* using a Scanning Electron Microscope (SEM) at 500x magnification D) Neck of *R. tetragona* using a Scanning Electron Microscope (SEM) at 500x magnification. Note: sl = scolex, un = unsegmented neck, rt = rostellum, sc = sucker, hk = hook. Scale bar = 200μm and 300 μm.

The suckers of each species appear to be prominent from the surface of the scolex. The suckers and rostellum of *R. echinobothrida* and *R. tetragona* also armed with hooks, it can also help the attachment of the worms on the host tissue (Lalchandama, 2009). The surface of the body/strobilla has a soft and velvety texture, forming ridges and grooves and there are small pores that can be saw from the surface. Meanwhile, on the posterior side, there is segmented body formation with porous surface. The morphological comparison of rostellum and surface body parts between *R. echinobothrida* and *R. tetragona* using a Scanning Electron Microscope (SEM) at 900x and 1200x can be seen in Figure 2.

Figure 2. Morphological Comparison of Scolex and Neck Parts between *R. echinobothrida* and *R. tetragona* using A Scanning Electron Microscope (SEM). A) Scolex of *R. echinobothrida* using a Scanning Electron Microscope (SEM) at 500x magnification B) Scolex of *R. tetragona* using a Scanning Electron Microscope (SEM) at 500x magnification C) Neck of *R. echinobothrida* using a Scanning Electron Microscope (SEM) at 500x magnification D) Neck of *R. tetragona* using a Scanning Electron Microscope (SEM) at 500x magnification. Note: sl = scolex, un = unsegmented neck, rt = rostellum, sc = sucker, hk = hook. Scale bar = 200μm and 300 μm.

Permin and Hansen (1998) stated that *R. echinobothrida* is more pathogen than *R. tetragona* using A Scanning Electron Microscope (SEM).
tetragona. It is presumably because the difference in morphological aspect of these two species. *R. echinobothrida* has double layers of sharp hooks surrounding its rostellarum, meanwhile *R. tetragona* only has one layer (Lalchhandama, 2009). Also in coordination with its name, echo which means spiny/prickly and bothrida which means the muscular structures that have function to pinch the host tissue, the suckers of *R. echinobothrida* are armed with 39 enourmous amount of spiny hooks (Lalchhandama, 2009). Because of this morphological structure, *R. echinobothrida* can damage the host tissue through its intestinal wall, by burying their scolex deep in to the wall up to muscularis mucosa and in the end it can lead to the formation of intestinal nodules (Al Marsomy and Al-Hamaadani, 2016).

**Conclusion**

Based on the morphological structure of tapeworms found in the 57 small intestines of native chickens taken from various markets in Sidoarjo Regency, the tapeworms found were *R. echinobothrida* and *R. tetragona*. *R. echinobothrida* has a higher prevalence (57.89%) than *R. tetragona* (21.05%) in native chickens from various markets in Sidoarjo Regency. Based on the morphological structure observed using a Binocular Microscope and Scanning Electron Microscope (SEM), *R. echinobothrida* and *R. tetragona* have difference in shape and size of the scolex, sucker, rostellarum, neck and body segments.

**References**


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