



Original Research



Prevalence of *Hymenolepis nana* in wild rats in Surabaya City, East Java

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ABSTRACT

Hymenolepiasis is one of the *neglected zoonotic* diseases in humans, caused by the cestodes *Hymenolepis nana* and *Hymenolepis diminuta*. Its definitive hosts are rodents (mostly rats). This study aims to identify the morphology both microscopically and macroscopically. This research is a laboratory exploratory research and the sampling method uses purposive sampling technique. The samples used in this study amounted to 100 samples of wild rats that had been trapped and then necropsied, worms obtained from the digestive tract were stained with dark red stain and observed with a microscope. The results showed that microscopic morphological examination showed that the anterior part of the *H. nana* worm had hooks and crowns. The overall prevalence of *H. nana* worms was found to be 18% (18/100). The prevalence of *H. nana* in wild rats in Surabaya City in *Rattus tanezumi* was 16% (12/77). *Rattus novergicus* in Surabaya area with positive samples was 26% (6/23). Morphology in *H. nana* worms has a distinctive difference. The anterior part of *H. nana* has hooks that surround the rostellum, the anterior part has a 'crown of hooks'.

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INTRODUCTION

Rats are animals that are easily infected with dangerous diseases because they like dirty environments, almost all organs of the rat body have been infected by dangerous diseases spread by rats, namely Hymenolepiasis, Strobilocerkosis, and Meningocephalitis (Habsari and Mulyowati, 2020). Hymenolepiasis is one of the *neglected zoonotic* diseases in humans, caused by the cestodes *Hymenolepis nana* (*H. nana*) and *Hymenolepis diminuta* (*H. diminuta*). The definitive hosts are mainly rodents (mainly rats) (Blecharz-klin *et al.*, 2022). Transmission of helminthiasis can be direct and indirect. Direct transmission is caused by consuming water or food contaminated with worm eggs, while indirect transmission occurs through vectors, one of which is beetles (Setyaningrum, 2016).

The life cycle of *H. nana* does not require an intermediate host under conditions of autoinfection is thought to be a major factor favoring higher infection rates in populations when infected by the worm. Whereas, in *H. diminuta*, rodents and humans are the definitive hosts. *H. diminuta* has intermediate hosts, namely rat lice and beetles (Widiastuti *et al.*, 2016). Generally, human cases of hymenolepiasis are mostly asymptomatic, however, humans infected with this parasite sometimes experience mild clinical symptoms, such as diarrhea, abdominal pain, anorexia, and gastrointestinal disorders. The most serious cases can cause severe illness, especially life-threatening conditions in immunosuppressed HIV patients (Yang *et al.*, 2017).

Based on the life cycle of *H. nana* and *H. diminuta*, the infective phase will leave the host's body through feces into the environment so that



contamination of food or water by feces will increase the chances of transmission of these two zoonotic cestodes (Widiastuti *et al.*, 2016).

The prevalence rate of hymenolepiasis cases that occurred in Indonesia in 2010 reported by Priyanto *et al.* (2014) stated that in 2010, thirty primary school students in Banjarnegara sub-district in Central Java Province were screened for helminth infection, the report revealed that five students (17%) were infected, one of which was infected by *H. nana*. In addition to cases occurring domestically, the incidence of hymenolepiasis also occurs abroad reported by Paramasvaran *et al.* (2009) that *H. diminuta* and *H. nana* infections in 0.7% of oil palm plantation workers were positive for hymenolepiasis, in addition to the same hymenolepiasis infection also occurred in Malay men from the fishing community in Teluk Bahang, Penang. The prevalence of this cestode infection is high in areas with poor hygiene and poor environment. These infections are usually more common in densely populated areas or in areas with large populations.

Based on the explanation above, researchers conducted a study to confirm the species characteristics of these two types of cestodes, macroscopically and microscopically. In addition to microscopic examination, the researchers also wanted to know the prevalence of *H. nana* infection in wild rats in Surabaya. Data from this study is expected to be used to determine the prevalence of wild rats infected with *H. nana* in Surabaya and the morphological characteristics of *H. nana* in wild rats in Surabaya.

MATERIALS AND METHODS

Sampling

Hymenolepis nana worm samples were collected from wild rats trapped in five areas of Surabaya (North, West, South, East, and Central Surabaya), with each sampling area including garbage dumps, markets, densely populated and sparsely populated residential areas, and industrial areas. This study was an exploratory laboratory study using purposive sampling techniques. The number of samples used in this study was 100 rats. Two species of wild rats were used in this study: *Rattus tanezumi* and *Rattus novergicus*. The wild rat samples consisted of 77 *Rattus tanezumi* and 23 *Rattus novergicus*. The study was conducted from July to December 2023.

Mice were captured using *life-traps* made of wire with a size of 21 cm x 12 cm x 10 cm. The 15 *life-traps* used were purposively distributed in 5 points such as landfills, markets, dense and sparsely populated settlements, and factory areas, located in 5 parts of Surabaya, namely West, East, North, Central, and South Surabaya. Captured rats were immediately taken to the Laboratory of Veterinary Parasitology Division, Faculty of Veterinary Medicine, Universitas Airlangga, East Java.

Microscopic examination of *Hymenolepis* sp.

The mice that had been obtained were anaesthetised with ketamine 50 mg/kg body weight in mice, then the unconscious mice were placed on the necropsy table in a dorsal lying position and the four legs were fixed with needles. The rats were then cleaned with alcohol and necropsied to take the gastrointestinal organs. Dissection was performed according to procedures based on the book Protocols for field and laboratory rodent studies by Auffray *et al.* (2011). Adult worms found were placed in Petri dishes and given physiological NaCl observed with a microscope. Examination of *H. nana* worms was observed directly with a microscope, and identified based on their morphology based on the book Veterinary Parasitology by Taylor *et al.* (2007). This study was approved by the ethics committee of the Faculty of Veterinary Medicine, Universitas Airlangga with ethics number 2.KEH.080.07.2022.

Carmine colouring

Hymenolepis sp. worms that were obtained intact continued to stain the worms using the *Semichen-Acetic Carmine* method referring to Khulmann (2006), namely the worms were fixed between two *glass objects*, both ends of the *glass* were tied with thread (*raffia*), then the worms were kept in 5% glycerin alcohol for 24 hours. The staining results were observed with a microscope to see the morphological characters.

Calculation of prevalence value

Positive results obtained from mice infected with *H. nana* were calculated for the prevalence rate in the Surabaya area, calculated using the formula below:

$$P = \frac{\text{Total of positive samples}}{\text{Total of samples taken}} \times 100$$

Description:

P = Prevalence (%)

N = Number of mice infected with parasites (mice)

n = Number of samples observed (mice).

RESULTS AND DISCUSSION

The results of helminth examination of *H. nana* in the gastrointestinal tract of wild rats in Surabaya City using carmine staining aims to determine the morphology of *H. nana*. The results of observations using a microscope with a magnification of 100x showed the anterior part equipped with a sucker, rostellum, hook, crown hook, and scolex.

The results of microscopic examination in this study found that the anterior part of *H. nana* has a characteristic feature, namely that the rostellum has a pointed and large hook. The observation results are in line with research conducted by Mahmoud *et al.* (2011) which explains that *H. nana* in its anterior part has a scolex that looks like a rectangle and is equipped with a larger hooked rostellum. The rostellum is located in the centre of the scolex and is equipped with a pointed hook and faces backwards.

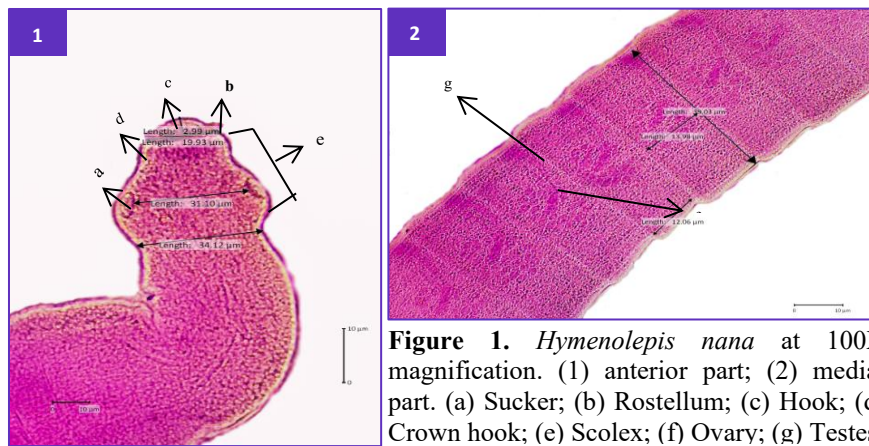


Figure 1. *Hymenolepis nana* at 100X magnification. (1) anterior part; (2) medial part. (a) Sucker; (b) Rostellum; (c) Hook; (d) Crown hook; (e) Scolex; (f) Ovary; (g) Testes

Hymenolepis nana on the medial part has proglottids scattered medially. *H. nana* is hermaphroditic, because in each proglottid there are male and female reproductive organs. This is in line with the research of Qawiem *et al.* (2022) which explained that adult *H. nana* worms have proglottids that have male and female reproductive organs, so *H. nana* is hermaphroditic. *H. nana* adult proglottids have a wider size than immature proglottids with visible testes, and gravid proglottids contain eggs.

Morphology in *H. nana* and *H. diminuta* worms have a distinctive difference between the two. This is known by the anterior part of *H. nana* has a hook that surrounds the rostellum, while *H. diminuta* does not have a hook on the rostellum. This is in accordance with the research conducted by Younis *et al.* (2021) in their research showed a comparison of the characteristics between the two morphologically using a light microscope. In the worm *H. nana* the anterior part has a 'crown hook', namely the crown on the hook there is a scolex.

The overall prevalence of *H. nana* was found to be 18% (18/100). This result was obtained from the prevalence in *R. tanezumi* of 16% (12/77). Prevalence in *R. norvegicus* was 26% (6/23). The most positive total samples in *R. tanezumi* were in the Central Surabaya area obtained (4/21) at 19%, while in *R. norvegicus* the most positive total samples in the East Surabaya area obtained a total positive sample (3/13) at 23%. *R. norvegicus* in North Surabaya was not found positive for *H. nana*.

Based on the prevalence results in Table 1. It is known that the prevalence of *H. nana* is more positive for infection in *R. norvegicus* because its life is dirtier and worse than *R. tanezumi* and *R. norvegicus* is everywhere, both sewers and garbage and basically rats live always side by side with humans. This is in accordance with research conducted by Fitte *et al.* (2017) which states that the prevalence of *H. nana* in *R. norvegicus* is higher than *R. tanezumi*. This is due to poor environmental and sanitation conditions and in the area there is close contact between rats and humans who are definitive hosts of *H. nana*.

Hymenolepis nana occurs mostly in areas classified as densely populated, this is known from the number of positive *R. tanezumi* samples that are

most infected in the Central Surabaya area than other areas in Surabaya. This is similar to the explanation of research conducted by Goudarzi *et al.* (2021) which states that *Rattus* sp. animals are the most widespread and adapt easily to humans, especially in urban areas and have an important role in the transmission of diseases transmitted by rats to humans. Research by Goudarzi *et al.* (2021) showed that the results of the study were 19.6% *Rattus* spp. infected with *H. nana* than *Mus musculus* which was 16.1%. This is because from the biological aspects of the two species, the enormous reproductive potential, synanthropic behaviour, and adaptation to urban environments make them potential reservoirs of *H. nana* and other rodent-borne pathogens that are harmful to humans.

Table 1. Prevalence of *H. nana* in Wild Rats in Surabaya

Species	Region	Total samples	Prevalence (%)
			<i>H. nana</i>
<i>R. tanezumi</i>	West Surabaya	12	3 (25%)
	East Surabaya	17	2 (11.8%)
	Central Surabaya	21	4 (19%)
	North Surabaya	14	2 (14.3%)
	South Surabaya	13	1 (7.7%)
	Total	77	12 (16%)
<i>R. norvegicus</i>	West Surabaya	2	1 (50%)
	East Surabaya	13	3 (23%)
	Central Surabaya	3	1 (33%)
	North Surabaya	2	0%
	South Surabaya	3	1 (33%)
	Total	23	6 (26%)

CONCLUSION

The results of observations using a microscope with a magnification of 100x n the worm *H. nana* showed the anterior part equipped with a sucker, rostellum, hook, crown hook, and scolex. the anterior part has a 'crown hook', namely the crown on the hook there is a scolex. Our findings show that wild rats in Surabaya city are mostly infected with *H. nana*, which has the potential to infect humans. Therefore, prevention and control is necessary, especially in good environmental and sanitation conditions. For further research, morphological

identification using SEM and other methods is needed to determine the detailed characteristics of *H. nana*.

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AUTHORS' CONTRIBUTIONS

LTS, M, and HP concept and design the proposal, coordinating research, and revised the manuscript. ES, PH and K contributed as a author and approved the final manuscript. DPR and MHA collected data, analyzed the data, and the literature review.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICAL APPROVAL

The experimental protocol was approved by the Animal Care and Use Committee of Faculty Veterinary Medicine, Universitas Airlangga No. 2.KEH.080.07.2022.

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