



Detection of Soil Transmitted Helminths (STH) Eggs Using Nail Test and Stool Test Among Scavengers at Jatibarang Landfill Semarang

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ABSTRACT

Helminthiasis or worm infection is caused by worm parasites belonging to Soil Transmitted Helminths (STH) group. Helminthiasis in human often occurs due to environmental factors and personal hygiene issue. The gold standard diagnosis of helminthiasis is done by identifying the worm eggs in the feces or stool. Based on its transmission, helminthiasis examination can be done by observing the nails. This study analyzed the prevalence of helminthiasis in nails and stool and examined the differences in the yield of helminth eggs between nail and stool. A simple random sampling technique was conducted to select 50 scavengers at Jatibarang Landfill, Semarang. Univariate analysis was used as data analysis tool to determine the prevalence of worm infections, while bivariate analysis in the form of Mann Whitney differential test was employed to determine the gap in the number of Soil Transmitted Helminth (STH) worm eggs in nail and feces samples. *Ascaris lumbricoides* worm eggs appear dominated the infections, with infection rates through nail of 2% and feces of 12%. $p = 0.049$ (<0.05) was obtained in the Mann Whitney test, indicating the presence of significant difference and implying that nail test cannot replace stool test as the gold standard of helminthiasis diagnosis.

Keywords : helminthiasis, stool test, nail test, Soil Transmitted Helminths (STH)

INTRODUCTION

Helminthiasis refers to an infectious diseases caused by worm parasites called Soil Transmitted Helminth (STH) (Natadisastra, 2009). *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* are helminths that are mostly found in Indonesia (Natadisastra, 2009). These helminths cycle of life requires soil with certain conditions to reach the infective stage (Safar, 2021). The transmission of Soil Transmitted Helminths (STH) is cosmopolitan (worldwide). WHO (2016) reported more than 1.5 billion people or 24% of the world's population are infected with *Soil Transmitted Helminths* (STH) throughout the world. Infection is widespread in tropical and subtropical regions, where

highest prevalence was found in sub-Saharan Africa, Americas, China and East Asia. In 2010, an estimated 819 million people were infected with *Ascaris lumbricoides* worm, 464.6 million with *Trichuris trichiura* and 438.9 million with hookworm around the world (Ramadhini, 2015). Within 2-4 weeks, the fertilized worm eggs become infective and can grow well in moist soil with optimal temperature and oxygen. These eggs are easily damaged under sunlight exposure. As a tropical country with high humidity, Indonesia is prone to the transmission of *Soil Transmitted Helminths* (STH) (Seja, 2015). The Regulation of the Minister of Health of the Republic of Indonesia Number 15 of 2017 concerning Prevention of Worms mentions that helminthiasis is an infectious disease that remains a public health problem in Indonesia because it spreads all over the nation, resulting in health, nutrition, intelligence and productivity issues.

The prevalence of helminthiasis in Indonesia is still high. The results of a survey done in 2013 showed that helminthiasis prevalence in Indonesia reached 85.9% with an average national rate of 28.12%. The most-frequently found parasites identified in helminthiasis were *Ascaris lumbricoides* 60%, *Trichuris trichiura* 16%, hookworms 7% and other worm species up to 17%. Helminthiasis in Semarang City is commonly found in the community, especially in school-aged children (5-14 years) which reached 38% in 2010 and decreased to 30% in 2011 (Ikawati, 2016).

Helminthiasis prevalence varies in different areas due to several factors, including dirty and slum areas such as landfills (Azmy, 2017). Waste is defined by Azmy (2017) as the by-product of various activities in human life and natural processes which often causes problems. Improper waste management will cause environmental health problem for waste emits unpleasant odor which helps the disease to spread (Dini, 2017). Semarang City has the largest and only waste management facility namely Jatibarang landfill. Domestic waste were transported from several sub-districts to be dumped there. Meanwhile, scavengers at Jatibarang Landfill are in direct contact with waste but they rarely wear proper personal protective equipment. They do their jobs from morning to evening, and they also have break time for meal, drink and toilet done in the landfill area. This situation poses the risk of disease transmissions, where piles of wet organic waste accelerate the process of waste decomposition into humus. Waste decomposition makes the soil hollow and wet which can encourage the growth of worms in the soil. Ikawati (2016) reported the prevalence of STH helminthiasis in Jatibarang landfill among waste scavengers reached 47.5%. Azmy (2017) also found that 38 (60.53%) of workers at the Jatibarang landfill are positive for helminthiasis.

Onggowaluyo in Ramadhini (2015) mentioned poor personal hygiene as one of risk factors that allow STH worms to penetrate the human body when humans are unable to keep their hands, nails and feet clean. Fingernails are a place for worm eggs to stick and can get into the human body while eating before hand washing. Nail hygiene as a risk factor in

helminthiasis has reached 89.5% because worm eggs often stick to dirty nails (Anggraini et al., 2020).

Diagnostic technique is an important aspect in diagnosing as shown by parasitic stage of the worm. Patients infected with worms often show no symptoms or only experiencing mild symptoms. Laboratory tests assists and supports the helminthiasis diagnosis since analysis of clinical symptoms is not thorough (Setya, 2015).

Helminthiasis can be diagnosed by finding and identifying worm eggs or adult worms in stool test. Stool test remains the gold standard examination for helminthiasis. Stool corresponds to the life cycle of *Soil Transmitted Helminths* (STH) worms that live, develop and lay eggs in the intestine. Helminthiasis diagnosis can be performed by observing the presence of worm eggs or adult worms in the feces. Stool test is often conducted using macroscopic and microscopic methods. Macroscopic examination observers the color, blood, mucus, consistency, odor, PH and food residue, while microscopic examination identifies the parasites such as protozoa, worm larvae and eggs (Setya, 2014). Regarding other mode of transmission of the disease, fingernail test can be performed to diagnose helminthiasis earlier. Fingernail examination can be used as an additional examination or initial examination in diagnosing helminthiasis. Dirty nails can be the place for worm eggs to stick which can be swallowed into the mouth and enter the digestive tract when eating (Renyaan, 2020). Fingernail and stool examinations are similar, except for the different materials to be examined. In several studies, worm eggs were found in nail excrement, including Renyaan's research (2020) whose study involved the farmers in Kaliwungu sub-district, Jombang. The study showed helminthiasis prevalence of 40%. Wintoko's research (2014) done in an elementary school in Bandar Lampung showed a helminthiasis prevalence of 88.2%. Futhermore, Dini (2017) who involved on scavengers at Sukawinatan Palembang Landfill found the incidence of STH egg contamination of 10.57%.

Helminthiasis still occurs frequently, especially in places with poor sanitation. Previous studies done at the Jatibarang Landfill Semarang identified helminthic infections through fingernail test and stool test. The use of these two examination materials has never been done before. Therefore, it is necessary to identify the *Soil Transmitted Helminths* (STH) in fecal and nail samples to be compared.

This study was performed to examine the incidence of helminthiasis by examining nails and feces of scavengers at TPA Jatibarang, Semarang. In addition, this study also determined the differences in the results of examination of *Soil Transmitted Helminths* (STH) in worm eggs between materials for examination of nails and feces in scavengers at TPA Jatibarang, Semarang.

METHOD

This study has passed the ethical permit as shown by the validation ethics numbered : 4080/B.1/KEPK-FKUMS/1/2022. Samples were 50 randomly selected scavengers at Jatibarang Landfill based on inclusion and exclusion criteria. Inclusion criteria were factors related to the objective of this study that was to examine Soil Transmitted Helminths eggs on nail and feces materials. Whereas, exclusion criteria were factors that could technically affect the results of the study.

Inclusion criteria included respondents who had completed informed consent and respondents who were willing to provide stool samples and nail clippings. Informed consent contains information about clean living habits and habits of keeping nails clean. Exclusion criteria in this study were scavengers who had been dewormed within 6 months.

Primary data were obtained from informed consent forms, feces and fingernail samples that were then examined in the laboratory. Some materials and tools were used, including nail clippers, plastic pots, centrifuge, microscope, dropper pipette, test tube, measuring cup, object glass, cover glass, stool pot and stir bar as well as 10% KOH, 10% Formalin, 2% Eosin and distilled water.

The stool and nail tests used a simple concentration method. The principle of operation of a simple concentration method is that there is a relatively heavy difference in the density of worm eggs that causes the eggs to settle after the centrifugation process. The operation of a simple concentration method is described as follows :

1. Nail Test Mechanism

Scavengers' nail clippings were cut using sterilized nail clippers to be placed into a sterile plastic pot and added with 10 ml of 10% KOH to be set still for 24 hours. The nail clippings were then transferred to a test tube and centrifuged at 2500 rpm for five minutes. The supernatant liquid was discarded and the sediment was taken using a pipette, then placed on a glass object and covered with a cover glass. The preparations were examined under a microscope with 40X objective lens magnification.

2. Stool Test Mechanism

The feces that had been collected in the stool pot were put into a 5 ml test tube and added with distilled water. The fecal solution was then stirred until homogeneous and then centrifuged at 3000 rpm for one minute. The supernatant liquid was discarded and the sediment was taken out using a pipette. The sediment was placed on the slide and added with one drop of 2% eosin to be covered with a coverslip. The preparations were examined under a microscope in 40X objective lens magnification.

Univariate analysis and bivariate analysis were employed in data analysis. Univariate analysis was performed to examine the prevalence of helminthiasis based on the transmission and percentage of worm infections using the following formula as proposed by Renyaan (2020).

$$X = \frac{f}{n} \times 100\%$$

Remarks :

X = Percentage

f = Frequency

n = Number of respondents

Bivariate analysis was carried out to determine differences in the results of Soil Transmitted Helminth (STH) worm eggs in nail and fecal samples in the forms of Kolmogrov Smirnov statistical normality test and the differential test. In the Kolmogrov Smirnov normality test, data are regarded normally distributed if the significance value (p) > 0.05 and abnormally distributed if the significance value (p) ≤ 0.05 . After that, a differential test was carried out according to the results of the distribution test. T-test is conducted when the data are normally distributed, while Mann Whitney test is carried out if the data are not normally distributed. The criteria for this differential test are:

1. Significant value (p) smaller than 0.05 ($p < 0.05$) :

There is a difference in the number of worm eggs found in fingernails and stool

2. Significant value (p) greater than 0.05 ($p > 0.05$) :

No difference is found the number of worm eggs found in fingernails and stool

RESULT AND DISCUSSION

Subjects of this study were 50 scavengers at Jatibarang Landfill who met the inclusion criteria and have agreed to participate in this study by filling out informed consent. The results of examination of Soil Transmitted Helminths eggs in nail samples found that 2% of 50 samples showed the presence of *Ascaris lumbricoides* eggs, while 98% or 49 samples did not show Soil Transmitted Helminths eggs (see Figure 1).

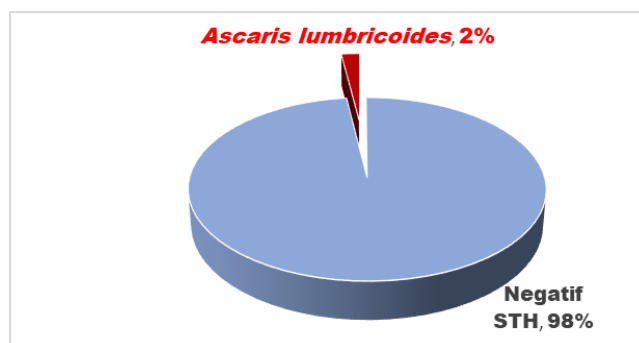


Figure 1. The Soil Transmitted Helminth eggs found in nails

The microscopic observation showed the presence of *Ascaris lumbricoides* worm eggs in the nail specimen with a magnification of 40x. They are somewhat elongated in shape and have three layers of walls as illustrated in Figure 2.

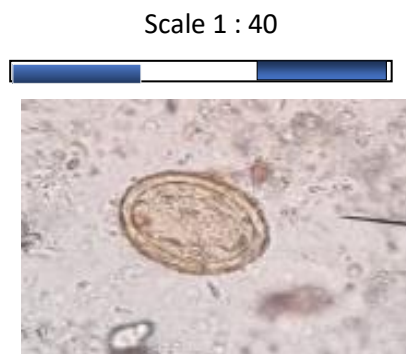


Figure 2. *Ascaris lumbricoides* eggs in nail specimen at 40x magnification.

The results of Soil Transmitted Helminths eggs from 50 fecal samples showed that 12% positive for Soil Transmitted Helminths eggs, 10% of which were identified as *Ascaris lumbricoides* species and 2% identified as *Trichuris trichiura* species. Meanwhile, 44 samples (88%) were negative for Soil Transmitted Helminths eggs. The results are shown in Figure 3.

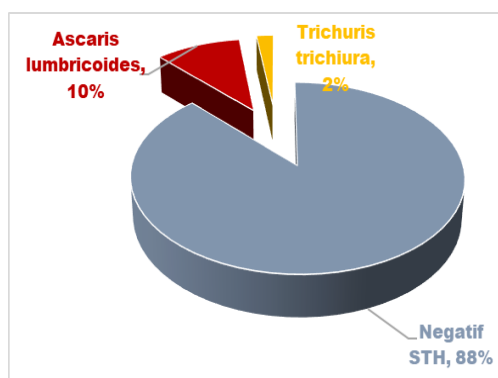


Figure 3. The *Soil Transmitted Helminth* eggs in fecal specimen

Ascaris lumbricoides and *Trichuris trichiura* worm eggs were found in the fecal specimen with a magnification of 40x in microscope. The *Ascaris lumbricoides* eggs showed elongated shape with three layers of walls, while the *Trichuris trichiura* eggs have a distinctive shape resembling a barrel with opercula at both poles as shown in Figures 4a and 4b.

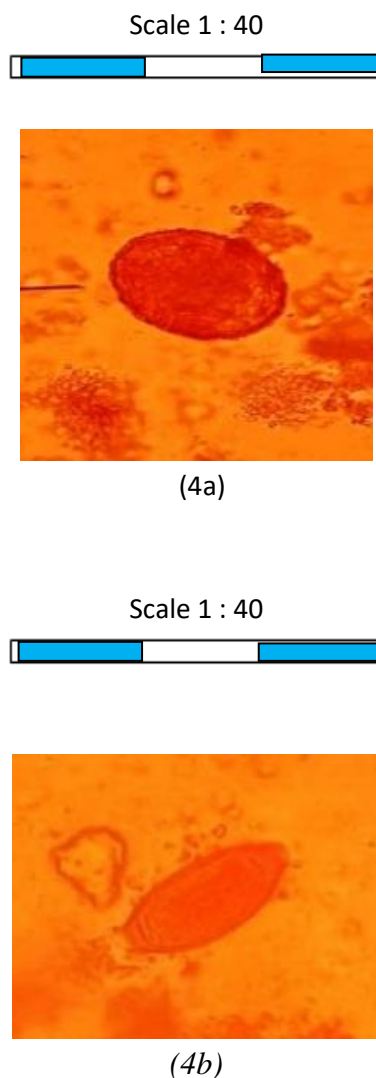


Figure 4 (a) *Ascaris lumbricoides* eggs found in the fecal specimen with 40x magnification and eosin dye.

(b) *Trichuris trichiura* eggs found in the fecal specimen with 40x magnification and eosin dye.

Ascaris lumbricoides had the highest percentage of eggs in both nail and fecal samples, likely due to their high egg production compared to other worm species. Female *Ascaris lumbricoides* can lay 100,000 to 200,000 eggs per day, while female *Trichuris trichiura* laid 3,000 to 4,000 eggs, 10,000 eggs laid by female *Ancylostoma duodenale*, and 9,000 eggs laid by female *Necator americanus* (Natadisastra, 2009; Rosdiana, 2021; Adrianti, 2020). The eggs of *Ascaris lumbricoides* are protected by a thick hyaline layer and a rough, lumpy albuminoid layer (Rahmadhini, 2015). Ikawati in 2016 showed that the incidence of helminthiasis reached 47.5%, with *Ascaris lumbricoides* eggs dominating by 52.6%.

Scavengers at Jatibarang Landfill were identified to be infected by both *Ascaris lumbricoides* and *Trichuris trichiura* because these eggs grow well in soil with a temperature of 30°C, high humidity, and poor sanitation in tropical and subtropical areas. Hookworm eggs from the species *Necator americanus* and *Ancylostoma duodenale* were not found in the scavengers' samples at the Jatibarang landfill as scavengers wore boots while working. The infective stage of hookworms for humans is the filariform larvae, which can penetrate the skin. Therefore, wearing foot gear is an important preventive measure (Adrianto, 2020).

Triyanti (2016) reported that the level of helminthic infection was not related to age or gender, and that anyone could develop helminthiasis depending on their lifestyle and risk factors for worm infection. Table 1 shows that the highest rates of worm infection were found in male respondents between the ages of 44 and 65.

Table 1. Helminthiasis prevalence based on sex and age

	Nail		Feces	
	Positive	Negative	Positive	Negative
Sex				
Male	1 (2%)	30(60%)	5(10%)	26(52%)
Female	0	19(38)	1(2%)	18(36%)
Age				
24-43	1(2%)	24(48%)	2(4%)	23(46%)
44-65	0	25(50%)	4(8%)	21(42%)

Environmental and personal hygiene seem to be the factors affecting the helminthiasis among scavengers in Jatibarang Landfill as shown in Table 2.

Table 2 Helminthiasis prevalence based on personal hygiene

	Nail		Feces	
	Positive	Negative	Positive	Negative
Wearing foot gear :				
Yes	1(2%)	49(98%)	6(12%)	44(88%)
No	0	0	0	0
Wearing gloves :				
Yes	0	18(36%)	0	18(36%)
No	1(2%)	31(62%)	6(12%)	26(52%)
Hand washing using soap :				
Yes	1(2%)	46(92%)	6(12%)	41(82%)
No	0	3(6%)	0	3(6%)
Handwashing before meal :				
Yes	1(2%)	49(98%)	6(12%)	44(88%)
No	0	0	0	0
Handwashing after defecating :				
Yes	1(2%)	49(98%)	6(12%)	44(88%)
No	0	0	0	0

Defecating in toilet :				
Yes	1(2%)	49(98%)	6(12%)	44(88%)
No	0	0	0	0
Nail trimming when nails are long :				
Yes	1(2%)	49(98%)	6(12%)	44(88%)
No	0	0	0	0
Nail trimming every 2 weeks :				
Yes	1(2%)	32(64%)	1(2%)	32(64%)
No	0	17(34%)	5(10%)	12(24%)

Table 2 shows lower helminthiasis rates among respondents who frequently wore shoes, did not wear gloves, washed their hands with soap, washed their hands before eating, washed their hands after defecating, defecated in a toilet, and cut their nails regularly, with only one person (2%) testing positive in nail samples and six people (12%) in fecal samples. However, five individuals (10%) who did not cut their nails regularly every two weeks tested positive for STH worm eggs. Inadequate hand hygiene practices, ingestion of contaminated food and drink, and not doing nail trimming once a week were found as the factors that cause accumulation of bacterial microorganisms and worm eggs.

The prevalence of helminthiasis in scavengers at TPA Jatibarang Semarang is regarded low. The Regulation of the Minister of Health of the Republic of Indonesia number 15 of 2017 mentions that the prevalence of helminthiasis can be classified as follows: prevalence $\geq 50\%$ is categorized high, prevalence $\geq 20\% - < 50\%$ is categorized moderate and prevalence $< 20\%$ is categorized low.

The results of nail and stool tests for Soil Transmitted Helminths (STH) eggs showed that 12% of the fecal samples were positive for STH eggs, while only 2% of the nail samples tested positive. This gap can be influenced by the number of STH eggs present in the soil at the study site, where greater number of eggs in the soil can increase the intensity of the search for worm eggs (Ramadhini, 2015). In addition, STH worms that grow and lay eggs in the intestine makes stool test the gold standard for helminthiasis diagnosis. Figure 5 shows the results of STH worm eggs examinations in nail and fecal specimen.



Figure 5. The outcome of STH eggs observation in nail and fecal specimen.

The normality test resulted in $p = 0.000$, indicating that the data were not normally distributed. Therefore, the Mann Whitney U test was employed, which results showed a significant difference ($p = 0.049$, $p < 0.05$) between the Soil Transmitted Helminths eggs examination in nail and fecal specimen as shown in Table 3.

Table 3 Statistical tests on the results of STH examination in nail test and stool test

	Normality test	Mann Whitney Test
Nail	P=0.000	P= 0.049
Feces	P=0.000	

A significant difference was found in the Soil Transmitted Helminths eggs examination between the nail test and stool test. Similarly, Kurniawan (2018) in a study entitled "Helminthiasis Diagnostic Test using Stool Test and Nail Test among Students of SDN 1 Krawangsari, Natar Lampung Selatan District" found that the results of the two tests are statistically different.

CONCLUSION

Based on the results of research that has been done *about Soil Transmitted Helminths* (STH) eggs examination using nail test and stool test among scavengers at Jatibarang Landfill Semarang, the following conclusions can be drawn:

1. The results of this study show that *Ascaris lumbricoides* species dominated the helminthiasis found among scavengers in Jatibarang Landfill. This study also found that the helminthiasis diagnosis in nail samples from *Ascaris lumbricoides* species infection was 2% and 12% in the fecal specimen with 10% *Ascaris lumbricoides* worm species and 2% *Trichuris trichiura*.
2. Helminthiasis is more common in male respondents by 10%, and those of 44-65 year old are more prone to it. Worm eggs were also found in 12% of respondents who often wore footwear, not wearing gloves, washing their hands after defecating, defecating in the toilet, and regularly cutting their nails. Whereas those who did not trim their nails every two weeks are more vulnerable to helminthiasis by 10%.
3. A significant difference was found in the examination of Soil Transmitted Helminths eggs between nail test and stool test.

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