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Contamination of helminth egg in raw vegetables from traditional markets in Gresik, East Java, Indonesia

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Abstract

Soil-transmitted helminth (STH) infections pose a serious threat to public health since they are spread through environmental fecal contamination. One of the main ways that intestinal parasites are acquired is through the consumption of raw vegetables without adequate washing. Thus, this study aims to examine helminth contamination of raw vegetables which are brought from traditional markets in Gresik Regency, East Java, Indonesia. Analytical observational study with cross sectional design were conducted for two months. A total of 125 raw vegetables including carrot, cabbage, lettuce, potato, mustard, leek, spinach were collected from traditional market in Gresik, East Java, Indonesia, between September and October 2023. The prevalence of STH eggs were calculated by comparing the number of positive sample and the number of vegetables which examined. The prevalence of STH eggs in raw vegetables collected from Gresik Regency are 17.6%. Spinach was the most frequently vegetables which contaminated by helmint egg (31.8%). Ascaris egg was the most frequently helmint egg which contaminated raw vegetables which collected from traditional markets in Gresik, East Java, Indonesia.

Keywords: Parasitic contamination; Helmint; Vegetables; Tropical disease

1. Introduction

Fruit and vegetables were included as dietary recommendations due to the its high level of vitamin, minerals, fiber, and antioxidants. Daily intake both of raw and cooked vegetables is an important diet pattern worldwide, due to the micro-nutrients composition in each vegetables (Jolly, 2013). Fruits and vegetables are high in vitamins, fiber, and minerals and have been shown to lower the risk of chronic disease and several forms of cancer (Sharma, 2020). Due to the rich source of valuable elements including vitamins, minerals, fiber, and antioxidant properties, vegetables are considered as crucial parts of the human diet. Eating raw and cooked veggies on a daily basis promotes human growth and development and helps prevent a variety of disease (Aysha, 2017)

Vegetables that have been heated, like many other foods, progressively lose components including vitamins and minerals that are good for human health. The quantity and length of heat exposure determine this loss. As a result, eating raw veggies has gained popularity as a way to live a healthy lifestyle. Because there are several harmful bacteria that can taint vegetables and endanger public health, it is important to consider the sources of water and fertilizer, as well as the movement of people and animals in places where vegetables are cultivated (Erez, 2022).

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Consuming vegetables without washing them properly is thought to be a major way that parasitic diseases can spread to people. It has been reported that eating contaminated vegetables may be a contributing factor to intestinal parasite outbreaks in developed or developing nations (Slifko, 2000). Vegetables that were not thoroughly cleaned could be a source of helminth transmission. This is because farmers use natural fertilizers and sewage water to irrigate their plants (Megrin, 2010). The likelihood of a contamination source and the survival of the parasite stages that transmit disease to humans are both increased by the increased global demand for food and the quick transportation of food, particularly soft fruit and vegetables (Slifko, 2000). Foodborne parasite outbreaks are predicted to have a significant effect on public health, especially in light of the world's growing meat consumption and the realization that billions of people lack access to clean water (Boireau, 2015).

Inadequate water supply infrastructure, poor sanitation, illiteracy, insufficient health services, and overcrowding have all been associated with the frequency of STHs (Olusola, 2013). According to Imalele et al., a significant amount of human excrement is dumped into the ground in Calabar, Nigeria, polluting the soil with STH ova and larvae (Imalele et al., 2021). Since the majority of STH infections are contracted by the fecal-oral route, infection can also be acquired from secondary sources such as food, water, vegetables, and fruits (Abe et al., 2019).

Helminthiasis can be mild in both tropical dan subtropical countries, going undiagnosed in some cases. As a result, the true cost and impact of helminthiasis are greatly underestimated. If prompt and effective treatment is not received, both asymptomatic and symptomatic cases develop chronic helminthiasis (Degarege, 2022). The elaboration of helminth egg in raw vegtables have been previously published, and showing various prevalence of heminth egg. Hookworm egg were commonly found in raw vegetables (lettuce parsley, carrots, green onion, spinach, and so on) that was collected from Afyonkarahisar, Turkey (Erez, 2022). The epidemiology of hookworm infection was reported in soil that used human feces as fertilizer (Centers for Disease Control, 2023). The occurrence of helminth ova in fertilizer can pose a threat to human health, specifically the possibility of STHs spreading.

A cross-sectional study was conducted on fertilizer in the market, and the results showed the positivity of helminth ova, which were predominated by *Trichuris* and *Ascaris* eggs (Figura et al., 2022). Not only the helminth ova, which were detected in fertilizer, but also the helminth larvae. *Toxocara* sp. Also present in commercial fertilizer (Figura et al., 2022). Most commercially fertilized crops show the presence of *Trichuris* eggs, which can pose a significant health threat (Else et al., 2020). The other report from Nigeria shows that Ascaris spp. eggs were found in 300 samples of lettuce, 250 samples of cabbage, 200 garden eggs, 150 carrots, 130 cucumbers, and 100 green peppers that were analysed (Adamu, 2012). This study aims to examine helminth contamination of raw vegetables which are brought from traditional markets in Gresik Regency

2. Material and methods

2.1. Study Area

This study was carried out in Kebomas and Cerme districts as part of Gresik Residence, East Java, Indonesia. Gresik Regency is located in the northwest of Surabaya City, which is the capital of East Java Province, with an area of 1,191.25 km^2 that is divided into 18 districts, 330 villages, and 26 sub-districts. Geographically, Gresik Regency is located between 112° to 113° East Longitude and 7° to 8° South Latitude and is a lowland with an altitude of 2 to 12 meters above sea level, except for Panceng District, which has an altitude of 25 meters above sea level.

Gresik Regency is a coastal area that extends from the districts of Kebomas, Gresik, Manyar, Bungah, Sidayu, Ujungpangkah, and Panceng, as well as the districts of Sangkapura and Tambak, which are located on Bawean Island. Gresik Regency is bordered to the north by the Java Sea, to the east by the Madura Strait and Surabaya City, to the south by Sidoarjo Regency and Mojokerto Regency, and to the west by Lamongan Regency. The geographical area for sampling site were shown in Figure 1.

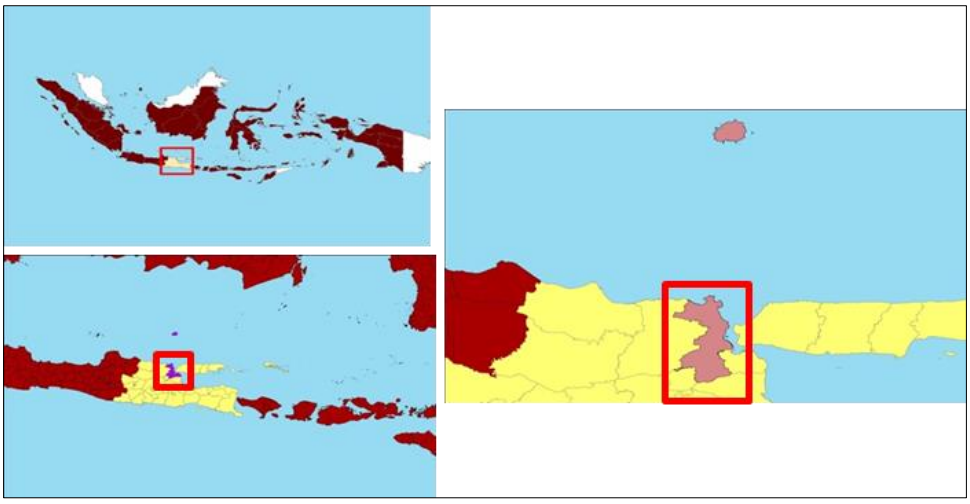

Figure 1 The geographical mapping of sampling site (Gresik Regency)

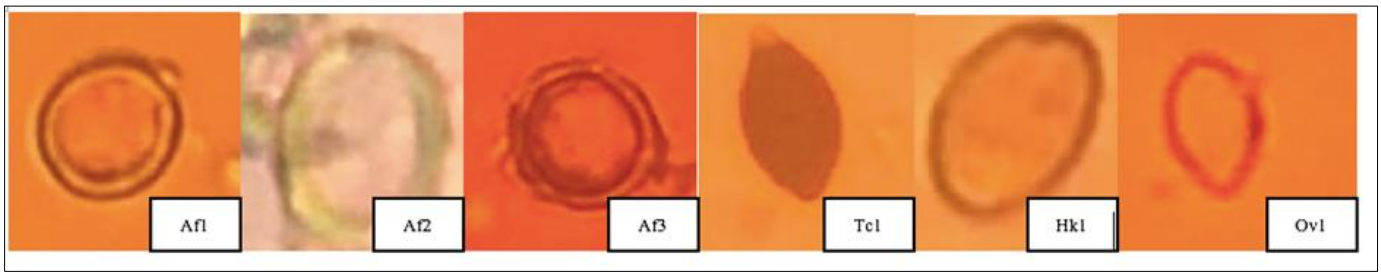

Figure 2 Representatives of helminth egg contamination in raw vegetables collected from traditional market in Gresik, East Java, Indonesia. Af1, Af2, Af3: Ascaris lumbricoides fertile egg, Tc1: Trichuris trichiura egg, Hk1: Hookworm egg, Ov1: Oxyuris vermicularis egg

2.2. Study Design

Analytical observational study with cross sectional design were conducted for two months. A total of 125 raw vegetables including carrot, cabbage, lettuce, potato, mustard, leek, spinach were collected from traditional market in Gresik, East Java, Indonesia, between September and October 2023. Sampling site were choosen based on the number of citizen in those districts. Cerme and Menganti were categorized as the crowded districts in Gresik Regency, East Java, Indonesia.

2.3. Sample Collection

A total of 125 raw salad vegetables from many types including carrot, cabbage, lettuce, green onion, mustard, potato, spinach, sweet potato, white mustard were purchased from six traditional markets in Gresik Regency, East Java, Indonesia. The collected vegetables were sealed by plastic bags and labelled according to the date collection and location.

2.4. Research Procedure

All of vegetables were transported to Parasitology Laboratory for direct examination to identify the occurrence of helminth eggs. A total of 200 gram of each vegetables were mixed with NaCl as saline solution, then the solution from those mixture were dropped into object glass, followed by dropping eosin 2% as the staining of helminth egg. Microscopical examination were conducted by two trained medical analyst. The occurrence of helminth egg were captured and summarized into the Table 1. Identification of egg morphology were done by following the identification guidelines for morphological egg of soil-transmitted helminth by CDC.

2.5. Data Analyst

The prevalence of STH eggs from raw vegetables was calculated through this formula:

- Prevalence of STH eggs : Number of positive sample/total sample x 100%

Table 1 The distribution of helminth eggs in raw vegetables collected from traditional markets in Gresik, East Java, Indonesia

No.	Vegetables	Number of Samples	Hookworm spp eggs	*Ascaris lumbricoides*	*Trichuris trichiura*	*Oxyuris vermicularis*	Total
1.	Carrot	27	0	1	0	1	2 (9.09%)
2.	Cabbage	26	1	0	1	0	2 (9.09%)
3.	Lettuce	10	0	1	0	3	4 (18.18%)
4.	Green Onion	21	0	1	1	1	3 (13.6%)
5.	Mustard	15	0	0	0	0	0 (0%)
6.	Potato	7	0	3	0	0	3 (13.6%)
7.	Spinach	11	0	2	3	2	7 (31.8%)
8.	Sweet Potato	7	0	1	0	0	1 (4.5%)
9.	White Mustard	1	0	0	0	0	0 (0%)
	Total	125	1	9	5	7	

3. Results

The detail of egg morphology were shown below in Figure 2. This figure consisting of many variants of helminth egg, including *Ascaris lumbricoides* fertile, *Trichuris trichiura*, Hookworm, and *Oxyuris vermicularis*.

Spinach was found to be the most frequently contaminated sample (31.8%), followed by lettuce (18.18%), green onion and potato (13.6%), carrot and cabbage (9.09%), and sweet potatao (4.5%). There's no helmint egg contamination in mustard and white mustard (0%). Both of potato and green onion have the same percentage of helmint egg contamination (13.6%). This also found in carrot and cabbage that showing the same percentage of helmint egg contamination (9.09%). The distribution of helminth eggs in raw vegetables collected from traditional markets in Gresik, East Java, Indonesia were shown in Table 2 and Figure 3.

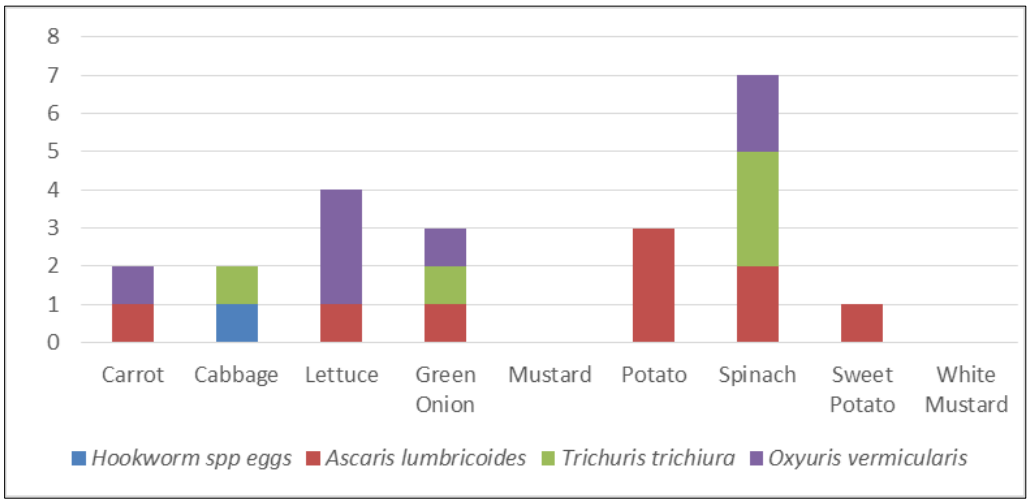

Figure 3 The distribution of helminth eggs in raw vegetables collected from traditional markets in Gresik, East Java, Indonesia

According to the number of sample and the number of positive sample, our study indicates that the prevalence of STH eggs were 22/125 x 100% : 17.6%.

4. Discussion

The epidemiology of hookworm ova was reported in some previous studies from other countries, such as Africa. One-third of pre-school children show positivity for hookworm infection, followed by Ascaris infection and Trichuris infection. Hookworm infection also shows significant outcomes in infected children in Africa, such as anemia and, in the worst case, morbidity (Brooker'* et al., 1999). Severe anemia was reportedly the specific outcome of hookworm infections. However, to differentiate the specific species of hookworm, molecular methods should be used. The two main species of hookworm that resulted in severe anemia are *Necator americanus* and *Ancylostoma duodenale (Clements & Addis Alene, 2022).* Interestingly, the main treatment for hookworm infections didn't need to know exactly which species were infected individuals (Murray et al., 2015). Another helminth species, such as *Enterobius vermicularis* and Strongyloides, was also detected in wastewater sludge. Surprisingly, it was not only helminth eggs that were detected but also protozoan cysts, such as *Entamoeba histolytica* and *Giardia* (Sabbahi et al., 2022). The presence of helminth ova in soil was caused by some environmental factors, such as temperature, humidity, and other microbes (Campos et al., 2013).

Our study shows that the most predominant of raw vegetables which contaminated by helmint egg was spinach (31.8%).Due to its distinct nutritional contents, spinach (*Spinacia oleracea* L.), a dark green leafy vegetable, is widely consumed worldwide and a member of the Chenopodiaceae family (Li et al., 2019). One of the naturally enriched vegetables that is particularly worthy is spinach, which contains a variety of phytonutrients and bioactive compounds with beneficial biological properties, such as lutein, zeaxanthin, ascorbic acid, flavonoids, and polyphenols (Manzoor et al., 2020; Salehi et al., 2019). The potential health benefits of utilizing technological advancements in formulation or preserving fresh vegetables are being explored in relation to the functional and health qualities of spinach. Additionally, spinach contains a variety of active ingredients, including flavonoids and other polyphenolic active ingredient compounds that work in concert to reduce inflammation and boost antioxidant levels (Chenping, 2016).

Some efforts to eliminate and prevent hookworm in children living in endemic areas have been studied; one of them is providing STHs drugs in public places (Taylor-Robinson et al., 2019). Indonesia is one of the endemic countries for helminthiasis, and the helminth infection was classified as a neglected tropical disease. During the COVID-19 pandemic, this disease didn't get much attention (Fauziyah et al., 2021). A study in northern Iran shows an asymptomatic individual that was infected by *Necator americanus* according to molecular examination (Sharifdini et al., n.d.).

Significant parasite loads, especially soil-borne parasites of raw vegetables and forage grown outdoors in the fields as well as on the motherland east of the Nile Delta in Egypt, were reported. This should be a concern, and the urgency of implementing soil control measures, especially in the pre-harvest period for raw vegetables and greens, is an important step in reduce the transmission of parasites from soil to humans (Yahia et al., 2023a). Parasite contamination in soil was not only reported in this study but also in other studies. An assessment of parasitic contamination from Kerala, India, shows the positivity of *Ascaris* sp. from raw vegetables that have morphological structure near the soil, such as tomatoes, potatoes, ginger, carrots, and bit root (Sunil et al., 2014). The occurrence of STH eggs in the environment is a sign of animal or human activity that involves unhygienic practices, including open defecation. STH requires soil as the medium for its development (Sunil et al., 2014).

Recent investigations of soil contamination with STH eggs have also been carried out in the Philippines. A total of 70% of the soil samples taken from rural Southern Luzon towns were positive for STH eggs. Furthermore, 31% of the soil samples from urban and rural regions of Southern Luzon were positive for STH eggs (Paller & Babia-Abion, 2019). Soil plays a crucial role in the transmission of intestinal parasites to human and animal hosts. Soil may get contaminated as a result of the detrimental habit of disposing of the feces of infected hosts either directly into the soil, which is a significant source of parasite contamination in growing vegetables, or onto human hands (Yahia et al., 2023b). One of the most prevalent illnesses affecting the poor and a major contributor to anemia and undernutrition in developing nations is human hookworm infection. Worldwide, an estimated 576 million individuals are infected, with sub-Saharan Africa having the highest rate of infections, followed by Southeast Asia, India, and the Americas (WHO, 2023).

Hookworms, which were discovered in this investigation, are zoonotic parasites that may infect humans. The possibility of using untreated manure and polluted wash water are two contemporary farming practices that both conventional and organic farms utilize. These activities represent serious risks for additional parasite transmission to people, animals, and the environment. Farm product consumers might potentially be in danger of illness. Helminthiasis infection is a serious public health threat. Individuals with helminth infections can show various signs and symptoms. People with mild STH infections often have no symptoms. Serious infections can cause a range of health problems, including abdominal pain, diarrhea, blood and protein loss, rectal prolapse, and delayed physical and cognitive development (Centers for Disease Control and Prevention, 2023).

5. Conclusion

The prevalence of STH eggs in raw vegetables collected from Gresik Regency are 17.6%. Spinach was the most frequently vegetables which contaminated by helmint egg (31.8%). *Ascaris* egg was the most frequently helmint egg which contaminated raw vegetables which collected from traditional markets in Gresik, East Java, Indonesia.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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