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# Identification of Intestinal Parasites in Spinach at Padamara Market, Padamara District

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**ABSTRACT**

**Introduction:** Vegetables are essential for maintaining human health because they are a great source of vitamins, minerals, fiber, and other nutrients. Most people consume raw vegetables to preserve the nutrients in them. However, raw vegetables have a great potential to spread intestinal parasitic infections. Raw vegetables consumed as fresh vegetables also have the potential to transmit geohelminth infection. Protozoan infections can be transmitted through food and some of these diseases.
**Objective:** This study aims to identify intestinal parasites in spinach at Padamara Market, Padamara District
**Methods:** A total sampling technique was used to collect 20 bunches of spinach at Padamara Market, Padamara District, and the identification of intestinal parasites using the 0.2% NaOH sedimentation method was carried out at the Laboratory of Microbiology and Parasitology, Faculty of Medicine, Universitas Muhammadiyah Purwokerto in April–May 2021.
**Results:** 14 samples (70%) of spinach were positive for intestinal parasites, while 6 samples (30%) were negative for intestinal parasites. The results of intestinal parasite identification found the presence of *Ascaris lumbricoides* eggs as many as 10 (30.3%), Minute Intestinal Fluke eggs as many as 6 (18.18%), and *Entamoeba coli* cysts as many as 17 (51.52%).
**Conclusions:** A. lumbricoides egg, Minute Intestinal Fluke eggs, and E. coli cyst were identified in spinach at Padamara Market, Padamara District.

## Introduction

Soil-transmitted helminths (STH) are intestinal nematodes that infect humans with helminthiasis through direct contact with soil that contains STH eggs or larvae. STH infections are endemic in many countries around the world (Asihka et al., 2014). The World Health Organization (WHO) reports that STH infections occur in more than 1.5 billion people (24%) of the world's human population. Most STH infections are known to occur in Sub-Saharan Africa, the Americas, China, and East Asia (WHO, 2023).

The incidence of intestinal parasites is high, especially in developing countries.

The eggs/ larvae of intestinal parasites can be found attached to vegetables, fruits, fingers, cutlery, door handles, and money (Kyi & Zin, 2014).

Vegetables play an important role in supporting human health because they contain many nutrients, fiber, minerals, and vitamins. Vegetables can be obtained at affordable prices when compared to meat and livestock. Most people consume raw vegetables to preserve the nutrients in them. However, raw vegetables have a great potential to spread intestinal parasitic infections (Yusof, 2020).

Raw vegetables consumed as fresh vegetables also have the potential to transmit geohelminth infection through food eaten by humans, larvae penetrating the skin, and inhalation (Bestari et al., 2020). *Ascaris lumbricoides*, Hookworm, and *Trichuris trichiura* are most commonly infected in humans. (Mumpuni et al., 2020).

Protozoan infections can be transmitted through food, and some of these diseases cause serious health and economic problems in many developing countries. *Cryptosporidium, Cyclospora, Giardia*, *Entamoeba histolytica, Entamoeba coli*, and *A. lumbricoides* are considered to be the most common parasitic contaminants of fruits and vegetables (Yusof et al., 2017).

The use of irrigation water for crop irrigation as well as animal fecal fertilizer contaminated with intestinal parasites causes the spread of disease infection to be more widespread (Loganathan et al., 2016).

Jusuf et al. showed that STH contamination in lettuce sold in traditional markets and modern markets in Medan City was indicated by as much as 85% of lettuce in traditional markets were positively contaminated with STH (*Strongyloides stercoralis* as much as 35%, rhabditiform larvae of *S. stercoralis* as much as 30%, Hookworm eggs as much as 15%). STH contamination in lettuce in the Modern Market was 90% with proportions (*Strongyloides* (35%) Hookworm eggs (20%) and *Toxocara* eggs (5%) (Jusuf et al., 2013).

Wardhana et al. also stated that there was STH egg contamination in the cabbage salad at the University of Lampung food stalls by as much as 26.19%. The presence of *A. lumbricoides* eggs was found at 14.28%, *T. trichiura* eggs at 7.14%, and as much as 4.76% of fresh cabbage was contaminated with these two types of STH (Wardhana et al., 2014).

Spinach sold in Padamara Market, Padamara District, is in high demand by buyers because the price is very economical. Padamara Market was chosen as the research location because the market floor is grounded, the location is very strategic, crowded and dense with visitors, the location is narrow, and there are still many vegetable traders selling on sacks so

that it is easily contaminated with dust or dirt.

Spinach was chosen as the research sample because it is the most demanded by buyers in Padamara Market and the selling location is still on the ground, which has the potential for STH transmission.

This study aims to identify intestinal parasites in spinach at Padamara Market, Padamara District.

## Methods

The study is a descriptive observational study with a cross-sectional design conducted during April–May 2021. The sampling involved 20 bunches of spinach, and a total sampling technique was applied at Padamara Market, Padamara District, Purbalingga Regency. The identification of intestinal parasites was conducted using the 0.2% NaOH sedimentation method at the Microbiology and Parasitology Laboratory, Faculty of Medicine, Muhammadiyah Purwokerto University.

### *Determination of Sampling Coordinates*

The determination of coordinate points for sampling locations was conducted at 20 points where spinach samples were collected. This was accomplished using the GPS Essential for Android application. Each coordinate point obtained was meticulously recorded for precise documentation and subsequent use in the study

### *The identification of intestinal parasites*

The process for identifying STH eggs in spinach using the 0.2% NaOH sedimentation method. Each spinach leaf sample was cut into small pieces. The pieces were placed in a beaker glass, and each sample was assigned a unique identity. The spinach samples were soaked for 30 minutes in a beaker containing a 0.2% NaOH solution. After soaking, spinach samples were separated from the 0.2% NaOH bath water. Surface water on the beaker glass was removed, and the water at the bottom was extracted using a volumetric pipette (10–15 ml). The bath water containing the extracted material was centrifuged at 1500 rpm for 5 minutes.

The supernatant (liquid above the sediment) was discarded. The sediment formed at the bottom of the centrifuged sample was carefully collected using a Pasteur pipette. A drop of the sediment was placed on a glass object. A dye, either eosin or lugol, was added to enhance visibility. The prepared slide was observed under a microscope with 40x10 magnification.

This detailed process ensures a systematic examination of spinach samples for the presence of intestinal parasites using a standardized method, and the identification is facilitated by microscopy and reference materials such as the Atlas of

Parasitology identification book (Jasman et al., 2019)**.**

***Data Analysis***

The data analysis for the results of intestinal parasites identification involved univariate tests, and the findings are presented in the form of frequency distribution tables. By conducting univariate tests and presenting the results in frequency distribution tables, the analysis provides a clear and detailed account of the prevalence and distribution of intestinal parasites in the studied spinach samples.

**Results and Discussion**

Based on Table 1, 14 samples (70%) of spinach were positive for intestinal parasites, while 6 samples (30%) were negative for intestinal parasites.

**Table 1**. Frequency distribution of intestinal parasites in spinach at Padamara Market, Padamara Subdistrict

| Intestinal Parasites | Frequency (n) | Percentages (%) |
|---|---|---|
| Positive | 14 | 70 |
| Negative | 6 | 30 |

**Table 2.** Prevalence of intestinal parasites in spinach at Padamara Market, Padamara Sub-district

| Species | Frequency (n) | Percentages (%) |
|---|---|---|
| *A. lumbricoides* | 10 | 30,3 |
| *Minute intestinal fluke* | 6 | 18,18 |
| *E. coli* | 17 | 51,52 |
| Total | 34 | 100 |

The results of intestinal parasites identification (Table 2) found the presence of *A. lumbricoides* eggs as many as 10 (30.3%), *Minute Intestinal Fluke* eggs as many as 6 (18.18%), and *E. coli* cysts as many as 17 (51.52%).

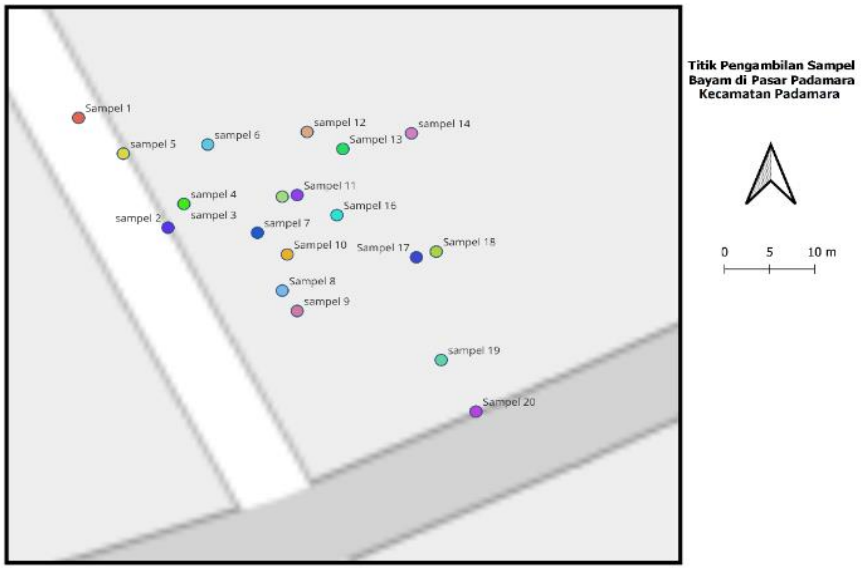



**Figure 1**. Sampling Point for Spinach in Padamara Market, Padamara Sub-district.

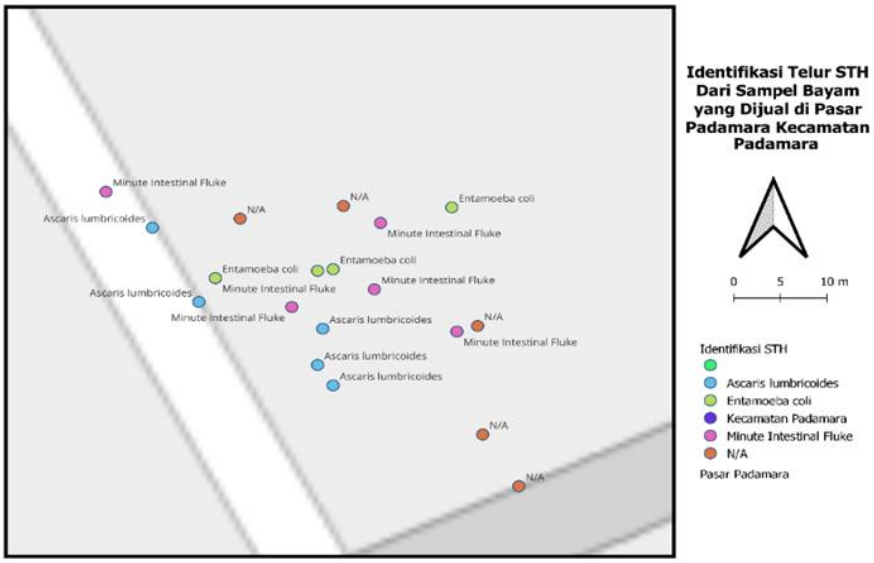
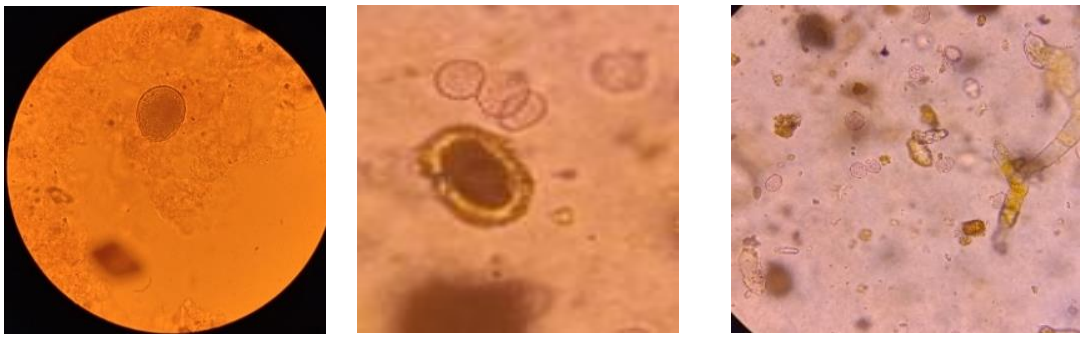



**Figure 2.** Identification of intestinal parasites from Spinach in Padamara Market, Padamara District



(A) (B) (C)

**Figure 3.** Identification of intestinal parasites

Note: *Minute Intestinal Fluke* magnification 40x10 (A), *A. lumbricoides* egg magnification 40x10 (B), *E. coli* cysts magnification 40x10 (C)

Padamara District Padamara Market was chosen as the research location because the market floor is on the ground, the location is very strategic, crowded, and dense with visitors, the location is narrow, and many vegetable traders sell vegetables on sacks so that they are easily contaminated by dust or dirt.

The results of this study are in line with the results of Hidayati et al., who found positive STH in 2 (9.5%) kale vegetables, 3 (14.3%) spinach vegetables, and 3 (14.3%) mustard greens. The STH eggs identified in all samples were positive for *A. lumbricoides* (Hidayati et al., 2016).

Also supported by the results of Satria & Yulfi's study, which found the most STH contamination in curly lettuce vegetables as much as 3 (14.29%), and radishes as much as 3 (14.29%), while the least was found in spinach, chicory, and pokchoy vegetables as much as 1 (4.76%) each. No contamination was found in cabbage and celery. The identification results showed the presence of *A. lumbricoides* eggs in as many as seven (11.11%) and hookworm eggs in as many as five (7.95%) (Satria & Yulfi, 2021).

The results of this study found the detection of MIF and *E. coli* in spinach vegetables sold at Padamara Market, Padamara District, Purbalingga Regency. Minute Intestinal Fluke is an infection caused by small intestinal fluke eggs that infect humans in South Korea. MIF species that frequently infect include *Metagonimus yokogawai, M. miyatai, M. takahashii, Heterophyes nocens, Heterophyopsis continua, Stellantchasmus falcatus, Stictodora fuscata, Pygidiopsis summa, and Gymnophalloides seoi*. MIF eggs are characterized by round, pyriform, elliptical body shapes with a size range of 21-35 x 12-21 µm. The surface ultrastructure of MIF eggs can be a diagnostic marker for *C. sinensis* eggs (Lee et al., 2012).

The results of this study are contrasted with the results of Yusof et al., who found no worms or protozoa in kale or fruit. Pegaga vegetables were positive for *Strongyloides* larvae, *Diphyllobotrium* eggs, and unidentified trematodes (flukes). *Entamoeba coli* cysts and *Blastocytis* spp. were also found in pegaga vegetables (Yusof et al., 2017).

The advantage of identifying STH eggs with the 0.2% NaOH method is that the specific gravity of the solution is smaller than the worm eggs, thus accelerating the worm eggs to settle at the bottom of the test tube and facilitating examination under a microscope. The 0.2% NaOH sedimentation method has a sensitivity of 66.67% and a specificity of 97.50% for the identification of *A. lumbricoides* eggs. The disadvantages of the 0.2% NaOH

sedimentation method are that it takes a long time because it must soak for 30 minutes for each sample. Debris carried in the soaking solution can interfere with the identification process because artifacts and garbage are often found during identification (Regina et al., 2018).

The contamination of STH eggs in spinach sold in Padamara Market, Padamara Subdistrict, is due to the lack of hygiene of each spinach seller, who often places spinach sold on tiles and only on dirty sacks. Vegetables that are arranged in an open and unclean place can be contaminated by STH eggs in the soil or dust attached to the spinach (Setiawan et al., 2017).

The causes of helminthiasis include consuming raw vegetables directly without washing and incomplete cooking of vegetables. Proper vegetable washing techniques include washing vegetables in running tap water, washing them sheet by sheet, then dipping them briefly in hot water and rinsing them with boiled water so that STH eggs that may be attached to vegetables can be removed with the flow of water (Hidayati et al., 2016). In addition, it can be prevented by not using manure or fertilizer made from animal manure as a medium for fertilizing the soil in vegetable planting (Asihka et al., 2014)

## Conclusion

The positive spinach containing STH eggs in Padamara Market, Padamara Subdistrict, was 14 (70%) while the negative STH eggs were 6 (30%). Species identified in spinach sold in Padamara Market include *Ascaris lumbricoides* as many as 10 (30.3%), *Minute Intestinal Fluke* as many as 6 (18.18%), and *Entamoeba coli* as many as 17 (51.52%).

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