The Identification of Soil-Transmitted Helminth Eggs With Different Methods on Basil Leaf in Catfish Pecel Sellers in East Purwokerto

Ayu Lestari[1, b)], Dita Pratiwi Kusuma Wardani[1, a),] Ikhsan Mujahid[1, c)], and Muhammad Luthfi Almanfaluthi[2,d)]

[1] *Medical Laboratory Technology, Faculty of Health Science, Universitas Muhammadiyah Purwokerto, Indonesia*
[2] *Medical Doctor, Faculty of Medicine, Universitas Muhammadiyah Purwokerto, Indonesia*

[a)]Corresponding author: dita.tiwhie@gmail.com
[b)] ayu.lestary1717@gmail.com
[c)] ikhsan_m83@yahoo.com
[d)] m.luthfi.a@ump.ac.id

Abstract. Soil Transmitted Helminthes (STH) is a group of worms that need soil to grow into infective form. STH infection can be transmitted through contact with soil contaminated with the helminth eggs and an attachment to soil-planted vegetables. This research aimed to determine the differences in STH's prevalence with NaCl flotation and MgSO4 sedimentation methods on basil leaves in catfish pecel sellers in East Purwokerto. This was analytical observational research with a cross-sectional design conducted on 12 catfish pecel sellers for three weeks. A total of 216 slides were observed in this study. The results of the examination showed that there were samples contaminated with Ascaris lumbricoides as much as 1 slide (0.9%) in the MgSO4 sedimentation method and 2 slides (1.9%) with the Minute Intestinal Fluke egg species using the NaCl flotation method. The results of Cohen's Kappa showed that there were differences in the identification of STH eggs between the NaCl Flotation method and MgSO4 on basil leaves (p= 0.019, K= 0.662).

Keywords: NaCl Flotation, Basil leaf, MgSO4 Sedimentation, STH.

INTRODUCTION

Soil-Transmitted Helminth (STH) is still a health problem concern in several developing countries, especially in Indonesia. Many Indonesian people like consuming raw or fresh vegetables to be used as a complementary food. Vegetables can be the source of STH infection in humans because they do not through a cooking process with the purpose to kill various microorganisms in food due to the influence of high temperature.[1] World Health Organization (WHO) states that there are more than 1.5 billion people or 24% of the world's population infected with STH.[2] The prevalence of STH in Indonesia is around 2.5-62%, especially in people with poor sanitation.[3]

Transmission of STH infection can occur through soil contaminated with helminth eggs. There are several risk factors for STH infection such as bad personal hygiene and poor environmental sanitation in residential areas.[4] This transmission can also accour through improper processing and washing of raw vegetables.[5] If we consumed vegetables without cooking, washing, or peeling them properly, they can be a high potential to transmit STH and has an effect to digestive disorders such as diarrhea, abdominal pain, malnutrition, and malaise.[6] Not only these factor, but also lack of kitchen hygiene, awareness, and lack of knowledge about food processing contribute to high prevalence of STH infections.[7]

Lobo et al.[5] reported that *Ascaris lumbricoides* eggs 26 samples (70.3%) Hookworm eggs 6 samples (16.2%), mixed infection *A. lumbricoides* and Hookworm eggs 4 samples (10.8%), mixed infection *A. lumbricoides* and *Trichuris trichiura* eggs 1 sample (2.7%) were identified from basil leaf in grilled fish seller in Palu. Yustika et al. [8] reported that *Oxyuris vermicularis* eggs, Hookworm eggs, adult Hookworm, Hookworm larvae, *A. lumbricoides* eggs were identified from fresh vegetables in traditional markets in Semarang City. Fane et al. [9] also reported that *A. lumbricoides* eggs 6 samples (24%), Hookworm eggs 1 sample (4%), and negative 18 samples (72%) were identified from basil leaf in Catfish pecel seller in Kebon Jeruk District, West Jakarta. Wantini and Sulistianingsih [10] reported that *A. lumbricoides* eggs found in fresh vegetables in Catfish Pecel sellers around Z.A Pagar Alam Street, Bandar Lampung. The aim of this research was to identify of Soil Transmitted Helminth eggs with different method on basil leaf in Catfish Pecel Seller in East Purwokerto.

METHOD

This study was conducted in May-June 2022 with cross-sectional design in Microbiology Laboratory, Medical Laboratory Universitas Muhammadiyah Purwokerto. Identification of STH eggs using different method between NaCl flotation and $MgSO_4$ sedimentation. The sample in this study was from basil leaves from 12 catfish pecel sellers in East Purwokerto with total sampling for three weeks observations.

NaCl Flotation Method

First, make a saturated NaCl solution with 500 mL of distilled water in a beaker glass then add 165 gr of NaCl and stirred well until becomes saturated. Basil 10 gr soaked into 50 mL of NaCl solution and allowed to stand for 25 minutes while stirring until homogenous. The saturated NaCl solution was immersed in a test tube until it filled the mouth of the tube and covered with a covered glass and then left for 45 minutes. Samples were observed under a microscope and identified with a parasitology atlas.[11], [12]

$MgSO_4$ Method

First, make a MgSO4 solution 1.2N/m3 with 400 mg MgSO4 dissolved in 1 liter of distilled water. Basil 2 gr cut into small pieces and put into 8 mL of distilled water then homogenized. Basil was soaked in a test tube then centrifuged at 1000 rpm for 5 rpm and centrifuged again at 2000 rpm for 10 minutes. The precipitate formed was dripped onto the object glass and covered with a covered glass. Samples were observed under a microscope and identified with a parasitology atlas.[13], [14]

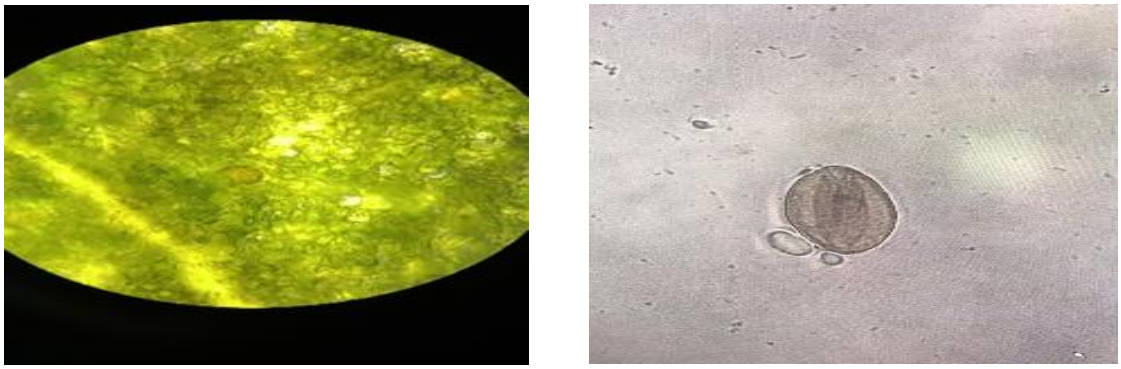
Data identification of STH eggs between NaCl Flotation and $MgSO_4$ in fresh basil leaf were analyzed by Cohen's Kappa.

RESULTS

A total of 216 slides were observed from the different methods in this research to identify STH eggs in basil leaves. The prevalence of STH eggs in basil leaves in catfish pecel sellers in East Purwokerto was presented in Table 1. Minute Intestinal Fluke found in basil leaf 2 samples (0.93%). The identification of STH eggs was illustrated in Figure 1.

TABLE 1. Prevalence of STH eggs in basil leaf in catfish pecel seller in East Purwokerto

Species	Frequency (n)	Percentages (%)
Ascaris lumbricoides	1	0,46
Minute Intestinal Fluke	2	0,93
Negative	213	98,61
Total	216	100



(a) (b)

FIGURE 1. Identification of STH eggs in basil leaf in catfish pecel seller in East Purwokerto
(a) *A. lumbricoides* (b) Minute Intestinal Fluke

TABLE 2. Differences identification of STH eggs between NaCl Flotation and $MgSO_4$ in Basil Leaf

		MgSO4 Sedimentation		Total	P value	Kappa
		Positive	Negative			
NaCl Flotation	Positive	1 (0.9%)	1 (0.9%)	2 (1,9%)	0.019	0.662
	Negative	0 (0%)	106 (98.1%)	106 (98.1%)		
Total		1 (0.9%)	107 (99.1%)	108 (100%)		

Based on Table 2, it is known that only 1 sample (0.9%) has positive results between NaCl and MgSO4 method whereas 106 (98.1%) samples have negative results. There were differences in the identification of STH eggs between the NaCl Flotation method and MgSO4 on basil leaves (p= 0.019, K= 0.662). The kappa value = 0.662 indicates a moderate relationship between the NaCl and MgSO4 Flotation methods.

DISCUSSION

Flotation and sedimentation methods are qualitative method to identify STH eggs. Each method has advantages and disadvantages. Flotation method takes a long time and is appropriate for preparations containing a few helminth eggs while the sedimentation method sometimes gives false negative results because there are damage particles or unable to form sediment due to centrifugation errors.[15] The sedimentation method allows the detection of various gastrointestinal parasites including small parasites whose microscopic identification requires a large lens such as the cyst of Entamoeba sp. or cysts of Giardia sp. However, this method has the disadvantage of accumulating dirt that can cover the parasites. The flotation method relies on the use of a solid solution using an appropriate specific gravity, less parasitic forms may float while larger, denser impurities sink to the bottom of the tube.[16]

The results of this study similar with Wantini & Sulistianingsih[10] study which showed that *A. lumbricoides* egg 1 sample (8.3%) were identified from basil leaf while *A. lumbricoides* eggs 4 sample (33.3%) and *T. trichiura* egg 1 sample (8.3%) were identified from cabbage. There was no *T. trichiura* egg were identified in this study, but we identified Minute Intestinal Flukes (MIF). In contrast to Suwondo et al.[17] reported that *Ancylostoma duodenale* eggs on basil at the Panam Market.

MIF is a zoonotic species with small eggs with ovoid, pyriform or elliptical body shapes measuring 21-35 x 12-21 µm. Their egg surface ultrastructure appears to be a good marker for the diagnosis of C. sinensis eggs. They can infect humans are *Metagonimus yokogawai, Metagonimus miyatai, Metagonimus taka hashii, Heterophyes nocens, Heterophyopsis continua, Stellantchas mus falcatus, Stictodora fuscata, Stictodora ran, Pygidiopsis summa, Gymnophalloides seoi*, and *Acanthotrema felis*.[18] Eggs of *A. lumbricoides* can survive in soil at freezing temperatures and are also resistant to chemical disinfectants and temporary immersion in various hard-class chemicals.[5]

The process of cooking vegetables that are undercooked, no more attention to process of washing vegetables so that it us possible that STH may be left in the vegetables and also consuming raw vegetables without washing them first are risk factors for causing STH infection. The correct washing technique is with running water, washed per sheet, then dip the vegetables in hot water then rinse again with boiled water so that the STH eggs that are still attached can be removed with a stream of water. Storage of vegetables in dirty and open places causes STH contamination because STH eggs found on the ground or dust can be carried by the wind and contaminate vegetables. In addition, the transmission of worm eggs can also be through flies that previously landed on soil/dirt infected with STH, then the fly's feet carry the eggs from the soil and then land on vegetables that are uncovered.[19]

Harnan et al. [20] reported that there was a relationship between the habit before serving fresh vegetables and STH infestion.Vegetables that were washed in running water before serving had a 2,329 chance of experiencing negative STH infections.

CONCLUSION

Ascaris lumbricoides and *Minute Intestinal Flukes* was identified in this study. There were differences in the identification of STH eggs between the NaCl Flotation method and MgSO4 on basil leaves

ACKNOWLEDGMENTS

Thank you to Medical Laboratory Technology Universitas Muhammadiyah Purwokerto for facilitating this research

REFERENCES

[1] B. Y. Onesiforus, “Prevalensi dan Hubungan Higiene Sanitasi Terhadap Kontaminasi Telur STH pada Sayur Kemangi (Ocimum basilicum L.) yang Dijual Sebagai Hidangan Lalapan di Kecamatan Semarang Barat,” *J. Anal. Med. Biosains*, vol. 8, no. 2, p. 82, 2021, doi: 10.32807/jambs.v8i2.227.

[2] WHO, “Soil-transmitted helminth infections,” *World Health Organization*, 2022. https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections (accessed Jan. 12, 2022).

[3] M. J. R. Tapiheru and N. Zain, “Prevalensi Infeksi Soil Transmitted Helminth Pada Murid Sekolah Dasar Negeri 105296 Kecamatan Percut Sei Tuan, Kabupaten Deli Serdang, Sumatera Utara,” *JIMKI J. Ilm. Mhs. Kedokt. Indones.*, vol. 8, no. 3, pp. 1–7, 2021, doi: 10.53366/jimki.v8i3.249.

[4] D. I. Hanif, M. Yunus, and R. W. Gayatri, “Gambaran Pengetahuan Penyakit Cacingan (Helminthiasis) Pada Wali Murid Sdn 1, 2, 3, dan 4 Mulyoagung, Kecamatan Dau, Kabupaten Malang, Jawa Timur,” *Prev. Indones. J. Public Heal.*, vol. 2, no. 2, p. 76, 2017, doi: 10.17977/um044v2i2p76-84.

[5] L. T. Lobo, J. Widjadja, Octaviani, and Puryadi, "Kontaminasi Telur Cacing Soil Transmitted Helminths (STH) PAda Sayuran Kemangi Pedagang Ikan Bakar di Kota Palu Sulawesi tengah," *Media Litbangkes*, vol. 26, no. 2, pp. 65–70, 2016, doi: 10.22435/mpk.v26i2.5442.65-70.
[6] R. Anindita, R. I. Arlinda, and M. Inggraini, "Identifikasi Telur Soil Transmitted Helminth (STH) Pada Kubis (Brassica oleracea) dan Kemangi (Ocimum basilicum) di Penjual Makanan Sepanjang Jalan Dasa Darma Kecamatan Rawalumbu Kota Bekasi," *J. Bioshell*, vol. 11, no. 1, pp. 25–31, 2022, doi: 10.56013/bio.v11i1.1352.
[7] M. Mustria and Sahidan, "Identifikasi Soil Transmitted Helminths Pada Kubis dan Lalapan Pedagang Pecel Lele di Kecamatan Gading Cempaka Kota Bengkulu Tahun 2021," *J. Fatmawati Lab. Med. Sci.*, vol. 2, no. 1, pp. 1–10, 2022.
[8] A. Yustika, A. Wijayanti, and S. A. Tjahjo P, "Identifikasi Cacing Dan Telur Cacing Pada Sayuran Lalapan Di Pasar Tradisional Kota Semarang," *J. Kesehat. Lingkung. J. dan Apl. Tek. Kesehat. Lingkung.*, vol. 19, no. 2, pp. 289–296, 2022, doi: 10.31964/jkl.v19i2.500.
[9] A. T. Fane, E. S. Majawati, and H. H. Liman, "Identification of 'Soil Transmitted Helminth' Contamination on The Raw Vegetables in Warung Pecel Lele in Kebon Jeruk District, Jakarta," *Indones. J. Biotechnol. Biodivers.*, vol. 5, no. 1, pp. 9–16, 2021, doi: 10.47007/ijobb.v5i1.64.
[10] S. Wantini and E. Sulistianingsih, "Hubungan Higiene Sanitasi Terhadap Telur Nematoda Usus Pada Lalapan Mentah di Warung Pecel Lele Sepanjang Jalan Z.A Pagar ALam Bandar Lampung," *J. Anal. Kesehat.*, vol. 8, no. 1, pp. 1–6, 2019, [Online]. Available: http://ejurnal.poltekkes-tjk.ac.id/index.php/JANALISKES.
[11] D. A. Anggraini, N. F. Fahmi, R. Solihah, and Y. Abror, "IDENTIFIKASI TELUR NEMATODA USUS SOIL TRANSMITTED HELMINTHS (STH) PADA KUKU JARI TANGAN PEKERJA TEMPAT PENITIPAN HEWAN METODE PENGAPUNGAN (FLOTASI) MENGGUNAKAN NaCl," *J. Ilmu Kesehat. Bhakti Husada Heal. Sci. J.*, vol. 11, no. 2, pp. 121–136, 2020, doi: 10.34305/jikbh.v11i2.166.
[12] F. Yulianti, T. Lasmini, B. N. R. S. Aritonang, and E. L. Batu, "Identifikasi telur cacing Soil Transmitted Helminths Pada Sayur Kubis di Pasar Kota Pekanbaru," *J. Sains dan Teknol. Lab. Med.*, vol. 8, no. 1, pp. 13–20, 2022, doi: https://doi.org/10.52071/jstlm.v8i1.95.
[13] N. H. Kholidah, Y. Armiyanti, D. A. Rachmawati, B. Hermansyah, and Y. Nurdian, "Perbandingan Penggunaan MgSO4 Jenuh dengan Sukrosa Jenuh untuk Identifikasi Telur dan Larva Cacing Soil-Transmitted Helminth di Tanah Perkebunan dengan Metode Flotasi," *J. Agromedicine Mecical Sci.*, vol. 7, no. 2, pp. 65–71, 2021, [Online]. Available: htttps://doi.org/10.19184/ams.v7i2.17093.
[14] M. A. Muttaqien, "Identifikasi Kontaminasi Tanah Oleh Telur dan Larva Soil-Transmitted Helminthes Di Daerah Perkebunan Gunung Pasang Kabupaten Jember," 2018.
[15] S. Suraini and A. Sophia, "Evaluasi dan Uji Kesesuaian Pemeriksaan Telur Cacing Soil Transmitted Helminths Menggunakan Metode Langsung, Sedimentasi Dan Flotasi," in *Prosiding Seminar Kesehatan Perintis*, 2020, vol. 3, no. 2, pp. 31–36.
[16] H. Pouillevet, S. E. Dibakou, B. Ngoubangoye, C. Poirotte, and M. J. E. Charpentier, "A Comparative Study of Four Methods for the Detection of Nematode Eggs and Large Protozoan Cysts in Mandrill Faecal Material," *Folia Primatol.*, vol. 88, no. 4, pp. 344–357, 2017, doi: 10.1159/000480233.
[17] Suwondo, E. Febrita, and L. Pratiwi, "Identifikasi Jenis Telur Nematoda Yang Terdapat Pada Sayuran," *J. Biog.*, vol. 12, no. 1, pp. 14–18, 2015.
[18] J.-J. Lee *et al.*, "Comparative morphology of minute intestinal fluke eggs that can occur in human stools in the Republic of Korea," *Korean J. Parasitol.*, vol. 50, no. 3, pp. 207–213, 2012, doi: 10.3347/kjp.2012.50.3.207.
[19] F. Hidayati, Rifqoh, and D. Nurmansyah, "Cemaran Telur Cacing Soil Transmitted Helminths (STH) Pada Sayur Bayam, Kangkung, dan Sawi Yang Dijual Di Pasar Banjarbaru Tahun 2015," *J. ERGASTERIO*, vol. 04, no. 01, pp. 25–33, 2016.
[20] H. Harnan, R. J. Sitorus, C. Anwar, H. Hermansyah, and H. Hernita, "Hubungan Lalapan dengan Kejadian Infeksi Soil Transmitted Helmiths (STH) pada Anak Sekolah di Kecamatan Gandus Tahun 2019," *J. Anal. Med. Biosains*, vol. 7, no. 1, p. 6, 2020, doi: 10.32807/jambs.v7i1.160.