Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage: https://journal.uii.ac.id/JKKI

The harada-mori technique: Revisited

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Article Review

ARTICLE INFO

Keywords:
Harada-Mori
Hookworm infection
Strongyloides stercoralis
Larvae

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DOI: 10.20885/JKKI.Vol13.Iss2.art11

History:
Received: May 7, 2021
Accepted: May 31, 2022
Online: August 31, 2022

ABSTRACT

More sensitive diagnostic methods for detecting worms' eggs are needed in areas with low soil-transmitted helminth (STH) prevalence. The Harada-Mori technique was first introduced by Mr. Harada and Mr. Mori. This technique is easy, simple, and does not require sophisticated equipment. The Harada-Mori technique is based on larvae tropism nature in the water to concentrate the larvae of hookworms and Strongyloides stercoralis. Since its first application in 1955, this technique has undergone several modifications. The Harada-Mori could complement the Kato-Katz technique for faeces examination in areas with low STH infection intensity.

Metode diagnostik yang lebih sensitif untuk mendeteksi telur cacing diperlukan di daerah dengan prevalensi soil-transmitted helminth (STH) yang rendah. Metode Harada-Mori pertama kali diperkenalkan oleh Bapak Harada dan Bapak Mori. Cara ini mudah, sederhana, dan tidak memerlukan peralatan canggih. Metode ini berdasarkan sifat tropisme larva ke air untuk mengkonsentrasi larva cacing tambang dan Strongyloides stercoralis. Sejak diperkenalkan pada tahun 1955, metode ini telah mengalami beberapa kali modifikasi. Penggunaannya dapat melengkapi metode Kato-Katz pada pemeriksaan feses di daerah dengan intensitas infeksi STH yang rendah.

INTRODUCTION

Soil-transmitted helminth (STH) infection is a major health problem worldwide, especially among impoverished people who live in rural areas.[1-3] This disease is considered as the most frequent infection among the neglected tropical diseases (NTDs), which infected 1 billion people worldwide.[4] It is estimated that 1.92 million disability-adjusted life years (DALYs) are lost due to STH infection.[5] In 2010, as many as 438.9 million people globally were infected with hookworm, 819 million were infected with *Ascaris lumbricoides*, and 464 million were infected with *Trichuris trichiura*.[6] Nasution et al. (2019) reported that the prevalence of infected elementary school students in Mandailing Natal Regency, North Sumatra, Indonesia, reached 87.2%.[7] School-aged children represent the age group which is susceptible to STH infection.[8,12] Approximately 70% of school-aged children in Indonesia infected by STH suffered from mild to moderate malnutrition.[13]

Several examination methods can diagnose STH infection, such as parasitologic and molecular methods. Parasitologic methods comprise direct smear, Baermann technique, formalin ethyl acetate concentration (FEAC), Harada-Mori filter paper, and agar plate culture

(APC).[14,15] The APC and the molecular methods with polymerase chain reaction (PCR) have up to 100% sensitivity. Unfortunately, both methods require more sophisticated equipment. The Harada-Mori technique could be an alternative since it is a sensitive, simple, and easy parasitologic examination.[16]

Controlling STH infection in children is important to reduce complications and improve quality of life. Mass antihelmintic drug administration (MDA) has been proved to reduce the disability caused by worms infection, increase the children's nutrition status, and reduce cases of anaemia.[17] Preventive chemotherapy is recommended as a public health intervention in areas with a prevalence of children's STH infection of ≥20%.[18] The reduction in STH infection was possible through a good control program and increased awareness of personal hygiene.[2,19]

STH control program is successful in several countries, as reflected by the reduction of STH infection prevalence. However, areas with a low prevalence of STH infection, especially in urban areas, require more sensitive diagnostic methods. The method should be able to detect light intensity infection on a community scale. The Harada-Mori technique is an easy method for detecting hookworm infection. This article discusses the Harada-Mori technique as a method of choice for hookworm infection compared to other current diagnostic methods. The development of this method over time will also be discussed.

METHODS

Literature sources were searched from Google Scholar, Pubmed, and Proquest, and were carried out with "soil-transmitted helminth", "hookworm", "diagnostic method", and "Harada-Mori" as keywords. The literature inclusion criteria were English or Indonesian writing literature published in 2011-2020. In addition, Indonesian Ministry of Health regulations, WHO guidelines, and country health profiles were also used as sources of epidemiological data on STH infection.

The Harada-Mori history

The Harada-Mori technique was performed to diagnose hookworms (*Ancylostoma duodenale* and *Necator americanus*) infection.[1] Hookworm has a unique life cycle that the detection requires a special method. A direct smear examination for hookworm eggs may give a negative result since hookworm eggs could hatch soon after defecation (1-2 days).[20-22] Dacombe et al. (2007) also found that hookworm eggs started to decrease as soon as 3 hours after defecation.[20] Moreover, the egg's morphology shows similarly, resulting in difficulties identifying worm species. The Harada-Mori technique permits the development of infective larvae stage three (L3), facilitates easier detection, and provides a clear image for the identification of worm species.[23] Therefore, this technique allows differentiation of A. *duodenale*, *N. americanus*, and even *S. stercoralis*.[24]

The Harada-Mori technique was first introduced in 1955 by Mr. Harada and Mr. Mori. This technique uses natural larval tropism to water to concentrate larvae.[16] At the beginning of its introduction, the Harada-Mori technique showed a high positive result. The larvae were concentrated in the liquid and free from faecal residue. In addition, the procedure does not require large rooms or sophisticated equipment.[25]

The Harada-Mori technique

The Harada-Mori technique is best performed using fresh faeces (2 hours after removal). Simple equipment, such as filter paper, test tube, and microscope, is used in this method. The use of gloves is essential to ensure the safety of the laboratory technician since filariform larvae could penetrate the skin and cause hookworm infection.[22] The steps are 1) The filter paper is cut into narrow strips about 5 inches long with one tapered end; 2) One gram of faeces that has not been stored in the refrigerator or preserved is smeared on the centre of the paper strip; 3) This paper strip is inserted into a 15 ml tube containing 4-5 ml of distilled water, with the tapered end touching the bottom of the tube;

4) The tube is closed, then placed in an upright position at room temperature (25-28°C) for up to 10 days; 5) The water level is checked every day to evaluate evaporation; 6) After 7-10 days, the liquid is dropped onto the slide and examined using a microscope (10× objective lens); 7) A positive result if stage three infective motile larvae (filariform larvae) of the hookworm or *S. stercoralis* are found.[24,26,27]

Several modifications have been made to the Harada-Mori technique (mHarada-Mori). Modifications include additional steps or material modifications. Martin-Rabadan et al. (1999) examined the water every day at small magnification to look for *S.stercoralis* larvae which should be distinguished from hookworms.[16] Kitvatanachai et al. (2019) utilised a higher amount of stool (2 grams) and plastic bags as containers instead of tubes.[27] Another additional procedure involves killing the larvae (to reduce the risk of infection to laboratory technician) after 10 days by adding 0.5 ml formaldehyde to the tube (placement of the tube in hot water for a while could be an alternative) followed by the centrifugation step (Figure 1).[26-28] Surja et al. (2020) combined these three steps, resulting in 16 out of 158 positive samples of hookworms that were not detected by the Kato-Katz method.[28]

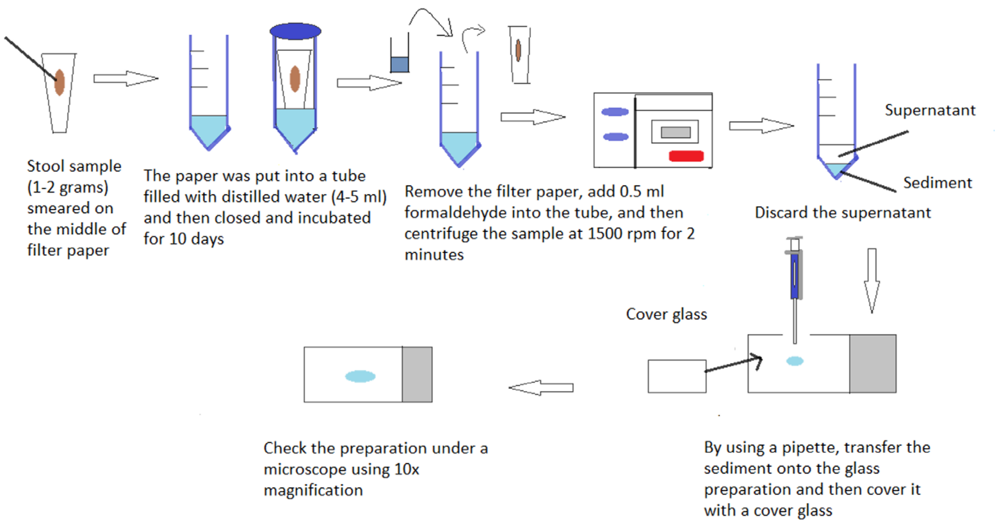

Figure 1. Steps of the Harada-Mori technique (Ngwese et al., 2020 with modification)[26]

Sensitivity and specificity of the Harada-Mori technique

The modified Harada-Mori (mHarada-Mori) technique showed a higher detection rate when compared to direct smear examination and the modified formalin-ether concentration technique (mFECT).[27,29] A previous study in Krabi, Thailand, in 2017 reported that the mHarada-Mori technique could detect hookworm infections up to 75%, while the mFECT and direct smear only detected 66.7% and 45.8% of hookworm infections. Although mFECT is a well-known concentration technique, its filtration process could cause numerous eggs and larvae netted on the gauze.[29] The Harada-Mori technique could also detect hookworm larvae and *S. stercoralis*, which are sometimes undetected on the Kato-Katz examination and the flotation, translation, and centrifugation (FLOTAC) technique.[26] The mHarada-Mori technique also has other advantages, which are simple and inexpensive (only using polyethene-based plastic bag materials available in the local market), economical (0.06 USD/test), non-invasive, and easy to perform.[29,30]

Another result was reported by Hailegebriel et al. (2017) that compared several modern examination methods. The Harada-Mori technique had a low examination sensitivity (45.2%) compared to the APC examination (97.7%) and the Baermann method (73.8%) for S.stercoralis examination.[24,31] This may be due to the inadequate amount of stool used in the Harada-Mori technique. Sultana et al.(2012) suggested that the sensitivity of the Harada-Mori examination could be increased by carrying out the triplicate examination.[32]

Application of Harada-Mori technique in Indonesia

The average prevalence of STH infection among school-age children in Indonesia between 2002-2009 was around 31.8%.[33] Yuwono. et al. (2019) found that the overall prevalence of STH infection in Sorong, West Papua was 30.6%. As many as 59.9% of them had mixed infections.[12] STH infection is dominated by hookworm and *S. stercoralis* infections.[34,35] Furthermore, STH infection is more common in Indonesia among women than men in both the population of children and adults.[12,34]

The Indonesian Ministry of Health in 2017 has carried out various programs to tackle STH infections in Indonesia. The STH prevention program aims to reduce the prevalence of STH infection in children under five, pre-school, and elementary school-age children to less than 10% and to increase the coverage of mass prevention drugs for STH infection to at least 75% among the target population. Case finding is part of the STH surveillance for managing worms infection. Case finding is carried out actively through screening primary school children and passively based on reports of patients visiting health care facilities. The test used to determine the prevalence of STH is the Kato-Katz technique.[36]

The Kato-Katz technique has a high examination sensitivity reaching 96.9% and a specificity of 95% for detecting STH infection. However, the sensitivity of the Kato-Katz technique to detect hookworm infection is only 65.2%. This low sensitivity is possible due to the rapid degeneration of hookworm eggs over time. Although preparations for the Kato-Katz examination could be made relatively quick, some laboratories often carry out microscopic readings too late.[37] Therefore, apart from using the Kato-Katz technique, the Harada-Mori technique can be an alternative.

CONCLUSIONS

The Harada-Mori technique is one optional technique for diagnosing hookworm and *S. stercoralis* infection. This technique can complement the results of the Kato-Katz technique, especially in light-intensity STH infection settings.

CONFLICT OF INTEREST

No conflict of interest

ACKNOWLEDGEMENT

None declare

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