Short Communications

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Misclassification of *Opisthorchis viverrini* and Minute Intestinal Fluke Eggs by Routine Laboratory Staff Using Images from the Kato-Katz Method

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Abstract

Background: The Kato-Katz method is a commonly used diagnostic tool for helminth infections, particularly in field studies. This method can yield inaccurate results when samples contain eggs that are similar in appearance, such as Minute Intestinal Fluke (MIF) and *Opisthorchis viverrini* (OV) eggs. The close resemblance of eggs can be problematic and raises the possibility of false diagnoses. The objectives were to compare the diagnostic performance of the Kato-Katz method for accurately identifying MIF and OV and to provide evidence of possible misclassification. **Methods:** Based on questionnaire responses from 15 (young parasitologists and public health staff), the test comprised 50 MIF egg images and 50 OV egg images, for a total of 100 Google Form questionnaires. **Results:** The morphology of MIF and OV eggs found size and shape similarity and found that the shoulder rims were small, while the OV egg found the knobs had disappeared. The opercular conjunction was apparent, the shoulder rims and miricidium were prominent. The average percentage of correctly classified infections was 61.6 ± 12.1%. The accuracy percentages for both public health staff and young parasitologists in identifying were found to be 59.0 ± 14.8 and 66.8 ± 2.8, respectively. There was no significant difference observed in both groups. **Conclusion:** These findings highlight the need for improving the accuracy of parasite identification. Preserving stool samples before the Kato-Katz method can help mitigate the potential degradation or distortion of parasite eggs. The incorrect classification of both eggs had an impact on treatment plans and the policy of parasite control programs.

Keywords: Kato-Katz method- minute intestinal fluke- misclassification- *Opisthorchis viverrini*

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Introduction

The main parasite problem in Thailand refers to liver fluke infections caused by *Opisthorchis viverrini* (OV). The most often observed infection in Northeast Thailand is the OV infection, which is highly prevalent [1]. The incidence of Minute Intestinal Fluke (MIF), on the other hand, is quite low [1]. Both parasites are typically found in freshwater fish, which is a popular dietary choice in Thailand. It is significant to notice that MIF and OV eggs resemble each other strikingly, which might cause confusion and misidentification [2]. The distinguishing features of OV eggs include their tiny size, oval shape, and presence of an operculum and opercular knob. However, despite being smaller and oval in shape, MIF eggs lack an opercular knob [3]. Therefore, prevention control include, regular screening, and treatment programs are essential to reduce the prevalence of the infection in endemic areas. However, the two parasites have different clinical manifestations. The clinical manifestations of MIF infection include abdominal pain, lassitude, and flatulence [4], while OV infection is increased risk of Cholangiocarcinoma (CCA) [5].

The Kato-Katz method is a commonly and widely used diagnostic tool for helminth infections [6]. The World Health Organization (WHO) recommends using the Kato-Katz method approach as the optimal and standard technique for the detection of soil-transmitted helminths and other helminth eggs in fecal samples [7]. Although it is a useful diagnostic tool, occasionally it may give inaccurate results. A misidentified diagnosis could result from comparable eggs in the sample. If helminth eggs, such as MIF and OV eggs, are closely related, it can be difficult to differentiate them apart. According to the Kato-

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Katz method, feces and egg shells are cleared by glycerol. So that is why similar shapes and sizes of eggs will cause misdiagnosis. Moreover, the overlapping of feces particles can interfere with visualization [8]. Specialized staining methods and extensive examination by an expert parasitologist are required for accurate identification.

Although it has been thought about in the past for technical staff to differentiate between OV and MIF eggs, no one has ever reported it. There is no formal report from Department of Disease Control, Ministry of Public Health, Thailand that MIF and OV were misdiagnosed. Therefore, the study aims to provide evidence of misdiagnosis and compare the diagnostic performance of the Kato-Katz method for diagnosed MIF and OV. This can be used to analyze or classify parasite eggs for accurate diagnosis and to design personnel weaving to assess potential and improve diagnostic methods.

Materials and Methods

To prepare positive references for Minute Intestinal Fluke (MIF) and *Opisthorchis viverrini* (OV) eggs, leftover adults fixed with 70% alcohol were used. Fresh stools from healthy humans were left over from Srinagarind Hospital, Thailand. All protocols were approved by the animal ethics committee (AEMDKKU0322) and the human ethics committee (HE6510003) of Khon Kaen University. The stool samples were then divided into two groups: Group 1, which had OV eggs added, and Group 2, which had MIF eggs added. The Kato-Katz examination was performed on each group. The procedure involved sieving fresh stool, covering it with a cellophane, and incubating it with a 3% malachite green glycerol solution at 25°C. Next, MIF and OV eggs were added to each stool slide. The eggs were then examined under a light microscope, and photos were taken with a magnification of 40X. The classification of helminth eggs using the Kato-Katz method was conducted using Google Forms, as shown in Figure 1A.

The questionnaire in the Google Form included pictures of MIF and OV eggs. The test comprised 50 MIF egg images and 50 OV egg images, total of 100 Google Form questionnaires. The study was conducted among a group of 15 staff (randomly representative parasitology expert staff from three regions of OV infection in Thailand: the north; Office of Diseases Prevention and Control (ODPC) 1 Chaingmai, OPDC 2 Phitsanulok, northeast; OPDC 7 Khon Kaen, OPDC 8 Udonthani, and OPDC 10 Ubonratchathani, and eastern regions; OPDC 6 Chonburi), including young parasitologists from the Graduate Degree Parasitology Department, Faculty of Medicine, Khon Kaen University, Thailand, and public health staff from the Microscopic Technicians of the Department of Disease Control in Thailand who work with parasitic infections. Their work involves the identification and characterization of parasites such as MIF and OV. The data were analyzed using a T-test was performed to compare the percentages of total accuracy (% Accuracy = 100- [(true value-measured value*100)/true value]) , MIF accuracy, and OV accuracy between public health staff and young parasitologists [9]. A P-value of less than 0.05 was considered to indicate statistically significant differences ($P < 0.05$). SPSS version 25 (SPSS Inc., IL, USA) was used.

Results

Examining the detection of MIF and OV, which are almost morphologically similar eggs, MIF groups found shoulder rims were negligible, as shown in Figure 1B, C. OV groups found knobs that disappeared. The opercular junction was distinct; the miricidium and shoulder rims were prominent, as shown in Figure 1D, E. Public health staff identified ranges of 15-40 eggs as MIF eggs and 16-44 eggs as OV eggs. But young parasitologists identified ranges of 24–49 eggs as MIF eggs and 18-42 eggs as OV eggs (Table 1).

Public health staff and young parasitologists showed MIF accuracy of 51.0 ± 19.0 and 61.2 ± 20.7, respectively ($P = 0.358$). Public health staff and young parasitologists had OV accuracy of 67.0 ± 19.8 and 72.4 ± 20.61, respectively ($P = 0.631$). MIF and OV identification

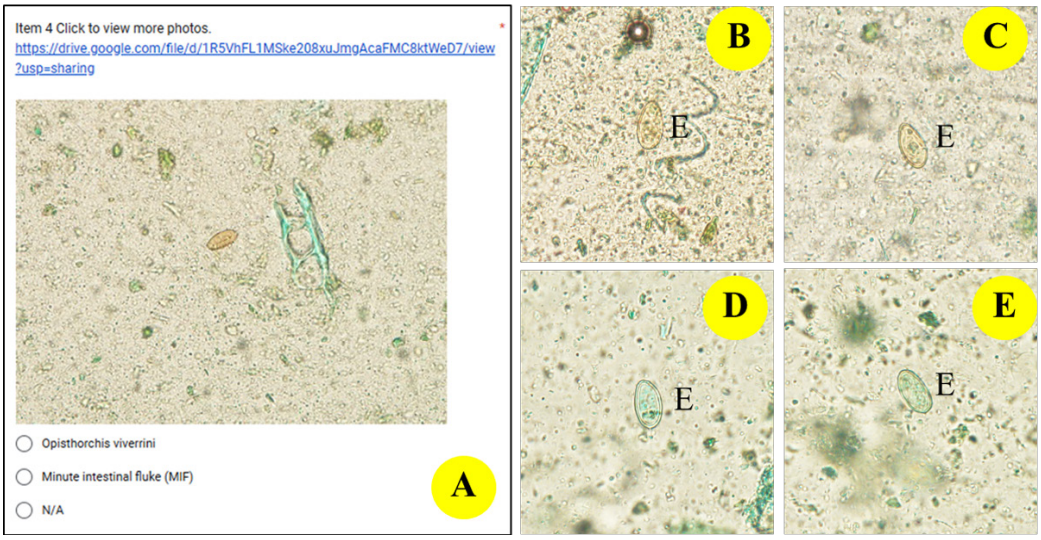

Figure 1. Representative of the Google Form Test (A) and representative of MIF and OV egg from the Kato-Katz method (B-E); MIF egg (B, C); OV egg (D, E); and E: egg.

Table 1. The Results of the Questionnaire Test

Groups	Order	% Accuracy	MIF (50 point)	OV (50 point)	% MIF	% OV
Public health Staff (N = 10)	1	59	15	44	30	88
	2	74	42	32	84	64
	3	44	17	27	34	54
	4	53	17	36	34	72
	5	43	24	19	48	38
	6	69	26	43	52	86
	7	69	28	41	56	82
	8	34	18	16	36	32
	9	68	28	40	56	80
	10	77	40	37	80	74
Young parasitologist (N = 5)	1	69	28	41	56	82
	2	68	26	42	52	84
	3	67	49	18	98	36
	4	68	26	42	52	84
	5	62	24	38	48	76

MIF, Minute Intestinal Fluke; OV, Opisthorchis viverrini

in public health staff and young parasitologists were not statistically significant. For type of staff, both the accuracy percentage of public health staff and young parasitologist identification were 59.0 ± 14.8 and 66.8 ± 2.8, respectively. The accuracy percentages of both groups were not statistically significant (P = 0.272).

Experienced staff still misdiagnoses as many as 50%. Of the 15 parasitologist-confirmed helminth eggs, among both groups, 27.2 ± 9.7 points were in MIF identification and 34.4 ± 9.7 points were in OV identification.

Discussion

Misclassification of egg pictures between OV and MIF using the Kato-Katz method is an occurrence among public health staff and young parasitologists. This is due to the similar size and shape of the eggs, which can be difficult to differentiate. In the present study, the problem of OV or MIF using the Kato-Katz detection may cause misdiagnosis and awareness of epidemic survey report that is misleading to controlling OV and CCA in endemic areas.

The formalin-ethyl acetate concentration technique (FECT) is a widely used and standard method for diagnosing certain types of parasites. It has the advantage of being able to detect a wide range of parasites, including both protozoa and helminths. However, one limitation of the FECT method is that it requires specialized equipment such as a centrifuge, which may not be available in all settings, especially in field studies [10]. The Kato-Katz method is often used in field studies for diagnosing helminth infections because of its relative simplicity and the ability to perform it on-site. However, this method has some limitations, particularly with different clearing times across different soil-transmitted helminth eggs. For example, hookworm eggs rapidly disappear in cleared slides, resulting in false negative test results if the interval between slide preparation and examination is too long (≥60 min) [11].

The previous studies showed that the combined results of Kato-Katz with Mini-FLOTAC fixed at 5% [12], Mini-FLOTAC fixed with sodium acetate fixative [13], or fixing the eggs with formalin [14] could fix the egg morphology, but there was no quality report. Therefore, improvement of the quality is needed, so our study of stool fixed with formalin plus glycerol [8] will be useful for the Kato-Katz technique. Other techniques, so far, include antigen detection in urine or anti-*Opisthorchis viverrini* Immunoglobulin G antibodies (IgG) (innovative lateral flow immunochromatographic test kit) in human blood samples, which are advantageous choices for accurate diagnosis [15 ,16]. Some limitations of them, such as the cost and special technician for blood or urine collection, are needed, and moreover, they are not suitable for field study but suitable for hospital [15 ,16]. However, it is essential to consider these limitations and explore alternative or complementary methods to improve the accuracy and reliability of helminth infection diagnosis in field settings. In conclusion, the study revealed evidence of misclassification of eggs between OV and MIF using images from the Kato-Katz method, specifically among routine staff responsible for OV and MIF diagnoses. Additionally, the number of representative parasitology expert staff in this project seems low, but that is the true message, which is very formative. Misclassifications of both eggs affect treatment plans and the policy of parasite control programs.

Author Contribution Statement

Data curation, investigation, methodology and formal analysis; AW, AA, TB, PL, OP, and OW. Conceptualization and project administration; TB. Supervision and visualization; TB. Writing-original draft and writing-review & editing; AW, AA, TB, PL, OP, and OW.

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Ethical approvals

All protocols were approved by the animal ethics committee (AEMDKKU0322) and the human ethics committee (HE6510003) of Khon Kaen University.

Conflict of Interest

The authors declare they have no conflict of interest

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