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Detection of a carcinogenic liver fluke among migrant workers by three coprological concentration methods

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Abstract. *Opisthorchis viverrini* is a major health problem in many part of Southeast Asia. Therefore, this study aimed to detect *Opisthorchis viverrini* infection among migrant workers in Thailand by using three coprological concentration methods. A cross-sectional study was conducted among 147 migrant workers in Thailand. Fecal samples were processed by the modified formalin ether concentration technique, the Kato Katz thick smear, and the mini-parasep solvent free parasite fecal concentrator. Specificities, sensitivities, negative predictive values, positive predictive values, Kappa indexes were analyzed with SPSS version 22. The infection rate with opisthorchiasis was 27.2%. The parameters measured for the modified formalin ether concentration technique, Kato Katz thick smear, and mini-parasep solvent free parasite fecal concentrator methods respectively were as follows: infection rates (23.1%, 12.9%, and 22.4%), sensitivities (85.0%, 47.5%, and 82.5%), specificities (100%, 100%, and 100%), positive predictive values (100%, 100%, and 100%), and negative predictive values (94.7%, 83.6%, and 93.9%). The kappa index value of diagnostic agreement between formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator showed substantial agreement for *O. viverrini*. In conclusion, the calculated analytical sensitivity, and negative predictive values indicate that modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator are more accurate in detecting *O. viverrini* infections. The study indicates that the mini-parasep solvent free parasite fecal concentrator method was the method with high potential to be used in routine laboratory and community diagnosis for opisthorchiasis.

INTRODUCTION

Opisthorchis viverrini is still a major health problem in many parts of Asia, particularly in Southeast Asia especially in Lao People's Democratic Republic (PDR), Thailand, Cambodia, and Vietnam (Jongsuksuntigul & Imsomboon, 2003; Sithithaworn *et al*., 2012). *O. viverrini* infection has been reported to be associated with hepatobiliary diseases associated with hepatomegaly, cholecystitis, gallstones, and cholangitis (Thamavit *et al*., 1978; Harinasuta *et al*., 1984). Epidemiological and animal studies have demonstrated that *O. viverrini* infection is strongly correlated with cholangiocarcinoma (Sripa *et al*., 2007; Sripa *et al*., 2008; Shin & Oh, 2010). Presently, *O. viverrini* infection has been classified by the International Agency for Research on Cancer, World

Health Organization, as a Type 1 carcinogen (IARC, 2010). Cholangiocarcinoma has been reported in high incidence in Thailand particularly in the northeast region. For this reason, *O. viverini* infection is still an important problem in many parts of this region and eradication of the liver fluke is urgently needed in these areas.

The microscopic examination of feces is essential for the laboratory diagnosis of *O. viverrini* infection. In some parasitic infections, the density may be small and the number of eggs the in the stool may be not enough, leading to misdiagnosis (Sithithaworn *et al.*, 1991). Concentration techniques particularly the modified formalin ether concentration and Kato Katz thick smear are used in routine laboratory examination and epidemiological studies for detecting *O. viverrini* infection (Sithithaworn *et al.*, 1991; Kaewpitoon *et al.*, 2016). The concentration technique has an increased the sensitivity and accuracy of microscopy for the diagnosis of *O. viverrini*. However, the ether used for the modified formalin ether concentration technique has some disadvantages such as it is a highly flammable and toxic agent. New improved diagnostic method, the mini-parasep solvent free parasite fecal concentrator has been used in the laboratory for detecting intestinal helminthic infections. This is a useful and simple technique for identifying and isolating parasitic's eggs, and larvae. This is a rapid and reliable tool (Saez *et al.*, 2012; Useh *et al.*, 2011; Zeeshan *et al.*, 2011). Previously, we used the mini-paraep solvent free parasite fecal concentrator for detecting *O. viverrini* in the hospital and found that it is useful for laboratory use, increasing the yield of parasitic infection as compared to direct smear (Kaewpitoon *et al.*, 2016). Therefore, this study aimed to detect *O. viverrini* infection among migrant workers in Thailand land by using the mini parasep solvent free parasite fecal concentrator, and two conventional methods; modified formalin ether concentration technique, and Kato Katz thick smear and compare their sensitivity.

MATERIALS AND METHODS

Study Populations

A total of 147 migrant workers in Nakhon Ratchasima Province, in northeastern region of Thailand, were enrolled in the study from October 2016 to February 2017. All participants provided informed consent before participating in the study. This study was approved by the Ethics Committee for Research Involving Human Subjects, Suranaree University of Technology; EC- 59-38. Permission was obtained from the managers of the companies where the migrants worked. Informed written consent was obtained from the study participants and guardians.

Diagnosis of *O. viverrini* infection

Fecal samples were collected and then examined by modified formalin ether concentration technique, Kato Katz thick smear and mini-parasep solvent free parasite fecal concentrator. Specimens were collected, processed and examined following the manufacturer's instructions for the mini-parasep solvent free parasite fecal concentrator and standard operating procedures (SOPs) for the modified formalin ether concentration technique (Perry *et al.*, 1990) and Kato Katz thick smear (Shimeles, 2015). Briefly, mini parasep solvent free parasite fecal concentrator was processed. 1 gram of feces was emulsified in 3.3ml of 10% formalin in saline and then was sealed by screwing in the filter/sedimentation cone unit. The fecal sample preparations were vortex, inverted and then centrifuged at 1,500 rpm for 2 minutes. The mixing chamber and the filter was then unscrewed and discarded for incineration. The supernatant in the sedimentation cone was decanted into disinfectant beaker. The deposit was soluble with 10% formalin in saline and then subsequently examined microscopically using ×40 objectives. Fecal samples were examined by two laboratory technologists and then confirmed by an expert parasitologist. Participants who were infected with

O. viverrini were treated with praziquantel, while who were infected other known intestinal parasites were treated with recommended anti-parasitic drugs and also attended health education.

Statistical Analysis

Data analysis was done using SPSS Inc., Chicago, America, for descriptive and inferential statistics. Fecal diagnosis results based on the combined modified formalin ether concentration technique, mini-parasep solvent free parasite fecal concentrator, and Kato Katz thick smear were used as gold standard to estimate specificity, sensitivity, positive predictive value, negative predictive value and infection rate of each method in detecting *O. viverrini*. Kappa Estimator was employed to determine the strength of agreement of each method with the combined result. Kappa values were interpreted as follows 0.01–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–0.99, were slight agreement, fair agreement, moderate agreement, substantial agreement, and perfect agreement, subsequently (Landis & Koch, 1977). Efficiency of modified formalin ether concentration technique, mini-parasep solvent free parasite fecal concentrator, and Kato Katz thick smear, for detecting *O. viverrini* was analyzed by the chi-square test (X^2-test). For all statistical analyses, p-value < 0.05 and 95% confidence intervals (95% CI) is considered significant.

RESULTS

One hundred and forty-seven (n=147) migrant workers provided stool sample for the possible diagnosis of *O. viverrini* and other known heminthes to be examed by modified formalin ether concentration technique, Kato Katz thick smear and mini-parasep solvent free parasite fecal concentrator. The majority of participants were male (55.1%), mean age was 31.3 years old (SD=9.3), primary school (68.7%), married (67.3%). Most of them were Cambodians (82.3%), employee (94.6%), and worked for a period of 3.9 years in Thailand .

Helminthic eggs were detected in 47 migrant workers, are shown in Table 1. Of the studied subjects 30.6% (45/147) were found to be infected by one or more helminthic infection. By taking the combined result (as a gold standard) the following parasites were detected; *O. viverrini* 27.2%, Hookworm 1.4%, *Taenia* sp. 1.4%, and *S. stercoralis* 0.7%. The following infection rate for *O. viverrini* was detected in 34.6% (9/26) Lao PDR and 25.6% (31/121) Cambodia migrant workers.

The intensity of infection was calculated based on Sithithaworn *et al.* (1991) guideline for *O. viverrini*. The intensity of infection after converting the amount of eggs counted in 1 g of stool by modified formalin ether concentration technique, mini-parasep solvent free parasite fecal concentrator,

Table 1. Frequency of parasitic infection in 147 migrant workers in Nakhon Ratchasima, Northeast, Thailand

Helminthic Infection	Modified formalin ether concentration technique (%)	Mini-parasep solvent free fecal concentrator (%)	Kato Katz thick smear (%)	Total (3 methods)
Opisthorchis viverrini	34 (23.1)	33 (22.4)	21 (14.3)	40 (27.2)
Strongyloides stercoralis	0	1 (0.68)	0	1 (0.7)
Hookworm	1 (0.7)	2 (1.4)	1 (0.7)	2 (1.4)
Taenia sp.	2 (1.4)	2 (1.4)	2 (1.4)	2 (1.4)
Total	**37 (25.2)**	**38 (25.8)**	**24 (16.3)**	**45 (30.6)**

and Kato Katz thick smear in to eggs per one gram of stool (EPG) is shown in Table 2. The intensity of *O. viverrini* infection was detected by modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator. However, positive results were lower detected in heavy, moderate, and low level of intensity by Kato Katz thick smear, compared to modified formalin ether concentration technique and min- parasep solvent free parasite fecal concentrator (Table 2).

For detection of all parasite species, the modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator yielded comparable positive rates. Positive detection rate by modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator were significantly higher than for Kato Katz thick smear method ($p = 0.005$). As for the detection of *Taenia* sp., there was no significant difference among all three methods. However, for the detection of Hookworm and *S. stercoralis*, there was an increased detection by mini-parasep solvent free parasite fecal concentrator. *O. viverrini* positive by the combined results of the three methods, was classified and found that the infection of *O. viverrini* detected by modified formalin ether concentration technique was 23.1% (34/147). The infection of *O. viverrini* detected by mini-parasep solvent free parasite fecal concentrator was 22.4% (33/147). Less egg count for *O. viverrini* was detected by Kato Katz thick smear 14.3% (21/147). For the detection of *O. viverrini*, the positive rate was highest with modified formalin ether concentration technique, followed by mini-parasep solvent free parasite fecal concentrator and Kato Katz thick smear. Difference between modified formalin ether concentration technique and Kato Katz thick smear is statistically significant (X^2-test=6.4, $p<0.01$), but not between modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator (X^2-test=0.2, $p>0.05$).

Modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator were found more sensitive in identifying participants who were infected with *O. viverrini* better in predicting participants who were negative for these *O. viverrini* than the Kato Katz thick smear. In addition, modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator were found highly negative predictive value and accuracy in identifying participants who were infected with *O. viverrini* better than the Kato Katz thick smear. Meanwhile, specificity and positive predictive value were highly values in all methods.

The agreement of modified formalin ether concentration technique, mini-parasep solvent free parasite fecal concentrator, and Kato Katz thick smear with the standard (combined results of the three methods). The agreement of modified formalin ether concentration technique, and mini-parasep solvent free parasite fecal concentrator, was substantial agreement in detecting *O. viverrini*. Meanwhile, the moderate agreement in detecting *O. viverrini* was found in the Kato Katz thick smear (Table 4). The agreement of the modified formalin ether

Table 2. Intensity of *Opisthorchis viverrini* infection in 147 migrant workers in Nakhon Ratchasima, Northeast, Thailand

Level of intensity (EPG)	Methods		
	Modified formalin ether concentration technique	Mini-parasep solvent free fecal concentrator	Kato Katz thick smear
Low (1-999)	12 (8.2%)	15 (10.2%)	8 (5.4%)
Moderate (1,000-9,999)	10 (6.8%)	10 (6.8%)	5 (3.4%)
Heavy (10,000-29,000)	3 (2.0%)	1 (0.7%)	0
Very heavy (above 30,000)	9 (6.1%)	7 (4.8%)	8 (4.1%)

Table 3. The efficacy of modified formalin ether concentration technique, Kato Katz thick smear and mini parasep solvent free parasite fecal concentrator for diagnosis *O. viverrini* infection using positive from combined 3 methods as gold standard

Parameters	Modified formalin ether concentration technique	Mini-parasep solvent free fecal concentrator	Kato Katz thick smear
Infection rate	23.13	22.45	12.93
Sensitivity	85.00	82.50	47.50
Specificity	100.00	100.00	100.00
Positive predictive value	100.00	100.00	100.00
Negative predictive value	94.69	93.86	83.59

Table 4. Values of agreement (kappa index) among the modified formalin ether concentration technique, Kato Katz thick smear and mini-parasep solvent free parasite fecal concentrator for diagnosis *O. viverrini* infection using positive from combined 3 methods as gold standard

Methods	Kappa	p-value	95% CI	
			Lower	Upper
Modified formalin ether concentration technique	0.74	0.01	0.60	0.86
Kato Katz thick smear	0.47	0.04	0.40	0.71
Mini-parasep solvent free parasite fecal concentrator	0.67	0.01	0.52	0.82

concentration technique slightly higher in detecting *O. viverrini*, compared to mini-parasep solvent free parasite fecal concentrator and Kato Katz thick smear, however this result was no statistically significant in mini-parasep solvent free parasite fecal concentrator (X^2-test=0.1, $p>0.05$) but difference in Kato Katz thick smear (X^2-test=1.2, $p<0.05$).

DISCUSSION

Liver fluke, *O. viverrini* is a cause of cholangiocarcinoma that remains a major public health problem in Thailand. *O. viverrini* is endemic in northeast and northern part of Thailand, where raw fish consumption is most common (Kaewpitoon *et al*., 2008; Sripa *et al*., 2010; Sithithaworn *et al*., 2012; Kaewpitoon *et al*., 2015). In the Mekong Basin sub-region, *O. viverrini*-induced cholangiocarcinoma ranks first in mortality among cancers for men and the second in women (Sithithaworn *et al*., 2012; Andrews *et al*., 2008). It is reported that Thailand, Lao PDR, Cambodia and Vietnam will have increased cases of *O. viverrini*-induced cholangiocarcinoma in the near future as a result of the demographic and economic factors (Sripa *et al*., 2010; Sithithaworn *et al*., 2012). The spread of *O. viverrini* infection in the region is due to increased migration among the ASEAN Economic Community countries (Bray *et al*., 2012). This results are the first reported studies on *O. viverrini* infection in migrant workers in Thailand. A total of 27.2% migrant workers were *O. viverrini* positive (by the combined results of the three methods). The infection rate was 34.6% and 25.6% in Laos and Cambodia Lao PDR, respectively. This result indicates that migrant workers have high infection and need to be treated. Therefore, active surveillance with rapidly and easily tool for diagnosis is required for detecting *O. viverrini* in a large scale of the migrant worker in Thailand.

Light microscopy is still used for examination of *O. viverrini* and is recognized for diagnosis confirmation with the demonstration of the ova in the fecal

sample. However, the probability of a positive result in the low density of *O. viverrini* obtained by direct smear is very poor (Sithithaworn *et al*., 1997). Concentration methods are therefore employed for better detection of *O. viverrini* particularly by use of Kato Katz thick smear and modified formalin ether concentration technique. The concentration methods are developed and commonly used in the laboratory diagnosis and epidemiological studies (Saengsawang *et al*., 2013; Suwannahitatorn *et al*., 2013; Chaiputcha *et al*., 2015; Chudthaisong *et al*., 2015; Yeoh *et al*., 2015). However, the concentration technique has some limitation. The conventional formalin-ether concentration method is an unpleasant and time-consuming technique involving high-risk due to gauze-filtration of fecal material (Won *et al*., 2015). Mini-parasep solvent free parasite fecal concentrator; a new diagnosis tool is developed for increasing the yield, simple and safety (DiaSys Europe, Berkshire, England). Mini-parasep solvent free parasite fecal concentrator represents a simple and useful method for identifying and isolating *O. viverrini*. Comparison of the diagnostic performance of these three methods with the combined results showed that the infection of *O. viverrini* detected by modified formalin ether concentration technique was 23.1% slightly high more than mini-parasep solvent free parasite fecal concentrator, and Kato Katz thick smear. However, the egg count of *O. viverrini* in modified formalin ether concentr This results of the study indicates that mini-paarasep solvent free parasite fecal concentrator have the performance to detect *O. viverrini* similarly to modified formalin ether concentration technique.

In case of the efficacy, these three methods showed different sensitivities for each detected parasite. However, the *O, viverrini* diagnosis using mini-paarasep solvent free parasite fecal concentrator showed similar sensitivity and negative predictive value to the modified formalin ether concentration technique. This result is similar to other studies that evaluated the efficacy of mini-parasep solvent free parasite fecal concentrator for the laboratory diagnosis of intestinal parasitism (Ikeh & Elujola, 2015). The intensity of infection from the *O. viverrini* for which the value for calculation was available in guideline criteria, *O. viverrini* is found in low, moderate, heavy, and very heavy levels of intensity. The three detection methods all showed better performance in detecting *O. viverrini* in the presence of very heavy levels of infection. However, modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator showed better performance in assessing intensity of infection for *O. viverrini* than Kato Katz thick smear in low, moderate, and heavy levels of infection. This agrees with previous studies reported from Ikeh & Elujola (2015) and Katagiri & Oliveira-Sequeira (2010) for detecting intestinal parasitic infections. Modified formalin ether concentration technique, and mini-parasep solvent free parasite fecal concentrator, showed substantial agreement with the combined value for detecting *O. viverrini*. Interestingly, the substantial kappa value revealed that these samples presented liver fluke infection and reinforces the importance of using these techniques in diagnosing *O. viverrini*. These two methods also showed similarities in other known intestinal parasitic infections. Katagiri & Oliveira-Sequeira (2010) reported that the kappa index value of diagnostic agreement between commercial assays for fecal concentration, centrifugation-sedimentation, and centrifuge floatation was high for intestinal parasitism in dogs. Meanwhile, Kato Katz thick smear has a moderate agreement for detecting *O. viverrini*. Thus indicating the need to perform tests in parallel in order to improve the diagnosing of *O. viverrini*. Mini-parasep solvent free parasite fecal concentrator has safety and health benefits that include: a completely sealed/enclosed process, reduced reagent volumes, no sample contamination, and ready to use systems (Kaewpitoon *et al*., 2016). Additionally, it has performance benefits including: a rapid four-step process, human resources optimized and easy infected cases identification (Kettelhut *et al*., 2008).

CONCLUSION

In conclusion, the calculated analytical sensitivity, negative predictive value, and accuracy indicated that modified formalin ether concentration technique and mini-parasep solvent free parasite fecal concentrator were more accurate in detecting *O. viverrini* infections. The study indicates that the mini-parasep solvent free parasite fecal concentrator method can be used in routine laboratory and community diagnosis of opisthorchiasis.

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