RESEARCH ARTICLE

Detection of the Carcinogenic Liver Fluke *Opisthorchis viverrini* Using a Mini Parasep SF Faecal Parasite Concentrator

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Abstract

The Mini Parasep SF fecal parasite concentrator (MPSFC) is a new modification of the closed concentration system, which can easily be adopted in any routine clinical pathology laboratory. Here we describe our experience with the system in diagnosing *Opisthorchis viverrini*. A total of 199 fecal samples was submitted for routine examination in the clinical pathology laboratory of Suranaree University of Technology hospital, Nakhon Ratchasima province, Thailand, during August to October 2015. Out of all samples examined, 10 (5.03%) were positive with intestinal parasites including *O. viverrini* (2.01%), followed by *Strongyloided stercoralis* (1.51%), Hookworm (0.5%), *Taenia spp.* (0.5%), and *Entamoeba coli* (0.5%). Regarding the distribution of intestinal parasites in relation to the methods used, and found that 4 samples (2.01%) were positive using the direct wet smear method while 10 (5.03%) were positive with the Mini Parasep SF method; the difference was statistically significant (X^2-test = 116.506, p-value =0.001). Mean time for processing using the Parasep system was 6.03 min/sample, the conventional direct wet smear method at 0.3 min/sample. Cost per test, conventional direct wet smear method costing less than the Parasep method at USD 0.74/sample versus USD 1.47/sample. This first report of *O. viverrini* detection using MPSFC indicates that Parasep concentration test is useful in the routine laboratory, increasing the yield of parasites as compared to direct microscopy, but with greater processing time and cost. Further comparisons between the Parasep concentration test and common methods for *O. viverrini* detection are required, particularly concerning use in epidemiological surveys.

Keywords: Detection - carcinogenic liver fluke - *Opisthorchis viverrini* - mini parasep SF faecal parasite concentrator

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Introduction

The carcinogenic liver fluke, *Opisthorchis viverrini* is an endemic in Thailand including Lao People's Democratic Republic, Cambodia and central Vietnam (Sripa et al., 2010). The infections are considered, more than 10 million people are infected with *O. viverrini* (Sithithaworn et al., 2012). In Thailand, it is estimated that 6 million people are infected with the *O. viverrini* (Jongsuksuntikul and Imsomboon, 2003). The *O. viverrini* infection is associated with hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, and gallstones (Harinasuta and Vajrasthira 1960; Thamavit et al., 1978; Harinasuta et al., 1984). Furthermore, *O. viverrini* has been classified as Type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (WHO) (IARC, 1994). This figure indicated that it is a serious public health problem in Thailand, particularly in northeastern and northern region (Kaewpitoon et al., 2008; Sripa et al., 2010).

Light microscopy examination is considered the main stay for confirmation of clinical diagnosis with demonstration of the ova stage in fecal sample essential for the recognition of *O. viverrini*. However, the probability of a positive result via direct microscopy is very poor due to the low density of *O. viverrini* in the specimens obtained (Sithithaworn et al., 1997). In order to improve the parasitic yield concentration methods are employed. Kato thick smear, modified Kato Katz thick smear, modified formalin ethyl-acetate concentration technique are common used in the epidemiological survey for *O. viverrini* including other known intestinal parasitic infections (Kaewpitoon et al., 2012b; 2012c; Saengsawang et al., 2013; Suwannahitatorn et al., 2013; Chaiputcha et al., 2015; Chudthaisong et al. 2015; Yeoh et al., 2015).

Recently, Mini Parasep SF Faecal Concentrator; a new diagnosis tool are used for detected the intestinal parasitic infection. Mini Parasep SF is the solvent-free faecal parasites concentrator represents a simple and useful method for isolating and identifying helminth's eggs and

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larvae, and protozoal cysts. The organisms eventually present without altering their morphological characteristics, concurring also to an easy acknowledgment of the same through microscope observation. The closed concentration system allows rapid, reliable, and safe detection of intestinal parasites by inexperienced technologists (Saez et al., 2011; Useh et al., 2011; Zeeshan et al., 2011). The Parasep filter concentrator tube is a newer modification of the closed concentration system, which can easily be adopted in any routine microbiology laboratory, however, no data reported that using of this method for detection of *O. viverrini*. Therefore, this study aimed to apply the Mini Parasep SF Faecal Concentrator method for diagnosing *O. viverrini* in Suranaree University of Technology hospital, Nakhon Ratchasima province, Thailand during August and October 2015. This data may able useful for further tool support the epidemiological survey in the community.

Materials and Methods

Stool specimens submitted for routine examination in the clinical pathology laboratory of Suranaree University of Technology hospital during August to October 2015 were included in this prospective study. Of all submitted samples, 199 specimens were purposive selected for inclusion in the study and were initially studied with concentration by experienced medical technologists and parasitologists and the results were recorded. The samples (0.3 g/each) were then concentrated using the Mini Parasep SF Faecal concentrator tube (manufactured by DiaSys Europe Ltd, Berkshire, England). Mini Parasep SF Faecal Parasite Concentrators are closed, single use tubes with built-in filter. The tubes and the sedimentation cones were labeled with the specimen identification numbers. They were pre-filled with 3.3 ml 10% buffered formalin, one drop Triton-X and 2 ml ethyl acetate using the spoon on the end of the Mini Parasep SF filter and used for the sedimentation technique in accordance with the manufacturer's instruction sheet. The Mini Parasep SF was sealed by screwing in the filter/sedimentation cone unit. This was then vortex to emulsify with the sedimentation cone pointing upwards. The Mini Parasep SF was then inverted and centrifuged at 1500 rpm for 2 minutes. The mixing chamber and the filter was then unscrewed and discarded for incineration while the supernatant in the sedimentation cone was decanted. The deposit was then examined microscopically using physiological saline and iodine for the eggs, trophozoites and larvae of intestinal parasites (one with saline and one with iodine). Each of the preparations was examined systematically for a minimum of 5 minutes microscopically. For comparison, all specimens was prepared and detected using direct wet smear and microscopic. Statistical comparisons were performed using X^2-test analysis and 'P' values of <0.05 were accepted as statistically significant. The study protocol was approved by Suranaree University Ethical Review Committee, EC58-48.

Results

Out of the 199 samples examined, 10 samples (5.03%) were positive with intestinal parasite including *O. viverrini* (2.01%), and followed by *Strongyloided stercoralis* (1.51%), and each 0.50% of *Hookworm*, *Taenia spp*., and *Entamoeba coli* (Table 1). *O. viverrini* infection using Mini Parasep SF method, was characterized with gender and age group, and found that female (3.70%) was slightly higher than male (0.85%); but the difference was not statistically significant (p-value >0.05). The majorities of *O. viverrini* infection, were found age group 41-50 years old (3.28%), and followed by age group 61-70 years old (2.78%), and 51-60 years old (1.35%), respectively (Table 2). Distribution of intestinal parasites in relation to the methods used, was investigated and found that 4 samples (2.01%) were positive using the direct wet smear method while 10 (5.03%) were positive with the Mini Parasep SF method; the difference was statistically significant (X2-test z-test = 116.506, p-value =0.001). The direct wet smear technique identified 3 different parasites while the Mini

Table 1. Intestinal parasitic infection in stool sample using Mini Parasep SF Faecal Parasite Concentrator

Intestinal Parasites	No. of infection/ examined	% of infection
Opisthorchis viverrini	4/199	2.01
Strongyloides stercoralis	3/199	1.51
Hookworm	1/199	0.5
Taenia sp.	1/199	0.5
Entamoeba coli	1/199	0.5
Total	10/199	5.03

Table 2. Characteristic baseline and *Opisthorchis viverrini* infection using Mini Parasep SF Faecal Parasite Concentrator

Characteristic Data	No. of examined	No. of infection	% of infection
Gender			
Male	118	1	0.85
Female	81	3	3.7
Age			
30-40	18	0	0
41-50	61	2	3.28
51-60	74	1	1.35
61-70	36	1	2.78
>70	10	0	0
Total	199	4	2.01

Table 3. Distribution of Intestinal Parasites in Relation to the Methods Used (n=199)

Intestinal Parasites	Number (%) Positive	
	Direct wet smear	Mini Parasep SF
Opisthorchis viverrini	1(0.50)	4(2.01)
Strongyloides stercoralis	2(1.01)	3(1.51)
Hookworm	0(0)	1(0.50)
Taenia sp.	1(0.50)	1(0.50)
Entamoeba coli	0(0)	1(0.50)
Total	4(2.01)	10(5.03)

*X^2-test = 116.506, p-value =0.001

Parasep SF method identified 5 different parasites. With the direct wet smear technique, the following parasites were identified - *O. viverrini* 1 (0.50%), *S. stercoralis* 2 (1.01%), and *Taenia spp* 1 (0.50%). With the Mini Parasep SF technique, the following parasites were detected - *O. viverrini* 4 (2.01%), *S. stercoralis* 3 (1.51%), *Hookworm* 1 (0.50%), *Taenia spp* 1 (0.50%), and intestinal protozoan; *E. coli* 1 (0.50%) (Table 3).

Processing times and test turnaround times were investigated. Both the Parasep method and conventional direct wet smear method were conducted on the 199 samples. Processing times were recorded by the independent observers and found that mean time for processing using the Parasep system was considerably higher at 6.03 min/sample when compared to the conventional direct wet smear method at 0.3 min/sample. Cost per test was calculated for conventional stool by direct wet smear method, and for the Parasep test; with conventional direct wet smear method costing less than the Parasep method at USD 0.74/sample versus USD 1.47/sample.

Discussion

The efficacy of Mini Parasep, a new faecal Parasite Concentrator developed by the company DiaSys Europe Limited (formerly Intersep Ltd). Here we describe our experience with the system in diagnosing *Opisthorchis viverrini*. Recent data indicates that Mini Parasep SF method is useful technique for *O. viverrini* detection in the routine laboratory. Out of the 199 samples examined, 10 samples (5.03%) were positive with intestinal parasite including *O. viverrini* (2.01%), and followed by S. stercoralis (1.51%), and each 0.50% of *Hookworm*, *Taenia spp*., and *E. coli*. *O. viverrini* infection using Mini Parasep SF method, was characterized with gender and age group, and found that female (3.70%) was slightly higher than male (0.85%); but the difference was not statistically significant (p-value >0.05). The majorities of *O. viverrini* infection, were found age group 41-50 years old (3.28%), and followed by age group 61-70 years old (2.78%), and 51-60 years old (1.35%), respectively. This figures is similar to that previously study a provincial wide of Nakhon ratchasima province in 2012, a cross-sectional survey was conducted during a one year period from October 2010 to September 2011, the infection was determined using a modified Kato's thick smear technique. A total of 1,168 stool samples were obtained from 516 males and 652 females, aged 5-90 years. Stool examination showed that 2.48% were infected with *O. viverrini*. Males were slightly more likely to be infected than females, but the different was not statistically significant (Kaewpitoon et al., 2012). Recent result is confirmed that *O. viverrini* still found in this province and using Mini Parasep SF method is useful for routine laboratory.

Out of 4 samples (2.01%) were positive using the direct wet smear method while 10 (5.03%) were positive with the Mini Parasep SF method; the difference was statistically significant (X^2-test = 116.506, p-value=0.001). The direct wet smear technique identified 3 different parasites while the Mini Parasep SF method identified 5 different parasites. This significant result is similar to other studies. The ether-based concentration, the Parasep Solvent Free (SF), the McMaster and the FLOTAC techniques were compared based on both validity and feasibility for the detection of Trichuris eggs in 100 fecal samples of nonhuman primates. Trichuris eggs were found in 47% of the samples. FLOTAC was the most sensitive technique (100%), followed by the Parasep SF (83.0% [95% confidence interval (CI): 82.4-83.6%]) and the ether-based concentration technique (76.6% [95% CI: 75.8-77.3%]). McMaster was the least sensitive (61.7% [95% CI: 60.7-62.6%]) and failed to detect low FEC. The quantitative comparison revealed a positive correlation between the four techniques (Rs=0.85-0.93; p-value = 0.0001) (Levecke et al., 2009). Furthermore, the evaluated alongside the modified Formol ether concentration and direct smear techniques using 120 stool samples in Calabar, Nigeria between May and June, 2011. Of the three methods, FECT was most efficient in the detection of intestinal parasite (57.5%) and was closely followed by the Min Parasep method (50.0%). There was no statistically significant difference at the rate which the two methods detected intestinal parasitic infections (P>0.05). On the contrary, the Mini Parasep and the FECT methods detected more intestinal infections than the direct smear (28.3%). The difference in both cases were statistically significant (P<0.05). Both the Min Parasep and the FECT detected 30.2% and 40.7% respectively of infections that were negative with the direct smear. The difference in the detection rate of infection by the two methods was not statistically significant (P>0.05). All the samples that were positive by direct smear for intestinal parasites were also positive by the Mini Parasep and FECT. The FECT was more efficient in detecting helminth infections while the Mini Parasep performed better in detecting protozoan infection. The Mini Parasep was simple, user-friendly and involves working in an enclosed system with little or no danger of acquiring infection while the FECT is more labour-intensive with potent danger of acquiring infection and outbreak of fire because of the use of ether in an "open" system. The result of this study has confirmed that the Mini Parasep is efficient for the detection of intestinal parasites. Researchers recommend the adoption of the Mini Parasep method because of its simple and safe technology and efficacy, since most of our laboratories are not currently using the FECT for the diagnosis of intestinal parasites (Useh et al., 2011). In addition, Zeeshan et al. (2011) used the Parasep O and P filter concentrator tubes (manufactured by DiaSys Ltd, Berkshire, England. Product Code 146000) along with direct microscopic techniques and found that Parasep filters enhanced the ability to detect intestinal parasites that would have been missed on routine microscopy. Furthermore, they found the Parasep filter concentration method to be easy, cost-effective and reliable for routine stool examinations.

Recently, mean time for processing using the Parasep system was considerably lower at 6.03 min/sample when compared to the conventional direct wet smear method at 0.3 min/sample. While, Zeeshan et al. (2011) indicated that the comparison the Parasep method and conventional formal-ether sedimentation methods were conducted

on the 25 samples negative by direct microscopy and processing times were recorded by an independent observer. Mean time for processing using the Parasep system was considerably lower at 6 min/sample when compared to the conventional formal-ether sedimentation method at 12 min/sample. Cost per test was calculated for conventional stool by direct wet smear method, and for the Parasep test; with conventional direct wet smear method costing less than the Parasep method at USD 0.74/sample versus USD 1.47/sample. This results are higher than Zeeshan et al. (2011) that cost per test was USD 0.30/sample by formal-ether method, and USD 0.9/sample for the Parasep test.

In conclusion, *O. viverrini* is still found in Nakhon Ratchasima province particularly pateints who underwent hospital. Parasep concentration test is useful in the routine laboratory, increase in the yield of parasites as compared to direct microscopy, processing time and cost. However, the efficacy of Mini Parasep SF method is need required more data regarding to *O. viverrini* detection with the comparison between the other known concentration methods mainly modified Kato Katz thick smear, modified formalin ethyl-acetate concentration technique.

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References

Chaiputcha K, Promthet S, Bradshaw P (2015). Prevalence and risk factors for infection by *Opisthorchis viverrini* in an urban area of Mahasarakham province, northeast Thailand. *Asian Pac J Cancer Prev*, **16**, 4173-6.

Chudthaisong N, Promthet S, Bradshaw P (2015). Risk factors for *Opisthorchis viverrini* infection in Nong Khai province, Thailand. *Asian Pac J Cancer Prev*, **16**, 4593-6.

Harinasuta C, Vajrasthira S (1960). Opisthorchiasis in Thailand. *Am J Trop Med Hyg*, **54**, 100-5.

Harinasuta T, Riganti M, Bunnag D (1984). *Opisthorchis viverrini* infection: pathogenesis and clinical features. *Arzneimittelforschung*, **34**, 1167-9.

IARC. (1994). Infection with liver flukes (*Opisthorchis viverrini*, Opisthorchis felineus and Clonrochis sinensis). *IARC Monogr Eval Carcinog Risks Hum*, **61**, 121-75.

Jongsuksuntigul P, Imsomboon T (2003). Opisthorchiasis control in Thailand. *Acta Trop*, **88**, 229-32.

Kaewpitoon N, Kaewpitoon SJ, Pengsaa P (2008). Opisthorchiasis in Thailand: review and current status. *World J Gastroenterol*, **14**, 2297-302.

Kaewpitoon SJ, Rujirakul R, Ueng-Arporn N, et al (2012). Community-based cross-sectional study of carcinogenic human liver fluke in elderly from Surin province, Thailand. *Asian Pac J Cancer Prev*, **13**, 4285-8.

Kaewpitoon SJ, Rujirakul R, Kaewpitoon N (2012). Prevalence of *Opisthorchis viverrini* infection in Nakhon Ratchasima province, Northeast Thailand. *Asian Pac J Cancer Prev*, **13**, 5245-9.

Levecke B, De Wilde N, Vandenhoute E, et al (2009). Field validity and feasibility of four techniques for the detection of trichuris in simians: a model for monitoring drug efficacy in public Health? *PLoS Negl Trop Dis*, **3**, 366.

Saez AC, Manser MM, Andrews N, et al (2011). Comparison between the midi parasep and midi parasep solvent free (SF) faecal parasite concentrators. J Clin Pathol, **64**, 901-4.

Saengsawang P, Promthet S, Bradshaw P (2013). Infection with *Opisthorchis viverrini* and use of praziquantel among a working-age population in northeast Thailand. *Asian Pac J Cancer Prev*, **14**, 2963-6.

Sithithaworn P, Pipitgool V, Srisawangwong T, et al (1997). Seasonal variation of *Opisthorchis viverrini* infection in cyprinoid fish in north-east Thailand: implications for parasite control and food safety. *Bull WHO*, **75**, 125-31.

Sithithaworn P, Andrews RH, Nguyen VD, et al (2012). The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitol International*, **61**, 10-6.

Sripa B, Kaewkes S, Intapan PM, et al (2010). Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. *Adv Parasitol*, **72**, 305-50.

Sripa B, Pairojkul C (2008). Cholangiocarcinoma: lessons from Thailand. *Curr Opin Gastroenterol*, **24**, 349-56.

Suwannahitatorn P, Klomjit S, Naaglor T, et al (2013). A follow-up study of *Opisthorchis viverrini* infection after the implementation of control program in a rural community, central Thailand. *Parasit Vectors*, **6**, 188.

Thamavit W, Bhamarapravati N, Sahaphong S, et al (1978). Effects of dimethylnitrosamine on induction of cholangiocarcinoma in *Opisthorchis viverrini*-infected Syrian golden hamsters. *Cancer Res*, **38**, 4634-9.

Useh MF, Asuquo AE, Otu-Bassey IB, OL Ubi (2011). Evaluation of the efficacy of the mini parasep sf faecal parasite concentrator; a new technique against the direct smear and formol ether concentration technique for the detection of intestinal parasites in Stool. *J Med Lab Sci*, **20**, 37-43.

Zeeshan M, Zafar A, Saeed Z, et al (2011). Use of Parasep filter fecal concentrator tubes" for the detection of intestinal parasites in stool samples under routine conditions. *Indian J Pathol Microbiol*, **54**, 121-3.

Yeoh KW, Promthet S, Sithithaworn P, et al (2015). Re-examination of *Opisthorchis viverrini* infection in northeast Thailand. *Asian Pac J Cancer Prev*, **16**, 3413-8.