PREVALENCE OF *OPISTHORCHIS VIVERRINI* INFECTION AMONG VILLAGERS HARBORING *OPISTHORCHIS*-LIKE EGGS

Kabkaew L Sukontason[1], Kom Sukontason[1], Somsak Piangjai[1],
Swangjai Pungpak[2] and Prayong Radomyos[2]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand;
[2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. The precise occurrence of *Opisthorchis viverrini* infection in humans, who were positive for *Opisthorchis*-like eggs in a stool examination, was determined using the potassium permanganate staining method. In the 68 specimens examined, there were more individual *O. viverrini* eggs (38.24%) than singular *Haplorchis taichui* eggs (29.41%). One-fourth of the total specimens contained a mixed infection of *O. viverrini* and *H. taichui* eggs. The median ratio of *O. viverrini*: *H. taichui* eggs in mixed infection cases was 2.29 (min = 1, max = 17.5). It is suggested that chemotherapy with praziquentel treatment should be given to patients who are positive for *O. viverrini*-like eggs.

INTRODUCTION

Opisthorchis viverrini (liver fluke; Opisthorchiidae) and *Haplorchis taichui* (minute intestinal fluke; Heterophyidae) are the most common flukes found in humans as well as in fish that are the second intermediate host of the parasite in northern and northeastern Thailand (Pungpak *et al*, 1997;1998; Radomyos *et al*, 1994;1998; Srisawangwong *et al*, 1997; Waikagul, 1998; Sukontason *et al*, 1999a). The former species is a parasite of medical importance that causes a varied degree of such conditions as cholangitis, choletithiasis and cholecystitis (Pungpak *et al*, 1985), and bile duct cancer (Parkin *et al*, 1991). There is no report of the latter species having any clinical importance. To date, the prevalence of *H. taichui* in fecal specimens after anthelminthic therapy has been reported as higher than that of *O. viverrini* in northern Thai people (Radomyos *et al*, 1994;1998).

Humans can serve as the definitive host of both *O. viverrini* and *H. taichui*. Since the eggs of both parasites are passed in feces, stool examination was commonly used for detecting infections. The formalin-ether sedimentation method has been used routinely for the examinations. The eggs of *O. viverrini* and *H. taichui*, including other minute intestinal flukes, are very similar in both size and shape, and indistinguishable from one another when using a routine stool examination with light microscope (Ditrich *et al*, 1990; 1992; Kaewkes *et al*, 1991; Tesana *et al*, 1991). The presence of *O. viverrini*-like eggs in a stool examination leads to a question of accuracy in the prevalence of *O. viverrini* infection. Recently, the staining technique using potassium permanganate solution was developed to solve this problem (Sukontason *et al*, 1999b). The objective of this study was, therefore, to determine the existence of *O. viverrini* infection in humans positive for *Opisthorchis*-like eggs in their stool by using the potassium permanganate staining technique.

Correspondence: Dr Kabkaew L Sukontason.
E-mail: klikitvo@mail.med.cmu.ac.th

MATERIALS AND METHODS

Fecal specimens were obtained from people residing in two villages (Huay Hug and Mae Luang Nuer) of Doi Sa Ket district, Chiang Mai Province in northern Thailand, 700 km north of Bangkok. These villages are adjacent to Mae Guang Udomthara Dam, a man-made reservoir of the Mae Guang River. Both villages are about 30 km east of Chiang Mai city center. Sixty-eight specimens with *O. viverrini*-like eggs were found in stools by using the formalin-ether sedimentation method (Ritchie, 1984). The potassium permanganate staining method with slight modification (Sukontason *et al*, 1999b) was performed for an appearance of a distinct musk-melon ridge pattern on the *O. viverrini* egg surface. Briefly, the sediment resulting from the formalin-ether sedimentation method was added with 1 ml of diethyl ether and shaken vigorously for 1 minute. The upper layer of ether was then discarded. A few drops of sediment were transferred, using a transparent pipette, onto a glass slide before one drop of 1% potassium permanganate solution was added prior to covering with a coverslip. The eggs of the parasite were examined under a light microscope at a magnification of 100 x and 400 x.

Every egg that appeared was assessed regarding its individual length, width, morphology (*O. viverrini*-like or *H. taichui*-like), presence of knob, shoulder and

distinct surface pattern. Eggs with a distinct musk-melon ridge pattern and those with a light striae pattern were diagnosed as *O. viverrini* and *H. taichui*, respectively.

As for the statistical analysis, the width and length of *O. viverrini* and *H. taichui* eggs were compared using the Student-*t* test. The presence of knob, shoulder and unique bulb-like feature on *O. viverrini* and *H. taichui* eggs was analyzed using the Chi square test.

RESULTS

Eight hundred and sixty-nine *O. viverrini*-like eggs were detected from the 68 specimens collected. When observed under a magnification of 100 x, the eggs of *O. viverrini* and minute intestinal flukes were easily seen due to a dark brown eggshell (with a relatively green inner rim) and its light brown background. However, the differentiation of eggs species could not be determined. At the magnification of 400 x in conjunction with an alteration of the diaphragm, a musk-melon ridge pattern on the *O. viverrini* egg surface was clearly seen, while a light striae pattern was present on the *H. taichui* egg surface.

Of the 68 people examined, 69.12% (47/68) had *O. viverrini* egg, 38.24% (26/68) had pure *O. viverrini* eggs, and another 30.88% (21/68) had a mixture of *O. viverrini* and minute intestinal fluke eggs. Pure *H. taichui* eggs were found in only 29.41% (20/68) (Table 1). The eggs of unknown species, probably of those other minute intestinal flukes, were found in 5 specimens.

In the mixed infection cases, the median ratio of *O. viverrini* with *H. taichui* eggs was 2.29 (min = 1, max = 17.5; n = 19) (Table 2).

O. viverrini eggs were significantly longer and wider than *H. taichui* eggs (Student-*t* test = 1.99 and 9.05; p = 0.048 and < 0.01, respectively) (Table 3). The presence of knob and shoulder were significantly higher in *O. viverrini* eggs than *H. taichui* eggs (χ^2 = 26.05 and 398.25, respectively; $p < 0.001$). The unique bulb-like feature of *O. viverrini* eggs was significantly higher in *O. viverrini* eggs ($\chi^2 = 508.91$; $p < 0.001$).

Table 1
Number of *Opisthorchis viverrini* and *Haplorchis taichui* cases positive for *O. viverrini*-like eggs re-examined with potassium permanganate staining.

Species	No. infected case	(%)
O. viverrini	26	(38.24)
H. taichui	20	(29.41)
Unknown	1	(1.47)
O. viverrini + *H. taichui*	17	(25.00)
O. viverrini + Unknown	2	(2.94)
O. viverrini + *H. taichui* + Unknown	2	(2.94)
Total	68	(100.00)

Table 2
Ratio in the number of *Opisthorchis viverrini*: *Haplorchis taichui* in each infected case previously positive for *O. viverrini*-like eggs.

	Ratio	
Statistic features	OV:HT	OV:UNK
Number of cases	19	4
Median	2.3	7.5
Minimum	1	1.5
Maximum	17.5	16.0

OV = *O. viverrini*, HT = *H. taichui*, UNK = Unknown

Table 3
Comparative measurement and character of *Opisthorchis viverrini* and *Haplorchis taichui* eggs.

Character of egg	*O. viverrini*	*H. taichui*	p-value
Length (Mean ± SD)[a]	27.2 ± 2.0	26.8 ± 2.3	0.034[b]
	(n = 696)	(n = 164)	
Width (Mean ± SD)[a]	14.9 ± 3.2	13.6 ± 1.1	0.000[b]
	(n = 696)	(n = 164)	
Knob (%)	93.82	80.49	<0.01[c]
	(653/696)	(132/164)	
Shoulder (%)	93.53	23.78	0.000[c]
	(651/696)	(39/164)	
Bulb-like feature (%)	95.55	18.29	0.000[c]
	(665/696)	(30/164)	

[a] Measurements are in micrometers.
[b] Student's *t*-test
[c] Chi-square test

DISCUSSION

In northern Thailand, *H. taichui* was recently found in abundance as a primarily fluke in both humans (Wijit *et al*, 1998; Pungpak *et al*, 1998; Radomyos *et al*, 1998) and fish that are the second intermediate host (Sukontason *et al*, 1999a). This result, however, indicated that *O. viverrini* was still a major fluke that parasitized humans in some areas of Chiang Mai. Autopsies carried out in Chiang Mai revealed adult *O. viverrini* and was supportive evidence of the prevalence of infection, which was 52.7% (68/129) (Wijit *et al*, 1998).

In this study, although the number of cases with pure *O. viverrini* (n = 26) was close to those with pure *H. taichui* (n = 20), the former fluke was more common. Since praziquentel has been proved as effective against both *O. viverrini* and *H. taichui* at the same dosage (40 mg/kg body weight at a single dose) (Pungpak *et al*, 1998), chemotherapy should also be applied to kill both worm species.

The presence of *O. viverrini*-like eggs by stool examination suggested that 69.12% (47/68) were *O. viverrini* cases, and treatment with praziquentel was indicated. Some side effects of praziquentel may occur, but they can be minimized by changing the time of drug intake from the morning to after dinner (Sornmani *et al*, 1984). However, for the specific treatment of *H. taichui*, niclosamide is the drug of choice because of its high efficacy, very low side effects and low cost (Sukontason *et al*, 2000). Moreover, to obtain the precise prevalence of this minute intestinal fluke species only, potassium permanganate staining in stool examinations is recommended.

REFERENCES

Ditrich O, Giboda M, Scholz T, Beer SA. Comparative morphology of eggs of the Haplorchiinae (Trematoda: Heterophyidae) and some other medically important heterophyid and opisthorchiid flukes. *Folia Parasitol* 1992;39:123-32.

Ditrich O, Giboda M, Sterba J. Species determination of eggs of opisthorchiid and heterophyid flukes using scanning electron microscopy. *Angew Parasitol* 1990;31:3-9.

Kaewkes S, Elkins DB, Sithithaworn P, Haswell-Elkins MR. Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and lecithodendriid trematodes. *Southeast Asian J Trop Med Public Health* 1991;22:623-30.

Parkin DM, Srivatanakul P, Khlat M, *et al*. Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. *Int J Cancer* 199;48:323-8.

Pungpak S, Radomyos P, Radomyos B, Schelp FP, Jongsuksuntigul P, Bunnag D. Treatment of *Opisthorchis viverrini* and intestinal fluke infections with praziquentel. *Southeast Asian J Trop Med Public Health* 1998;29:246-9.

Pungpak S, Riganti M, Bunnag D, Harinasuta T. Clinical features in severe opisthorchiasis viverrini. *Southeast Asian J Trop Med Public Health* 1985; 16:405-9.

Pungpak S, Viravan C, Radomyos B, *et al*. *Opisthorchis viverrini* infection in Thailand: studies on the morbidity of the infection and resolution following praziquentel treatment. *Am J Trop Med Hyg* 1997;56:311-4.

Radomyos P, Radomyos B, Tungtrongchitr A. Multi-infection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. *Trop Med Parasitol* 1994;45:133-5.

Radomyos B, Wongsaroj T, Wilairatana P, *et al*. Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Public Health* 1998;29:123-7.

Ritchie LS. An ether sedimentation technique for routine stool examination. *Bull US Army Med Dept* 1984;8:326.

Sornmani S, Schelp FP, Vivatanasesth P, *et al*. A pilot project for controlling *O. viverrini* infection in Nong Wai, northeast Thailand, by applying praziquentel and other measures. *Arzneimittelforschung* 1984;34(9B):1231-4.

Srisawangwong T, Sithithaworn P, Tesana S. Metacercariae isolated from cyprinoid fishes in Khon Kaen district by digestion technic. *Southeast Asian J Trop Med Public Health* 1997;28 (suppl 1):224-6.

Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U. Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao District, Chiang Mai Province, northern Thailand. *Southeast Asian J Trop Med Public Health* 1999a;30:365-70.

Sukontason K, Piangjai S, Sukontason K, Chaithong U. Potassium permanganate staining for differentiation the surface morphology of *Opisthorchis viverrini*, *Haplorchis taichui* and *Phaneropsolus bonnei* eggs. *Southeast Asian J Trop Med Public Health* 1999b;30:371-4.

Sukontason K, Sukontason K, Muangyimpong Y, Piangjai S. Treatment of *Haplorchis taichui* in *Mus musculus* mice. *Exp Parasitol* 2000;94:48-50.

Tesana S, Srisawangwonk T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C. Eggshell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian J Trop Med Public Health* 1991;22:631-6.

Waikagul J. *Opisthorchis viverrini* metacercariae in Thai freshwater fish. *Southeast Asian J Trop Med Public Health* 1998;29:324-6.

Wijit A, You-sukh A, Sribanditmongkol P, Toriyama K, Morakote N. Prevalence of *Opisthorchis viverrini* infection in human autopsy cases in northern Thailand. *Commun Dis J* 1998;24:113-9. (In Thai with English abstract).