# COPRO-DIAGNOSIS OF *HAPLORCHIS TAICHUI* INFECTION USING SEDIMENTATION AND PCR-BASED METHODS

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**Abstract.** Fifty-one human fecal specimens were collected from villagers inhabiting along Mae Kuang River, Ban Sob Tha, Pa Sang District, Lamphun Province, Thailand. By the formalin-ether sedimentation technique (FEST) under a light microscope, eggs of 3 helminth species, *Haplorchis taichui*, *Ascaris lumbricoides* and unidentified hookworm species were detected with prevalences of 22, 14 and 4%, respectively. PCR amplification with *H. taichui* specific primers showed that *H. taichui* specific amplicon 260 bp was generated in all FEST-positive specimens, and also in some FEST negative specimens. This *H. taichui* specific PCR method can be used to detect this parasite in all developmental stages and in both definitive and intermediate hosts, which should be useful in prevention and control programs.

## INTRODUCTION

Helminthiasis is a public health problem in developing countries. In Thailand, opisthorchiasis and heterophyiasis are common trematode infection endemic in northeastern and northern Thailand, respectively (Manning *et al*, 1971; Radomyos *et al*, 1998). *Haplorchis taichui* is a minute intestinal fluke in the family Heterophyidae. Several kinds of mammals including humans, rats, cats, dogs and chicken can serve as definitive hosts. (Pearson, 1964; Pearson and OW-Yang, 1982). Larval stages, miracidium, sporocyst, redia and cercaria, are found in freshwater snails, *Melanoides tuberculata, Stenomelania newcombi* and *Thiara granifera* (Martin, 1958; Noda, 1959), which represent first intermediate hosts. Metacercaria (infective stage) is found mainly in mud carp (*Henicorhynchus siamensis*) (Kumchoo *et al*, 2005), which has a high infection rate and is endemic in nortern Thailand. People acquire this parasite by consuming undercooked foods prepared from fish containing metacercariae. Symptoms of *H. taichui* infection has not been clearly reported. The first clinical report revealed that *H. taichui* was found by autopsy with many embryonated eggs remaining in blood vessel of cardiac muscle (Africa *et al*, 1935). *H. taichui* can be considered as food-borne zoonotic trematode.

In northern Thailand, the prevalence of parasitic infection is still high. A previous report indicated that trematode eggs found in humans were *O. viverrini* and *H. taichui* was not detected (Chiang Mai Provincial Public Health Office, 2001). However, a subsequent report showed that *H. taichui* was found to infect villagers inhabiting Mae Ping River, Chom Thong District, Chiang Mai

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Province (Chuboon and Wongsawad, 2003). Several survey data showed that no *O. viverrini* were found in secondary intermediate hosts in northern region, whereas most of metacercariae found belong to heterophyid trematodes especially the minute intestinal fluke, *H. taichui* (Wongsawad *et al*, 2004; Kumchoo *et al*, 2005; Boonchot and Wongswad, 2005). Hitherto, the actual trematode species endemic in this area is still not clearly determined.

DNA-based approaches are the most efficient and accurate tools used for detection and identification of organisms and for screening of genetic variations among any population. High annealing temperature-random amplified polymorphic DNA (HAT-RAPD) analysis is one of these approaches. It was developed by Anuntalabhochai *et al* (2000) and has been adapted for use in a trematode, *Stellantchasmus falcatus*, identification (Sripalwit *et al*, 2003), analysis of DNA quality and quantity of some trematodes (Wongsawad *et al*, 2006; Wongsawad and Wongsawad, 2007) and intraspecific variation analysis of three paramphistome flukes in Thailand (Sripalwit *et al*, 2007).

This study was aimed to identify the species of trematode eggs found in fecal specimens from villagers inhabiting Mae Kuang River, Lamphun Province using HAT-RAPD method and formalin-ether sedimentation technique. Since several reports have revealed that both *H. taichui* and *O. viverrini* are found in the same fish host (Wongratanacheewin *et al*, 2001). Therefore, possible mixed infection with *O. viverrini* was also determined using HAT-RAPD PCR.

## MATERIALS AND METHODS

### Formalin-ether sedimentation technique (FEST)

Fifty-one fecal specimens were collected from villagers inhabiting Mae Kuang River, Lamphun Province, Thailand. One portion was subjected to DNA extraction and the rest was used for FEST by homogenizing with 10% formalin and filtering through two layers of gauze. The filtrate was subjected to FEST (Chai *et al*, 1998).

### Genomic DNA extraction

Genomic DNA of fecal specimens was extracted and purified using Dneasy Tissue Kit (QIAGEN) according to instructions of the manufacturer. Adult worms of *H. taichui* and *O. viverrini* were also subjected to DNA preparation. Extracted genomic DNA was diluted to a working concentration of 30 ng/µl and stored at -20ºC until use.

### *H. taichui* specific PCR amplification

PCR amplification for detection of *H. taichui* was performed using *H. taichui* specific primers, Hapt-F and Hapt-R, as desribed previously (Wongsawad *et al*, 2008).

### HAT-RAPD PCR for detection of contamination and/or mixed infection

HAT–RAPD PCR was performed to test for contamination/mixed infection with liver fluke (*O. viverrini*) using OPP-11, random 10-mer primer (Operon technology, USA) as previously described (Wongsawad *et al*, 2008).

## RESULTS

### FEST examination of fecal specimens

FEST showed that 3 egg types of helminth parasites, namely, *H. taichui*, *Strongyloides stercoralis* and an unidentified hookworm species with prevalences of 22, 14 and 4%, respectively (Table 1).

### PCR detection of *H. taichui*

A 260 bp amplicon was generated in all fecal specimens in which trematode eggs were found based on FEST (Fig 1). The 260 bp amplicons were obtained in FEST negative specimens.

Table 1
Prevalence of parasitic infections among villagers inhabiting Mae Kuang River, Pa Sang district, Lamphun Province using FEST.

| Parasite egg | No. infected /examined | % prevalence |
|---|---|---|
| *Haplochis taichui* | 11/51 | 22 |
| *Strongyloides stercoralis* | 7/51 | 14 |
| Hookworm[a] | 2/51 | 4 |

[a]Species of hookworm was not determined.

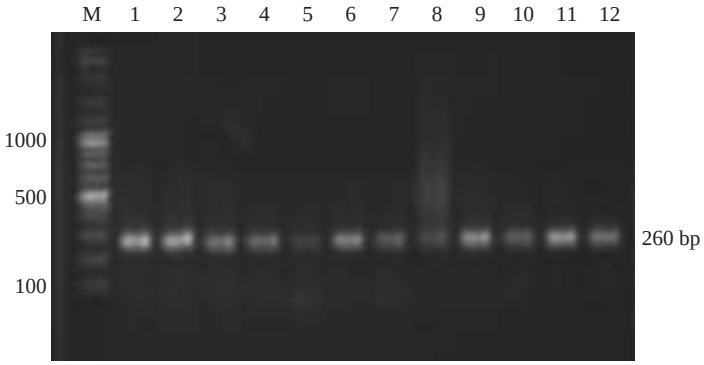


Fig 1–*H. taichui* specific PCR amplification. The 260 bp *H. taichui* specific amplicon was generated in all fecal specimens in which trematode eggs were found. (Lane M: 100 bp marker; lane 1: *H. taichui* positive control; lanes 2-12 : *H. taichui* positive specimens from fecal examination.

**Contamination/mixed infection using HAT-RAPD PCR**

No *O. viverrini* HAT-RAPD profile was observed among *H. taichui*-positive fecal specimens (Fig 2).

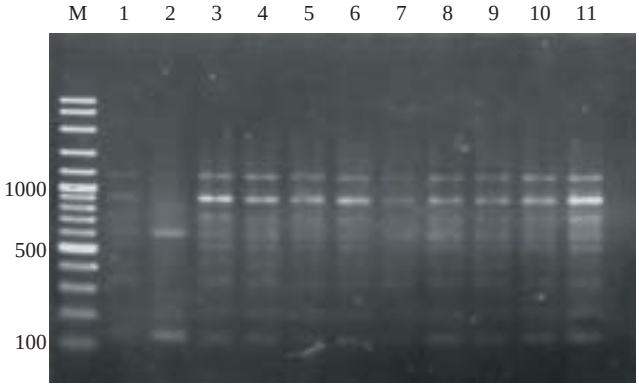


Fig 2–HAT-RAPD PCR was conducted as described in Materials and Methods, demonstrating that there was no contamination/ mixed infection of *O. viverrini* among *H. taichui* positive specimens from fecal examination. Lane M: 100 bp marker; lane 1: *H. taichui* positive control; lane 2 : *O. viverrini* positive control; lanes 3-11 : *H. taichui* positive specimens.

## DISCUSSION

This study shows that trematode infecting humans in Chiang Mai Province was identified as *H. taichui*. This is in contrast to a previous report indicating that trematode eggs found in human stool are *O. viverrini* and none of *H. taichui* (Chiang Mai provincial Public Health Office, 2001). Heterophyiasis, mostly *H. taichui*, was the common trematode infection endemic in northern Thailand (Radomyos *et al*, 1998) and a subsequent report revealed that *H. taichui* was found to infect villagers inhabiting Mae Ping River, Chom Thong District, Chiang Mai Province (Chuboon and Wongsawad, 2003). Several survey data showed that no *O. viverrini* were found to be infecting secondary intermediate hosts in northern region, whereas most of the metacercariae found were identified as heterophyid trematode with *H. taichui* showing highest prevalence and intensity (Wongsawad *et al*, 2004; Boonchot and Wongsawad, 2005; Kumchoo *et al*, 2005). Eggs of minute intestinal flukes are similar in shape and size to that of a liver fluke, *O. viverrini* (Tesana *et al*, 1991; Radomyos *et al*, 1998), which makes it difficult to identify the actual species by using only egg morphology.

Moreover, the 260 bp *H. taichui* specific amplicon was obtained only in *H. taichui* positive samples and there was no mixed infection with *O. viverrini* when using HAT-RAPD PCR. Interestingly, the 260 bp amplicon was also generated in negative specimens based on FEST. The advantages of copro-diagnosis using a combination of FEST and PCR methods were the significantly decrease in cost and time consumption, while it is accurate.

Two nematodes, *S. stercolaris* and hookworm, were also found in this study, as in the report of Chuboon and Wongsawad (2003). However, in this study, hookworm eggs were observed but not in the report of Chuboon and Wongsawad (2003). Different environmental conditions affecting *S. stercolaris* and hookworms, which are members of soil transmitted helminths whose infection rates are dependent on humidity, precipitation and soil composition.

In summary, our research demonstrated that PCR methods showed a high sensitivity and specificity for the detection of *H. taichui* DNA in fecal specimens. These techniques should be applied for the detection of this parasite in all larval stages in both definitive and intermediate hosts, which will be useful in prevention and epidemiological control programs.

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## REFERENCES

Africa CM, De Leon W, Garcia EY. Heterophyidiasis II. Ova in sclerosed mitral valves and other chronic lesions in the myocardium. *J Phil Med Assoc* 1935; 15: 583.

Anuntalabhochai S, Chiangda J, Chandet R, Apawat P. Genetic diversity within Lychee (*Litchi chinensis* Soonn.) based on RAPD analysis [Abstract]. Caihns, Australia: International Symposium on Tropical and Subtropical Fruit, 26 November-1 December 2000: 45.

Boonchot K, Wongsawad C. A survey of helminthes in cyprinoid fish from the Mae Ngad Somboonchon Reservoir, Chaing Mai Province, Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 103-7.

Chai JY, Song TE, Han ET, *et al*. Two endemic foci of heterophyid and other intestinal fluke infection in southern and western coastal areas in Korea. *Korean J Parasitol* 1998; 36: 155-61.

Chiang Mai Provincial Public Health Office. Annual report: helminthiasis. 2001: 19-24 (in Thai).

Chuboon S, Wongsawad C. Helminthic infection of human in Ban Thalook, Tumbol, Sobtia, Chom Thong District, Chaing Mai Province [Abstract]. Bangkok, Thailand: The 4th Seminar on Food and Water-borne Parasitic Zoonoses, and 2nd International Meeting on Gnathostomiasis and Joint International Tropical Medicine Meeting, 2003: 205.

Kumchoo K, Wongsawad C, Chai JY, Vanittanakom P, Rojanapaibul A. High prevalence of *Haplorchis taichui* metacercariae in cyprinoid fish from Chaing Mai Province, Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 451-5.

Manning GS, Lertprasert P, Watanasirmkit K, Chetty C. A description of newly discovered intesfinal parasites endemic to northeastern Thailand. *J Med Assoc Thai* 1971; 54: 466-75.

Martin WE. The life histories of some Hawaiian heterophyid trematodes. *J Parasitol* 1958; 44: 305-23.

Noda K. The larval development of *Stellantchasmus falcatus* (Trematoda: Heterophyidae) in the first intetmediate host. *J Parasitol* 1959; 45: 635-42.

Pearson JC. A revision of the subfamily Haplorchinae Looss, 1988 (Trematoda : Heterophyidae). *Parasitology* 1964; 54: 601-76.

Pearson JC, OW-Yang CK. New species of *Haplorchis* from Southeast Asia, together with keys to the *Haplorchis*-grop of Heterophyid trematodes of the region. *Southeast Asian J Trop Med Publ Health* 1982; 13: 35-60.

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.

Sripalwit P,Wongsawad C, Chai JY, Rojanapaibul A, Anantalabhochai S. Development of Hat-RADP technique for the identification of *Stellantchasmus falcatus* [Abstract]. Bangkok: 4th Seminar on Food and Water borne Parasitic Zoonoses, 2nd International Meeting on Gnathostomiasis and Joint International Tropical Medicine Meeting, 2003: 289.

Sripalwit P, Wongsawad C, Wongsawad P, Anuntalaphochai S. High annealing temperature – random amplified polymorphic DNA (HAT-RAPD) analysis of three paramhistome flukes from Thailand. *Exp Parasitol* 2007; 115: 98-102.

Tesana S, Srisawangwonk T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C. Egg-shell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 324-6.

Wongratanacheewin S, Phumidonming W, Sermsawan RW, Maleewong W. Development of a PCR- based method for the detection of *Opisthorchis viverrini* in experimentally infected hamster. *J Parasitol* 2001; 122: 175-80.

Wongratanacheewin S, Phumidonming W, Sermsawan RW, Pipitgool V, Maleewong W. Detection of *Opisthorchis viverrini* in human stool specimens by PCR. *J Clin Microbiol* 2002; 40: 3879-80.

Wongsawad C, Rojtinnakorn J, Wongsawad P, Rojanapaibul A. Helminths of vertebrates from Maesa stream. *Southeast Asian J Trop Med Public Health* 2004; 35(suppl 1): 140-6.

Wongsawad C, Wongsawad P, Chai JY, Paratasilpin T, Anantalaphochai S. DNA quantities and qualities from various stages of some trematodes using optical and HAT-RAPD methods. *Southeast Asian J Trop Med Public Health* 2006; 37(suppl 3): 62-8.

Wongsawad P, Wongsawad C. Application of HAT-RAPD markers as the diagnostic tool on minute intestinal fluke, *Haplorchis taichui* identification [Abstract]. Bangkok: Joint International Tropical Medicine Meeting, 2008: 138 p.

Wongsawad P, Wongsawad C. DNA fringerprints of some heterophyid trematodes from adult and metacercarial stages in Thailand. *Southeast Asian J Trop Med Publ Health* 2007; 38: 110-3.