

INFECTION DYNAMICS AND MOLECULAR IDENTIFICATION OF METACERCARIAE IN CYPRINOIDS FROM CHIANG MAI AND SAKON NAKHON PROVINCES

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Abstract. The infection dynamics of metacercariae were assessed in cyprinoid fish (cyprinoids) from Chiang Mai and Sakon Nakhon Provinces, Thailand, during October 2008 to September 2009. The samples were collected during 3 seasons from rivers and local markets. Metacercarial infection was determined by acid-pepsin digestion and confirmed using HAT-RAPD PCR method. Thirteen and 16 species of cyprinoids were collected from Chiang Mai and Sakon Nakhon with overall prevalences of metacercarial infection of 84.5 and 47.6%, respectively. *Haplorchis taichui*, *Haplorchoides* sp, and *Centrocestus caninus* were found in Chiang Mai and 4 species of metacercariae: *H. taichui*, *Haplorchoides* sp, *O. viverrini* and an unknown trematode species in Sakon Nakhon. *H. taichui* and *Haplorchoides* sp metacercariae in 3 species of cyprinoids (*Henicorhynchus siamensis*, *Cyclocheilichthys armatus*, *Amblyrhynchichthy truncatus*) had the highest prevalence (100%) in Chiang Mai, while the highest prevalence (100%) of metacercaria in Sakon Nakhon was *Haplorchoides* sp in 1 species of cyprinoids (*Cyclocheilichthys armatus*). The overall prevalence from Chiang Mai Province was highest in the rainy season (95.6%), lower in the hot-dry season (88.1%) and lowest in the cool season (72.5%). In Sakon Nakhon Province the highest prevalence was in the hot-dry season (52.7%), and lower in the rainy and cool season, 44.4% and 43.5%, respectively. The HAT-RAPD profiles confirmed the identity of metacercariae and adult stage of *H. taichui*, *Haplorchoides* sp, *C. caninus* and *O. viverrini*.

Keywords: metacercariae, cyprinoids, infection dynamic, molecular identification

INTRODUCTION

The infective metacercarial stage is found in cyprinoids with high prevalence in Thailand (Wongsawad *et al*, 2000; Kumchoo *et al*, 2003, 2005). The heterophyid trematodes are commonly distributed in northern and northeastern regions caus-

ing a health problem. These worms are found in various definitive hosts such as birds, cat, dog, rat and man through eating uncooked food from cyprinoids. The liver fluke, *O. viverrini* (Opisthorchiidae), is also shown highly prevalent in north-eastern region (Sithithaworn and Elkins, 2003) and at least 15 species of cyprinoids serve as intermediate hosts of *O. viverrini* metacercariae (Kaewkes, 2003). Infection with the other heterophyid, a minute intestinal fluke, is highly prevalent in northern region (Sukontason *et al*, 1999; Boonchot and Wongsawad, 2005).

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The metacercariae of *C. caninus*, *H. taichui* and *Stellantchasmus falcatus* are found in cyprinoid fish collected from Mae Sa stream, Mae Rim District, Chiang Mai Province (Wongsawad *et al*, 2000). Sukontason *et al* (1999) reported the presence of *Centrocestus* metacercariae in 6 species of cyprinoid fish in northern Thailand. Recently metacercariae of *H. taichui* and *Haplorchoides* sp were reported in freshwater fish from Mae Ngad and Mae Kuang Udomtara reservoirs, Chiang Mai Province (Nithikathkul and Wongsawad, 2008).

Although heterophyids can be distinguished at both metacercariae and adult stages, using morphological criteria, the taxonomy of heterophyids in general is difficult and complicated (Pearson and Ow-Yang, 1982). PCR assays have been developed for identifying these trematodes. Sripalwit and Wongsawad (2007) used high annealing temperature-random amplified polymorphic DNA (HAT-RAPD) to identify three paramphistome flukes in Thailand. Wongsawad *et al* (2009) have reported the detection of a minute intestinal fluke, *H. taichui* using HAT-RAPD. Maleewong *et al* (2003) used DNA profiling to study *O. viverrini* from north-eastern region of Thailand. Sripalwit and Wongsawad (2007) used HAT-RAPD technique for identification of *S. falcatus*.

This study investigated the seasonal variation of metacercarial infection in cyprinoids from Chiang Mai and Sakon Nakhon Provinces to compare metacercarial occurrence in north and northeastern Thailand. HAT-RAPD was used to confirm the species identified.

MATERIALS AND METHODS

Metacercaria detection

Cyprinoid fish were collected from

rivers and local markets in Chiang Mai and Sakon Nakhon Provinces, Thailand, during October 2008 to September 2009. Metacercariae were removed using 1% pepsin solution (99 ml of 0.85% sodium chloride solution, 1 g of pepsin and 1 ml of concentrated hydrochloric acid) at 37°C for 1½ hours. The digested materials were filtered through grading sieves to remove large particles and rinsed twice with normal saline solution. Metacercariae were collected and counted under a stereomicroscope. They were identified based on morphological observation and the data were analyzed for prevalence.

Molecular identification

Metacercariae and the adult stage of *H. taichui*, *Haplorchoides* sp, *C. caninus* and *O. viverrini* were identified by HAT-RAPD PCR method. Adult worms of *H. taichui*, *C. caninus* and *O. viverrini* were obtained from experimentally infected hamsters, and *Haplorchoides* sp was removed from the intestine of *Hemibagrus nemurus*. Genomic DNA of adult worms and metacercariae from cyprinoid fish were extracted and purified using a commercial tissue DNA extraction kit (Vivantis, Oceanside, CA). Genomic DNA was diluted to a working concentration of 50 ng/µl and stored at -20°C until used. Arbitrary primers OPN09 (Operon Technology, Alameda, CA) were used for HAT-RAPD PCR and the reaction was carried out in a final volume of 20 µl in MyCycler™ Thermocycler (Bio-RAD, Hercules, CA). The PCR thermal cycling conditions were as follows: 1 cycle at 95°C for 5 minutes; 30 cycles at 95°C for 45 seconds, 48°C for 45 seconds, and 72°C for 1 minute; and 1 cycle at 72°C for 7 minutes. HAT-RAPD final amplicons were separated by 1.4% TBE agarose gel electrophoresis, stained with ethidium bromide and photographed using a Kodak Digital Camera Gel Logic 100.

RESULTS

Thirteen species of cyprinoids were collected from Chiang Mai and 16 species from Sakon Nakhon Provinces, with metacercarial infection of 84.5% and 47.6%, respectively. The prevalence in Chiang Mai Province was highest in the rainy season (95.6%), slightly decreased in the hot-dry season (88.2%) and lowest in the cool season (72.5%). In Sakon Nakhon Province, the highest prevalence was in the hot-dry season (52.7%), while in the rainy and cool seasons similar results were found 44.4% and 43.5%, respectively.

In Chiang Mai, 3 species of metacercariae were identified: *H. taichui*, *Haplorchoides* sp, and *C. caninus*. Infection by *H. taichui* and *Haplorchoides* sp metacercariae of 3 species of cyprinoids (*Henicorhynchus siamensis*, *Cyclocheilichthys armatus*, *Amblyrhynchichthys truncatus*) showed the highest prevalence (100%). The prevalence of *H. taichui* metacercariae was highest in the rainy season (79.4%) and decreased in the hot-dry season (76.3%) and the cool season (52.5%). *Haplorchoides* sp infection showed the highest prevalence in the rainy season (77.9%) and also decreased in the hot-dry season (61.0%) and the cool season (37.5%). Infection of *C. caninus* metacercariae had the highest prevalence in the rainy season (4.4%), decreased in the hot-dry season (1.7%) and increased again in the cool season (2.5%).

Four species of metacercariae were found in Sakon Nakhon Province: *H. taichui*, *Haplorchoides* sp, *O. viverrini* and unknown trematode species, with the highest prevalence (100%) of *Haplorchoides* sp in

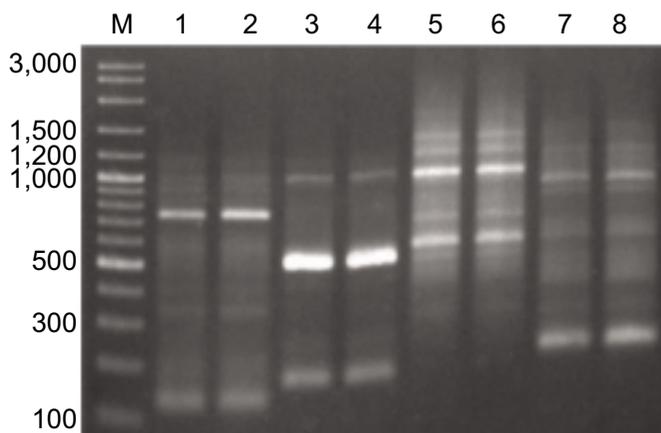


Fig 1—HAT-RAPD profile of adult worms and their metacercarial stage. The profiles were generated using OPN-09 primer (Operon Technology, USA). Lane M, 100 bp molecular markers; 1, *H. taichui* (adult); 2, *H. taichui* (metacercaria); 3, *Haplorchoides* sp (adult); 4, *Haplorchoides* sp (metacercaria); 5, *C. caninus* (adult); 6, *C. caninus* (metacercaria); 7, *O. viverrini* (adult); 8, *O. viverrini* (metacercaria).

1 species of cyprinoids (*Cyclocheilichthys armatus*). The prevalence of *H. taichui* metacercariae in cool and rainy seasons was 2.9% and 1.2%, respectively, whereas no infection was found in the hot-dry season. The prevalence of *Haplorchoides* sp metacercariae was highest in the hot-dry season (51.7%), and decreased in the rainy season (44.4%) and the cool season (30.4%), whereas the prevalences of *O. viverrini* (5.8%) and the unknown species (7.3%) was recorded in only the cool season.

The identification of adult worms and metacercaria were confirmed by HAT-RAPD PCR method. HAT-RAPD profiles of metacercariae were compared with their respective adult stages. *H. taichui* showed 5 polymorphic fragments of 120-1,000 base pair (bp) in size, *Haplorchoides* sp showed 3 fragments of 150-1,000 bp, *C. caninus* 6 fragments of 600-1,400 bp, and *O. viverrini* 4 fragments of 240-1,200 bp (Fig 1).

DISCUSSION

The infection dynamics of metacercariae infection were investigated among cyprinoid fish collected from rivers and local markets during 3 seasons (hot-dry, rainy and cool). Thirteen species of cyprinoids were collected from Chiang Mai and 16 species from Sakon Nakhon, with a lower prevalence of metacercarial infection in the northeast region. Three species of metacercariae were found in Chiang Mai and 4 species including an unknown trematode species in Sakon Nakhon. All metacercariae found are common in northern and northeastern regions. Boonchot and Wongsawad (2005) reported their *C. caninus*, *H. taichui* and *Haplorchoides* sp metacercariae were present in Mae Ngad Somboonchon reservoir, Chiang Mai Province and *H. taichui* and *Haplorchoides* sp metacercariae were found in fish from Mae Kuang Udomtara Dam (Nithikathkul and Wongsawad, 2008).

In Chiang Mai, prevalence was highest in the rainy season, quite high in the hot-dry season and lowest in the cool season, whereas in Sakon Nakhon, the highest prevalence was in the hot-dry season, and lower in the rainy and the cool seasons. These findings are similarly to the finding of Faust and Nishigori (1926) who reported that rainfall may force fecal material, including parasite eggs, into natural water resources. Consequently, high populations of the snails are exposed to those parasites. Most trematodes require approximately a 2-month developmental period from egg to metacercaria, and 1½ months for development to cercarial stage in snail and nearly 1 month for metacercariae in fish (Sukontason *et al*, 1999). In contrast, Sukontason *et al* (1999) indicated that the prevalence of metacercariae of *Haplorchis taichui* found in the Ban Pao,

Chiang Mai, was highest in the cool and lowest in the rainy season. The massive water body in the rainy season can decrease the accumulation or deposition of trematode's eggs.

The HAT-RAPD profiles of metacercaria and adult stage of *H. taichui*, *Haplorchoides* sp, *C. caninus* and *O. viverrini* confirmed the correct identification of the metacercariae in cyprinoids. Sato *et al* (2009) reported that PCR can discriminate among *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui*. These findings show the development of molecular markers for specific detection of each trematode is beneficial for epidemiological studies and for prevention control programs.

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