RESEARCH NOTE

VIABILITY OF METACERCARIAE IN NORTHERN THAI TRADITIONAL FOODS

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In the northeastern and northern region of Thailand, fishborne trematodes particularly *Opisthorchis viverrini* and the minute intestinal flukes of the family Heterophyidae are commonly found (Radomyos *et al,* 1994, 1998; Srisawangwong *et al,* 1997). *Opisthorchis viverrini* infection has been known as the most important trematode infection with the serious manifestations of obstructive jaundice and cholangiocarcinoma (Preuksaraj, 1984) while the minute intestinal flukes are regarded as being a much less important public health problem. Life cycles of these trematodes are close together, with several kinds of freshwater fish in the cyprinoid group being the second intermediate host. Exposure to infection occurs by consumption of raw and/or undercooked fish harboring the infective stage or metacercariae. Sometimes economic, environmental and social factors impose the consumption of raw or insufficiently cooked fish on the community or on individuals. Traditional eating habits are part of the deeply rooted culture of a community and are therefore resistant to change. Fermentation is a traditional preservation procedure for freshwater fish in Thailand and consumption of semi-fermented fish shortly after its preparation is increasing the risk of human infections. The northerners are believed to acquire the infection from eating raw fish local dishes called "pla-som" (salted semi-fermented fish) and "lab-pla" (raw fish in spicy salad) prepared from wild caught fish (WHO, 1995). In this regard, the viability of metacercariae of *O. viverrini* and minute intestinal flukes in the muscle of fish hosts is an important factor in acquiring the infection. As far as is known, there has been no information regarding the viability of trematode metacercariae in these two traditional foods prepared by local people of northern Thailand, thus this study was undertaken.

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A total of 30 freshwater fish (*Thynnichthys thynoides,* mean weight = 32 g, range = 26-39 g) were collected from Mae Ngud Reservoir, Chiang Mai Province, northern Thailand. These fish were placed in an ice box at 10°C and transported to the laboratory. Trematode metacercariae were immediately collected from 5 fish as the control group using acid pepsin solution [1% hydrochloric acid 1 ml : pepsin (Sigma®) 1 g : 0.85% sodium chloride solution 99 ml] in a mixer blender at the ratio of 1 g of fish : 10 ml acid pepsin solution. The digested material was transferred into shaking water bath for 1 1/2 hour at 37°C, then it was subsequently passed through 2 layers of wet gauze. The sediment was rinsed with 0.85% sodium chloride solution and examined for metacercariae with the stereo microscope. The identification of metacercariae was carried out by morphological examination based on Scholz *et al* (1991) and the identification of sclerites on the ventrogenital sac of *Haplorchis* species was based on Pearson and Ow Yang (1982).

Another 25 fish were prepared for the food "pla-som" by the traditional method. Individual fish was rinsed using tap water until clean and the internal organs were removed and then mixed thoroughly with sodium nitrate 20 g, salt 15 g, garlic 40 g and steamed sticky rice 70 g. In all 5 groups of mixed fish were then put in separated plastic bags and tightly packed with elastic. They were kept at room temperature until used. Trematode metacercariae were collected from each batch daily using acid pepsin solution as previously described and identification was undertaken.

The local food dish "lab-pla" was customarily prepared by mixing the chopped raw fish with "lab" recipe (mainly comprising of chili powder, local herbs and spices) which can be bought from any local market in northern Thailand. However, this traditional preparation is not suitable for metacercarial determination because the residuum of "lab"

recipe could not be digested by acid pepsin solution. In this regard, the supernatant of "lab" recipe was used instead of the whole recipe. Ten g of "lab" recipe was stirred in 50 ml of distilled water and placed in room temperature for 1 hour. The solution was then diluted to obtain 25 and 50% solution. Sixty active metacercariae digested from *T. thynoides* by acid pepsin solution were exposed to 2 ml of each solution (25, 50 and 100% of "lab" recipe solution; 20 metacercariae per solution) in a plastic plate (1.7 cm in diameter and 2 cm in height). The movement of metacercariae in each group was determined immediately and hourly for another 3 hours under converted microscope using 10x objective lens.

The effects of sodium chloride and acetic acid against metacercarial viability were also examined. Ten percent sodium chloride, 5 and 10% acetic acid were studied while distilled water was used as the control group. Twenty active metacercariae digested from *T. thynoides* by acid pepsin solution were exposed to 2 ml of each solution in a plastic plate (1.7 cm in diameter and 2 cm in height). The movement of metacercariae in each group was determined immediately and hourly for another 3 hours under converted microscope using a 10x objective lens.

The viability of metacercariae recovered from traditional "pla-som" preparation during various periods of fermentation is shown in Fig 1. In the initial study in the control group (immediately examined), 161 metacercariae were found and 94 (58.4%) were active. No degenerate metacercariae appeared. All metacercariae found were identified as being *Haplorchis taichui*. The proportion of active metacercariae dropped to 9.9% (9/91 metacercariae) after 1 day of fermentation, and they were not found after 2, 3, 4 and 5 days of fermentation. Degenerate metacercariae increased from 13.19% (12/91 metacercariae) on day 1 to 100% by day 2 to 5 of fermentation (197/197, 205/205, 188/188 and 245/245, respectively).

Regarding the viability of metacercariae in fish prepared as "lab-pla" dish, all of the metacercariae moved actively in the overall of "lab" supernatant during the period of 3 hours investigation. No degenerate or non-active metacercariae was found.

In 10% sodium chloride and 5% acetic acid solutions, all larvae actively moved in the first 2 hours, then the movement of larva decreased. At the

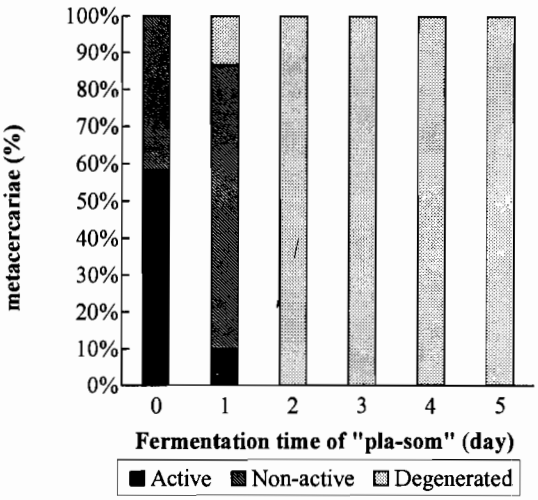

Fig 1–Viability of metacercariae recovered from traditional "pla-som" preparation in various period of fermentation.

third hour of investigation, only 13 (65%) and 14 (70%) active metacercariae were found from the former and the latter solutions, respectively. In 10% acetic acid solution, the movement of metacercariae was reduced to become quiescent after 1 hour exposure. All metacercariae were inactive after 2 and 3 hours of exposure period, but no degenerate metacercariae were found.

The metacercariae found in *T. thynoides* were all *H. taichui*. This was in accordance with Radomyos *et al* (1998) that the most frequent trematode found in gastrointestinal tract of northern people was *H. taichui*. The traditional "pla-som" preparation is not consumed if the period of fermentation less than 3 days. On the other hand, the taste of this food is also not sour enough for eating until the third day of fermentation. The method of "lab-pla" preparation believed by local northerners to kill the parasites is not correct. All metacercariae exposed to "lab" recipe solution were active, and they may be capable of transmission. Ten percent sodium chloride, 5 and 10% of acetic acid solutions can not be used to immediately kill metacercariae in raw fish preparations. From this study, it is evident that the local dish "lab-pla" may be a major source of infection of fishborne-trematode infection of northerners in Thailand.

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