SOME FACTORS AFFECTING *STELLANTCHASMUS FALCATUS* METACERCARIA IN LABORATORY

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Abstract. Killing factors, various temperatures and solutions were studied in the laboratory on *Stellantchasmus falcatus* metacercaria in half-beaked fish (*Dermogenus pusillus*). Killing criteria followed the Movability Index from ≤ 1.000 within 24 hours. The metacercariae were collected from Chiang Mai moat. They were incubated in 0.85 % NaCl at -20˚C, room temperature, 4˚, 37˚, and 65˚C. The *in vitro* investigation showed that at -20˚C and 65˚C, the worms were killed within 18 and 2 hours, respectively, while other temperatures produced no effect. The solutions investigated were NaCl (10, 20, 30, and 40%), lemon juice (25, 50, 75, and 100%), acetic acid (5, 10, 20, and 30%), vinegar (1, 3, and 5%) and water as a control. The worms were killed in NaCl at 20, 30, and 40% within 12, 6, and 2 hours, respectively. Acetic acid at 5% and 10% killed the metacercaria within 12 and 6 hours while at 20% and 30%, within 2 hours. The killing effect of 3% vinegar was found within 18 hours and of 5% vinegar within 12 hours. Lemon juice showed no killing effect.

INTRODUCTION

Food-borne trematode infections have been an important public health problem throughout the world (WHO, 1995). In Thailand, the opisthorchid fluke (*Opisthorchis viverrini*) and heterophyid fluke are common trematodes infecting humans (Tantachumrun and Kliks, 1978; Waikagul, 1991; Waikagul *et al*, 1997; Radomyos *et al*, 1998). *Stellantchasmus falcatus* is one of the heterophyid flukes reported in human in northern Thailand, with the half-beaked fish, *Dermogenus pusillus*, serving as the important second intermediate host (Kliks and Tantachumrun, 1974; Wongsawad *et al*, 2000). In addition, former reports showed that natural half-beaked fish were infected with *S. falcatus* in Chiang Mai Province, northern Thailand (Wongsawad *et al*, 1998; Sripalwit *et al*, 2003). Consumption of uncooked or partially cooked fish harboring infective stage metacercariae is the major cause of infection. In northern Thailand there are traditional foods such as Koi-Pla and Lab-Pla which are made of raw fish. The recipes of such dishes were reported to kill the metacercariae (Keittivuti *et al,* 1986). However, lemon juice at 4.4% at 70˚C within 24 hours could not kill the liver fluke (*O. viverrini*) metacercariae (Waikagul, 1998), and they could survive in 0.85% NaCl solution at 4˚C for 5 weeks (Sithithaworn *et al*, 1997). The higher concentration of NaCl at 13.6% was also unable to kill the metacercariae within 24 hours (Rodthong, 1999). Keittivuti *et al* (1986) studied the survival of metacercariae in traditional food and found that 24-hour seasoning with recipes could kill all metacercariae, and that lemon juice and fish sauce decreased the survival rate of the liver fluke metacercariae. Although some food recipes could kill the metacercariae, it remained uncertain how metacercariae responded to various recipes. The present study was performed to determine the Movability Index (MI) of *S. falcatus* metacercariae incubated in certain killing factors namely, different temperatures and various solutions known to be the ingredients of many traditional fish recipes (NaCl, acetic acid, vinegar, and lemon juice).

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MATERIALS AND METHODS

The metacercariae of *Stellantchasmus falcatus* were collected from half-beaked fish (*Dermogenus pusillus*) in Chiang Mai moat and divided into 2 treatments. Treatment 1 was temperature. Metacercariae were incubated in 0.85 % NaCl at -20˚C, 4˚C, room temperature (25˚C), 37˚C, and 65˚C. Treatment 2 was solution treatments with water as the control. The metacercariae were divided into 4 groups : the first group was NaCl solution at 10, 20, 30 and 40%, second group was acetic acid solution at 5, 10, 20, and 30%, the third group was lemon juice at 25, 50, 75, and 100%, and fourth group was vinegar solution at 1, 3 and 5%. All groups were incubated at room temperature. Thirty worms were used in each experiment and examined at 2, 4, 6, 12, 18 and 24 hours, respectively. The metacercariae of all treatments were examined using the Movability Index (MI) since the value at ≤1.000 within 24 hours refers to the death and > 1.000 refers to the survival of the worms (Prawang, 2001).

RESULTS

Killing solutions together with various temperatures were studied in the laboratory on *Stellantchasmus falcatus* metacercariae collected from half-beaked fish. The killing criterion followed the MI from ≤ 1.000 within 24 hours. The scores (N) of MI indicating the pattern of movement (Fig 1) as active, slightly active, non-active and degenerated were 3, 2, 1, 0, respectively. The results of the temperature treatments are shown in Fig 2. The *in vitro* investigation revealed that at -20˚C and 65˚C, all worms were killed within 18 and 2 hours, respectively, while other temperatures produced no effect (Fig 2).

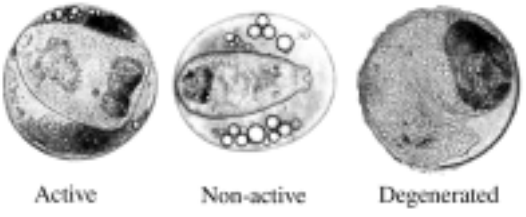

Fig 1- The metacercariae of *Stellantchasmus falcaltus*.

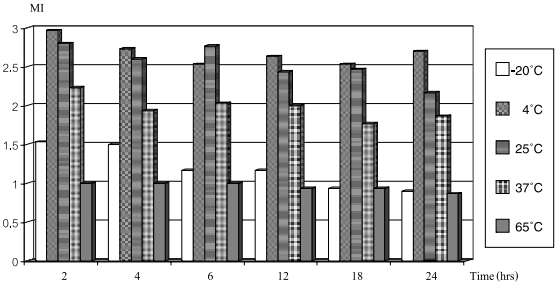

Fig 2- MI of metacercariae of *S. falcaltus* at 5 temperature levels.

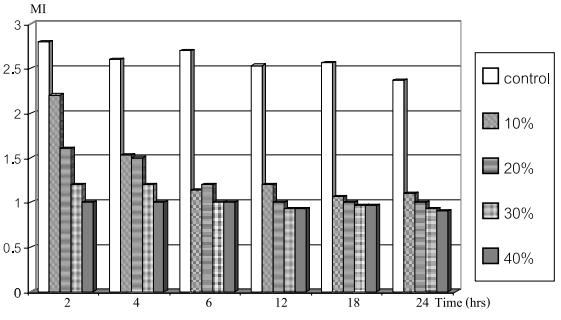

Fig 3- MI of metacercariae of *S. falcaltus* in 4 concentrations of NaCl.

The results of the solution treatments are shown in Figs 3-6. Only 3 out of 4 solutions tested showed a positive effect at different time intervals. The worms were killed in NaCl at 10, 20, 30, and 40% within 12, 6, and 2 hours, respectively (Fig 3). Again, the metacercariae in 5 and 10 % acetic acid were killed within 12 and 6 hours, respectively, while 20% and 30% within 2 hours (Fig 4). The vinegar solution at 3% and 5% also showed the killing effect within 18 and 12 hours, respectively (Fig 5). Only lemon juice produced negative results; none of the concentrations tested could kill the metacercariae in this treatment (Fig 6).

DISCUSSION

The *in vitro* investigation showed that at -20˚C and 65˚C , the worms were killed within 18 and 2 hours. This means that low-temperature cooking could not kill the metacercariae, while higher-temperature cooking can be more effective. At temperatures higher than 65˚C, however, it would be necessary to study the exact temperature that produces 100% killing effect for safe cooking. Waikakul (1998) examined the killing effect of similar factors on *Opisthorchis viverrini* and

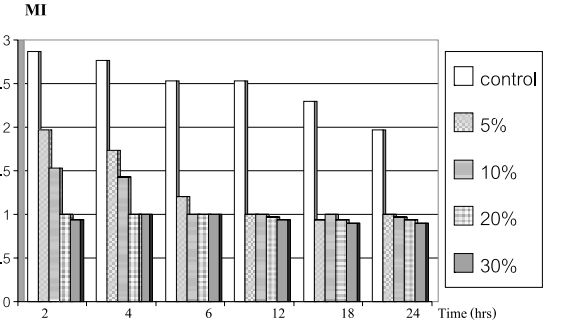

Fig 4- MI of metacercariae of *S. falcaltus* in 4 concentrations

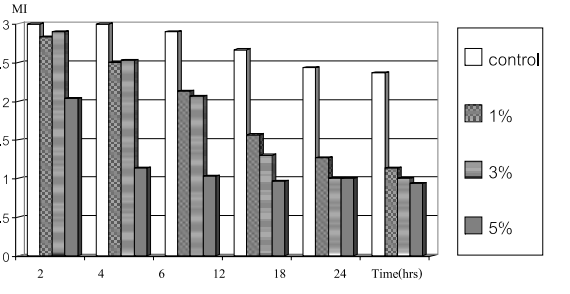

Fig 5- MI of metacercariae of *S. falcaltus* in 3 concentrations of vinegar.

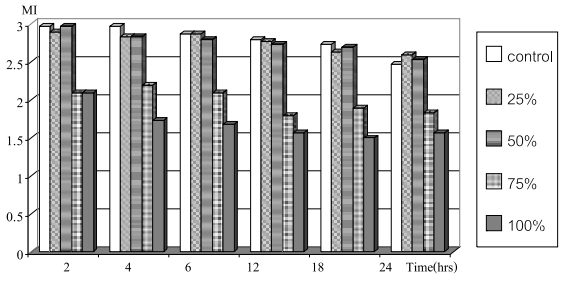

Fig 6- MI of metacercaria of *S. falcaltus* in 4 concentrations of lemon juice.

found that in 4% lemon juice, the metacercariae were killed at higher temperature up to 70˚C.

Rodthong (1999) reported that 13.6% NaCl within 24 hours was unable to kill the metacercariae while 20% NaCl in this study could within 12 hours. Keittivuti *et al* (1986) showed that all metacercariae from traditional food were killed at 24 hours after seasoning, but each solution in this study showed killing effect at least 2 hours after treatments except lemon juice. Although these solutions could kill the worms, it took a lot of time to kill them while real cooking required a much shorter time. The important factor is that traditional food such as Koi-Pla and Lab-Pla are commonly eaten immediately after seasoning, thus worms can survive and infect humans.

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