

# Motility Of Intestinal Fluke, Echinostoma Spp, Metacercariae In Fish Dishes Prepared By Different Uncooked Methods

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## Abstract

Fish-borne trematode is a worldwide problem. In addition to the major liver flukes, several types of intestinal flukes also use minnows as the intermediate host. Traditional ingestion of undercooked and uncooked fish preparations is a major factor in pathogen acquisition. Several freshly killed freshwater fish, *Cylocheilichthys armatus*, were purchased from the local market and sent to the laboratory to be examined under a stereoscopic microscope for active *Echinostoma* spp metacercariae. A total of 10 fish were infected with many active metacercariae and used in the preparation of uncooked fish dishes: 1) left to dry at room temperature; 2) frozen; 3) refrigerated; 4) marinated in saline; and 5) marinated in 5% acetic acid solution. The motility of the metacercariae in each of these dishes was examined under a stereoscopic microscope. Motility was estimated as the level of activity or degeneration of the metacercariae at the start of the experiment and then every 30 minutes until all the parasites appeared degenerate (and therefore, presumably not viable). Degeneration of the parasites was slowed by cooling: degeneration of all metacercariae took approximately 10 hours in the refrigerated or frozen fish, compared with 4 hours in all other dishes left at room temperature. Of interest, this period is longer than reported times for the metacercariae of the intestinal flukes in recent published works. In conclusion, the studied uncooked preparation mimicking the traditional practice cannot induce degeneration to the contaminated metacercariae of in the short period. Since the real practice of the Thais is rapidly eating after preparation, hence, the active strategy to change this rooted behavior is necessary.

## INTRODUCTION

Fish-borne trematode is a worldwide problem, with the number of people infected by liver flukes alone estimated at 21 million. In addition to the diseases caused by major liver flukes, *O viverrini* and *C sinensis*, infections by the other types of intestinal flukes such as *Heterophyidae* spp, *Haplorchinae* spp and *Echinostoma* spp are fished-borne diseases as well. Presently, these fluke diseases are a common public health problem in the northeastern region of Thailand.<sup>(1,2)</sup> Because the minnows are the source of the infective stage of these flukes, ingestion of undercooked and uncooked fish is a major factor in pathogen acquisition. Traditional Thai dishes such as Pla Som and Lab Pla are believed to be the source of infection to many people.<sup>(3)</sup> However, there have been only a few reports concerning the metacercariae in Thai traditional dishes. The first report was restricted to monitoring the viability of *Haplorchis taichui* metacercariae in only 2 specific dishes, Pla Som and Lab Pla.<sup>(4)</sup> The second and third report studied the viability of *Heterophyidae* spp and *Haplorchinae* spp metacercariae in

matched minnows using a number of preparation methods.<sup>(5, 6)</sup> In order to fulfill the knowledge on the metacercariae study, the another study concerning the motility of *Echinostoma* spp metacercariae after different preparation methods that mimic traditional practices was performed.

## MATERIALS AND METHODS

### PREPARATION OF UNCOOKED FISH DISHES

The protocol of this study is the same as the previous reports<sup>(5, 6)</sup>. Several freshly killed freshwater fish, *C armatus*, were purchased from the local market and examined under a stereoscopic microscope for active *Echinostoma* spp metacercariae. The entire fish was examined, including the fins and flesh. A total of 10 fish were infected with many active metacercariae, in many parts. These infected fish were used further in the study. Five different raw fish dishes that mimicked traditional meals (2 fish/dish) were prepared using the following methods: 1) left to dry at room temperature (about 37 ° Celsius in Thailand) ; 2) frozen at -20° Celsius; 3) refrigerated at 4° Celsius; 4) marinated in saline (5%

sodium chloride solution); and 5) marinated in 5% acetic acid solution to mimic traditional Thai vinegar. All experiments were performed at the Veterinarian Parasitology Laboratory, Department of Pathology, Faculty of Veterinarian Science, Chulalongkorn University under the routine laboratory quality control.

**MOTILITY STUDY OF THE METACERCARIAE**

The motility of the metacercariae in each of these dishes was estimated under the stereoscopic microscope. Since non motile parasites may still be viable, the researchers excysted the immature fluke to study their motility. Motility was estimated as the level of activity or degeneration of the metacercariae at the start of the experiment and then every 30 minutes until all the parasites appeared degenerate (Table 1).

**Figure 1**

Table 1: Change with Time, Postpreparation, in the Viability of Metacercariae in Raw Fish Dishes

Preparation Method	Time Postpreparation (hour)*		
	Longest time which all active metacercariae	Longest time which any active metacercariae	Time at which all metacercariae were degenerate
1) Dried in room air	0	2	4
2) Frozen	0	5	10
3) Refrigerated	0	5	10
4) Marinated in 5% sodium chloride	0	2	4
5) Marinated in 5% acetic acid	0	2	4

\*The fish were checked immediately after they were prepared and then every 30 minutes until all metacercariae were degenerated.

**RESULTS**

All metacercariae were active and motile at the start of observation. Degeneration was observed in all dishes after 4 hours, except for the dishes that were frozen or refrigerated, in which degeneration was observed after 10 hours.

**DISCUSSION**

Fluke infection is an important infectious disease in Thailand. Health education promoting cooked fish consumption for preventing further infection is one of the the main strategy for fluke control in Thailand(7). A recent survey indicated that ingestion of uncooked fish dishes was a risk factor for fluke infection.(8) An important factor in transmission of fluke infection via eating fish dishes is viability of the metacercariae possibly living in the fish.

Not only the liver fluke diseases but also the intestinal fluke diseases are endemic in Thailand. Here, we focus our study on the *Echinostoma spp*, which is an important group of intestinal flukes. According to the recent study of Radomyos

et al (1), the *Echinostoma spp* infection is still prevalent among the Thais in rural area. Since the life cycle of spp is similar to the liver flukes and other intestinal flukes that the mollusk is its first intermediate host and the minnows is its second intermediate host. Hence, the tradition ingestion of uncooked and undercooked fish dishes is the major factor for pathogen acquisition. Here, the motility of the metacercariae of *Echinostoma spp* was studied in different uncooked fish dishes prepared to mimic the traditional practice. According to Thai tradition, people buy the freshly killed fish from the market, unlike populations in some countries who prefer to buy live fish. Most Thai people in this area believe that the addition of salt or vinegar to raw fish rapidly kills the metacercariae. Unfortunately, similar to results of the recent studies (5, 6), without being well cooked, the fluke metacercariae can be motile for a long time before degeneration.

Like the previous studies of the Haplorchinea spp and Heterophyidae spp metacercariae (5, 6), the metacercariae in this study remained viable and infective for many hours of freezing. However, the uncooked frozen or refrigerated fish dish is not a favorite dish among the Thai people. This is the first report of the viability of the metacercariae of this species in prepared food. As anticipated, degeneration of the parasites was slowed by cooling: degeneration of all metacercariae took approximately 10 hours in the refrigerated or frozen fish, compared with 4 hours in all other dishes left at room temperature. Of interest, this period is longer than reported times for the metacercariae of the other intestinal flukes in recent published works (5, 6). In conclusion, the studied uncooked preparation mimicking the traditional practice cannot induce degeneration to the contaminated metacercariae of in the short period. Since the real practice of the Thais is rapidly eating after preparation, hence, the active strategy to change this rooted behavior is necessary.

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