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Excystation of *Haplorchis taichui* metacercariae could be elicited by change in pH

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Abstract

The effect of different component of the intestinal fluid on the excystation of *Haplorchis taichui* metacercariae was examined *in vitro*. Encysted metacercariae obtained by pepsin digestion of fish muscle showed that the highest percentage of metacercariae excystation could be obtained after being treated with trypsin at pH 7.5. However, it was also observed that excystation of the metacercariae could also be effected by changing the pH to the basic condition. The most optimum pH for the metacercariae excystation was found to be 7.5. This method of obtaining the excysted metacercariae could prove to be useful for taxonomical study of trematode in general.

Key words : *Haplorchis taichui*, metacercariae, excystation, pH effect

Haplochis taichui is a food-borne zoonotic parasite. Human infection by this parasite had been reported in Bangladesh⁵⁾, Egypt⁶⁾, Laos¹³⁾, Thailand¹³⁾ and the Philippines^{1,2)}. Man is infected through eating raw or improperly cooked freshwater fishes that harbored the metacercariae. This parasite has recently become a major trematode species found in fishes in several Asian countries, replacing *Clonorchis sinensis*⁹⁾. In fact more

was found to be that of *H. taichui*¹⁰⁾.

For identifying the species of encysted metacercariae, the most common method was to pipette the metacercariae onto a microscopic slide, cover it with a cover slip and gently apply pressure on the cover slip to let the metacercariae come out of its cyst. Another commonly used method for releasing the metacercariae from their cyst is to prick the cyst with optical forceps under the dissection

The following reagents were added to each of the groups and treated as described above.

Groups	Reagents added
Group F :	HCl was added to change the pH value to 2

Group A had the same condition, that is pepsin, which was used to digest the fish muscle, excystation of the metacercariae was not observed. However, it was observed that excystation of the metacercariae could also be effected by changing the pH value as seen for Group B (Fig. 1). Excystation of the metacer-

stroyed in the process. Development of an effective method of excystation would be helpful for the study on the identification of metacercariae. We report herein the effect of intestinal fluid component and pH changes on the excystation of *H. taichui* metacercariae *in vitro*.

Fishes used in this study were caught with nets in the Sun Moon Lake, 71 km east of Taichung, Taiwan. Nets, which were cast at different location in the lake, were left overnight and retrieved the next morning. Only *Hemiculter leucisculus* caught with the net were used as the source of the metacercariae. The metacercariae of *H. taichui* used in this study were isolated from several dozens of *H. leucisculus* by pepsin digestion. The metacercariae were still encysted after pepsin digestion of the fish muscle.

The fishes were weighed, their scales scraped off, fin snipped and eviscerated. Their muscles were cut with scissors and then digested using a magnetic stirrer at 37°C for about 1 hr in 0.5% pepsin (1 : 10,000) (Sigma Chemical, St. Louis, MO) solution. Concentrated hydrochloric acid was added to the pepsin solution to bring the pH down to less than 2. Approximately 10-15 ml of the pepsin solution was added to each gram of the fish muscle.

Metacercariae obtained after digestion of the fishes were found to consist of *C. sinensis*, *H. taichui* and *Haplorchis pumilio*. The encysted metacercariae were identified under a light microscope with the criteria of having a conspicuous ventral sucker for *C. sinensis*, shorter prepharynx length, comparatively lesser transparency and generally larger size for *H. taichui* than for *H. pumilio*. *H. taichui* has 14 hooklets at their comparatively small ventral sucker, whereas *H. pumilio* has 32 smaller ventro-genital sucker hooklets. These hooklets were not seen in *C. sinensis* metacer-

cariae¹⁴). Only *H. taichui* metacercariae were selected for the *in vitro* excystation experiment.

The encysted metacercariae obtained after pepsin digestion of the fish muscles were first pooled together and then randomly aliquoted for each group. A total of 100 encysted metacercariae were prepared for each group (Groups A-E) and were treated as follows :

Groups	Reagents added
Group A :	1 % pepsin ; HCl was added to change the pH value to 2
Group B :	saline ; 1 % NaOH was added to change the pH value to 7.5
Group C :	1 % pancreatin (Sigma Chemical, St. Louis, MO) ; 1 % NaOH was added to change the pH value to 7.5
Group D :	1 % pancreatin and 1 % bile salt (50% Sodium cholate and 50% sodium deoxycholate) (Sigma Chemical, St. Louis, MO) ; 1 % NaOH was added to change the pH value to 7.5
Group E :	1 % trypsin (Sigma Chemical, St. Louis, MO) ; 1 % NaOH was added to change the pH value to 7.5

The above 5 groups of metacercariae were incubated at 40°C while being stirred with a magnetic stirrer. Excystation of the metacercariae was observed every 30 min under a dissection microscope. Excystation of the metacercariae was carried out by incubation at 40°C because birds such as night heron⁷, cattle egret^{1,12} and chicken¹¹ had been reported to serve as definitive host for *H. taichui*.

A total of 100 metacercariae in saline were prepared for each group (Groups F-J).

The following reagents were added to each of the groups and treated as described above.

Groups	Reagents added
Group F :	HCl was added to change the pH value to 2
Group G :	HCl was added to change the pH value to 4
Group H :	HCl was added to change the pH value to 6
Group I :	1 % NaOH was added to change the pH value to 7.5
Group J :	1 % NaOH was added to change the pH value to 9

From the results of the effect of different intestinal fluid component on the excystation of metacercariae, Group E, which was treated with trypsin, showed the highest percentage of excysted metacercariae (Fig. 1). Since

Group A had the same condition, that is pepsin, which was used to digest the fish muscle, excystation of the metacercariae was not observed. However, it was observed that excystation of the metacercariae could also be effected by changing the pH value as seen for Group B (Fig. 1). Excystation of the metacercariae by the changing only the pH to the basic was confirmed by the second experiment (Fig. 2). The most optimum pH for excystation was 7.5 as seen for Group I.

In vitro excystation of the metacercariae of aquatic food-borne zoonotic parasites such as *C. sinensis*⁸⁾, *Paragonimus heterotremus*⁴⁾ and *P. westermani*³⁾ had been reported. In these reports, emphasis had been placed on the effect of intrinsic and extrinsic factors such as cysteine protease, trypsin and bile salts as the effector mechanism of excystation of the metacercariae. In our study with *H. taichui*, we also similarly observed that addi-

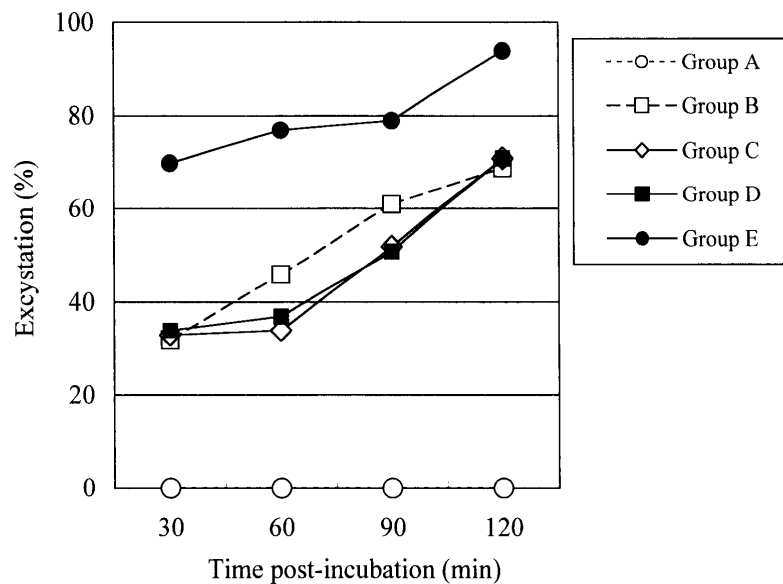


Fig. 1. Effects of intestinal fluid component on excystation of *Haplorchis taichui* metacercariae *in vitro*. Metacercariae were treated as follows : Group A : Pepsin, pH 2, Group B : Saline, pH 7.5, Group C : Pancreatin, pH 7.5, Group D : Pancreatin & bile salt, pH 7.5, Group E : Trypsin, pH 7.5

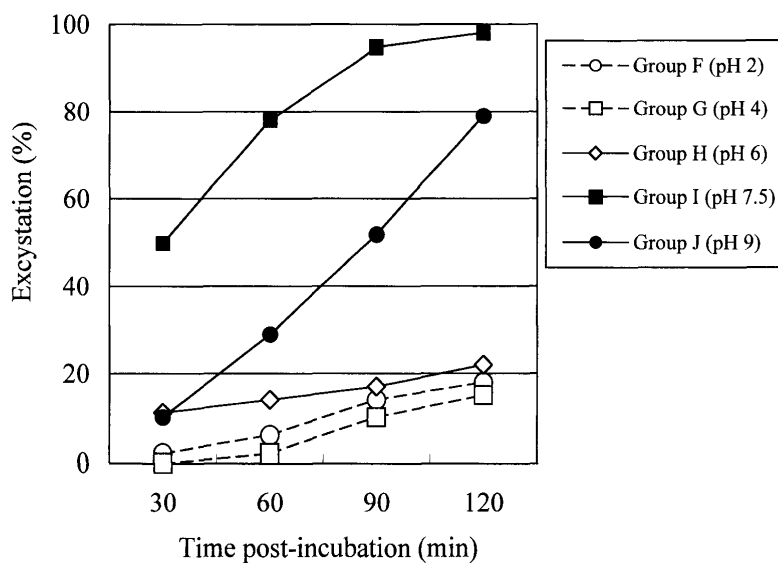


Fig. 2. Effects of pH on excystation of *Haplorchis taichui* metacercariae *in vitro*.

tion of intestinal fluid components did accelerate and facilitate the metacercariae to excyst *in vitro*.

However, an unexpected finding of ours is that excystation of *H. taichui* metacercariae could also take place by just changing the pH of the solution in which the worms were kept in. We postulated that *in vivo*, the metacercariae will excyst immediately as they enter from the stomach into the small intestine where there is a change in pH value from acidic to the basic. This change in pH might be the trigger for activating the endogenous worm protease that acts within to release the metacercaria from the cyst. However, further experiments will be needed to prove this postulation. Thus, our finding will provide a very convenient method for the collection of metacercariae for research where addition of intestinal enzymes must be avoided.

In our study, it was observed that pepsin inhibited the excystation of *H. taichui* metacercariae. This phenomenon bodes well for the metacercariae in that they will still be protected from the gastric digestive juice

while in the stomach. The excysted metacercariae were very active but they lost their vigor 1-1.5 hr post excystation. Probably the incubation medium that we used did not contain any nutrient that might be essential for sustaining the mobility of the excysted metacercariae. We supposed that the worms must adhere to the mucosa of the small intestine and start feeding as soon as possible in the intestinal environment after they have excysted.

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