

RESEARCH NOTE

A SIEVING METHOD FOR COLLECTING THE METACERCARIAE OF TREMATODE PARASITES FROM FRESHWATER FISH

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Abstract. This study describes a sieving method for the collection of metacercariae from frozen (-20°C) freshwater fish. Digested fish tissue is filtered through a series of sieves; the crude filtrate is then centrifuged. Centrifugation produces a sediment from which metacercariae can be removed. Half of the metacercariae that were obtained from the fish meat that had been frozen for 10 days (-20°C) were dead; the other half were alive and some larvae were moving slowly.

Komiya (1966) reported on the distribution of species of metacercariae: 38 species may be recovered from freshwater fish; 27 from brackish-water fish; 13 from marine water fish; 8 from shrimp; 16 from crabs; 8 from molluscs, and 2 from frogs. Many species of metacercariae are pathogens: transmitting trematode parasites to human hosts; yet more species of metacercariae lead to important parasitic zoonoses. The recovery of metacercariae from freshwater fish is important for the epidemiological, immunological, chemotherapeutic, and prophylactic study of their related parasitoses. Since it is usually difficult to isolate quickly a great number of metacercariae from fish using a dissection technique, we used a sieving method to extract metacercariae. This method can be applied to both fresh and frozen freshwater fish. This report describes how our sieving method may be used to isolate pure metacercariae from fish.

Seventy-five freshwater fish (*Hemiculter leucisculus*) were collected from Chengching

Lake, Kaohsiung County. The fish were placed immediately into an ice box (Tag Along 24) at 0°C and brought back to our laboratory, where they were kept in a freezer (-20°C) until examination.

After thawing, the body cavity of each fish was opened and the viscera and the head were removed. The fish tissue was disrupted in a Waring Blendor. Two hundred and fifty ml of artificial digestive fluid (HCl 0.1%; pepsin 0.5%) were then added to the disrupted tissue, which had been put into a 250 ml conical flask. After adding glass beads, the conical flask was placed in an orbital shake incubator (Hottech Instrument Co Model 718) at 37°C for 2 hours.

The digested fish meat suspension was allowed to pass through a series of 10 cm sieves (Kuang Yang Co, Taiwan) arranged, from top to bottom, as follows: #40 (sieve pore = 420 µm), #80 (117 µm), and #140 (105 µm). The metacercariae passed through all but the lowest filter, where they accumulated; they were then reverse washed with saline off of the #140 sieve and into a 1,000 ml flask. After centrifugation, numerous metacercariae were collected with a plastic pipette from the bottom of the 15 ml centrifuge tube. Half of the meta-

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cercariae that were obtained from the fish meat that had been frozen for 10 days (-20°C) were dead; the other half were alive and some larvae were moving slowly.

This is the first time that this sieving method has been used to recover metacercariae of *Clonorchis sinensis*; similar sieving methods have been employed in the collection of schistosome eggs from mouse intestines (Dresden and Payne, 1981) and have, more recently, been utilized for determining the resistibility of *C. sinensis* metacercariae in frozen and salted freshwater fish (Fan *et al*, 1998). Our sieving method offers several advantages: it is simple, practical, and quick – it can be completed within two hours. The method can be applied to the recovery of metacercariae from both frozen freshwater fish and ethanol- or formalin-fixed fish; moreover, the method can be employed in the collection of metacercariae from crabs, crayfish, and shrimp. Forty-two species of *Paragonimus* have been reported, many of which infect humans; our sieving method could be useful when studying the prevalence and infectivity of crab hosts in areas with endemic paragonimiasis. Our method

may be extended to the recovery of the following metacercariae (hosts): *Opisthorchis* spp (fishes), intestinal flukes, *Heterophes* spp, and *Echinostoma* spp (snails). The technique may also aid the collection of *Capillaria hepatica* eggs (rat liver) and *Trichinella spiralis* larvae (rat and pig muscle).

ACKNOWLEDGEMENTS

The authors wish to thank the National Science Council, Republic of China, for a research grant (NSC89-2320-B010-039).

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