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In vitro excystation of metacercarial cysts of *Echinostoma trivolvis* from *Rana* species tadpoles

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Abstract: *In vitro* excystation studies were done on the metacercarial cysts of *Echinostoma trivolvis* obtained from the kidneys of naturally infected *Rana* species tadpoles. Cysts were excysted in an alkaline trypsin-bile salts medium and the percentage of excystation was compared with that from previous studies done on cysts obtained from the kidneys of snails. The percentage of excystation of *E. trivolvis* metacercariae from tadpole kidneys was similar to that reported for previous studies on cysts obtained from experimentally infected gastropod hosts. The possible role of tadpoles as an agent for the transmission of *Echinostoma* and echinostomiasis to humans is discussed.

Key words: *Rana* species, tadpoles, trematoda, *Echinostoma trivolvis*, metacercarial cysts, *in vitro* excystation

INTRODUCTION

Echinostoma trivolvis is a ubiquitous 37-collar-spined echinostome in North America (Kanev *et al.*, 1995) The first intermediate host is *Helisoma trivolvis* (Planorbidae) and a variety of invertebrates and cold blooded vertebrates serve as second intermediate hosts. Definitive hosts include a variety of avian and mammalian species (see review in Huffman and Fried, 1990).

Whereas considerable information is available on gastropods as natural and experimental second intermediate hosts of *E. trivolvis*, much less information is available on the role of tadpoles in the genus *Rana* as second intermediate hosts. Beaver (1937) using the name of *E. revolutum* for this North American species discussed the role of various species of tadpoles and frogs as second intermediate hosts and Martin and Conn (1990) studied various aspects of the biology of echinostomatid cysts in the kidneys of *Rana clamitans* and *Rana pipiens*.

We recently found cysts of *E. trivolvis* in the kidneys of *Rana* species from a farm pond in Northampton County, PA, USA. The exact location of this farm pond has been given in Schmidt and Fried (1997). In the present study we report our observations on in vitro excystation of metacercarial cysts of *E. trivolvis* from the kidneys of tadpoles and compare our observations with previous studies on excystation of this species from cysts obtained from gastropod kidneys. Fried and Butler (1978) studied chemical excystation of *E. trivolvis* (referred to as *E. revolutum* in that paper) metacercariae from experimentally infected *Physa heterostropha* snails and Smoluk and Fried (1994) made similar studies on the metacercriae of this echinostome from experimentally infected *Biomphalaria glabrata* snails.

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MATERIALS AND METHODS

Rana species tadpoles were collected from a farm pond in Northampton County, PA, USA (Schmidt and Fried, 1997), and maintained in the laboratory at 12°C in 38 liter aquaria, 100 tadpoles per tank, for up to three months. Prior to dissection, tadpole length and wet weight were taken. Some kidneys were removed from tadpoles, placed in Locke's solution (Ursone and Fried, 1995), and examined under the low power of a compound microscope to determine the incidence of infection per kidney. Other kidneys were cut into three portions, each with about 100 cysts, and used for in vitro excystation studies. Excystation was done as described in Fried and Roth (1974). Portions of infected kidneys were rinsed in Locke's solution and then transferred to a petri dish containing 3 ml of 0.5% trypsin plus 0.5% bile salts in Earle's BSS, pH 8.1 (henceforth referred to as the TB medium), and maintained at 41°C and excystation was determined at 1 and 2 hr. To determine the effects of an acid pepsin pretreatment on excystment, some infected kidney tissue was pretreated in 1% acid pepsin in 0.85% saline (adjusted to pH 2 with 6N HCl) for 1, 2, or 3 hr, rinsed in Locke's, and then transferred to the TB medium, and maintained at 41C. Rates of excystation were observed at 1, 2, and 3 hr intervals (Fried and Roth, 1974). In some studies additional cysts were maintained in acid saline (pH 2) for 1 to 3 hr prior to treatment in the TB medium. The TB medium is made up as follows. Prepare a 0.5% trypsin (1-250, pig pancreas, United States Biochemical Co., Cleveland, Ohio) plus 0.5% bile salts (Bacteriological Bile Salts 3, also from USB) in Earle's Balanced Salt Solution; add sufficient 7.5% $NaHCO_3$ to adjust the pH of the medium to 8.0 ± 0.2; filter the medium prior to use.

RESULTS

Tadpoles were successfully maintained in the laboratory as described in this paper for at least 3 months with minimal mortality. The incidence of natural infection per tadpole kidney with *Echinostoma trivolvis* cysts is shown in Table 1. There was no correlation in cyst infection with length or weight of the tadpoles. Presumably most of the echinostomatid cysts in the tadpole kidneys were those of *E. trivolvis*. Infections in domestic chicks using these cysts (data not reported in this paper) produced only *E. trivolvis* adults which looked identical to that shown in Fig. 1 of Fried *et al.* (1997). Most of the tadpoles examined in this study were identified as *Rana catesbiana* (bullfrogs). However, some tadpoles of other species may have been present in our pool and therefore we are referring to our tadpoles as *Rana* species.

Table 1. Incidence of infection of *Echinostoma trivolvis* cysts in randomly selected *Rana* species tadpole kidneys

Tadpole #	Length (cm)	Wet weight (gm)	Number of cysts in one kidney
1	7.6	3.6	150
2	8.0	5.1	300
3	8.1	4.1	50
4	7.8	4.9	270
5	4.9	3.1	175
6	8.0	4.1	125
7	8.1	5.3	200
8	5.1	2.5	325
9	9.5	5.8	200
10	8.2	4.6	200

Results of the excystation experiments are shown in Table 2. The percentage of excystation in the TB medium without treatment more than doubled at 2 hr (43%) compared to 1 hr (18%). Although various pretreatments (pepsin or acid saline) caused a greater percentage excystation in the TB medium at 1 hr (Exper C vs. Exper A and Exper E vs. Exper A), by 2 hr in TB, excystation with or without pretreatment was quite similar.

DISCUSSION

Most cysts in the tadpole kidneys were viable based on the excystation studies. Moreover, the percentage of excystation obtained in this study on cysts from tadpoles is quite similar to that reported previously on

Table 2. Percentage excystation of *Echinostoma trivolvis* metacercarial cysts from tadpole kidneys

Exper.	Pretreatment[a)]	Treatment	Treatment time in hr.	Excysted (%)
A	none	TB	1	18
B	none	TB	2	43
C	P[b)]	TB	1	30
D	P	TB	2	41
E	AS	TB	1	33
F	AS	TB	2	46

[a)]Pretreatment time was always 1 hr.

[b)]Key: P = pepsin; AS = acid saline; TB = trypsin-bile salts medium; observations based on 100 cysts per experiment.

excystation of *E. trivolvis* metacercariae obtained from various gastropod hosts (Fried and Butler, 1978; Smoluk and Fried, 1994). Fried and Butler (1978) reported that after 1 hour about 25% of the metacercariae from *Physa heterostropha* excysted in the TB medium, whereas Smoluk and Fried (1994) reported that about 28% of the metacercariae from *Biomphalaria glabrata* excysted in the TB medium at 1 hour.

Although *Echinostoma* and echinostomiasis is a minor medical problem in the USA, eating habits of individuals are quite diverse and should North Americans feed on raw, marinated or inadequately cooked tadpoles, the possibility exists that these individuals may become infected with echinostomes. Human cases of echinostome infections have been reported from other parts of the world and are often associated with eating raw, marinated, or inadequately cooked fresh water invertebrates, fishes and frogs. By way of example, Seo *et al.* (1983) reported a human case of *Echinostoma hortense*; presumably the infection was obtained by eating raw fresh water fish.

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