Infectivity and Excystation of the Metacercaria of Echinoparyphium Flexum (Trematoda)

Bernard Fried and Kenneth L. Grigo

Department of Biology
Lafayette College
Easton, Pennsylvania 18042

ABSTRACT

Observations were made on infectivity and chemical excystation of the metacercaria of Echinoparyphium flexum (Trematoda) obtained from naturally infected Physa heterostropha snails in Cumberland County, Pa. Domestic chicks fed large numbers of cysts revealed an infectivity rate of about 2%. Regardless of the worm burden in chicks, flukes localized only in the upper region of the small intestine. Attempts to increase infectivity by treating cysts in various pretreatment solutions were unsuccessful. Cloacal inoculation of chicks with chemically excysted metacercariae also failed to produce infection. In vitro excystation experiments revealed that the synergistic effect of bile salts + trypsin in an alkaline pH produced maximal excystation at 42°C within 30 min, and cyst pretreatment in acidified-pepsin was not a prerequisite for excystation.

INTRODUCTION

Wills et al. (1) reported an avian schistosome cercaria, Trichobilharzia sp., and echinostome larvae in naturally infected Physa heterostropha Say snails collected in Cumberland County, Pa. in the summer of 1974. Based on infectivity studies in the domestic chick they identified the echinostome as Echinoparyphium flexum (Linton, 1892) Dietz, 1910. Najarian (2, 3, 4) has done extensive work on the systematics, biology, development, growth and life history of E. flexum. Based on observations on three experimentally infected domestic chicks, he (3) reported that the percentage infection in domestic chicks is very low. Larval stages of this parasite are available in commonly occurring fresh water snails (3) and therefore could provide a source of trematode material for teaching or research purposes. The literature has revealed that information on chemical excystation of the metacercaria of this species is not available.

The purpose of this report is to present our observations on infectivity of E. flexum metacercaria in the domestic chick and on chemical excystation of the cysts. The results are reported herein.

MATERIALS AND METHODS

Snails naturally infected with E. flexum larvae were maintained in laboratory aquariums (1), dissected in pond water, and cysts removed from the kidney were transferred to Locke’s solution. Most snails contained from 25 to 500 cysts, and larger snails were usually more heavily infected than smaller ones. Cysts were used in most experiments within one hour of removal from snails. Actual counts were made in studies that employed less than 100 cysts. When large numbers of cysts were required estimates were made based on the size of a cyst packet. With practice such estimates are reliable within ±20%.

During infectivity studies, unless otherwise stated, cysts were fed to chicks in a minimal amount of Locke’s solution. The usual inoculum consisted of either 25, 50, 100, 200, 400 or 1500 cysts/chick. In some studies cysts were pretreated in various solutions (5, 6) and fed to chicks in the same solution in an attempt to increase infectivity rates. Thus, 11 chicks were each exposed to 400 cysts pretreated in 7.5% NaHCO₃ and 5 chicks were each exposed to 400 cysts pretreated in 0.5% bile salts + 0.5% trypsin in Earle’s BSS, pH 8.1. Following the development of successful chemical excystation procedures, 10 chicks were exposed cloacally (7) to either 25, 50, 100 or 200 excysted metacercariae to determine if the cloacal route could achieve infection.

White leghorn domestic chicks, 1 or 2 days old, were used in infectivity studies. They were not given food or water on the day of exposure or necropsy. Chicks were killed by decapitation from 2 to 14 days postexposure and total worm counts and worm location in the small intestine were determined.

Excystation experiments were based on a previous study on Parorchis acanthus cysts and methods for preparing various excystation media are described in that paper (8). Cysts were removed from snails, transferred to Locke’s solution and studied in 3.5 cm plastic petri dishes containing 2 or 3 ml of the test solution. All tests were conducted at 42°C for 30 min unless otherwise stated. Each test utilized 25 cysts and was replicated four times.

Preliminary experiments revealed that successful excystation of E. flexum cysts occurred without acid-pepsin pretreatment and with an excysting medium consisting of 0.5% bile salts + 0.5% trypsin in Earle’s BSS adjusted to pH 8.1 with 7.5% NaHCO₃, essentially as previously described for P. acanthus cysts (8). To determine the effects of pH on excystation, cysts were studied in bile salts + trypsin adjusted with NaHCO₃ to achieve pHs of 7.0, 7.2, 7.4, 7.7, 8.1, 8.5, and 9.0. To determine the possible detrimental effects of acid-pepsin pretreatment, cysts were maintained for 0.5, 1, 2 and 3 hr in acidified pepsin (8), rinsed in Locke’s solution and then transferred to bile salts-trypsin, pH 8.1 for 30 min. Controls consisted of cysts maintained similarly in Locke’s solution prior to transfer to the bile salts-trypsin.

RESULTS

None of 9 chicks each exposed to 25 or 50 cysts was infected. Of 11 chicks each exposed to 400 cysts pretreated in NaHCO₃, only 2 were infected with a total of 10 worms. Of 5 chicks each exposed to 400 cysts pretreated with bile-salts-trypsin, only one was infected with 6 worms. None of 10 chicks exposed cloacally with chemically excysted metacercariae was infected. Results of feeding large numbers of cysts to chicks are summarized in Table 1. Total worm recovery based on either 400 or 1500 cysts/chick was approximately 2%.

Worms localized either singly or in clusters within the first 25 cm of the small intestine. The distribution of worms in 5 infected chicks that had each received 400 cysts and were necropsied 13 days postexposure is summarized in Table 2.

Excystation in E. flexum is active since the cyst wall is not dissolved by excysting fluids. Details of cyst structure were not studied and only organisms fully emerged from cysts were scored as excysted. Excystation never occurred in Locke’s solution or acid-pepsin, or in the absence of either bile salts or trypsin from the excysting medium. The effects of pH on excystation in trypsin-bile without acid-pepsin pretreatment is shown in Fig. 1. The results indicate maximal excystation within 30 min. at 42°C at approximately pH 8. The deleterious effects of prolonged pretreatment in acid-pepsin, prior to treatment in trypsin-bile is shown in Fig. 2. Beyond 30 min. acid-pepsin pretreatment has a marked detrimental effect on subsequent excystation in trypsin-bile. Controls pretreated in Locke’s solution for up to 3 hr. prior to treatment in trypsin-bile showed approximately 90% excystation.

DISCUSSION

Our results indicate that cyst pretreatment, useful for increasing infectivity in other avian trematodes (5, 6), was not effective for E. flexum. The cloacal inoculation procedure, useful for establishing
PROCEEDINGS OF THE PENNSYLVANIA ACADEMY OF SCIENCE Vol. 49, Issue 1, 1975

TABLE 1
Summary of infectivity data using approximately 400 or 1500 E. flexum cysts/chick.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>No. of chicks</th>
<th>No. of chicks</th>
<th>No. of days</th>
<th>Total No. of Worms</th>
<th>Range &amp; (Avg.) Worm Recovery/chick</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21</td>
<td>18</td>
<td>5-14</td>
<td>185</td>
<td>0-50 (9)</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>6</td>
<td>2-12</td>
<td>195</td>
<td>1-120 (33)</td>
</tr>
</tbody>
</table>

A = 400 cysts/chick; B = 1500 cysts/chick

FIGURE 1: Excystation of E. flexum cysts in 0.5% trypsin + 0.5% bile salts in Hanks BSS adjusted to various alkaline pH's with 7.5% NaHCO3. Observations were made on cysts maintained at 42C for 30 min.

FIGURE 2: Effects of 0.5 to 3 hr pre-treatment of E. flexum cysts in 1% acid-pepsin (pH 3-5) on subsequent excystation in trypsin-bile, pH 8.1, at 42C for 30 min.

TABLE 2
Location of 13-day-old E. flexum adults in the small intestine of 5 chicks each exposed to 400 cysts.

<table>
<thead>
<tr>
<th>Chick</th>
<th>No. of Worms</th>
<th>Worm location (cm)</th>
<th>Location of most worms (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>4-10</td>
<td>7-10</td>
</tr>
<tr>
<td>III</td>
<td>11</td>
<td>0.8-13</td>
<td>9-13</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>3-14</td>
<td>4-8</td>
</tr>
<tr>
<td>V</td>
<td>50</td>
<td>3-25</td>
<td>7-17</td>
</tr>
</tbody>
</table>

*First 12 cm of the small intestine is the duodenum. The region beyond that with limits that are not well-defined, is considered the jejunum or upper ileum. (12).

Our chemical excystation experiments have demonstrated that excystation in E. flexum is active and is facilitated by the synergistic effects of trypsin + bile salts at an alkaline pH, and a temperature which simulates the avian environment. Using complex methodology, Howell (13, 14) has successfully excysted the metacercaria of an Australian echinostome species, E. ovinum, and has discussed the need for the synergistic effect of bile salts + trypsin for successful in vitro excystation.

ACKNOWLEDGMENTS
This work was supported, in part, by funds from The Committee on Advanced Study and Research, Lafayette College.