Infectivity of *Amblossoma suwaense* (Trematoda: Brachylaimidae) in the Domestic Chick

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ABSTRACT: Anesthetized domestic chicks were infected per cloaca with free metacercariae of *Amblossoma suwaense* removed directly from *Campeloma decisum* snails (fresh metacercariae) or metacercariae maintained for 24 hr in sterile Locke's at 38 ± 1°C (conditioned metacercariae). Worms from fresh metacercariae established only in the cloaca and were not recovered after 24 hr postinoculation. Worms from conditioned metacercariae established mainly in the bursa of Fabricius and were recovered up to 4 days postinoculation. Ovigerous worms were not obtained. Worms from both sites fed on host mucosa and blood, caused hemorrhagic spots where suckers contacted host tissue, and showed a tendency to pair. The mean body area of worms from chicks was markedly greater than that of metacercariae. However, the area of the ovary of worms from chicks was markedly less than that of metacercariae.

Shimazu (1974) described the free (unencysted) brachylaimid metacercaria of *Amblossoma suwaense* from the viviparid snail *Sinotaia quadrata* in Japan, and Font (1980) recently found this metacercaria in *Campeloma decisum* snails in Wisconsin, USA. Adults of this parasite have not been described from natural or experimental hosts, although Shimazu (1974) and Font (1980) grew metacercariae to ovigerous adults in chick embryos. Schnier and Fried (1980) obtained ovigerous adults in vitro within 4 days in NCTC 135 plus 20% hens' egg yolk. Shimazu (1974) and Font (1980) were unable to infect domestic chicks with metacercariae and recent unpublished studies in our laboratory using unanesthetized chicks and per cloacal exposure were also unsuccessful. Fried and Harris (1971) used the cloacal drop procedure on anesthetized chicks to obtain infection with *Leucochloridiorhynchus constantiae* and Herman and Bacha (1978) used this procedure to infect chicks with *Himasthla quissetensis* cercariae. The purpose of this study was to infect anesthetized chicks per cloaca with *A. suwaense* metacercariae.

**Materials and Methods**

*Amblossoma suwaense* metacercariae were removed from the visceral epithelium of *C. decisum* snails (Schnier and Fried, 1980), rinsed in sterile Locke's solution and used immediately for infection studies (fresh metacercariae), or were used following maintenance for 24 hr at 37 ± 1°C in Locke's (conditioned metacercariae). Conditioned metacercariae were live and active at 24 hr, but showed no postmetacercarial growth or development.

Chicks, 1 and 2 days old, were anesthetized with Equi-Thesin (Fried and Berry, 1961) and infected individually per cloaca with either five fresh or five conditioned metacercariae. A petri dish containing Locke's was placed under each chick to collect worms released in the stool, and chicks that defecated worms within 15 min were reinfected with an equal number of worms. Chicks remained anesthetized for 2–3 hr and were observed for the loss of worms in the stool up to 5 hr postinoculation.

Chicks were necropsied 1 to 5 days postinoculation and the lower ileum, caeca, rectum, bursa of Fabricius, and cloaca were examined. Metacercariae and worms
Table 1. Summary of Amblosoma suwaense infectivity data in chicks.

<table>
<thead>
<tr>
<th>No. of chicks exposed to</th>
<th>No. of chicks infected with fresh (F) or conditioned (C) metacercariae</th>
<th>Total no. of worms in cloaca (Cl) or bursa of Fabricius (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh (F) or conditioned (C)</td>
<td>Age of chicks (days) at necropsy</td>
<td>Cl</td>
</tr>
<tr>
<td>F</td>
<td>C</td>
<td>F</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>1</td>
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<tr>
<td>–</td>
<td>5</td>
<td>1</td>
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<td>–</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>–</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>19</td>
</tr>
</tbody>
</table>

from chicks were studied live, then prepared as stained whole mounts, and measured following the model of Berntzen and Macy (1969). Measurements were made of relative body area (product of length times midacetabular width) and area of the gonads and vitellaria.

To determine if conditioning of a brachyляемid metacercaria had subsequent effects on growth and development, additional chicks were each inoculated per cloaca with five metacercariae of L. constantiae maintained for 24 hr at 37 ± 1°C in Locke’s. L. constantiae metacercariae were obtained from the uterus of Campeloma decisum snails as described previously (Harris et al., 1972). Chicks were necropsied 4 days postinoculation and worms from the bursa of Fabricius were examined as described above.

Results

Of 14 chicks inoculated with fresh metacercariae, seven (50%) expelled one to three worms in the stool, whereas seven (37%) of 19 chicks inoculated with conditioned metacercariae expelled one to two worms in the stool within 5 hr postinoculation. Results of infection in the chick with A. suwaense metacercariae are summarized in Table 1. Worms were recovered only in the cloaca or bursa of Fabricius. Of five worms recovered in the cloaca, four were from fresh metacercariae and all worms from the bursa were from conditioned metacercariae. In one chick inoculated with fresh metacercariae, two worms were recovered 1 day later, 2 mm from each other in the urodaeum of the cloaca. Another chick inoculated with conditioned metacercariae and necropsied 3 days later contained two worms within 2 mm of each other on adjacent folds of the bursa. Only single worms were recovered in the remaining infected chicks. In all infections worms attached tenaciously to the mucosal surface with the oral sucker and the acetabulum. Hemorrhagic areas were observed where the suckers attached to the mucosa. Worms intestinal contents were cream-colored suggesting that they fed on the mucosal surface. However, microscopic examination of worms also showed the presence of host erythrocytes in the intestinal caeca. Worms from chicks never contained black pigment seen in the intestinal caeca of metacercariae (Schnier and Fried, 1980).
Day-old *A. suwaense* from the cloaca increased their mean body area by 51%, their testicular area by 37%, and their vitelline area by 79%. No change in ovarian area was noted. Conditioned metacercariae showed no change in mean body or testicular areas, but showed a 14% decline in vitelline area, and a 60% decline in area of the ovary. As compared with fresh metacercariae, *A. suwaense*, 3 to 4 days old, from the bursa showed an increase in mean body area of 45%, in testicular area of 82%, and vitelline area of 7%, but showed a 67% decline in ovarian area.

Conditioned metacercariae of *L. constantiae* developed into ovigerous adults in the bursa of Fabricius in 4 days and were identical in size and development to adults of the same age from fresh metacercariae (Harris et al., 1972).

**Discussion**

Anesthetized chicks became infected following per cloacal administration of *A. suwaense* metacercariae. The use of anesthesia probably reduced chick defecation and helped in the establishment of worms as described by Herman and Bacha (1978) for *H. quissetensis*. Our study is the first to provide infection data on *A. suwaense* in an experimental definitive host, although Pojmanksi (1972) found young adults of *Amblosoma exile* in the cloaca of naturally infected ducks, *Aythya fuligula*.

*A. suwaense* became ovigerous within 4 to 5 days in chick embryos (Shimazu, 1974; Font, 1980) and in 4 days in vitro in NCTC 135-20Y (Schnier and Fried, 1980). However, the chick is apparently a poor host for *A. suwaense* since infections did not last beyond 4 days and ovigerous worms were not obtained. These observations are similar to those of Fried and Foley (1970) on *Clinostomum marginatum*, in which worms showed some postmetacercarial development, but did not survive in the mouth of the chick beyond 4 days.

Conditioned metacercariae established mainly in the bursa of Fabricius and survived longer in the chick than fresh metacercariae. Fresh metacercariae established only in the cloaca. Reasons for these findings are unclear. *L. constantiae* metacercariae establish only in the bursa of Fabricius of the avian host (Allison, 1943; Harris et al., 1972), whereas *Leucochloridium variae* metacercariae establish in both the cloaca and bursa (Fried and Guy, 1974). Cercariae of *H. quissetensis* establish in the lower ileum and bursa of the chick, but worms from the latter site often show gonadal atrophy (Herman and Bacha, 1978). As discussed for *H. quissetensis* by Herman and Bacha (1978), immunologic factors associated with the bursa may inhibit ovarian development in *A. suwaense*.

The body area of *A. suwaense* grown in vitro decreased (Schnier and Fried, 1980), whereas that of worms grown in chicks increased. The area of the ovary of worms grown in vitro increased (Schnier and Fried, 1980), whereas that of worms grown in chicks decreased. These findings are unusual since most trematodes show more marked somatic and reproductive growth in vivo than in vitro (Fried, 1978).

**Acknowledgment**

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Literature Cited


