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Life Cycle of Alaria Marcianae in Louisiana (Trematoda, Transmammary, Milk-Borne).

Wesley Lawrence Shoop
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Life Cycle of *Alaria marcianae* in Louisiana

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology and Physiology

by

Wesley L. Shoop
B.S., University of Nebraska, 1977
M.S., Louisiana State University, 1980
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>Chapter I</td>
<td></td>
</tr>
<tr>
<td>The Life Cycle of <em>Alaria marcianae</em></td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>4</td>
</tr>
<tr>
<td>Results</td>
<td>6</td>
</tr>
<tr>
<td>Discussion</td>
<td>17</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>21</td>
</tr>
<tr>
<td>Chapter II</td>
<td></td>
</tr>
<tr>
<td>Transmammary Infection of Paratenic and Definitive Hosts with <em>Alaria marcianae</em></td>
<td>24</td>
</tr>
<tr>
<td>Introduction</td>
<td>26</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>27</td>
</tr>
<tr>
<td>Results</td>
<td>27</td>
</tr>
<tr>
<td>Discussion</td>
<td>28</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>30</td>
</tr>
<tr>
<td>Chapter III</td>
<td></td>
</tr>
<tr>
<td>Migration of <em>Alaria marcianae</em> in Domestic Cats</td>
<td>31</td>
</tr>
<tr>
<td>Introduction</td>
<td>32</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>32</td>
</tr>
<tr>
<td>Results</td>
<td>33</td>
</tr>
<tr>
<td>Discussion</td>
<td>34</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>37</td>
</tr>
</tbody>
</table>
Chapter IV

Tegumental Changes of *Alaria marcianae* during migration in the Domestic Cat ........................................ 38

Introduction ................................................................................................................. 39

Materials and Methods ......................................................................................... 41

Results ........................................................................................................................... 42

Discussion ...................................................................................................................... 58

Literature Cited ........................................................................................................ 61

Vita ..................................................................................................................................... 63
ABSTRACT

The life cycle of *Alaria marcianae* (Trematoda) as found in Louisiana involves the planorbid snail, *Helisoma trivolvis*, as first intermediate host; tadpoles of *Rana catesbiana*, *R. clamitans*, *R. utricularia*, *Hyla cinerea*, and *Gastrophryne carolinensis* as second intermediate hosts; a wide range of amphibian, reptilian, and mammalian paratenic hosts; and the bobcat (*Lynx rufus*), domestic cat (*Felis domesticus*), and juvenile raccoon (*Procyon lotor*) as definitive hosts. Eggs defecated by the definitive host require an incubation period of approximately 20 days; a free swimming miracidium hatches from each egg and must penetrate a snail within 24 hr or die; the miracidium transforms into a mother sporocyst within the anterior blood sinuses of the snail; mother sporocyst germ cells produce a generation of daughter sporocysts which migrate to the snail digestive gland; daughter sporocyst germ cells give rise to free swimming cercariae as early as 61 days postexposure to miracidia; cercariae must penetrate tadpoles within 48 hr or die and those that are successful metamorphose to the mesocercarial stage within 14 days; mesocercariae in tadpoles that are consumed by a paratenic host remain mesocercariae, but if consumed by a definitive host they begin a complex 15-day migration involving both direct and circulatory pathways to the lungs where they metamorphose to diplostomula and then a return trip to the small intestine via the bronchi, trachea, and esophagus where they mature to the adult. Electron microscopical studies on these stages suggest that the migration from the lungs to the small intestine is orchestrated by the host's defense mechanisms rather than an active migration on the part of the parasite. Transmammary infection with mesocercariae
is described experimentally in mice, domestic cats, and raccoons. Mesocercariae are diverted from their normal pathway to the mammary gland only during lactation, thereby implying a hormonal stimulus. No prenatal infection occurs. Three distinct patterns of mesocercarial behavior in lactating mammals are presented: (1) in euparasitic hosts the mesocercariae remain undifferentiated in both the parent and offspring; (2) in amphiparasitic hosts the mesocercariae remain undifferentiated in the parent, but they mature to adults when transmitted to the offspring; and (3) in definitive hosts both the parent and offspring possess the physiological requirements for development of the mesocercariae to adult worms.
Chapter I

The Life Cycle of Alaria marcianae
INTRODUCTION

To date, there exists a number of life history studies on members of the genus *Alaria* and they are among the most complex within the Trematoda (Bosma, 1934; Odlaug, 1940; Potekhina, 1950; Pearson, 1956; Johnson, 1968; 1979). The life cycles reported heretofore consist of: an adult residing in the small intestine of a carnivore; a defecated egg embryonating in water; a miracidium penetrating a planorbid snail with subsequent sporocyst generation; free-swimming furcocercous cercariae penetrating larval amphibians; paratenic residence in a wide spectrum of amphibian, reptilian, and mammalian hosts; and ingestion of the intermediate or paratenic host with a somatic migration and development of mecosercariae, diplostomula, and adults within the definitive hosts.

Not only do life cycle data establish the ecology of the parasite and place in perspective its evolutionary biology, but they also are the fundamental information used by epidemiologist when reconstructing the origin of human infection. Within the last decade, members of the genus *Alaria* have been recognized as significant human health hazards (Shea *et al.*, 1973; Freeman *et al.*, 1976; Beaver *et al.*, 1977). However, the available life cycle data neither predicted nor were adequate for explaining the etiology of human infection. This inadequacy was most apparent in an autochthonous infection in Louisiana in which the source traced by epidemiologists (Beaver *et al.*, 1977) had been reported previously to be an unsuitable host (Pearson, 1956; Johnson, 1968). Shoop and Corkum (1981) investigated the epidemiology of that infection and identified the infectious agent as *Alaria mar-
cianae and confirmed the source of infection suggested by Beaver et al., (1977).

Also noted in the report by Shoop and Corkum (1981) was the possibility of maternal transmission. Mesocercariae were located in the subcutaneous fat of mammalian paratenic hosts, but a marked preference for the mammary glands was observed in lactating females. Not only had maternal transmission not been suggested in any prior studies on *Alaria marcianae*, but neither had it been determined experimentally for any other trematode.

In light of these new findings it was decided to reinvestigate the life cycle of *Alaria marcianae* as it occurs in the swamps of Louisiana. In this chapter, the basic life cycle of *A. marcianae* is investigated experimentally and verified with field data. In Chapter 2, a new mode of transmission is described that changes the way we view the *A. marcianae* life cycle and adds a significant new dimension to our understanding of trematode life cycles in general. In the third chapter, the unique somatic migration within a mammalian host is documented chronologically and anatomically. The final chapter concerns the morphological transformations that *A. marcianae* undergoes during its migration in a mammalian host and presents new thoughts on its evolution.
MATERIALS AND METHODS

Experimental life cycle studies began with the collection of mesocercariae from water moccasins, Agkistrodon piscivorus. These larvae were fed to laboratory-reared cats and raccoons. The hosts were necropsied when eggs began to appear in their feces. Diplostomula from the lungs and adults from the small intestine were recovered. Eggs from the feces were incubated in tap water at room temperature. When miracidia hatched, each was placed singly with a snail in 20 ml of tap water for 24 hr and then transferred to 100 ml containers. Snails of Helisoma trivolvis, Physa gyrina, and Viviparus intertextus, collected from localities previously determined not to have Alaria marcianae infection, were used in the experimental infections. After exposure to miracidia, the snails were examined daily for the emergence of cercariae. When cercariae began to appear, colonies of laboratory-reared tadpoles of Rana catesbiana, R. clamitans, and R. utricularia were exposed to infected snails for a 24-hr period. Five tadpoles of each species were examined weekly until fully developed mesocercariae were found. All stages were fixed in steaming 10% formalin. Measurements are in micrometers unless indicated otherwise; ranges in parentheses.

The life-cycle stages occurring naturally were monitored by continuous collection at Head of Island, Louisiana, from 1978 to 1983. The following snails were examined as potential first intermediate hosts: 126 Helisoma trivolvis; 200 Physa gyrina; 200 Pseudosuccinea columella; 87 Viviparus intertextus; 12 Promenetus exacuous; 30 Laevalex fuscus; 30 Ferrisia fragilis; and 30 Hebetancylus excentricus.
Snails were brought to the laboratory, isolated singly in 100 ml containers, and examined daily for the emergence of cercariae. If emergent cercariae were similar to those of Alaria marcianae, they were placed with uninfected tadpoles, reared to the next stage, and identified. If snails exhibited no cercarial emergence after a two-week period, they were crushed and examined for sporocysts.

The following amphibians were collected and examined for mesocercariae: 23 Rana catesbiana; 170 R. clamitans; 22 R. utricularia; 30 Hyla cinerea; 6 Gastrophyne carolinensis; and 3 Notophthalmus viridescens. Mesocercariae collected from the hosts were fed to laboratory cats, reared to adulthood, and identified.

A list of paratenic hosts from Head of Island, Louisiana has been published by Shoop and Corkum (1981).

Through the efforts of the writer as well as others, the following mammals were trapped and examined for diplostomula and adult worms: 85 raccoons, Procyon lotor; 43 minks, Mustela vison; 11 river otters, Lutra canadensis; 37 opossums, Didelphis virginiana; 5 muskrats, Ondatra zibethica; 4 bobcats, Lynx rufus; 3 red foxes, Vulpes fulva; 1 gray fox, Urocyon cinereoargenteus; and 1 striped skunk, Mephitis mephitis.
RESULTS

First Intermediate Host

Miracidia placed with snails of *Helisoma trivolvis* became very active and were observed to penetrate the mantle region of the foot. In all *H. trivolvis*, penetration took place within 30 min postexposure. Miracidia showed no hyperactivity in the presence of *Physa gyrina* or *Viviparus intertextus* and no penetration was observed in either species of snail even after a 24 hr period.

Cercariae were first observed at day 61 in *Helisoma trivolvis* and all of these snails demonstrated cercarial emergence by 78 days. No cercarial emergence was noted in either *Physa gyrina* or *Viviparus intertextus*, and when necropsied after 150 days they were negative for sporocysts.

Infected *Helisoma trivolvis* shed cercariae for more than one year. As snails died, they were immediately examined for sporocysts. Daughter sporocysts with cercariae were recovered from all *H. trivolvis*, but only in two snails were mother sporocysts with daughter sporocysts observed.

Of the snails collected from Head of Island, Louisiana, only two specimens of *Helisoma trivolvis* shed *Alaria marcianae* cercariae. These cercariae were morphologically indistinguishable from those in experimental infections. When these snails were necropsied, daughter sporocysts were recovered and they were also morphologically indistinguishable from those in experimental infections.

Second Intermediate Hosts

Tadpoles of all three species of *Rana* became infected with meso-
cercariae after experimental exposure to cercariae. However, of the 20 R. clamitans examined, no tadpole was found to have more than two mesocercariae even though each was exposed to several hundred cercariae. In 20 R. utricularia tadpoles, no more than 10 mesocercariae were observed in a single tadpole and in 20 R. catesbiana, a mean of 110 (91-150) mesocercariae were recovered from each tadpole. Fully developed mesocercariae were recovered from R. catesbiana and R. utricularia tadpoles within two weeks, whereas in R. clamitans this stage was not fully developed until the fourth week. All mesocercariae recovered from tadpoles were unencysted and located in the muscles of the mylohyoid, sternum, and tail regions.

Twenty recently metamorphosed frogs of R. catesbiana that were exposed to cercariae never became infected.

The following adult amphibians were found naturally infected with mesocercariae of Alaria marcianae (mean number of mesocercariae recovered is in parentheses): 20 of 23 Rana catesbiana (58); 33 of 170 R. clamitans (3); 7 of 22 R. utricularia (5); 16 of 30 Hyla cinerea (5); 1 of 6 Gastrophryne carolinensis (1); and 0 of 3 Notophthalmus viridescens. Mesocercariae recovered from these natural infections were morphologically indistinguishable from those in experimental infections. The mesocercariae were unencysted and were recovered from the muscles of the mylohyoid, sternum, and hindlegs.

Paratenic Hosts

A list of paratenic hosts both natural and experimental from Head of Island, Louisiana, has been published by Shoop and Corkum (1981). In experimental infections every amphibian, reptilian, and mammalian host that was inoculated with mesocercariae became infected. Those
species found naturally infected include: 82 of 85 *Agkistrodon piscivorus*; 48 of 52 *Nerodia cyclopion*; 4 of 4 *N. erythrogaster*; 41 of 41 *N. fasciata*; 3 of 7 *N. rhombifera*; 12 of 17 *Thamnophis proximus*; 2 of 2 *Coluber constrictor*; 3 of 3 *Lampropeltus getulus*; 6 of 10 *Didelphis virginiana*; and 3 of 5 *Procyon lotor*.

It is noteworthy that, in the laboratory, bullfrogs were observed to cannibalize small frogs and could possibly serve as paratenic hosts.

Mesocercariae recovered from reptiles were found in most organs, but large concentrations were found in the fat bodies. Mesocercariae from mammals were found in the lungs, subcutaneous fat, and mammary glands.

**Definitive Hosts**

Thirteen laboratory cats, two juvenile raccoons, and two adult raccoons were fed mesocercariae. All of the laboratory cats and both juvenile raccoons became infected. Eggs appeared in their feces between 19-20 days postexposure. When the cats and juvenile raccoons were necropsied after 20 days, diplostomula were found within the alveoli of the lungs and mature adults were recovered from the duodenum.

The two adult raccoons that did not shed eggs were necropsied after thirty days. It was found that the mesocercariae had migrated to the subcutaneous fat and remained undifferentiated. No diplostomula or adult worms were present.

Eggs recovered from the feces of experimentally infected hosts were incubated in tap water at room temperature. When exposed to strong light, miracidia ruptured the opercular membrane of the egg as early as 20 days postincubation.
In natural infections, all bobcats examined were infected with *Alaria marcianae* diplostomula in the lungs and adult *A. marcianae* in the duodenum. These stages were morphologically indistinguishable from those in experimental infections. Of 81 adult and four juvenile raccoons examined, none of the adults and only two of the juveniles were found to be infected with diplostomula and adult worms. This corroborates the experimental results in which worms were found to mature in juvenile, but not in adult raccoons.

**Description of Stages**

**Miracidium** (Fig. 1)

Eggs incubated for 20 days ruptured to give rise to the ephemeral, free-swimming miracidium. No miracidium survived beyond a 24-hr period if it had not penetrated a suitable snail host.

Description (based on ten fixed specimens): The body is elongate, broadly rounded anteriorly, and tapered posteriorly. It measures 162 (149-183) long by 42 (35-50) wide. The body is covered with cilia and possesses two prominent eyespots. An apical papilla is located on the anterior tip of the body. Immediately posterior to the apical papilla is a saclike structure, the apical gland, which presumably aids in penetration of the snail. Paired lateral processes protrude from the body wall at the level of the posteriormost extent of the apical gland. Immediately posterior to the eyespots is a mass of cells that represent the neural ganglion. Immediately posterior to the neural ganglion is a mass of large cells representing the germinal cells. Two pairs of flame cells are present. The flame cell ducts empty to the outside via a pore in the posterior third of the
Figures 1-7. Stages of *Alaria marcianae* from Louisiana.


Scales are in micrometers.
body. At the posterior end, a glandular sac-like structure, resembling the apical gland, empties terminally.

**Mother Sporocyst** (Fig. 2)

Only two mother sporocysts were recovered from experimentally infected *Helisoma trivolvis*. Both were recovered from the anterior blood sinuses of the snails, one on day 81 and the other on day 103 postexposure to miracidia.

Description (based on two fixed specimens): The mother sporocyst is unbranched, elongate, tubular, and 3.8–4.7 mm long by 0.05–0.2 mm wide. Daughter sporocysts and germinal cells are present inside the mother sporocyst.

**Daughter Sporocyst** (Fig. 3)

Hundreds of daughter sporocysts developed in the digestive gland of all experimentally infected *Helisoma trivolvis*.

Description (based on ten fixed specimens): The daughter sporocyst is unbranched, elongate, tubular, and threadlike. It measures 1.3 (1.1–1.6) mm long by 45 (40–55) wide. The birth pore is present near the anterior end. Various stages from germinal cells to fully developed cercariae are present inside the daughter sporocysts.

**Cercaria** (Fig. 4)

Emergent cercariae from *Helisoma trivolvis* swam to the top of the water column, turned upside down, and parachuted to the bottom of the container. Their descent was so slow that at times they appeared suspended in the water. When a tadpole was in the vicinity, they swam excitedly and attached to the tadpole via the acetabulum and oral sucker. The cercaria is short-lived; more than half of those
that did not attach to a tadpole died within 24 hr and all were dead by 48 hr.

Description (based on ten fixed specimens): The cercaria is furcocercous, longifurcous, distomate, and pharyngeate. The body measures 140 (120-160) long by 27 (25-30) wide; the tail stem 155 (150-160) long by 25 (20-30) wide; the furca 150 (145-160) long; the oral sucker 26 (24-27) long by 20 (19-22) wide; the pharynx 10 (9-11) in diameter; and the acetabulum 20 (19-22) in diameter.

The oral hood is covered with rows of small spines. The spines are less densely distributed posteriorly, but cover the entire venter with the exception of areas anterior and posterior to the acetabulum. Three to four rows of spines are present on the acetabulum. A pair of unpigmented eyespots occurs laterally, midway between the oral sucker and the acetabulum. Two pairs of penetration glands are present, located just anterior to the acetabulum; their ducts empty into the oral sucker. A spherical mass of cells representing the genital primordium is located between the acetabulum and the posterior end of the body. The flame cell formula is $2[(2+2+2)+(2+2)+2]$. A pair of sensory hairs is located on the margins of the body in the posterior fourth of the worm. Numerous hairlike extensions of the tegument extend laterally on the tail stem.

Mesocercaria (Fig. 5)

All mesocercariae removed from the various host species were indistinguishable from one another.

Description (based on ten fixed specimens): The body appears to be an enlarged cercaria and measures 432 (380-525) long by 173 (150-195) wide. The oral sucker is 79 (65-99) long by 59 (55-66)
wide; the prepharynx is 8 (7-9) long; the pharynx is 25 (23-28) long by 22 (20-28) wide; and the genital anlage is 37 (33-44) in diameter.

The oral sucker is covered by 14 or 15 rows of simple spines. Spines extend onto and cover the body except for areas anterior and posterior to the acetabulum. The acetabulum has 3 or 4 rows of spines. Eyespots are absent. The most conspicuous structures in this stage are the four unicellular penetration glands located anterior and lateral to the acetabulum. These glands empty into the oral sucker via individual ducts. The flame cell formula is $2[(2\cdot6)+(2\cdot6)+(2\cdot6)] + 2[(2\cdot6)+(2\cdot6)]$.

Diplostomulum (Fig. 6)

Diplostomula were recovered from the lungs of domestic cats, bobcats, and juvenile raccoons. They formed 1-2 mm in diameter, fluid-filled vesicles in the lungs. Upon removal from these vesicles, the ceca of the worms appeared golden brown; this color reflected by the partially digested red blood cells on which the worms had been feeding.

Description (based on ten fixed specimens): The body is spatulate and 550 (480-600) long by 215 (205-240) wide; the forebody is 435 (410-460) long by 215 (205-240) wide; the hindbody is 435 (410-460) long by 215 (205-240) wide; the oral sucker is 55 (50-61) long by 60 (55-63) wide and surrounded on either side by earlike lappets; the prepharynx is 10 (6-12) long; the pharynx is 45 (41-48) long by 38 (33-40) wide; the paired ceca are 365 (330-380) long by 35 (26-40) wide; the acetabulum is 63 (54-69) long by 61 (59-67) wide; and the tribocytic organ is 120 (114-132) long by 71 (50-99) wide.

The entire forebody is covered with a dense array of spines; the
hindbody is devoid of spines. Four unicellular penetration glands are present in all stages of degeneration. The ducts to the oral sucker are the first to be absorbed, then the glands atrophy, and eventually all evidence of these structures is lost. The genital primordium remains undifferentiated in young diplostomula, but becomes a lobate structure in older forms.

**Adult (Fig. 7)**

Adults were recovered from the duodenum of domestic cats, bobcats, and juvenile raccoons. Each adult was individually attached to a host intestinal villus.

Description (based on ten gravid, fixed specimens): The body is elongate, distinctly bisegmented, and 1375 (1000-1600) long by 478 (350-600) at the widest point. The forebody is scoop-shaped and 883 (650-1050) long by 478 (350-600) wide. The entire forebody serves as an organ of attachment. Lappets are present on either side of the oral sucker; they rarely invaginate to form pseudosuckers. The hindbody is conical, 535 (280-500) wide and contains the reproductive organs. The forebody tegument is covered with small spines; the hindbody tegument smooth. The oral sucker is 90 (60-105) long by 73 (60-81) wide; the acetabulum is 74 (60-95) long by 75 (60-95) wide and is rarely covered by the tribocytic organ; the tribocytic organ is 453 (310-550) long by 200 (155-225) wide when evaginated. The prepharynx is 5 (4-6) long; the pharynx is 102 (75-215) long by 64 (55-85) wide; the esophagus is 6 (4-10) long; and paired ceca extend to the posterior end of the body. The testes are tandem, but not equal; the anterior testis is asymmetrical, typically wedge-shaped, laterally disposed on either side of the midline and 160 (128-
215) long by 225 (175-300) wide; the posterior testis is symmetrical, dumbbell-shaped, much wider than the anterior testis, 210 (165-276) long by 340 (275-425) wide, and has a ventro-medial groove to allow passage of the ceca and uterus; a muscular ejaculatory pouch lies posterior to the testes and empties into the genial atrium; the genital atrium is located in the posterior end of the body and opens on the dorso-subterminal side. The ovary is reniform, located anterior to the anterior testis and is 72 (60-99) long by 167 (100-180) wide; the Mehlis' Gland lies opposite the ovary; the uterus courses briefly into the forebody, turns immediately posteriad, and opens into the genital atrium; the vitellaria are located only in the forebody, from just anterior to the acetabulum to the forebody-hindbody juncture; the vitelline reservoir is prominent, located in the hindbody at the level of the anterior testis. Eggs are few (1-6), large, operculate, and 122 (110-128) long by 65 (60-75) wide. The excretory pore is terminal.
DISCUSSION

The significance of this report lies in the fact that it is the first description of an *Alaria* spp. life cycle from the southern United States and more importantly it is the only complete description of all stages of *A. marcianae* currently available. Adults of *A. marcianae* have been described by Hall and Wigdor (1918), La Rue and Fallis (1936), Burrows and Lillis (1965), and Shoop and Corkum (1982). In a classic study, Pearson (1956) described in detail the sporocyst generations, mesocercaria, and diplostomulum of *A. canis* (*A. marcianae*). In another excellent contribution, Johnson (1968) described all stages except the miracidium of *A. marcianae*, although it was evident from his work that he had seen it.

The first intermediate host for *Alaria marcianae* is *Helisoma trivolvis*. This was the only species of snail accepting experimental infection. This was corroborated by five years of field collecting in which *H. trivolvis* was the only snail species found naturally infected. Experimentally, the miracidium was observed to penetrate actively the mantle region of the snail. Two sporocyst generations occur in the snail with a culmination of cercarial emergence 61 days postexposure to miracidia. *H. trivolvis* survived in the laboratory for more than one year and continuously shed cercariae.

Cercariae liberated into the water column have but a brief time (24-48 hr) to find a suitable second intermediate host. Penetration only occurred among tadpoles, inasmuch as recently metamorphosed bullfrogs were refractory to infection. Tadpoles of all three *Rana* species became infected, but a marked predilection for the bullfrog was apparent both in terms of number and time of development. Simi-
lar results were obtained in the study by Pearson (1956), who also found *R. clamitans* to be a less suitable host.

Massive natural infections in bullfrogs in which several thousand mesocercariae were found in the hindlegs alone have been reported (Freeman et al., 1976). A likely reason the bullfrog may accumulate large infections naturally is that it often remains a tadpole for several seasons, thus increasing the time of exposure to cercariae. However, it must also be noted that bullfrogs were observed to cannibalize smaller frogs and the possibility of accumulating large numbers of mesocercariae in this manner can not be dismissed.

Two ecological pathways are available to the mesocercariae; the second intermediate host can be consumed by a paratenic host or either of those can be consumed by a definitive host. Present knowledge indicates that it is the second intermediate and paratenic hosts that are epidemiologically significant. A paratenic host is a species in which the parasite gains access, but within which it undergoes no development. Thus, paratenicity is an ecological bridge that links two stages of a life cycle that, by themselves, have a low probability of transmission. In this case, tadpoles are rarely eaten by domestic cats, bobcats, or raccoons, but they are common prey items of other predators such as amphibious water snakes. These snakes act as accumulators of mesocercariae and when they enter the food web, either as prey or carrion, they transmit massive infections. Adult raccoons and opossums also serve as paratenic hosts. Humans may serve as paratenic hosts either through mishandling a game animal or through direct consumption (Shea et al., 1973; Shoop and Corkum, 1981).

The life cycle of *Alaria marcianae* is completed when the second
intermediate host or paratenic host is ingested by a domestic cat, bobcat, or juvenile raccoon. Field studies indicate that it is the bobcat and juvenile raccoon that maintain the life cycle in Louisiana. The reason adult raccoons are refractory to further development of mesocercariae, and juvenile raccoons are not, is enigmatic. Mesocercariae apparently undergo a migration within the definitive host and reach maturity in the duodenum. Cuckler (1940) reported that the migration involves a direct somatic route to the lungs where diplostomula are found and then a return trip via the trachea and esophagus to the duodenum. My observations appear to confirm this. A detailed study of this phenomenon will do much to clarify the biology of this organism.

The basic life cycle and the morphology of the stages reported herein for Alaria marcianae do not differ significantly from information presented for A. canis by Pearson (1956), A. marcianae by Johnson, (1968), or A. alata by Potekhina (1950). Dubois (1970), in fact, synonymized A. canis under A. marcianae. To date, no comparison between A. marcianae and A. alata has been attempted. Historically, A. alata has been regarded as a Palearctic infection and A. marcianae as a Nearctic one. Based upon life cycle and morphology, these two species are indistinguishable. Interestingly, several workers have reported A. alata from mammalian carnivores in North America (Swales, 1933; Erickson, 1944). However, the observations and species designations of Swales and Erickson were ignored and the Palearctic-Nearctic split maintained. The only support for the continued separation of these two populations are: (1) A. marcianae infects snails of the genus Helisoma, whereas A. alata infects
the genus _Planorbis_; and (2) the migration of _A. marcianae_ in a definitive host takes as little as three weeks, whereas in _A. alata_ it has been reported to require five weeks (Savinov, 1953). Of these two objections the second must be regarded as the more significant. Given the close relationship of the two planorbid genera it would not be surprising if _A. alata_ and _A. marcianae_ could infect both. However, until the reported differences in migration time in the definitive hosts are understood the continued recognition of the two taxa is advisable.
LITERATURE CITED


Chapter II

Transmammary Infection of Paratenic and Definitive Hosts with

*Alaria marcianae*
Mr. Wesley L. Shoop  
Department of Zoology and Physiology  
Louisiana State University  
Baton Rouge, Louisiana 70803

Dear Mr. Shoop,

As Editor of The Journal of Parasitology, I hereby authorize your use of the following papers by Wesley L. Shoop and Kenneth C. Corkum:

"Transmammary Infection of Paratenic and Definitive Hosts with Alaria marcianae (Trematoda) Mesocercariae". J. Parasit. 69: 731-735.

"Migration of Alaria marcianae (Trematoda) in Domestic Cats" J. Parasit. 69: 912-917.

"Tegumental Changes of Alaria marcianae (Trematoda) during Migration in the Domestic Cat" J. Parasit. (in press).

to be re-microfilmed after use in your doctoral dissertation, with a proper credit line as to the original publication in The Journal of Parasitology, including Volume number.

Yours sincerely,

[Signature]

David F. Mettrick, Editor.
TRANSMAMMARY INFECTION OF PARATENIC AND DEFINITIVE HOSTS WITH ALARIA MARCIANAEE (TREMATODA) MESOCERCARIAE

Wesley L. Shoop and Kenneth C. Corkum
Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

ABSTRACT: Transmammary infection with Alaria marciannae mesocercariae was demonstrated using mice as model, paratenic hosts. Prenatal transmission was ruled out because neonates removed immediately postpartum from infected dams were never infected. Mesocercarial distribution in virgin females and in females examined immediately postpartum showed no marked preference for the mammary glands. In contrast, infection of neonates that were allowed to suckle on infected dams was absolute, and the number of mesocercariae in the mammary glands of postparturient dams that suckled their young was increased significantly. These experimental observations were coupled with other observations on paratenic hosts to outline the pathways open to mesocercariae in gravid hosts. The term amphiparatenic host is coined for those host species that are paratenic hosts as adults, but as juveniles can serve as definitive hosts.

We have noted the predilection of Alaria marciannae mesocercariae for the body fat and mammary glands of gravid, paratenic hosts (Shoop and Corkum, 1981). This led us to believe that maternal transmission of these highly migratory stages may be possible. This supposition was further complicated when we fed mesocercariae to neonatal raccoons, adults of which act only as paratenic hosts, and recovered adult A. marciannae from the small intestine. This behavior of the mesocercariae in neonatal raccoons was unexplainable at that time.

Additional evidence of maternal transmission became apparent during maintenance of A. marciannae in our laboratory. We fed mesocercariae to a pregnant cat, but fecal smears failed to show patency at the time normally presumed for maturity (19 days) of A. marciannae. Fecal smears from the other cats in the vivarium one month later showed, however, that large, operculate eggs were being passed by several young cats. Our records revealed that these cats were the offspring of the female that had failed to reach patency. At necropsy, these offspring were found to have adult A. marciannae in the small intestine.

Though maternal transmission was indicated in the infections of cats, it was not possible to determine whether it resulted from prenatal or postnatal pathways. Extraneous sources of infection were precluded as these cats were kept in a sixth-floor vivarium, fed only commercial cat chow, and there was no access to any intermediate host of A. marciannae.

Maternal transmission of helminths has been reviewed by Stone and Smith (1973) and Miller (1981). Though not uncommon in helminths such as nematodes, reports of it among trematodes are rare. Miller's own studies on the genus Pharyngostomoides have provided the only evidence for maternal transmission in trematodes (Harris et al., 1967; Miller, 1981). Pharyngostomoides and Alaria are closely-related, diplodostomid trematodes that have similar life-cycles. The stage involved in the Pharyngostomoides cycle is also a mesocercaria and transmission is thought to occur during lactation between a female raccoon and her pups (Miller, 1981).

To date, there exist many excellent studies on Alaria life histories and they have proven to be among the most complex (Bosma, 1934; Pearson, 1956; Johnson, 1968, 1979). The life-cycle components reported heretofore are as follow: adults reside in the small intestine of a carnivore; defecated eggs embryonate in water; miracidia penetrate a heliosomatid snail with subsequent sporocyst generations; free-swimming, furcocercous cercariae penetrate larval amphibians; paratenic residence occurs in a wide spectrum of amphibian, reptilian, and mammalian hosts; and ingestion of the intermediate or paratenic host is followed by an intricate somatic migration and maturation of mesocercariae, diplostomula, and adults within the definitive host. This generalized schema of the Alaria cycle failed, however, to account for our observations on the gravid cat and the subsequent infection of her young. Thus we examined the pathways of Alaria marciannae mesocercariae in gravid, paratenic hosts in an effort to better understand the ability of this stage to infect a subsequent generation. Those observations form the basis of the following report.
The distribution of A. marcianae mesocercariae in dams examined immediately postpartum.

<table>
<thead>
<tr>
<th>Dam no.</th>
<th>Mammary glands</th>
<th>Mesenteric fat</th>
<th>Subscapular fat</th>
<th>GI tract</th>
<th>Liver</th>
<th>Heart</th>
<th>Lung</th>
<th>Uterus</th>
<th>Ant. trunk</th>
<th>Post. trunk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 (23)</td>
<td>16 (42)</td>
<td>0</td>
<td>2 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16 (42)</td>
<td>1 (3)</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>5 (11)</td>
<td>15 (34)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>15 (34)</td>
<td>7 (16)</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>7 (17)</td>
<td>14 (33)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>17 (40)</td>
<td>3 (7)</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>6 (12)</td>
<td>20 (41)</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>15 (31)</td>
<td>5 (10)</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>4 (12)</td>
<td>14 (42)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13 (39)</td>
<td>2 (6)</td>
<td>33</td>
</tr>
</tbody>
</table>

*Numbers of mesocercariae recovered from the locations are followed by the percentage (in parentheses) of the total number recovered from that dam.

**Materials and Methods**

**General**

Wistar outbred mice were used in all experiments. They were maintained in stainless steel cages that were changed daily. Babco and Purina Rodent Chow were supplied ad lib. Alaria marcianae mesocercariae were collected from the fat bodies of cottonmouth snakes, Agkistrodon piscivorus. The fat bodies were ground for 1 to 2 sec in a Waring Blender and strained through a single layer of cheesecloth. After several washings with 0.7% NaCl the mesocercariae were segregated into lots of 150 worms and given p.o. to mice. Adult mice to be examined for distribution of mesocercariae were first skinned and the following parts were isolated: mammary glands, subscapular fat, mesenteric fat, liver, heart, lungs, gastrointestinal tract, uterus, anterior trunk musculature, and posterior trunk musculature. Young mice also were examined for mesocercariae after grinding for 1 to 2 sec in a blender.

**Exclusion of prenatal infections**

Five virgin females were infected with mesocercariae, mated, and isolated with that mate on the same day. At parturition, neonates were removed before suckling and examined for mesocercariae. Sires were kept in the cages as tracers to determine if infection would occur from excretions or secretions of the females.

**Mesocercariae in dams examined 21 days postpartum**

The five dams from the previous experiment were examined 21 days postpartum to determine the distribution of remaining mesocercariae in their somatic tissues.

**Onset of maternal passage**

Three dams were infected immediately postpartum and a single offspring from each dam was examined daily to determine when mesocercariae could first be detected in the nurslings.

**Results**

**Exclusion of prenatal infections**

Fifty-three young were born to the five infected dams and were examined immediately at parturition. None of the 53 young was infected with mesocercariae.

**Mesocercariae in dams immediately postpartum**

The five dams from the previous experiment were examined within 3 hr postpartum to determine the distribution of mesocercariae in their somatic tissues.

**Mesocercariae in virgin female mice**

Five virgin female mice were infected with mesocercariae and examined 21 days later for distribution of larvae in their somatic tissues.

**Mesocercariae in young during lactation**

Five virgin female mice were infected with mesocercariae and examined 21 days later for distribution of larvae in their somatic tissues.

**Mesocercariae in young during lactation**

Thirty-eight young were nursed by dams infected with mesocercariae postpartum. All 38
TABLE II. Distribution of A. marcianae mesocercariae in virgin female mice.

<table>
<thead>
<tr>
<th>Dam no.</th>
<th>Mammary glands</th>
<th>Mesenteric fat</th>
<th>Subscapular fat</th>
<th>G.I. tract</th>
<th>Liver</th>
<th>Heart</th>
<th>Lung</th>
<th>Uterus</th>
<th>Ant. trunk</th>
<th>Post trunk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 (18)</td>
<td>0</td>
<td>3 (8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td>24 (63)</td>
<td>3 (8)</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>16 (14)</td>
<td>0</td>
<td>20 (18)</td>
<td>0</td>
<td>7 (6)</td>
<td>0</td>
<td>3 (3)</td>
<td>0</td>
<td>43 (36)</td>
<td>21 (19)</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>20 (21)</td>
<td>0</td>
<td>14 (15)</td>
<td>0</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>46 (49)</td>
<td>10 (11)</td>
<td>94</td>
</tr>
<tr>
<td>4</td>
<td>18 (23)</td>
<td>0</td>
<td>20 (25)</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>29 (37)</td>
<td>10 (13)</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>23 (27)</td>
<td>0</td>
<td>17 (20)</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>37 (44)</td>
<td>7 (6)</td>
<td>85</td>
</tr>
</tbody>
</table>

* Numbers of mesocercariae recovered from the locations are followed by the percentage (in parentheses) of the total number recovered from that dam.

young examined at 21 days were infected with mesocercariae and the results are summarized in Table III.

The three neonates removed from each litter immediately postpartum without suckling were all negative for mesocercariae. The three neonates substituted into each litter and allowed to suckle all became infected.

The five sires that were used as tracers to determine if extraneous infection could occur from excretions or secretions from the females were negative for mesocercariae.

**Mesocercariae in dams examined 21 days postpartum**

Five dams were successfully infected postpartum. The distribution of mesocercariae in their somatic tissue after 21 days of nursing is summarized in Table IV.

**Onset of maternal passage**

At parturition, three dams were successfully infected with mesocercariae. The first mesocercariae detected in young from each group was at postpartum days 4, 5, and 5. After the initial passage at these days no correlation between length of nursing time and additional acquisition of mesocercariae was noted. Results are summarized in Table V.

**DISCUSSION**

This study revealed that *Alaria marcianae* mesocercariae did not infect prenatal mice. Not only were 53 uninfected neonates born to infected dams, but no mesocercariae were found in the uteri of virgin, parturient, or postparturient females. This is contrasted by the absolute postnatal infection in offspring that were allowed to nurse from infected dams.

Distribution of mesocercariae in virgin and parturient females was found to be concentrated in the anterior trunk musculature and body fat. A significant increase of mesocercariae in the mammary glands was seen in the dams examined 21 days postpartum. Migration of mesocercariae, then, appears to be directed to the mammary glands by design rather than by random movement.

All of the young mice used in this study had terminated their nursing by 21 days. This indicates that mesocercariae remaining in the mammary glands of the dams may be capable of infecting subsequent litters. Miller (1981) has indicated that this is true for mesocercariae of the genus *Pharyngostomoides*.

Our data show that mesocercariae introduced to dams may reach the sucklings as early as 4 days PI. The significance as to why there appears to be no correlation between suckling time and additional acquisition of mesocercariae is unknown. However, these observations demonstrate that transmission can be milkborne as colostral secretion terminates by day 1 in mice.

The concept of paratenicity was introduced by Baer (1951) to connote extension of a parasite in space and time. The ability of the mesocercarial stage to bridge ecological gaps from an
aquatic intermediate host to a terrestrial definitive host is well known. However, the extent to which they persist in time is just becoming apparent. Rau and Gordon (1978) found that *Alaria* sp. mesocercariae overwinter in poikilothermous hosts even in the harsh winters of Canada, and Miller (1981) presented evidence that mesocercariae of *Pharyngostomoides* may live for as long as 11 years. On the basis of these studies, the potential longevity of the mesocercarial stage far exceeds that of its second intermediate and paratenic hosts, thus insuring the continued passage of mesocercariae through a variety of paratenic hosts, e.g., a frog eaten by a snake, which is eaten by an alligator, which dies and is scavenged by an opossum.

We view transmammary passage of *Alaria marcianae* mesocercariae as a further modification of this paratenicity. The predilection of these larvae for the fat bodies of all paratenic hosts preadapt them for the additional step into the mammary glands of a gravid, mammalian host. This newly recorded behavior of the mesocercariae obfuscates the classical delimitations of paratenic and definitive hosts. For in fact, the roles of these hosts may change when gravid females are involved. Our observations indicate that mesocercariae introduced to gravid mammals result in three responses: (1) if introduced to a paratenic host (sensu Odening, 1976), such as a mouse, some of the larvae are retained by the female and others are transmitted to the offspring where they also remain mesocercariae; (2) if introduced to an amphiparatenic host (our terminology), such as a raccoon, some of the larvae remain undifferentiated in the female, but others are transmitted to the offspring where they mature to adults (Johnson, pers. comm., has indicated that he has also observed this phenomenon involving *A. marcianae* in raccoon in his laboratory); and (3) if the larvae are introduced to a definitive host, such as the domestic cat, some larvae mature to adults in the female while some undifferentiated mesocercariae are transmitted to the offspring where they also reach adulthood.

The mesocercarial stage is found only in the diplostomid genera *Alaria, Pharyngostomoides*, and *Procyotrem*, as well as the strigeid genus, *Strigea*. Because the only documented cases of transmammary transmission among trematodes involve mesocercariae it may be that this mode of transmission in trematodes is peculiar to this stage. Of the four genera listed, only *Alaria* exhibits a wide range of paratenic hosts, humans included. The role a pregnant human would play if infected is, as yet, unassessed.

### Table IV. Distribution of *A. marcianae* mesocercariae in dams examined 21 days postpartum.

<table>
<thead>
<tr>
<th>Location of larvae</th>
<th>Dam no.</th>
<th>Mammary glands</th>
<th>Mesenteric fat</th>
<th>Subscap. fat</th>
<th>G.I. tract</th>
<th>Liver</th>
<th>Heart</th>
<th>Lung</th>
<th>Uterus</th>
<th>Ant. trunk</th>
<th>Post trunk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>37 (73)</td>
<td>0</td>
<td>2 (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (6)</td>
<td>7 (14)</td>
<td>2 (4)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>41 (71)</td>
<td>0</td>
<td>2 (3)</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td>1 (2)</td>
<td>10 (17)</td>
<td>3 (5)</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23 (40)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22 (47)</td>
<td>1 (2)</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>31 (53)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15 (26)</td>
<td>7 (17)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>43 (56)</td>
<td>0</td>
<td>7 (9)</td>
<td>0</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
<td>21 (27)</td>
<td>4 (5)</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers of mesocercariae recovered from the locations are followed by the percentage (in parentheses) of the total number recovered from that dam.

### Table V. Onset of transmammary passage of *A. marcianae*.

<table>
<thead>
<tr>
<th>Postpartum days at which young were removed and examined</th>
<th>Dam no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>---------------------------------------------------------</td>
<td>---------</td>
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<tr>
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<td>–</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

We express our gratitude to Dr. Allen D. Johnson, University of South Dakota, for reading this manuscript and allowing us to cite his unpublished observations.

LITERATURE CITED

Chapter III

Migration of *Alaria marcianae* in Domestic Cats
MIGRATION OF *ALARIA MARCIANAE* (TREMATODA) IN DOMESTIC CATS

Wesley L. Shoop and Kenneth C. Corkum
Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803

**ABSTRACT:** The migration of *Alaria marcianae* was studied in domestic cats. Mesocercariae penetrated the stomach wall, entered the abdominal cavity, and penetrated the diaphragm within 3 hr. Direct penetration of the lungs via the thoracic cavity occurred within 6 hr. Also observed was a circulatory route to the lungs when mesocercariae were recovered from both the liver and the chambers of the heart. Upon entrance into the lungs, mesocercariae began to enlarge with sequential lappet formation, holdfast development, and penetration gland atrophy. By day 7, they were recognized as fully formed diplostomula. The diplostomula resided a minimum of 4 days in the lungs as they first appeared in the duodenum by day 11. Diplostomula were found in both the trachea and stomach, indicating that they reach the duodenum after being coughed up from the lungs and swallowed. Diplostomula recovered from the duodenum were indistinguishable morphologically from the most advanced lung forms. Maturation of the reproductive organs occurred in as little as 4 days as the first ovigerous specimens were seen on day 15. The bidirectional route to the lungs may be significant when viewed in light of the recent discovery of transmammalian transmission. If a hormonal stimulus is involved, it is conceivable that those mesocercariae in the circulatory system may be more readily influenced than those undergoing a somatic migration. This may account for why some larvae are diverted to the mammary glands in a pregnant mammal instead of the normal, maturative migration to the duodenum.

Cuckler (1940) was the first to report an obligate, three-host life-cycle for members of the genus *Alaria*, and that a complex, somatic migration occurs in the definitive host. He stated that ingested mesocercariae pass through the intestinal wall, and, via the peritoneal cavity, penetrate the diaphragm and develop to diplostomula in the lungs within 13 days. The diplostomula then migrate up the trachea and are reswallowed, finally establishing as adults in the duodenum by 21 days. This basic migrational pattern has been supported by studies from Pearson (1956) on *A. arisaemoides* and *A. canis* (= *A. marcianae*); Savinov (1953) on *A. alata*; and Johnson (1968) on *A. marcianae*.

Though a somatic route is generally believed to represent the pattern of migration, a few reports indicate that it may occur via the circulation. This possibility was suggested by Pearson (1956) for *A. canis* (= *A. marcianae*) as a likely alternative to the somatic route when he found mesocercariae in the blood of the liver from an experimentally infected fox. In their discussion of *A. arisaemoides* infection in dogs, Allen and Mills (1971) stated that migration of mesocercariae from gut to lungs does occur through the circulatory system. It was their interpretation that larvae reached the lungs via the posterior vena cava and the right side of the heart.

In recent years *Alaria* spp. have been found to infect humans (Beaver et al., 1977). Shoop and Corkum (1981) examined the epidemiology of *A. marcianae* and presented reasons for believing that this species was responsible for an autochthonous human infection in Louisiana. Also noted was the preference of *A. marcianae* mesocercariae for the fat bodies and mammary glands of some pregnant mammalian hosts. In a subsequent study, Shoop and Corkum (1983) demonstrated experimentally that the normal migration of *A. marcianae* in a mammalian host is, in fact, disrupted if that host becomes pregnant. The mesocercariae are diverted to the mammary glands by an as yet undetermined mechanism and ultimately are passed to the young sucklings. As a prelude to further studies on transmammary transmission it became desirable to reinvestigate the migration within a nonpregnant definitive host with the hope of establishing the baseline sequence of events. In this paper we present the chronological sequence, anatomical locations, and gross morphological changes that occur during migration of *Alaria marcianae* in the domestic cat.

**MATERIALS AND METHODS**

*Alaria marcianae* mesocercariae were isolated from the fat bodies of cottonmouth snakes, *Agkistrodon piscivorus*. They were segregated into lots of 200 in 0.8% saline and given p.o. to 6- to 9-wk-old weanling kittens. The kittens were necropsied 3, 6, 12, and 24 hr later, and then daily for 19 days. At each examination, a midventral incision was made...
in the abdominal wall of a kitten (killed with CO₂) and the abdominal cavity was flushed with saline and the washings collected. An incision was then made in the thoracic wall and the thoracic cavity was also flushed with saline and collected. The stomach was clamped at the pyloric sphincter and removed along with the small intestine. Each was isolated, scraped, comminuted in a Waring Blender for 2 to 3 sec, and washings examined. The liver was examined macroscopically for hemorrhages and then removed along with the posterior vena cava which had been clamped from the heart. The liver was then comminuted for 2 to 3 sec. The posterior vena cava was perfused with saline and the washings added to those from the liver. The heart was removed and minced. The lungs were examined macroscopically for hemorrhages, removed, and comminuted for 2 to 3 sec. The esophagus and trachea were then removed, separated, slit along their lengths, and washed in saline. Finally, the diaphragm was removed, rinsed in saline, and then comminuted for 2 to 3 sec. Parasites recovered from the kittens were fixed immediately in glutaraldehyde. Some were stained in Semichon's aceto-carmine and mounted to determine by light microscopy the sequence of morphological transformations. Others were examined with scanning electron microscopy and those data will be presented later.

RESULTS

Migration

Results are summarized in Table I. Within 3 hr, mesocercariae presented p.o. to kittens penetrated the stomach wall, entered the abdominal cavity, and penetrated the diaphragm. Within 6 hr, the mesocercariae were first detected in the lungs. Some mesocercariae were still found in the stomach wall after 12 hr and others were free in the abdominal cavity until day 4. Active penetration of the diaphragm was observed until day 2 and mesocercariae were also free in the thoracic cavity at that time. The number of mesocercariae in the lungs increased until day 3, then remained constant. Mesocercariae were observed in the lungs until day 9.

On days 1 and 2, mesocercariae were also found in the liver, but no petecchie nor any compromises of the capsule were observed. That this was an alternative route to the lungs was further corroborated by observing mesocercariae in the chambers of the heart on days 1 and 3.
Immediately upon entering the lungs, the mesocercariae began a transformation to the diplo stomula. They were recognized as fully-formed diplo stomula by day 7. Within the lungs, diplo stomula were found in clear, fluid-filled vesicles, 2 mm in diameter. There was a four day lag between the first appearance of a fully-formed diplo stomulum in the lungs and those appearing in the duodenum. Although numbers of diplo stomula in the lungs began decreasing at approximately the same time as they began increasing in the duodenum, substantial numbers were still present in the lungs at the termination of the study.

During the increase in numbers of diplo stomula in the small intestine we observed similar juveniles in the trachea on days 13, 18, and 19 and in the contents of the stomach on days 15 and 17. Washings from the thoracic cavity from day 11 onward also revealed viable diplo stomula; however, none was observed in the diaphragm or abdominal cavity and no further development occurred in the thoracic cavity.

The first gravid adults were observed in the duodenum by day 15, but eggs were not seen in the feces by formalin-ether sedimentation until day 19.

**Development**

There were no morphological changes in the mesocercariae after penetration of the stomach wall or diaphragm (Fig. 1). However, upon entrance into the lungs the mesocercariae immediately began to enlarge in length and width. By day 4, the anterior end showed increased lateral growth on either side of the oral sucker, presumably indicative of the rudimentary lappets. No indication of a holdfast organ was present. Day 5 showed contents of the four unicellular penetration glands, that were so readily apparent in young mesocercariae, to be exhausted and the large nuclei were often lost (Fig. 2). A depression on the ventral surface behind the acetabulum suggested the rudimentary holdfast organ. By day 7, the forms were recognized as fully-formed diplo stomula (Fig. 3). The forebody was foliaceous with the margins folded ventrally to form a scoop. The lappets on either side of the oral sucker were capable of evertion, and the holdfast organ was elongated and fully formed. The hind body was rudimentary, but the spherical germinal mass of the mesocercariae now varied in development from a four-lobed structure to that in which the ovary, two testes, and ejaculatory pouch were discernible. By days 7 to 9, the penetration glands atrophied further and many lost their connection to the ducts in the oral sucker.

On day 11, the first duodenal forms were observed and they were indistinguishable morphologically from the most advanced lung forms. The most apparent developmental change in the duodenum was the rapid growth of the hind body with concomitant maturation of the reproductive organs (Fig. 4). The first ovigerous specimens were observed on day 15 (Fig. 5). At that time the foliaceous margins of the forebody were observed to meet each other at the ventral mid-line allowing the entire forebody to wrap around a host intestinal villus.

**DISCUSSION**

It is evident that a migration of mesocercariae out of the gut and to the lungs must occur before development to the diplo stomula stage. Likewise, the lungs do not possess the requirements to cause maturation to the adult and the diplo stomula must migrate to the duodenum. Less clear are the routes that are taken to get to the lungs and duodenum.

Mesocercariae migrating through the stomach wall entered the abdominal cavity and actively penetrated the diaphragm. Subsequently, these stages entered the thoracic cavity and directly penetrated the lungs. However, we also found mesocercariae in both the liver and the blood of the heart, suggesting that an avenue via the circulation is possible. The circulatory route would seem, a priori, to be a quick and direct means to the lungs. The mesocercariae could enter the liv-

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**Figures 1-5.** Stages of *Alaria marcianae* during migration in a cat. 1. Two-day-old, undifferentiated mesocercaria from the thoracic cavity. Note the four, large, unicellular penetration glands. 2. Intermediate form taken from the lungs at day 5. Note the lateral growth on either side of the oral sucker suggesting the lappets, the exhausted penetration glands, and the developing holdfast immediately posterior to the acetabulum. 3. Fully formed diplo stomulum from the lungs. The lappets are eversible, the holdfast is fully formed, and the reproductive organs are discernible. 4. Immature worm from the duodenum on day 12. 5. Adult worm from the duodenum on day 15. All figures are to the same scale which is in micrometers.
cr. pass through the hepatic vein and posterior vena cava to the right side of the heart, allowing direct access to the lungs. However, we never observed any petechial hemorrhage in the liver nor any compromises of the liver capsule which might suggest a mass migration through this organ. It would seem reasonable to expect demonstrable hemorrhage of the liver if several hundred larvae were migrating through it. Moreover, if the circulatory system is the route of preference we should be able to recover the larvae in large quantity from the soft tissues of the liver and lung. We had good success in recovering larval stages from the lungs, but not from the liver nor from the chambers of the heart.

Migration through the circulation may account for the very quick arrival in the lungs of some of the larvae during the first 2 days. However, the large increase in number of mesocercariae in the lungs by day 3 indicated a somatic migration. If the circulatory route was preferred, the mesocercariae could conceivably reach the lungs in almost negligible time. The large increase of numbers of larvae in the lungs at such a late time as day 3 and onward indicated a route other than circulatory. The progressive transition of larvae in the stomach wall, abdominal cavity, diaphragm, thoracic cavity, and lungs also indicated a somatic migration. We believe, therefore, that although some forms may get to the lungs via the circulation, it is not the preferred route.

Upon reaching the lungs metamorphosis to diplostomula occurred in as little as 4 days. The buildup of mesocercariae in the lungs appeared by day 3 and all were fully-formed diplostomula by day 7. Lappet formation appeared first, then elaboration of the holdfast organ, and finally loss of the penetration glands. By day 11 the diplostomula were migrating and the first forms were seen in the duodenum. We found diplostomula in the trachea and stomach, thereby indicating that they do migrate into the trachea, are coughed up, and swallowed. This most likely is a very quick event with the opportunity of observing them in the esophagus quite remote.

Diplostomula were consistently demonstrated in the thoracic cavity from day 11 on. These apparently came out of the lungs. Diplostomula were never observed in the diaphragm or abdominal cavity and this was consistent with the fact that their penetration glands atrophied by days 7 to 9. Those diplostomula that entered the thoracic cavity from the lungs need not be viewed as reaching a dead end. If they migrate out of the lungs without the aid of penetration glands, presumably they have the ability of re-entering the lungs by the same means.

Our results indicated that the fundamental pattern of migration in the cat reported by Cuckler (1940) to be correct. Because we examined more hosts at more frequent intervals we were able to refine the time diplostomula develop from 13 days reported by Cuckler to 7 days and the time that sexual maturity is reached in the small intestine from 21 days to 15. We believe that migration may also occur through the circulatory system as suggested by Pearson (1956) and Allen and Mills (1971). This alternative route to the lungs may be the link to resolving our previous observation on pregnant cats. We (Shoop and Corkum, 1983) reported that some mesocercariae in a pregnant cat undergo a migration and become adults in the intestine while others are diverted to the mammary glands and subsequently enter the young sucklings. If Miller's (1981) hypothesis that a hormonal stimulus of mesocercarial migration to the mammary glands is valid, it is conceivable that mesocercariae in the circulatory system would be more readily influenced than those undergoing somatic migration.

In the present study A. marcianae matured in kittens within as little as 15 days. Savinov (1953) observed that A. alata from Eurasia matured in the canid host within 35 days. Except for the time frame the sequence of development for A. alata agrees closely with that of A. marcianae in cats reported here. Savinov also found mesocercariae in the liver and heart, but made no mention of its significance. Pearson (1956) has demonstrated that when A. canis (= A. marcianae) is fed to a canid host those worms mature in 34 days. The similarities between A. alata and A. marcianae in morphology, life-cycle, and now in the sequence of migration may suggest that the only reasons for their continued separation as distinct species are historical and not biological.

The significance of this study rests on the fact that a baseline sequence of events in a nonpregnant host is outlined. It is possible to now determine at which point, chronologically and anatomically, the migration is diverted when the host becomes pregnant. The fact that we were able to distinguish a bidirectional route to the lungs may be important in explaining why, in pregnant hosts, some mesocercariae divert to the mammary glands and others to the duodenum.
LITERATURE CITED


Chapter IV

Tegumental Changes of *Alaria marcianae* During Migration

In the Domestic Cat
The intestine-lung-intestine migration in trematodes is unique to the diplostomid genera *Alaria* Schrank, 1788 and *Pharyngostomoides* Har-kema, 1942. The precise chronological events of this migration have yet to be demonstrated for members of the genus *Pharyngostomoides*, but they have been elucidated for *Alaria alata* by Savinov (1953) and for *A. marcianae* by Shoop and Corkum (1983b). The generalized migration begins with the ingestion of mesocercariae that penetrate the stomach wall, pass through the diaphragm, and enter the lungs. Some mesocercariae may also reach the lungs via the circulatory system. Once in the lungs, the mesocercariae metamorphose into diplostomula. The mobile diplostomula remain briefly in the lungs before migrating into the trachea and then they are coughed up and swallowed. Upon reaching the small intestine the diplostomula attach to villi and mature to adulthood.

A most intriguing peculiarity has resulted from this complex migration in both genera. Miller and his associates concluded that the infection of raccoon litters with *Pharyngostomoides procyonis* was the result of a transmammary route of mesocercariae (Miller, 1981). In a recent report, Shoop and Corkum (1983a) demonstrated experimentally that transmammary infection also occurs in *Alaria marcianae*. Apparently, the normal cyclic migration of the mesocercariae is diverted to the mammary glands in lactating mammalian hosts and the young become infected as they nurse. Miller (1981) suggested that the diversion of mesocercariae to the mammary glands may be directed by hormonal cues.

As a further contribution to understanding the biology of these organisms we aim to document the external tegumental changes of the
mesocercaria, diplostomulum, and adult of *Alaria marcianae* as they undergo their somatic migration in domestic cats.
MATERIALS AND METHODS

Mesocercarial stages of *Alaria marcianae* were recovered from the fat bodies of the water moccasin, *Agkistrodon piscivorus*. Some mesocercariae were fixed immediately for scanning electron microscopy. Twenty-two kittens were inoculated p.o. with 200 mesocercariae each and necropsied 3, 6, 12, and 24 hr later, and then at daily intervals for 19 days. The following regions were examined at each necropsy: stomach wall, intestinal wall, abdominal cavity, liver, heart, diaphragm, thoracic cavity, lungs, trachea, esophagus, stomach, and small intestine. The abdominal and thoracic cavities were flushed with 0.8% saline and the fluids examined. The lungs, liver, and diaphragm were comminuted for 2-3 sec in a Waring Blender with saline, decanted, and parasites recovered. The trachea and regions of the intestinal tract were slit along their lengths, washed in saline, decanted, and the parasites recovered. Additionally, the stomach and intestinal walls were comminuted for 2-3 sec in a blender, decanted, and the parasites recovered. The heart was minced with scissors in saline and the parasites recovered.

All stages used in this study were fixed in 4.0% glutaraldehyde, dehydrated in an ethanol series, critically point dried, coated with Au/Pd, and viewed with a scanning electron microscope.
RESULTS

Prepulmonary Phase

Mesocercaria recovered from the stomach wall, abdominal cavity, diaphragm, liver, and heart of domestic cats were indistinguishable morphologically from those fixed immediately after removal from the snake paratenic host. The mesocercarial body was flattened dorso-ventrally with a slight concavity in the posterior half (Fig. 1). The anterior end tapered depending on the state of extension or retraction. The posterior end was broadly rounded with a small, dorsally reflected nub that represented the rudimentary hindbody. The excretory pore was located on the hindbody.

The oral sucker opened subterminally and was encircled by 14 or 15 rows of simple, posteriorly directed spines (Fig. 2). Body spines were located along the margins of the venter to the posterior end. At the level of the acetabulum these spined areas converged medially and surrounded the sucker (Figs. 1, 4). This pattern left two areas of the venter devoid of spines: one anterior to the acetabulum and one posterior to it. The acetabulum was usually retracted within the body wall and had 3 or 4 staggered rows of spines except for the anterior fifth where they were associated with the oral sucker. Where spines were absent, the tegument had a cobblestonelike appearance (Fig. 6).

Associated only with the spined areas were uniciliate papillae (Fig. 3). A series of six of these papillae circumscribed the base of the oral sucker. In addition, two arcs comprised of at least three papillae each extended posteriorly along the margins of the venter (Fig. 5).
Figures 1-6. Mesocercarial tegument during the prepulmonary phase of migration. 1. Ventral view of mesocercaria. Note the two areas anterior and posterior to the acetabulum that are devoid of spines. Bar = 50 um. 2. Oral sucker with simple spines. Bar = 5 um. 3. Uniciliate papilla on the ventral surface. Bar = 5 um. 4. Acetabulum with 3 or 4 rows of spines. Note the area immediately anterior which is devoid of spines. Bar = 5 um. 5. Ventral view with the arrows indicating an arc of three uniciliate papillae on the left side. Bar = 50 um. 6. Ventral view of the typical cobblestone tegument. Bar = 5 um.
LEGENDS FOR FIGURES

Figures 7-12. Diplostomulum tegument during the pulmonary phase of migration. 7. Ventral view of the diplostomulum. Bar = 50 um.
**Pulmonary Phase**

Only in the lungs did the mesocercaria begin a transformation to the diplostomulum. The anterior end showed increased lateral growth on either side of the oral sucker and formed short, conical projections (Fig. 9). These projections represented the rudimentary lappets. Within several days, a longitudinal patch on the ventral side of each projection differentiated into microvilliform processes (Figs. 10, 20).

Tegumentary changes included the acquisition of uniciliate papillae over the ventral surface. Spines were lost from the acetabulum (Fig. 12) and the oral sucker (Fig. 8), but on the remainder of the body they divided to give rise to three-pronged serrations (Fig. 11). Concomitant with these changes was the appearance of a depression in the body wall midway between the acetabulum and the posterior end of the body (Fig. 13). Within 1-2 days this depression developed into an elongate invagination of the body wall. This represented the rudimentary tribocytic organ (Fig. 14). As the tribocytic organ developed this previously spineless area became covered with simple, stout spines.

By 7 days PI, the worms within the lungs were fully developed diplostomula (Fig. 7). The nublike hindbody of the mesocercaria had grown to a cylindrical shape devoid of spination. With lateral growth of the forebody the worm became distinctly scoop-shaped in appearance.

Subsequently, worms indistinguishable from pulmonary phase diplostomula were also found in the trachea, stomach, and duodenum.

**Postpulmonary Phase**

Presumably, the diplostomula were coughed up from the lungs and swallowed. They were detected as early as day 11 PI in the upper
LEGENDS FOR FIGURES

Figures 13–14. Diplostomulum tegument during the pulmonary phase of migration. 13. Depression in the ventral body wall of a 3 day PI stage that represents the first indication of the tribocytic organ. Bar = 50 um. 14. Tribocytic organ at 5 days PI. Note the number of small spines. Bar = 100 um.
1-5 cm of the duodenum. Within 4 days they matured into adults (Fig. 15). All worms attached to a host villus by means of the forebody (Fig. 16). The lappets on either side of the oral sucker continued to grow in length (Figs. 17, 19) and embedded deeply into the duodenal mucosa. The area covered by the microvilliform processes expanded to include the entire ventral side of the lappet (Fig. 17), whereas the dorsolateral sides were covered with serrated spines (Fig. 19). These spines covering the dorsum and the lappets divided a second time to give rise to five- or six-pronged serrations (Fig. 25).

The tribocytic organ continued to elongate until it was approximately six times the length of the acetabulum (Figs. 15, 22). Within the tribocytic organ two distinct tegumental regions were recognizable. The lateral fleshy lobes were characterized by stout, outwardly directed spines (Fig. 23) while the inner folds were comprised of a labyrinthine array of tegumental processes (Fig. 24).

Uniciliate papillae were abundant over the ventral part of the forebody. Those papillae that were enclosed within the forebody possessed a longer cilium than those exposed on the remainder of the body (Fig. 26). Several circles of papillae were visible on the oral sucker (Fig. 18) as well as on the acetabulum (Fig. 21).

The hindbody of the adult grew rapidly, reflecting the development of the reproductive organs located there. In a freshly incised duodenum, only the hindbody of the adult can usually be observed above the villar mucus. The hindbody was completely devoid of spines. A sparse array of papillae was observed over most of the hindbody (Fig. 28) with the exception of a large concentration on the posterior end. These papillae surrounded a dorsal, subterminal cleft that represented
LEGENDS FOR FIGURES

the opening of the common genital pore (Fig. 27). Sperm were occasion­
ally observed in the genital pore. The excretory pore was located
terminally (Figs. 16, 27).
Figures 27-28. Adult tegument in the postpulmonary phase of migration. 27. Side view of the posterior end of the hindbody showing a concentration of papillae around the genital opening. Note the sperm within the opening. The excretory pore is located terminally. Bar = 50 um. 28. Uniciliate papilla and the tegument of the hindbody. Bar = 5 um.
DISCUSSION

The ontogeny of *Alaria marcianae* from mesocercaria to adult was described briefly by Shoop and Corkum (1983b) at the light microscopic level and is amplified in this report using scanning electron microscopy.

The mesocercarial stage is a highly active larva that exhibits a wide range of paratenicity (Shoop and Corkum, 1981). The morphology and behavior of the mesocercaria resembles those of an enlarged cercarial body. The expansion of this postcercarial stage, both in terms of time and potential hosts, appears to be a modification of the life cycle that compensates for the high host specificity observed in the definitive host. This is an alternative strategy to a life cycle in which there exists narrow specificity for the second intermediate host, but a wide range of available definitive hosts.

The retension in the mesocercaria of cercarial characters such as the four unicellular penetration glands and the rows of spines on the oral sucker give this stage remarkable ability to penetrate tissue. Through a combination of enzymatic and mechanical mechanisms the mesocercaria is able to migrate through the stomach wall, diaphragm, and lungs of paratenic and definitive hosts.

Upon entrance into the lungs, the mesocercarial stage metamorphoses into the diplostomulum. Earlike lappets appear early on either side of the oral sucker. Close inspection reveals that the ventral area on these lappets have differentiated into microvilliform processes similar to those reported by Erasmus (1969; 1970b) in *Diplostomum phoxini*. Histochemical studies by Johnson *et al.* (1971) and Bhatti and...
Johnson (1972) on adult A. marciianae demonstrated hydrolases in gland cells associated with these processes. The gland cells are believed to be the site of enzyme synthesis and the microvilliform processes are where these enzymes are liberated. Further, they showed that the lappets penetrated deeply into the crypts of the small intestine where destruction of the host tissue in contact with them was observed. They concluded that the lappets function in extracorporeal digestion.

From the present study, it is apparent that the microvilliform processes on the lappets are well developed in the diplostomula, but it is not known if they are functional at that stage or whether they must await migration of the worm to the duodenum. It could be speculated that these appendages are an adaptation to penetrate the crypts of the small intestine and that early development in the diplostomulum simply prepares them to take up residence in the gut as soon as they arrive.

The elaboration of the tribocytic organ was shown to arise from a depression on the ventral surface with subsequent invagination of the tegument. Simple, stout spines arose de novo on this organ. The specialization of the tribocytic organ into a region of spines and that of a labyrinthine tegument has also been shown by Erasmus (1970a,b) in D. phoxini. Johnson et al. (1971) and Bhatti and Johnson (1972) suggested that the function of the tribocytic organ, as well as the lappets, is in extracorporeal digestion. This organ may also function as a holdfast especially in light of the small size of the adult acetabulum (Fig. 15). However, as suggested by Öhman (1965), Johnson et al. (1971), and the present study (Fig. 16), attachment is probably accomplished by neither the tribocytic organ nor the acetabulum, but actually is effected by the scoop-shaped forebody. It was observed in the present study to
clamp tightly to an intestinal villus with the lappets buried deeply into the mucosa.

During transformation from mesocercaria to diplostomula the simple spines of the former modified to more complex serrated spines. Kie (1977) recognized that simple, posteriorly directed spines occur in highly migratory forms and that serrated spines are characteristic of non-migratory stages. A case could be presented against this hypothesis because diplostomula with serrate spines migrate out of the lungs, into the bronchi, up the trachea, and down the esophagus to the duodenum. However, the presence of serrated spines may actually indicate that migration of diplostomula of *A. marcianae* is a passive occurrence rather than an active one. Conceivably, diplostomula that enter a bronchus could be carried by means of cilia to the upper third of the trachea where the cough reflex would propel them into the buccal region. Once there, a simple swallow reflex would take them to the stomach and ultimately to the duodenum. In effect, the postpulmonary phase may be completely orchestrated by the reactions of the host, rather than an active migration on the part of the diplostomula.

*Alaria* is one of the few trematode genera that undergoes a somatic migration in mammals, including man. This study reveals some of the tegumental modifications that allow *A. marcianae* to adapt to the various habitats it encounters during migration in a host. Most spectacular are those of the adult that permit it to attach to a host villus. It appears that the specialized adaptations of the adult are the consequence of the high host- and habitat specificity, whereas the simplified morphology of the mesocercaria is a reflection of its low host specificity and paratenic habits.
LITERATURE CITED


VITA

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Major Field: Zoology

Title of Thesis: Life Cycle of Alaria marcianae in Louisiana

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