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Quantitative assessment of Myxobolus cerebralis viability and infective success in the salmonid host
by Crystal Jean Hudson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Veterinary Molecular Biology
Montana State University
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Abstract:
Whirling disease in trout is caused by a myxozoan parasite, Myxobolus cerebralis. The infectious stages of this parasite move from the skin, through the nerves and into the cartilage of young fish where the destructive effects of the parasite are seen. Very little is known about the immune response or mechanisms of resistance of different salmonids when exposed to M cerebralis. The triactinomyxon (TAM) parasite stage appears to have a limited time when released from the oligochaete (Tubifex tubifex) to infect the salmonid host. Limited data has been obtained regarding the age-related viability of the TAM and its ability to infect fish after release from the worm host.

To quantitatively assess TAM viability and infectivity, scanning electron microscopy was used to count TAM attachments at various time intervals after TAMs were harvested from oligochaete cultures. Both phase-contrast microscopy and vital staining protocol were used to enumerate TAMs and determine viability at increasing TAM age. In addition, sagittal whole fish sections were prepared for histological observation of parasite migration in fish epidermis.

Results with freshly harvested, 24, and 48 hour TAMs documented consistency between TAM attachment, phase-contrast, vital staining, and sporoplasm migration data which indicated a significant reduction in TAM viability and infectivity over time. At 72 hours post-harvest, phase-contrast microscopy and the vital staining protocol documented 38% and 39% parasite viability, but TAM attachment and sporoplasm migration data indicated few attachments or infective stages were present in the epidermis. These results suggest visual observation of TAM morphology may indicate viable parasites at increasing TAM age, but actual infectivity may be dramatically reduced in the first 48 hours after release from oligochaetes. Results also suggest this brief period of peak TAM viability may be the most susceptible to disruption of the M. cerebralis lifecycle for prevention of disease expression in early salmonid life stages.

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INFECTIVE SUCCESS IN THE SALMONID HOST

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APPROVAL

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Crystal Jean Hudson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Abstract

Whirling disease in trout is caused by a myxozoan parasite, *Myxobolus cerebralis*. The infectious stages of this parasite move from the skin, through the nerves and into the cartilage of young fish where the destructive effects of the parasite are seen. Very little is known about the immune response or mechanisms of resistance of different salmonids when exposed to *M. cerebralis*. The triactinomyxon (TAM) parasite stage appears to have a limited time when released from the oligochaete (*Tubifex tubifex*) to infect the salmonid host. Limited data has been obtained regarding the age-related viability of the TAM and its ability to infect fish after release from the worm host.

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INTRODUCTION

Salmonid whirling disease was discovered in Europe in 1893. The causative agent was identified as the protozoan parasite *Myxobolus cerebralis* (*Mc*). It is believed the parasite originally developed in association with brown trout, *Salmo trutta,* in central Europe and Asia and was non-pathogenic. Hofer initially reported the disease in rainbow trout, *Onchorynchus mykiss,* in Germany. The infected fish had recently been imported from the United States. Since 1893, *Mc* has been shown to infect numerous salmonid species, (Hoffman 1990; Hedrick 1998). Over time, the parasite has spread worldwide to over 21 countries due to the stocking of infected fish, discarding non-consumable fish carcasses, or from avian droppings (Hoffman 1990; Taylor and Lott 1978). Whirling disease was first diagnosed in the US in Pennsylvania in 1958. Circumstantial evidence strongly suggests the origin of the disease in this country to be from imported frozen European rainbow trout. At approximately the same time, whirling disease was also found in Nevada. Importations of European fish ceased and no further positive sites were reported until 1961. However, whirling disease has now been confirmed in 22 other states. It has been surmised that the spread of whirling disease was largely due to three major vectors: (1) transfer of live fish; (2) movements of infected fish in streams; and (3) parasite transfer from the feces of fish eating birds. Whirling disease is currently one of the most serious threats to wild and captive salmonids throughout the country (Rognlie and Knapp 1998), and has been associated with significant rainbow trout population declines in both Colorado and Montana (Vincent 1996).

<u>*Myxobolus cerebralis* - Taxonomy, Life Cycle</u>
<u>and Parasitic Characteristics</u>

Myxobolus cerebralis possesses unique phenotypic and genotypic characteristics

when compared with other Myxozoan parasites. Myxozoans are a diverse group of

multicellular organisms, although they were previously considered members of the

Protozoa, more recent comparisons of ribosomal and Hox genes suggest relationships

with the Bilateria or the Cnidaria (Smothers et al. 1994; Siddall et al. 1995; Schlegel et al.

1996; Anderson et al. 1998; Kent et al. 2001). The distinct structural features of *Mc*

strongly suggest a close similarity to the Cnidaria. The Cnidaria utilize differentiated

cells with extrusive filaments (cnidocysts) that are capable of trapping or attaching to

their host or prey (Siddall et al. 1995). *Mc* similarly utilizes extrusion of polar filaments

for attaching to the salmonid and oligochaete hosts. It falls within the order

Bivalvulidae, suborder Platysporina, and finally the genus *Myxobolus* (Kent et al. 2001).

Genetically based studies have documented a branching by *Mc,* separating it from other

Myxobolus species infecting fish. Several *Mc* isolates have been studied genetically and

the lack of variation between the isolates from diverse geographic regions appears to

validate the theory that the parasite was recently introduced to North America (Andree et

al. 1999). Wolf and Markiw (1984) originally described a model for a two host life cycle

of *Mc* which included an obligate oligochaete, *Tubifex tubifex* (Figure 1). Sequential

development of *Mc* in both the salmonid and the oligochaete hosts have been further

illustrated through intensive studies conducted by researchers from the University of

Munich (El-Matbouli 1998) (Figure 2).

HEALTHY SUSCEPTIBLE TROUT
ARE INFECTED VIA EPITHELIUM OR INFECTED
TUBIFICIDS ARE INGESTED BY TROUT

MATURE Triactinomyxon,
THE INFECTIVE STAGE
IS RELEASED INTO WATER

INITIAL FORM OF MYXOSPOREAN
INFECTION IN HOST EPITHELIUM

IN
TUBIFICID

INFECTED TROUT DEVELOP BLACK TAIL
AND SHOW WHIRLING BEHAVIOR
AT 1 TO 1.5 MONTHS.
AT 3 TO 4 MONTHS, CONVERSION
TO MYXOSPOREAN OF GENUS
Myxobolus IS COMPLETE

IN
FISH

AT 3 TO 4 MONTHS, CONVERSION TO
Triactinomyxon OF Myxobolus cerebralis
NEARS COMPLETION.
SPOROCYSTS CONTAIN EIGHT
ACTINOSPOREANS

TUBIFICID OLIGOCHAETES
Tubifex tubifex ARE INFECTED
BY Myxobolus cerebralis SPORES

MYXOSPOREAN CONVERSION TO
ACTINOSPOREAN TAKES PLACE
IN MUCOSA OF TUBIFICID GUT

SPORES OF Myxobolus cerebralis
ARE RELEASED FROM INFECTED FISH
AT DEATH OR AFTER INGESTION
BY PREDATOR

4   3   2   1   0   1   2   3   4

Markiw 1992

MONTHS

Figure 1. Two host life cycle of *Myxobolus cerebralis* describing salmonid and
oligochaete host sequential development. (illustration from Markiw 1992).

Extrasporogonic

Dermis   Nerves

Sporogonic

Cartilage

Epidermis
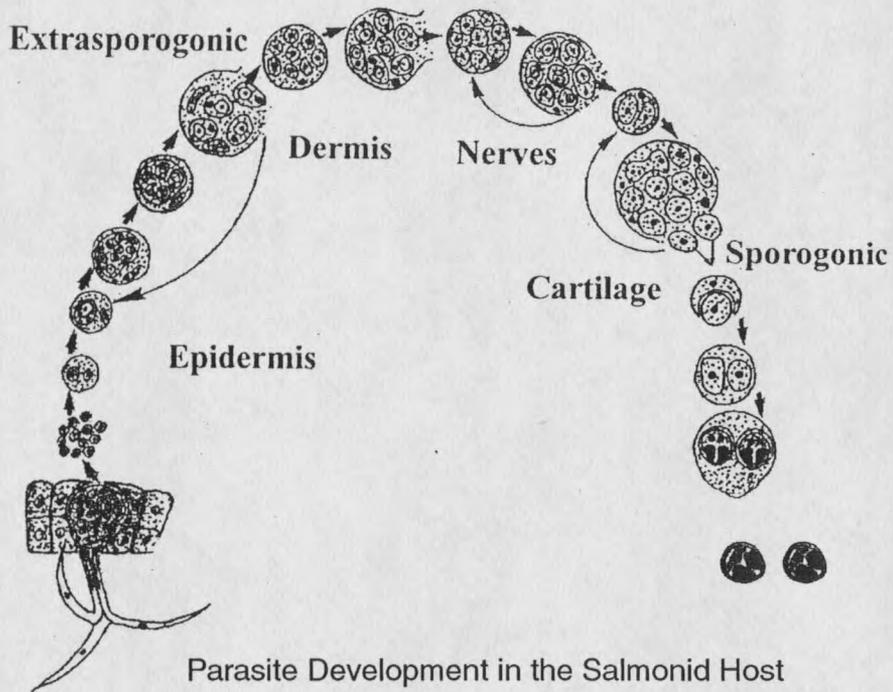
Parasite Development in the Salmonid Host

Gametogamy

Sporogony

Merogony
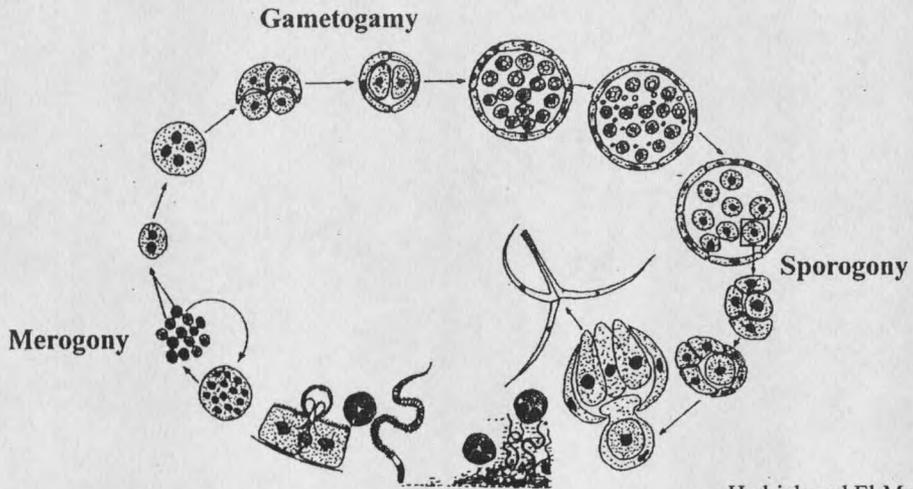
Hedrick and El-Matbouli 2001

Parasite Development in the Oligochaete Host

Figure 2. Life cycle stages of *Myxobolus cerebralis* in the salmonid and
oligochaete hosts. (illustration from 2001 Whirling Disease Symposium)

The initial development of *Mc* in the salmonid host begins with parasite attachment and penetration of the host by the triactinomyxon stage of the parasite. Triactinomyxons (TAMs) are released into the water column by the oligochaete worm *T. tubifex*, and may remain viable for periods of 6 to 15 ds at water temperatures of $7-15^0 C$ (Markiw 1992; El-Matbouli et al. 1999). Movement of TAMs is primarily a function of drifting in the water column and is facilitated by the buoyancy of the TAM structure. The TAM must locate a salmonid host to continue the parasites life cycle. The waterborne TAM consists of an epistyle containing 3 polar capsules, each with a polar filament and a sporoplasm aggregate with 64 germ cells (34 μm in height). The associated stylus is approximately 134 μm long and the 3 rays or processes are approximately 193 μm each in length (El-Matbouli and Hoffman 1998) (Figure 3). The salmonid phase of the life cycle of *Mc* begins with parasite attachment and penetration of the host by the TAM. TAM attachment causes significant epidermal damage by three mechanisms: (1) extrusion of polar filaments, (2) migration of the sporoplasm between cells, and (3) intracellular development and release of parasite daughter cells from infected host cells.

Evidently, distinct chemical and mechanical stimuli must be present on the salmonid host to facilitate firing of polar filaments for TAM attachment (El-Matbouli et al. 1999). TAMs do not extrude their polar filaments in the presence of non-living salmonid hosts, indicating that both mechanical and chemical stimuli are necessary for infection. Invading sporoplasm packets have a dense coat of villi combined with preformed proteases which facilitates entry between cells in the epidermis (Speer 2000).