Efficacy of fumagillin and TNP-470 in preventing experimentally induced whirling disease in rainbow trout, Oncorhyncus mykiss
by Linda Sue Staton

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University
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Abstract:
Fumagillin, an antibiotic derived from the fungus Aspergillus fumagatus, and TNP-470, a super active analog of fumagillin, were tested for efficacy to prevent Myxobolus cerebralis (Me) infection in rainbow trout. Six trials (2 laboratory and 4 field trials) were conducted in which fumagillin or TNP-470 medicated feed was fed to juvenile rainbow trout (~36 mm) exposed to Mc triactinomyxons. Fish were fed medicated feed at various dosages, at various times with respect to the time of exposure, and for varying lengths of time. Fumagillin was either top-dressed on feed or incorporated in feed. Presence and level of Mc infection was determined by polymerase chain reaction, operculum blots, histology, spore counts, and electron microscopy evaluation conducted 90-240 d post-exposure. Hematology samples were also collected to evaluate potential toxic effects of treatment. Fumagillin and TNP-470 were top-coated treatment was not efficacious in preventing or reducing Mc infection. Although incorporated fumagillin and TNP-470 administered for 10 d or 26 d did reduce the level of Me infection, results were not significantly different from positive controls. No treatment group was effective in preventing Mc infection. Although fumagillin treatment and TNP-470 fed for 10 d did not appear to negatively impact fish performance, toxicity was observed in fish fed TNP-470 for 26 d. These fish became lethargic 30 d post exposure, and blood samples revealed low hematocrits, severely decreased lymphocytes, and reduced numbers of blast cells. Electron microscopy revealed spore deformations resulting from both to fumagillin and TNP-470 treatment. Fumagillin affected the polar capsules and vacuolated the sporoplasm, and TNP-470 inhibited proper spore valve shell production. In general, results were very inconsistent, both within and between studies. Fumagillin and TNP-470 were not effective in the prevention or control of Mc infection in rainbow trout.

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Linda Sue Staton

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

Fumagillin, an antibiotic derived from the fungus *Aspergillus fumagatus*, and TNP-470, a super active analog of fumagillin, were tested for efficacy to prevent *Myxobolus cerebralis* (*Mc*) infection in rainbow trout. Six trials (2 laboratory and 4 field trials) were conducted in which fumagillin or TNP-470 medicated feed was fed to juvenile rainbow trout (~36 mm) exposed to *Mc* triactinomyxons. Fish were fed medicated feed at various dosages, at various times with respect to the time of exposure, and for varying lengths of time. Fumagillin was either top-dressed on feed or incorporated in feed. Presence and level of *Mc* infection was determined by polymerase chain reaction, operculum blots, histology, spore counts, and electron microscopy evaluation conducted 90-240 d post-exposure. Hematology samples were also collected to evaluate potential toxic effects of treatment. Fumagillin and TNP-470 were top-coated treatment was not efficacious in preventing or reducing *Mc* infection. Although incorporated fumagillin and TNP-470 administered for 10 d or 26 d did reduce the level of *Mc* infection, results were not significantly different from positive controls. No treatment group was effective in preventing *Mc* infection. Although fumagillin treatment and TNP-470 fed for 10 d did not appear to negatively impact fish performance, toxicity was observed in fish fed TNP-470 for 26 d. These fish became lethargic 30 d post exposure, and blood samples revealed low hematocrits, severely decreased lymphocytes, and reduced numbers of blast cells. Electron microscopy revealed spore deformations resulting from both to fumagillin and TNP-470 treatment. Fumagillin affected the polar capsules and vacuolated the sporoplasm, and TNP-470 inhibited proper spore valve shell production. In general, results were very inconsistent, both within and between studies. Fumagillin and TNP-470 were not effective in the prevention or control of *Mc* infection in rainbow trout.

INTRODUCTION

Since 1893, *Myxobolus cerebralis* (*Mc*), the causative agent of whirling disease has been known to infect rainbow trout, *Onchorynchus mykiss* (Hoffman 1990; Hedrick 1998; El-Matbouli et al. 1995). This parasite is thought to have developed as a non-pathogenic organism associated with brown trout *Salmo trutta* in central Europe and Northern Asia. It has since spread worldwide to over 21 countries due to the stocking of fish, discarding non-consumable carcass parts, or from avian droppings (Hoffman 1990; Taylor and Lott 1978). The parasite was first found in the United States in Pennsylvania waters in 1956, and is currently one of the most serious threats to wild and captive salmonids throughout the country (Rognile and Knapp 1998). The disease has now spread to 22 other states. Recent rainbow trout population declines in Colorado and Montana blue ribbon streams have been attributed to *Mc* (Vincent 1996).

Living organisms are continually faced with a barrage of antagonistic invaders that continually challenge their immune defense system. Unfortunately, with respect to many invaders, including *Mc*, immunological responses often occur too late to combat infection and prevent disease. Hence, without treatment intervention abnormal behavior, lesions, morbidity, and/or mortality occurs (Hedrick et al. 1999). In a *Mc* infection, a number of factors play a role in the initiation and management of infection. Numerous studies have concentrated on the worm host, evaluating such parameters as genetics, life stage, environment, temperature, etc. (Wolf et al. 1986; Hamilton and Canning 1988; El-Matbouli and Hoffman 1998; El-Matbouli et al. 1999; Antonio et al. 1999). Other studies have concentrated on the fish host, examining such factors such as age susceptibility,

species susceptibility, and parasite exposure level in an attempt to find the weak link in the parasite's life cycle (El-Matbouli and Hoffman 1991; Halliday 1974; Baldwin et al. 2000; Thompson et al. 1999). The goal of my study was to experimentally induce whirling disease in rainbow trout and to evaluate the efficacy of two antimicrobial therapeutants, fumagillin and TNP-470 (a superactive analog of fumagillin) to prevent or control infection.

Myxobolus cerebralis - Taxonomy, Life Cycle and Parasitic Characteristics

The phylum *Cnidaria* is characterized by metazoan organisms which have both somatic and reproductive cells, complex life cycles and cellular organelles. This phylum includes oceanic hydras, sea anemones, corals and jellyfish. The Myxozoa, including *Mc* is taxonomically grouped with these organisms (Rognlie and Knapp 1998). *Myxobolus cerebralis* has a two host life cycle consisting of the oligochaetae worm *Tubifex tubifex* and a salmonid fish. It also has two intermediate water borne life stages, the spore and the triactinomyxon (TAM) (Hedrick et al. 1998). The spore, which is the encysted stage of the parasite, is ingested from a decaying infected fish or filtered from the substrate and consumed by the *T. tubifex*. The spore embeds in the intestinal epithelium of the worm and rapidly proliferates. In the intestinal epithelium, the spore replicates into numerous TAMs which are shed by the *T. tubifex* either through the digestive tract or by death of the worm (El-Matbouli and Hoffman 1998). Triactinomyxons are free floating organisms that consist of three rays and an epistylist (El-Matbouli and Hoffman 1998; Hedrick et al. 1998). Triactinomyxoans remain viable in the water column for 7 d at 7° C (Hedrick et

al. 1998). The epistylist harbors the polar capsules and the infective sporoplast. The polar capsules anchor the TAM to the fish host, usually via the epidermis, buccal cavity, or the respiratory epithelium. The infective sporoplast fires the sporoplasm into the fish tissue (Figure 1). It has been reported that TAMs are drawn to salmonid fishes by chemical and mechanical stimuli (El-Matbouli et al. 1999). Within 5 minutes of TAM attachment, the sporoplasm begins to migrate through dermal tissue and 4 to 24 d post exposure the parasite reaches the nervous system. Once in nervous tissue, *Mc* again proliferates, transforms into a precartlaginous vegetative state, and migrates toward cartilaginous tissue. After at least 20 d in nervous tissue, *Mc* migrates toward cartilaginous tissue where it transforms into a trophozoite and begins feeding on the cartilage. The majority of trophozoites are found in the cranial cartilage, where they feed and reproduce until their eventual transformation into spores. The spore remains in the cartilage cavity surrounded by bone until death of the fish host. Decay or digestion by a predator returns the spore to the environment where it is available for ingestion by *T. tubifex* to perpetuate the parasitic life cycle (Hedrick et al. 1998). Spores may remain viable for years (Lom 1995).

The level and severity of *Mc* infection has been reported to be dose and temperature dependant (Markiw 1992; El-Matbouli et al. 1999; Hedrick et al. 1999). At 15 °C an optimal relationship appears to exist between *T. tubifex* and *Mc*. However, at 25 °C the development and release of mature TAMs is hindered and production ceases after 96 h. At 5 °C TAM production occurs, but at a slower rate of maturation (El-Matbouli et al. 1999).

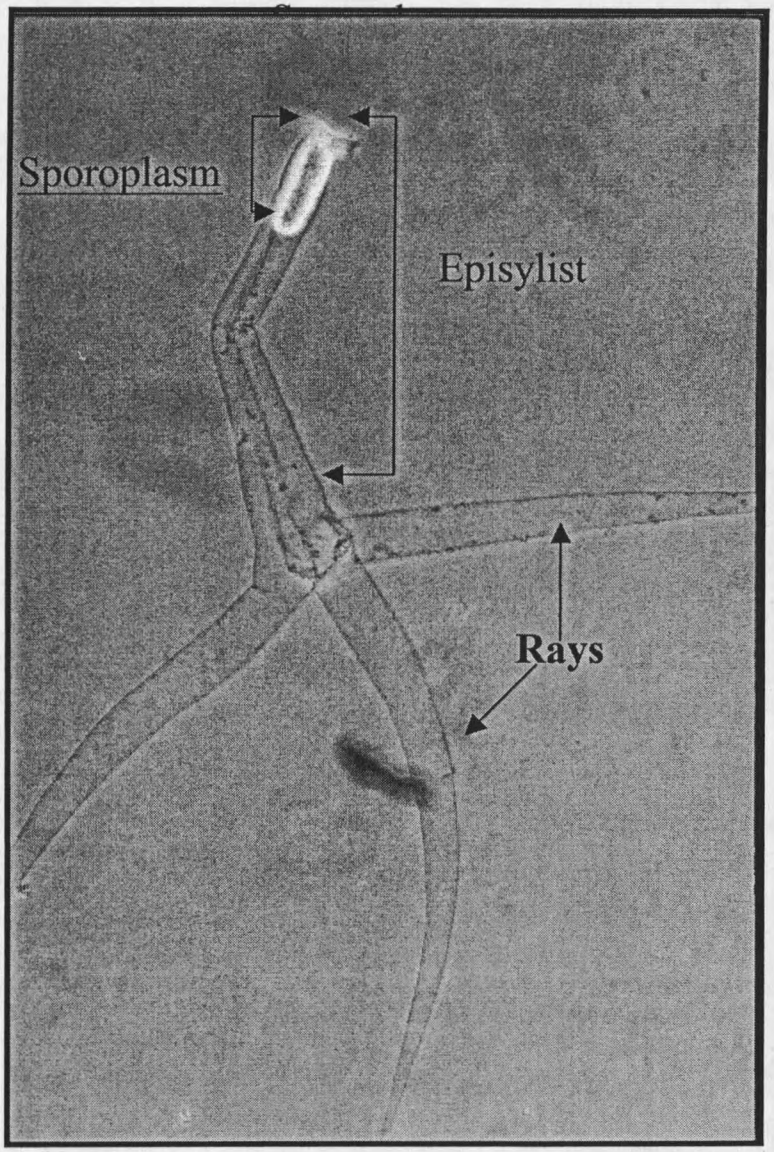

Figure 1.-A wet mount of a triactinomyxon (TAM). At the apical end of the epistylist is the sporoplasm containing 64 sporoplasts. The remaining three structures are rays that aid in buoyancy. Picture compliments of Beth MacConnell.