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Effects of Whirling Disease on Selected Hematological Parameters in Rainbow Trout

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ABSTRACT: Hematological responses to whirling disease in rainbow trout (*Oncorhynchus mykiss*) were investigated. Two-mo-old fingerling rainbow trout were exposed to cultured triactinomyxon spores of *Myxobolus cerebralis* at 9,000 spores/fish in December, 1997. Twenty-four wks post-exposure, fish were taken from infected and uninfected groups for peripheral blood and cranial tissue sampling. Histological observations on cranial tissues confirmed *M. cerebralis* infection in all exposed fish. Differences in hematological parameters between the two groups included significantly lower total leukocyte and small lymphocyte counts for the infected fish. No effects on hematocrit, plasma protein concentration, or other differential leukocyte counts were noted.

Key words: Lymphocytes, lymphopenia, *Myxobolus cerebralis, Oncorhynchus mykiss,* rainbow trout, salmonid, whirling disease.

Myxobolus cerebralis, a myxosporean parasite with an indirect life cycle, is the causative agent of whirling disease in salmonid fish. Whirling disease is clinically evident in infected salmonids through neurological and skeletal abnormalities including spinning or tail-chasing behavior, caudal hyperpigmentation (blacktail), and deformities of the cranium, operculum, and axial skeleton (Markiw, 1992a). Lesions associated with whirling disease are primarily associated with parasite infiltration of host neurological and skeletal tissues, and the degenerative and inflammatory changes that accompany them (Hedrick et al., 1991). As whirling disease can be chronic and potentially impact host bioenergetics (Hoffman, 1974; Markiw, 1992b), it is likely that additional systemic changes may also occur. The objective of this study was to examine the effects of whirling disease on peripheral blood parameters among rainbow trout infected with *M. cerebralis.*

Several thousand *Tubifex tubifex* worms, the aquatic oligochaete intermediate host of *M. cerebralis,* were obtained from creek sediment in the West Virginia (USA) panhandle in spring 1997 and were brought to the National Fish Health Research Laboratory (NFHRL) (Leetown Science Center, Kearneysville, West Virginia). Worms were acclimated and maintained in aquaria with spring water (12 C, pH = 7.05, total hardness = 250 ppm). Repeat samples of filtered water from these tubificid worm stocks did not reveal the presence of quantifiable levels of any triactinomyxon spores. In September 1997, *M. cerebralis* spores (the infective stage for worm intermediate host) were obtained from the cranial skeletal tissues of adult rainbow trout with clinical whirling disease as described by Markiw and Wolf (1980). Approximately eight hundred of the tubificid worms were indefinitely exposed to aqueous solutions of the spores added to their aquaria at 1,000 spores/worm. Approximately 100 days post-exposure, triactinomyxon spores (TAMs) were released from the worm hosts and collected from the water with a 50 μm wire mesh sieve for fish exposures. Microscopic examination of an aliquot of the filtered TAM suspension confirmed that the appearance of the TAMs was morphologically consistent with *M. cerebralis* (Markiw, 1986).

In December 1997, fingerling rainbow trout (2 mo post-hatch, 50 mm average total length, 1.8 g average weight) hatched and maintained at the NFHRL were divided into two groups of 80 fish each. The treatment group was bath-exposed to har-

vested TAMs at 9,000 TAMs/fish for 30 minutes in 16 l gently aerated spring water. The control group was sham-exposed in spring water. Following exposure, fish were removed to separate 75 l holding tanks supplied with flow-through spring water (3.8 l/min. flow, water quality parameters as above). Fish were maintained on a commercial pelleted trout diet at 2.5% body weight/day for the 24 wk duration of the experiment. Fish were arbitrarily removed from each tank simultaneously throughout the experiment for histological sampling (results not reported here).

At 24 wk post-exposure, 17 fish from each of the two groups were removed for sampling. All fish were anesthetized with an aqueous buffered solution of MS-222 anesthetic (250 mg/l; Finquel, Argent Chemical Laboratories, Redmond, Washington, USA), weighed, measured, and exsanguinated via venipuncture of the caudal vessels with a 22 gauge needle attached to a heparinized tuberculin syringe. Fish were then euthanized by cervical separation, and the heads bisected longitudinally and preserved in a formalin-based fixative.

Heparinized blood was transferred to microhematocrit capillary tubes (Chase Instruments, Glens Falls, New York, USA) and centrifuged to determine hematocrit (n = 16 fish/group). Plasma from the capillary tubes was used to determine plasma protein concentration with a hand held refractometer (Aloe-Hitachi, Japan). An additional aliquot of uncentrifuged blood was used to determine total leukocyte counts via hemacytometer, as described for fish (Klontz, 1994; n = 15 fish/group). Differential blood cell counts were also done for these fish with blood smears prepared on microscope slides and stained with Leukostat staining solution (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Leukocytes were categorized as small lymphocytes, large lymphocytes, polymorphonuclear cells (PMN's), or monocytes (Yasutake and Wales, 1983). Two hundred leukocytes were counted to determine the differential cell counts. Total leukocyte counts were adjusted for thrombocytes using the ratio of leukocytes to thrombocytes observed on the differential blood smears (Hrubec et al., 1996). Cranial tissue specimens were transferred to 70% ethyl alcohol after 72 hr in formalin based fixative. Specimens were processed, and 5 μm tissue sections were stained with hematoxylin and eosin for histological evaluation by light microscopy. Two sections per fish, both control and treatment, were examined to ascertain the presence or absence of *M. cerebralis* and to describe any associated tissue damage.

Statistical analyses were performed using S-PLUS software (MathSoft Inc., Seattle, Washington, USA). The exposed and control groups were compared with respect to total length, weight, plasma protein concentration, and counts of total leukocytes, small lymphocytes, and PMNs using a two-sided t-test. Differences between the two groups for the remaining responses (hematocrit, large lymphocyte and monocyte counts) were assessed with a two-sided permutation because the significant differences in sample variances (Levene's test, $P < 0.05$) between the two groups violated the assumptions necessary for the t-test. For both the t-test and the permutation test, the two groups were considered significantly different for any response that resulted in $P < 0.05$.

Mature *M. cerebralis* spores were observed histologically in cranial tissue from all TAM-exposed fish. Infections were rated as moderate to severe, based on the presence of spores, trophozoites, and associated degenerative and inflammatory changes (cartilage lysis, inflammatory cell infiltration, granuloma formation), using the histological rating system described by Hedrick et al. (1999). Spinning behavior was consistently noted among the infected fish beginning six wk post-exposure. No *M. cerebralis* spores, trophozoites, evidence of associated tissue changes, or clinical indications of whirling disease were evident in any of the unexposed control fish. Both body weight and total length were signifi-

TABLE 1. Weight, length and hematological parameters for uninfected and *M. cerebralis*-infected groups of rainbow trout.

	n =	Uninfected	Infected
Body weight (g)*	17	30.9 ± 2.0[a]	30.0 ± 1.1
Total length (mm)*	17	154 ± 3.1	146 ± 2.2
Hematocrit (%)	16	45.4 ± 0.8	46.3 ± 0.6
Plasma protein (g/dl)	16	5.1 ± 0.1	5.0 ± 0.2
Total leukocytes (10^6/ml)*	15	16.9 ± 1.8	11.8 ± 1.3
Small lymphocytes (10^6/ml)*	15	16.1 ± 1.8	10.6 ± 1.0
Large lymphocytes (10^6/ml)	15	0.23 ± 0.06	0.42 ± 0.11
PMNs (10^6/ml)	15	0.46 ± 0.09	0.73 ± 0.16
Monocytes (10^6/ml)	15	0.07 ± 0.02	0.08 ± 0.03

* Designates a statistically significant ($P < 0.05$) difference between uninfected and infected fish.
[a] Mean ± standard error.

cantly lower for the infected fish compared to uninfected control fish. Inhibition of growth of rainbow trout with whirling disease has been previously reported (Hoffman, 1974). Significantly lower total leukocyte counts and small lymphocyte counts were observed from the infected fish as well. No statistical difference in any other hematological parameters was noted between the two groups (Table 1). As small lymphocytes consistently represented a large percentage of the total leukocytes among both infected and uninfected fish, the lower total leukocyte counts among the infected fish were most likely attributable to the lower numbers of small lymphocytes.

Leukopenia, and lymphopenia in particular, have been associated with a variety of infectious and non-infectious disease conditions in fish, including heavy metal toxicities (Murad and Houston, 1988), ammonia toxicity (Wlason and Dabrowska, 1989), and bacterial infections (Noya et al., 1995). In salmonid fish, lymphopenia has been observed in brown trout (*Salmo trutta*) infected with *Saprolegnia* (Alvarez et al., 1988), rainbow trout following parenteral injection with *Vibrio anguillarum* or its extracellular products (Lamas et al., 1994), and rainbow trout following acute exposure to copper (Dick and Dixon, 1985). Lymphopenia has also been observed in association with exogenous administration of corticosteroids in salmonid fish, mimicking a physiological response to stress (McLeay, 1973; Pickering, 1984). Alterations in lymphocyte population dynamics due to direct interaction of the immune system with a pathogen is also a potential cause of lymphopenia in fish. Migration of lymphocytes from peripheral blood to affected tissues, lymphocyte destruction mediated by the pathogenic agent, and decreased lymphopoietic capability are all mechanisms suggested to cause lymphopenia in fish (Murad and Houston, 1988, Lamas et al., 1994). It is therefore not surprising that clinical whirling disease can produce lymphopenia, mediated either by the stress induced by the physical host responses (i.e., excessive spinning, inability to eat or function normally) or as a direct result of the host immune response reacting to the parasite.

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