Gross and Microscopic Pathology Associated with Large Cavernous Lesions in Muscle of Chinook Salmon from Lake Ontario

Authors: Meseck, Emily K., French, Tracy W., Grimmett, Stephanie G., Bartlett, Susan L., Wooster, Gregory A., et al.

Source: Journal of Wildlife Diseases, 43(1) : 111-115

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.1.111

Journal of Wildlife Diseases, 43(1), 2007, pp. 111–115

Gross and Microscopic Pathology Associated with Large Cavernous Lesions in Muscle of Chinook Salmon from Lake Ontario

Emily K. Meseck,[1] Tracy W. French,[2] Stephanie G. Grimmett,[3] Susan L. Bartlett,[3] Gregory A. Wooster,[3] Rodman G. Getchell,[3] John H. Schachte, Jr.,[4] and Paul R. Bowser[3,5] [1] Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA; [2] Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA; [3] Aquatic Animal Health Program, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA; [4] Fish Disease Control Unit, New York State Department of Environmental Conservation, 8314 Fish Hatchery Road, Rome, New York 13440, USA; [5] Corresponding author (email: prb4@cornell.edu)

ABSTRACT: Since 1999, eight adult Chinook salmon (*Onchorhynchus tshawytscha*) from Lake Ontario with large, focal, cavernous, fluid-filled muscle lesions have been examined in our respective laboratories. Gross and microscopic examination, cytology, and bacteriology were performed. Microscopically the lesions were consistent with chronic abscesses. Cytologic evaluation revealed diplomonad flagellate *Spironucleus* within these lesions. We provide a description of the gross and microscopic pathology associated with the cavernous lesions.

Key words: Abscess, Chinook salmon, pathology, *Onchorhynchus tshawytsch*, *Spironucleus*.

We document the pathology associated with large cavernous lesions in the muscle of Chinook salmon (*Onchorhynchus tshawytscha*) from Lake Ontario. We have observed these lesions only in adult Chinook salmon (4.5–9.1 kg). Fish with lesions were submitted for evaluation, either by the sport fishing public or by the staff of the Salmon River Fish Hatchery (Altmar, New York, USA) of the New York State Department of Environmental Conservation (NYSDEC) during the spawning season (October to December) between 1999 and 2004. Eight fish were presented to our respective laboratories (four to the NYSDEC Fish Disease Control Unit and four to the Aquatic Animal Health Program, Cornell University [CU]). All fish examined had been collected alive and originated from Lake Ontario. We are not aware of fish presenting with this lesion from any other body of water.

Necropsy procedures consisted of description of any gross abnormalities in each fish followed by immediate microscopic examination of fresh gill biopsies and skin scrapings, to identify parasites, bacteria (e.g., *Flavobacterium columnarae*), or fungi (e.g., *Saprolegnia* sp.). When a fluid-filled cavity was detected, typically by palpation of a raised area on the caudolateral body wall, aspiration of fluid from the lesion was attempted with a sterile syringe using aseptic technique. Bacterial culture from the lesion fluid was attempted on blood and brain-heart infusion agar (Becton Dickinson and Company, Sparks, Maryland, USA). Direct and sediment smears of the fluid were prepared for cytologic evaluation by staining air-dried slides with Wright's stain. Blood samples collected from the caudal artery were used to prepare smears that were also stained with Wright's stain. The abdominal cavity of the fish was aseptically opened, and samples of the posterior kidney were obtained for bacterial cultures on blood and brain-heart infusion agar. Bacterial cultures were incubated at 25 C for at least 21 days. The cavernous lesions were then opened, and tissues along the margin and into the surrounding muscle, along with gill, liver, kidney, spleen, gonad, stomach, and intestine, were sampled for histopathologic evaluation. Following fixation in 10% neutral buffered formalin, representative sections of tissues were embedded in paraffin, sectioned 6 μm thick, and stained with hematoxylin and eosin stains (Luna, 1968).

Fish presented for evaluation typically had large, raised, poorly demarcated, oval

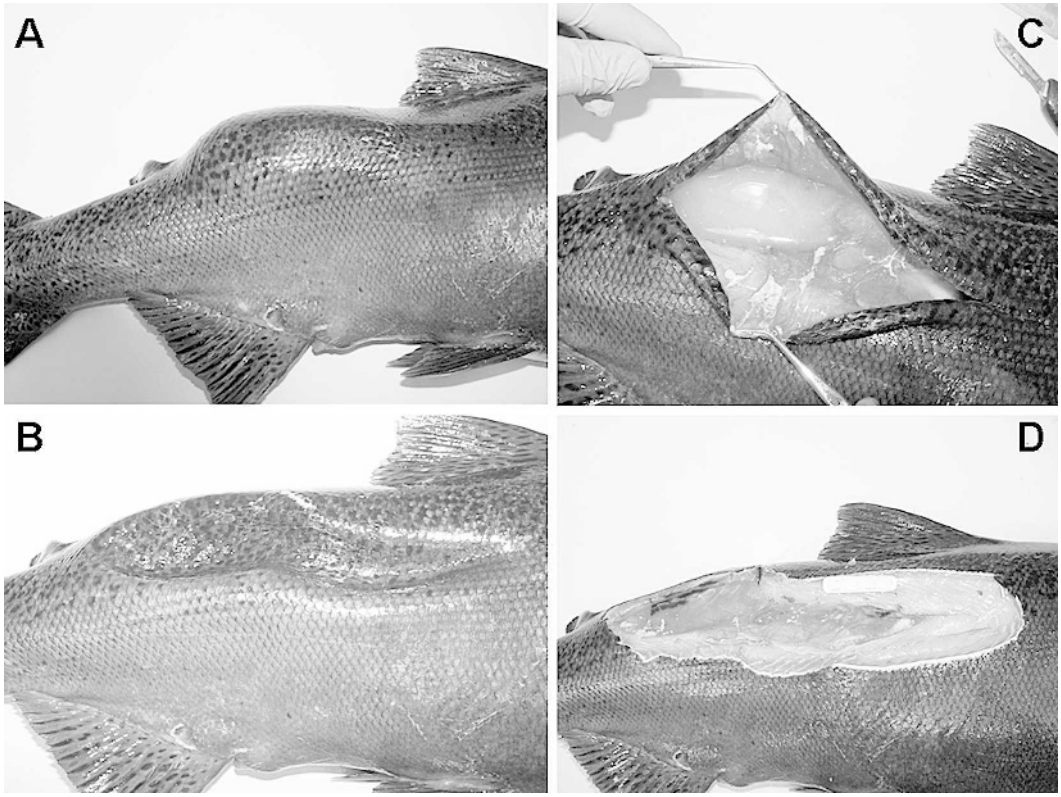

FIGURE 1. Adult Chinook salmon with a large cavernous lesion in the left dorsal musculature posterior to the dorsal fin. A. Lesion with fluid. B. Lesion with 300 ml of fluid removed. C. Lesion opened with some residual fluid remaining. D. Lesion with skin removed to show the margins of the cavernous lesion in the musculature.

areas in their epaxial musculature adjacent and posterior to the dorsal fin (Fig. 1). These lesions ranged in size from 70 to 100 mm long × 40 to 60 mm wide × 20 to 30 mm deep. Some lesions contained as much as 300 ml of variably clear to slightly turbid, yellow to yellow-brown, and odorless fluid. Occasionally aggregates of firm-to-friable yellow gelatinous necrotic material and fibrin were present within the fluid of these lesions. When opened, the margins of the lesions were often irregularly thick, up to 10 mm, and composed of firm, mottled-yellow and off-white granulation tissue with tags of fibrillar tan fibrin adhered to the luminal surface. The cavities often extended into the epaxial muscle anterior and/or posterior to the externally apparent margins (i.e., the raised areas on the skin). There were no connections between the cavernous lesions and the abdominal cavities. The lesions were limited to the muscle. No gross abnormalities were observed in any of the internal organs, in any of the fish.

Evaluation of Wright's stained preparations of the fluid from the cavernous lesions revealed varying numbers of neutrophils, lymphocytes, and macrophages along with varying amounts of necrotic debris. In addition, a pyriform protozoan (Fig. 2), measuring approximately 8–10 μm long × 2–3 μm wide with flagella originating from both the anterior and posterior was observed. These protozoa had characteristics, based on microscopic examination, of the genus *Spironucleus*. Examination of Wright's stained blood smears also revealed the parasite in

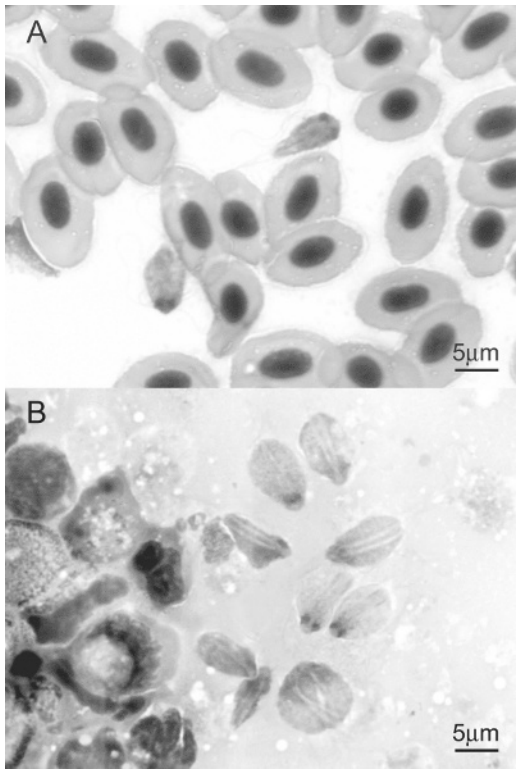

FIGURE 2. A. Blood smear showing two *Spironucleus* organisms. B. Smear of lesion fluid with *Spironucleus*. Numerous organisms are seen centrally, adjacent to degenerating inflammatory cells.

relatively low numbers as compared to fluid from lesions (4–5 parasites per 400× field in lesion fluid). Microscopic examination of fresh gill biopsies and skin scrapings did not reveal any pathogens considered associated with the large cavernous lesions in the muscle. The protozoan *Ichthyophthirius multifiliis* was observed on the gills and skin of one fish (at CU) and on the gills of one fish (at CU). In both cases the infestation was considered light.

Results of bacterial culture from the lesions and from the posterior region of the kidneys were variable. Of the eight fish processed, *Aeromonas salmonicida* was isolated from two fish (from the lesion and posterior kidney of one fish [CU], from the lesion of one fish [NYSDEC]), *A. hydrophila* was isolated from the posterior kidney of one fish (NYSDEC), *Klebsiella* sp. was isolated from the posterior kidney of one fish (CU), and no bacteria were isolated from four fish (two at CU, two at NYSDEC). These bacteria may have contributed to the development of these lesions in some fish, in association with *Spironucleus*.

Sections of the cavernous lesions and associated skeletal muscle were evaluated microscopically. Typically, cavernous lesion was surrounded by a capsule composed of mixed granulation tissue and well-vascularized fibrous connective tissue between 400 and 600 µm thick, with fascicles of granulation and fibrous connective tissues that extended into the adjacent skeletal muscle (Fig. 3). Perivascular and interstitial macrophages, lymphocytes, and segmented granulocytes were present within the capsule and the adjacent granulation/fibrous connective tissue and skeletal muscle. Microscopically, there were small-to-moderate amounts of mixed necrotic, karyorrhectic, and mineralized debris with variable amount of fibrin and hemorrhage in the lumen of the lesion. The lesions, composed of a fibrous connective tissue capsule with luminal necrotic debris, characterized these lesions as chronic abscesses, despite a paucity of neutrophils in the lumen.

In adjacent skeletal muscle, there were moderate perivascular to interstitial infiltrates of degenerate neutrophils and lymphocytes that occasionally surrounded skeletal muscle fibers. These often were mixed with scattered fine karyorrhectic debris and multifocal aggregates of fibrin (myositis). Rare skeletal muscle fibers surrounded by inflammatory cells were degenerate, with vacuolation and swelling, loss of normal cross-striations, and internalized nuclei. Rarely, there was fibrinoid necrosis of small blood vessels adjacent to the lesion. No significant microscopic abnormalities were observed in gill, liver, kidney, spleen, gonad, stomach, or intestine.

Information collected from the eight

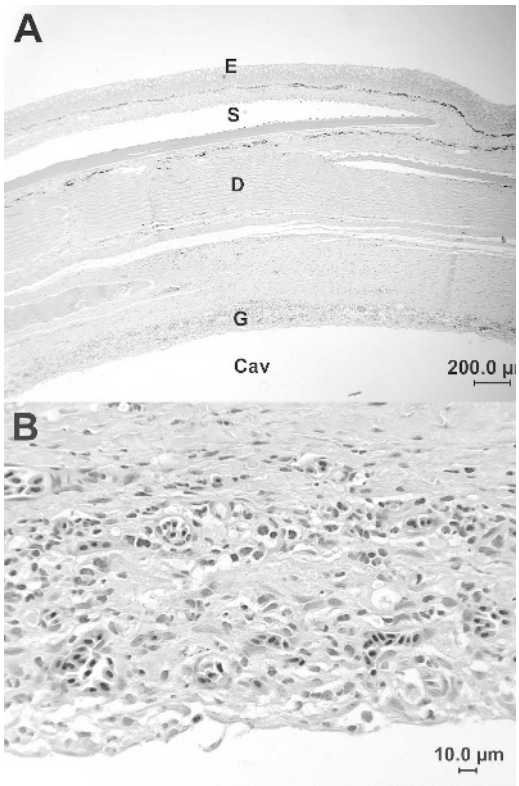

FIGURE 3. Photomicrographs of tissue along the border of a large cavernous lesion. A. Low magnification showing epidermis (E), scale (S), dermis (D), mixed granulation tissue of the lesion capsule (G), and the lesion cavity (Cav). B. High magnification of mixed granulation tissue of the lesion capsule.

adult Chinook salmon suggests that *Spironucleus* may be the causative agent of the cavernous lesions (abscesses). Neither the light external infestation of *I. multifiliis* nor the variable results of bacterial culture support a conclusion these findings had a causative role in the development of the abscesses. The disease process was concluded to be localized to the cavernous lesions in the epaxial muscle, based on negative microscopic findings in the gill, liver, kidney, spleen, gonad, stomach, and intestine. Controlled transmission studies are needed to establish *Spironucleus* as the cause of these lesions.

Within family Salmonidae, systemic spironucleosis has been reported in Chinook salmon (Kent et al., 1992), Atlantic salmon (*Salmo salar*) (Poppe et al., 1992), Arctic char (*Salvelinus alpinus*) (Sterud et al., 1998, 2003), and grayling (*Thymallus thymallus*) (Sterud, 1998). Common reported lesions included edema, congestion, and hemorrhage in kidney and liver. Large granulomatous lesions also were reported in the kidney and liver. Parasites have been found in a number of organs including the kidney, liver, gall bladder, intestine, eye, heart, brain, and gonads. Poppe et al. (1992) reported finding large numbers of the *Spironucleus* in the fluid contained in "large boils" in the muscle of the posterior region of the cultured Atlantic salmon. Of four different farms investigated by those researchers, large lesions were limited to muscle and were identified only in the largest fish (4–5 kg) on one farm.

In this report, gross lesions were limited to large fluid-filled cavernous lesions in the epaxial muscle, posterior and adjacent to the dorsal fin. In contrast to other reports of systemic spironucleosis of salmonids, gross lesions were not identified in the internal organs. Systemic *Spironucleus* infection in wild Chinook salmon is reported here, in contrast to previous documentations of *Spironucleus* infection in cage-cultured salmonids. To the best of the authors' knowledge, this is the first report of large cavernous lesions in muscle of wild Chinook salmon associated with *Spironucleus* infection. This condition previously has not been observed in the Chinook salmon population in Lake Ontario since its introduction into the lake in the early 1970s.

The Chinook salmon is an extremely important sport fish in New York State, as it constitutes a major part of the recreational salmonid fishery of Lake Ontario that contributes in excess of $100 million to the economy of the New York State each year. The impact of this condition to the Chinook salmon sport fishery is open to question. While the fish submitted were collected alive by sport fishing or during the salmonid-spawning program conducted by the New York State

Department of Environmental Conservation, the eventual impact on individual fish or the Chinook salmon population has yet to be determined. One must also speculate on why this disease appears to be emerging, some 30 yr after the introduction of Chinook salmon into Lake Ontario. While the Lake Ontario environment does not have the inherent stressors of an aquaculture production environment, there are factors that could impact on the resident salmonids. Changes in food supply, changes in the overall fish population structure, newly introduced invasive aquatic species, and increased human impact are but a few factors that could adversely affect the Chinook salmon. Evaluation and consideration of these factors will be an important component of any management strategies designed to limit the potential impact of this condition on the salmonid population of Lake Ontario.

LITERATURE CITED

KENT, M. L., J. ELLIS, J. W. FOURNIE, S. C. DAWE, J. W. BAGTSHAW, AND D. J. WITAKER. 1992. Systemic hexamitid (Protozoa: Diplomonadida) infection in seawater pen-reared Chinook salmon *Onchorhynchus tshawytscha*. Diseases of Aquatic Organisms 14: 81–89.

LUNA, L. G. (ed.). 1968. Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill, New York, New York, 258 pp.

POPPE, T. T., T. A. MO, AND L. IVERSEN. 1992. Disseminated hexamitosis in sea-caged Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 14: 91–97.

STERUD, E. 1998. In vitro cultivation and temperature-dependent growth of two strains of *Spironucleus barkhanus* (Diplomonadida: Hexamitidae) from Atlantic salmon *Salmo salar* and grayling *Thymallus thymallus*. Diseases of Aquatic Organisms 33: 57–61.

———, T. A. MO, AND T. T. POPPE. 1998. Systemic spironucleosis in sea-farmed Atlantic salmon, caused by *Spironucleus barkhanus* transmitted from feral Arctic char *Salvelinus alpinus*? Diseases of Aquatic Organisms 33: 63–66.

———, T. T. POPPE, AND G. BORNO. 2003. Intracellular infection with *Spironucleus barkhanus* (Diplomonadida: Hexamitidae) in farmed Arctic char *Salvelinus alpinus*. Diseases of Aquatic Organisms 56: 155–161.

Received for publication 13 December 2005.