OPERCULAR CYST FORMATION IN TROUT INFECTED WITH *Myxosoma cerebralis*[1]

ROBERT E. L. TAYLOR, Animal Science Department, University of Nevada, Reno, Nevada 89507, U.S.A.

MERYL H. HABER, Division of Laboratory Medicine, School of Medical Sciences, University of Nevada, Reno, Nevada

Abstract: External opercular cysts were observed in cutthroat trout, *Salmo clarki henshawii* affected with whirling disease. Microscopic examination of the cysts revealed numerous spores of *Myxosoma cerebralis* in their lumen and walls. Rupture of these cysts may provide a method of whirling disease transmission from live infected fish.

INTRODUCTION

Myxosoma cerebralis is the cause of whirling disease in salmonids. The life cycle of the organism has not been completely established although the disease is worldwide and has been known for over 70 years.[1] Hoffman, *et al.*[2] and others theorized that transmission was accomplished by ingestion of spores liberated from cartilage after death of infected fish. Rydlo[4] and Uspenskaya[5] suggested that spores were liberated from living fish through the intestinal tract. This paper reports the results of observations made of external cysts located in the operculum of whirling disease-affected fish.

MATERIALS AND METHODS

Observations were made on Lahontan Cutthroat trout, *Salmo clarki henshawii* raised at the Nevada State Hatchery, Verdi, Nevada. The fish varied in age from 6 to 18 months and were raised in water contaminated with *Myxosoma cerebralis*. Very few showed symptoms of whirling disease but microscopic examinations of wet mounts of macerated head cartilage demonstrated myxosomal spores in approximately 90% of the fish.

Approximately 300 fish, randomly picked from rearing ponds were anesthetized and examined grossly on several occasions during the year. Fish with cysts were killed and photographed. The affected tissue was removed and preserved in Bouin's fixative for tissue sectioning. Wet mounts were prepared from material expressed from the cysts. The spores were measured and tested for an iodinophilic vacuole with Lugol's iodine.

RESULTS AND DISCUSSION

Approximately 1-5% of all fish examined had opaque white raised cysts 1-3 mm in diameter on the medial surface of the operculum. The cyst location varied along the length of the operculum (Fig. 1). Some fish had as many as three cysts on one side. The gross features on these cysts have similarities to those of *Myxosoma cartilaginis* in bluegills[3] (*Lepomis macrochirus*).

[1] Supported by Dingle-Johnson (Federal Aid in Sport Fish Restoration Act) funds, Nevada Project F-20-9.

Contribution of the Nevada Agricultural Experiment Station, University of Nevada, Journal Series No. 272.

FIGURE 1. **M. cerebralis** cyst on medial aspect of operculum at base of gill arch. Operculum is viewed looking ventrally.

The inflammatory response produced by *M. cerebralis* was of the granulomatous type. The organism, in its trophozoite state, had invaded the cartilage of the operculum, resulting in necrosis and destruction of the cartilagenous cells (Fig. 2). Trophozoite forms were easily seen in cartilage cells. Within the cartilagenous matrix itself, there was no concomitent inflammatory reaction.

As the cartilage was broken down, inflammatory cells, predominantly mononuclear leukocytes and macrophages, had infiltrated the surrounding soft tissues and formed granulomas. These granulomas were composed predominantly of epithelioid and mononuclear cells, proliferating fibroblasts, and occasional multinucleate giant cells, (Fig. 5). Some had necrotic centers. Rarely, a myxosomal spore was found within a granuloma. Granulomas occurred in the soft tissues immediately adjacent to the cartilage of the gill and under the epidermis.

Opercular cysts formed in the subepidermal regions between the superficial squamous epithelium and the bone and cartilage of the operculum. The cyst wall was composed of proliferating fibroblasts, rapidly growing capillaries, and numerous mononuclear cells and macrophages, (Fig. 3). Rarely, acute inflammatory cells (neutrophils) were observed. Multinucleate giant cells were occasionally present in the cyst wall, but were uncommon. Special stains revealed numerous spore forms of *M. cerebralis*, within the cyst wall, (Fig. 4) and occasionally in a giant cell. The cyst wall had no epithelial lining. Instead, cells comprising the wall were seen to abut directly into the lumen.

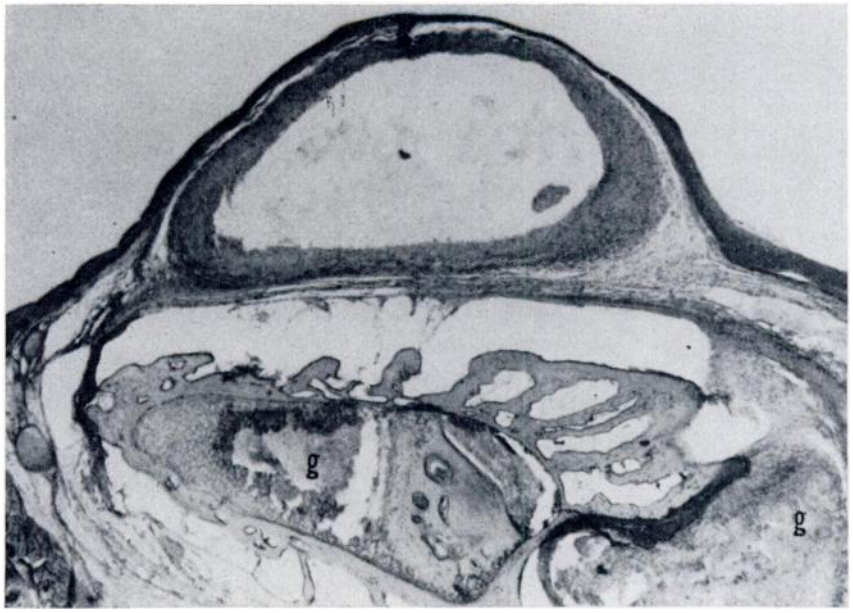

FIGURE 2. Low power photomicrograph of opercular cyst just underlying the surface epithelium. Beneath the cyst, the cartilage is involved by a granulomatous inflammatory reaction (g) caused by **M. cerebralis** H & E X25.

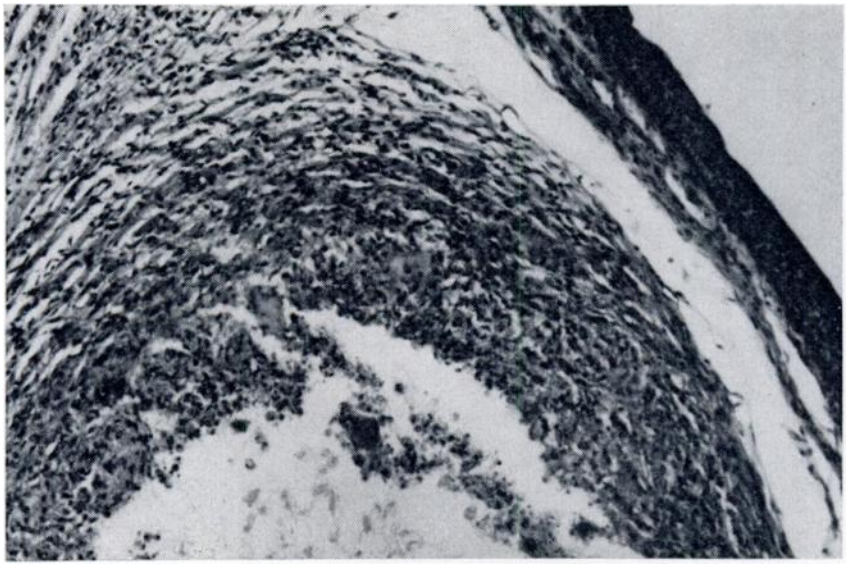

FIGURE 3. Higher power view of cyst wall showing its inflammatory nature and the absence of an epithelial lining. H & E, X200.

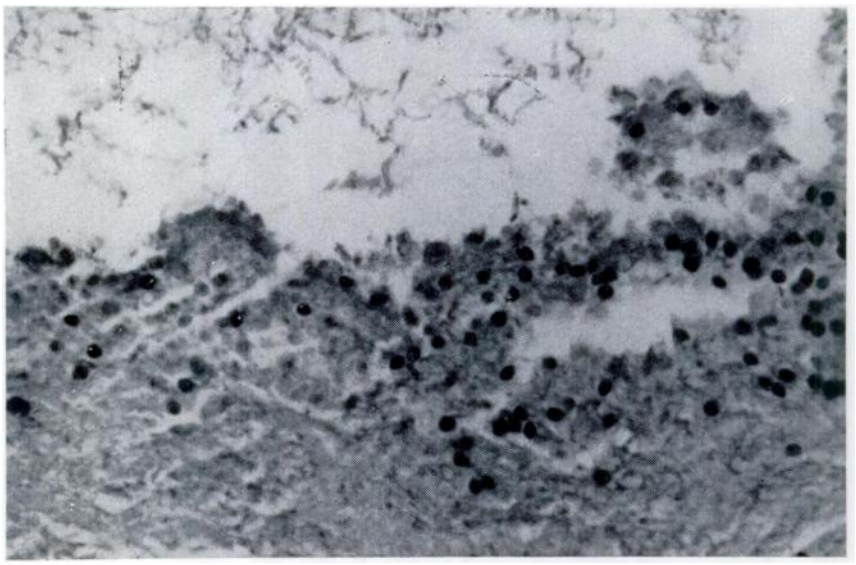

FIGURE 4. **M. cerebralis** spores in the cyst wall. Saffranin & Fast green, X400.

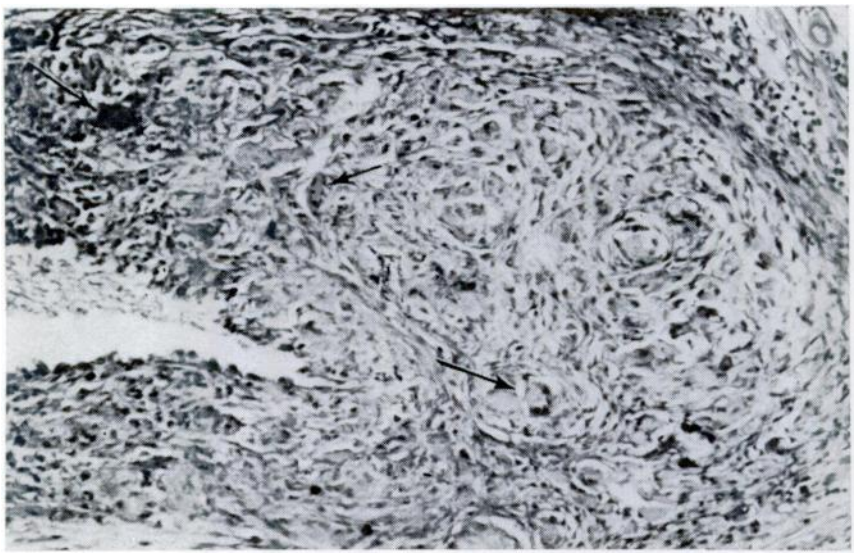

FIGURE 5. Photomicrograph of an area of cartilagenous destruction caused by **M. cerebralis.** Note the granulomatous nature of the inflammatory response. Multinucleated Langhan's type giant cells are present in the reaction (arrows). H & E, X250.

The lumen itself was partially filled with myxosomal spores and amorphous debris.

Fresh spores taken from head cartilage and opercular cysts averaged 8.8 μm by 8.15 μm and did not possess an iodinophilic vacuole. On this basis they were identified as *M. cerebralis.*

Rupture of these spore containing cysts into the environment could transmit whirling disease from live trout.

Acknowledgement

The authors express appreciation to the Nevada Fish and Game Department for their help in obtaining specimens and to Marley Lott for preparing the tissue sections.

LITERATURE CITED

1. HOFER, B. 1903. Uber die Drehkrankheit der Regenbogenforelle. Allgem. Fischereizeitung. 28: 7-8.
2. HOFFMAN, G. L., C. E. DUNBAR and A. BRADFORD. 1962. Whirling disease of trouts caused by *Myxosoma cerebralis* in the United States. Spec. Sci. Rep. Fish. 427: 5-7.
3. HOFFMAN, G. L., R. E. PUTZ and C. E. DUNBAR. 1965. Studies on *Myxosoma cartilaginis* n. sp. (Protozoa: Myxosporidea) of centarchid fish and a synopsis of the Myxosoma of North American freshwater fishes. J. Protozool. 12: 319-332.
4. RYDLO, M. 1971. Nachweis von Sporen der *Myxosoma cerebralis* in verschiedenen Organen drehkranker Regenbogenforellen. Osterreichs Fischerei. 7: 97-99.
5. USPENSKAYA, A. V. 1957. The ecology and spreading of the pathogen of *Myxosoma cerebralis* (Hofer, 1903; Plehn, 1905) of trout in the fish ponds of the Soviet Union. In, *Parasites and Disease of Fish,* ed. by G. K. Petrusheveski. Vo. 42, Bull. USSR Sci. Inst. Freshwater Fish (English translation, Office of Technical Services, U.S. Department of Commerce, Washington, No. 60-51169, 47-55).

Received for publication 7 December 1973