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Abstract: Granulomatous oral masses were observed in two chinook salmon (*On-corhyncus tschwytscha*) smolts with systemic nocardiosis. Gross and histopathological lesions are described indicating the proliferative inflammatory nature of this diesase.

INTRODUCTION

The actinomycete *Nocardia* has been reported as a pathogen of both elasmo-branchs and teleosts. The first verified report of nocardiosis occurred among neon tetras (*Hyphenessobrycon innesi*) in Argentina during the early nineteen-sixties.[13] At about the same time (1962-63) an outbreak of the disease was observed in rainbow trout (*Salmo gairdneri*) at the National Fish Hatchery in Lees-town, West Virginia.[11]

Nocardiosis was diagnosed in seven other species of fish in 1965 and 1966,[3,4] and the disease has been reproduced experimentally.[1,11]

Two species of organism have been identified, *N. asteroides*, which is also a pathogen of homeotherms, and *N. kam-pachi*, which was isolated from Japanese yellow-tail (*Seriola quinqueradiata* and *S. purpurascens*).[7,13]

The purpose of this paper is to describe two cases of the disease in aqua-culture-reared chinook salmon with large oral granulomata.

MATERIALS AND METHODS

Two 15 cm formalin-fixed chinook salmon, 16 months of age were submitted to the Marine Pathology Laboratory as part of a weekly monitoring system of Sea Grant and Agricultural Experiment Station aquaculture projects at the Uni-versity of Rhode Island. Both fish had been raised from eggs in a biologically filtered water re-use system and were taken from a 2850 liter tank containing approximately 1000 other salmon of simi-lar size and age.

Tissues for light microscopic exami-nation were embedded in paraffin, cut at 6 μ and routinely stained with hema-toxylin and eosin (H & E). Sections containing bone were decalcified in an 18 percent solution of formic and hydro-chloric acids and neutralized in a satura-ted solution of lithium carbonate.

Special stains used to identify and characterize the bacteria included the Brown-Bren and Lillie's Gram methods, Ziehl-Nielsen method for acid fast bac-teria and the Fite-Faraco modification for Nocardia.[2,9]

Gross Pathology

The mouths of both fish were held open by protruding oral masses. The mass from the first fish examined (C240) was 1.0 x 0.75 cm and was attached to the floor of the mouth to the right of the tongue near the anterior end of the jaw (Fig. 1). The second fish (C254) had a mass similar in size to the first (1 cm in diameter) attached to the anterior floor of the mouth to the left of the tongue. The point of attachment (C254) was broad-based and encompassed the tumor diameter.

In both instances the sides of the masses were surrounded by a white tissue resembling the gingiva; the dorsal aspects were ulcerated and roughened. The masses were of friable consistency and the cut section was mottled greyish-white. The second fish examined (C254) had a second ulcerated mass, approximately 1.0 x 1.5 cm, on the right lateral-most gill raker. No other gross lesions were observed.

Histopathology

In both cases the masses were composed primarily of reticulo-endothelial (RE) cells resembling the mammalian histiocyte. These cells were 10-20 μ in diameter, polygonal to round, with acidophilic cytoplasm and a large vesicular eccentric nucleus that was occasionally bean-shaped. A fine sparse network of collagen fibers was present within the RE cells. The mass was surrounded by a fibrous capsule, this in turn bounded by an oral mucosa composed of an epithe-lium containing many mucus cells and resembling piscine epidermis. The outer epidermal covering was absent on the dorsal aspect of the mass.

In addition to the RE cells, small lymphocytes and erythrocytes were diffusely distributed throughout the masses. Multi-nucleated giant cells of the foreign body type were rarely present.

Many of the inflammatory cells were necrotic in both masses but this was especially true of the larger mass (C254) in which the majority of the cells were necrotic.

Within the uniform sheet of RE cells were randomly distributed foci of necrosis. These were characterized by central zones of sparsely distributed necrotic debris and inflammatory cells surrounded by histiocytic cells whose long axis appeared to lie at right angles to the central necrotic areas.

Colonies of weakly basophilic bacteria were present on the ulcerated surface of one of the tumors (C254), extending for a short distance into its
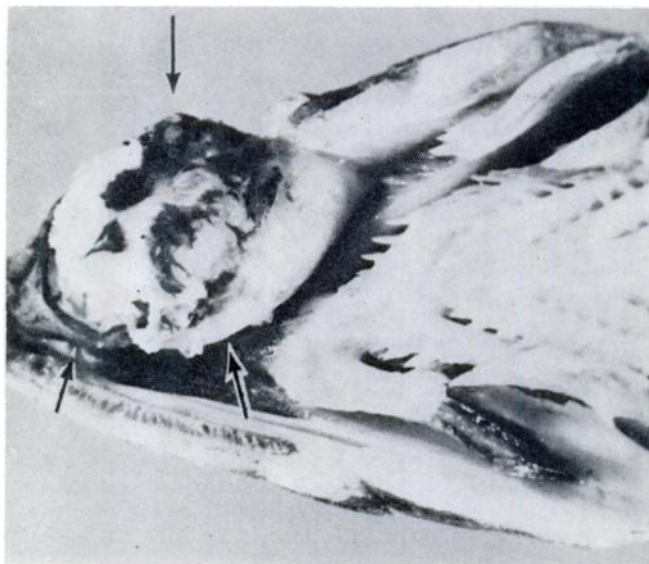

FIGURE 1. Granulomatous mass on the floor of the oral cavity, anterior end of lower jaw of Chinook salmon. Note ulcerated dorsal aspect (arrow).

parenchyma. The morphologic characteristics of the organisms could not be readily determined with H & E. Occasional filamentous structures were present about the edges of the colonies, but with H & E no other organisms were seen within the parenchyma of the masses.

Granulomatous inflammatory tissue, necrosis and bacterial colonies were found in the gill, myocardium, pericardium, spleen, kidney, pancreas, mesentery, pyloric caeca, and anterior gut.

Granulomatous proliferation was especially severe in the mesenteric tissue between the pyloric caeca. The inflammatory tissue had replaced a great deal of pancreatic tissue and was present in the subserosal musculature of the anterior gut as well as the submucosa of the pyloric caeca (Fig. 2).

While the response was primarily chronic and proliferative, focal areas of necrosis were present in a greater or lesser degree in all affected tissue. This more acute reaction was especially prevalent in the kidney and myocardium in which colonies of bacteria were surrounded by zones of necrosis and the proliferative granulomatous tissue.

Proliferative lesions were present within the gill filaments and beneath the epithelium at the base of some lamellae. Masses of bacteria occupied the lumena of filament vessels.

The bacteria present superficially on the second mass examined (C254) were both gram negative and gram positive and included bacilli as well as filamentous forms. Bacteria deeper within both masses were gram positive and filamentous. With the Fite-Faraco method for *Nocardia* the organisms were sharply outlined, magenta in color, filamentous, branched and beaded. They varied in size; 5 to 40 μ in length (from point of branch or isolated segments) and 0.5 to 1 μ in width. Organisms not apparent on H & E were easily seen with the Fite-Faraco method. In many instances the
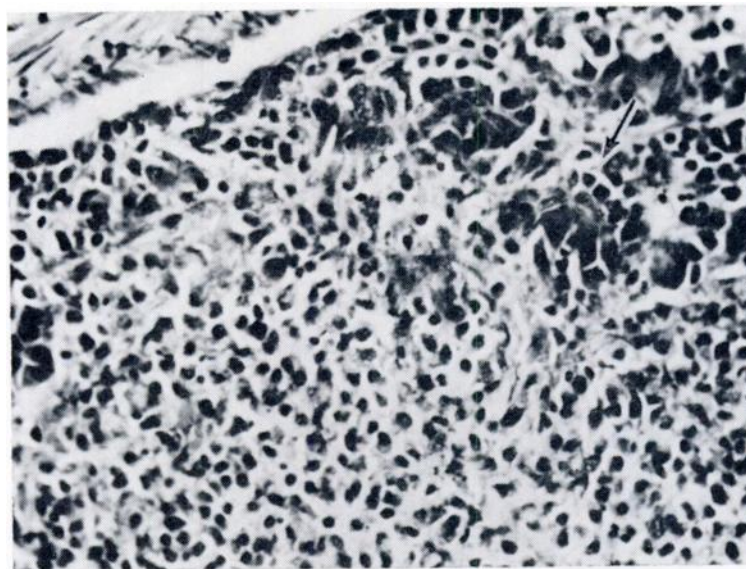

FIGURE 2. Diffuse granulomatous response within interpyloric cecal mesenteric pancreatic tissue. A few islands of exocrine pancreatic tissue remain at upper right (arrow). X160 H & E.

organisms were diffusely distributed throughout the granulomatous lesion (Fig. 3). The bacteria were not acid fast when stained by the Ziehl-Nielsen method.

DISCUSSION

On the basis of present knowledge, the differential diagnosis of oral masses in salmonids include ondotogenic tumors, leiomyoma, and thyroid hyperplasia or neoplasia.[6,9,10] To this list should be added granulomatous proliferations in association with Nocardia species. It would seem logical, however, to assume the list far from complete.

While differentiation of these four entities might be difficult upon gross examination, the histopathological appearance of each is diagnostic. The inflammatory response elicited by Nocardia and characterized by a diffuse granulomatous reaction with foci of necrosis is easily differentiated from the mesenchymal neoplasms or the glandular proliferation associated with thyroid hyperplasia and adenoma. Furthermore, the

presence of typical gram-positive, acid-fast, filamentous organisms within the tumor mass and within other lesions systemically allows etiological diagnosis.

The response to Nocardia in these fish is similar to the response in higher vertebrates.[5,12] There were, however, no instances of suppurative granulomata which may be present in pulmonary nocardiosis of man and domestic animals. Lack of suppurative response is due to the inability of fish to produce a true 'pus'. The diffuse granulomatous reaction coupled with necrotic foci and the presence of organisms observed in these cases is similar to that described in cases of canine nocardiosis.[12]

This combination of lesions may explain the conflicting reports in the literature of piscine response to nocardial organisms. Both focal granulomata and focal areas of necrosis have been described.[1,7,11] It seems probable that the focal areas of necrosis may be more prevalent in acute cases or may become confluent as in higher vertebrates and thus the lesions appear more destructive than proliferative.
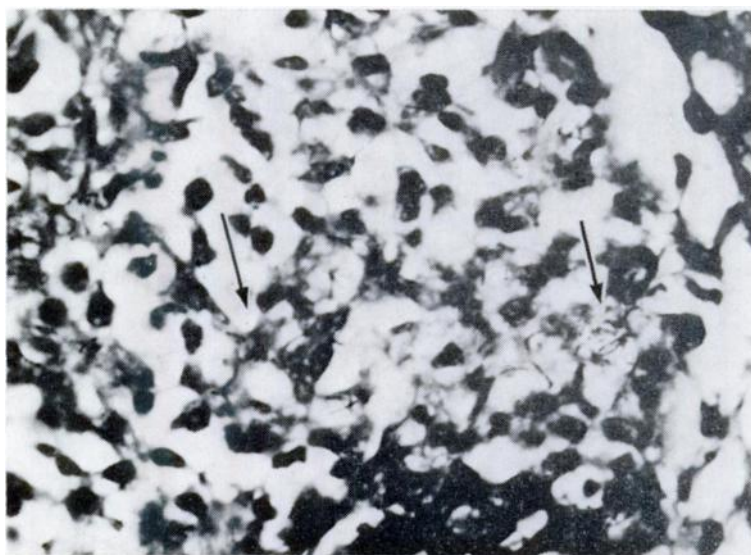

FIGURE 3. Filamentous, branched, beaded acid-fast nocardial organisms diffusely distributed within granuloma (arrows). X400 Fite-Faraco.

The tendency to undergo necrosis may, however, be absent in other species. A suspected case of nocardiosis in a brown shark (*Carcharhinus milberti*) submitted by O'Gara and Oliverio to the Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, D.C. (RTLA 524) was examined by one of the authors (REW). The lesions were characterized by diffuse infiltration of epithelioid cells and no foci of necrosis. The organisms within the lesions were gram-positive, acid-fast, filamentous, beaded and identical in appearance to those seen in the chinook.

Etiological diagnoses based on characteristic tissue response and organism morphology in tissue section is a common procedure employed by the histopathologist examining abnormal tissue from higher vertebrates. This procedure applies as well to the diagnosis of disease in fish. Histopathologic diagnosis of such diseases as infectious pancreatic necrosis, lymphocystis, corynebacterial kidney disease and ichthyophthyriasis are but a few examples of piscine infections which lend themselves well to diagnosis by gross and microscopic pathological techniques. The proliferative tissue response, unique morphology and staining characteristics of the nocardial organism will allow the comparative pathologist to arrive at a proper etiologic diagnosis when it is impossible to recover the causative agent.

The pathogenesis of piscine nocardiosis is as yet unknown. In this case the fish had been kept in a water re-use system for over 12 months. Water purification was achieved by biological nitrification and denitrification. Since the bacteria involved in these two processes are widely distributed in nature and particularly in soils, it is possible that *Nocardia* sp. may have been introduced at the time the filters were activated with bacteria obtained from soil sources.

Cultures of the filters on Lowenstein-Jensen medium were negative. It is interesting to speculate that the *Nocardia* may have caused systemic infection after having gained access via wounds of the buccal cavity. This might account for the apparent low incidence of cases; however, the exact incidence of the disease cannot be determined as all the fish were sacrificed for economic reasons shortly after the infected individuals were submitted for necropsy. Previous weekly monitoring of this group had revealed only a low incidence of kidney disease due to *Corynebacterium* sp.

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