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LESIONS OF TUBERCULOSIS IN MOUNTAIN WHITEFISH (*Prosopium williamsoni*)[□]

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Abstract: Lesions of tuberculosis in mountain whitefish (*Prosopium williamsoni*) were present in all visceral organs. The tubercles were composed of large rounded macrophages which contained numerous intracytoplasmic acid-fast bacilli. The lesions were not encapsulated and mineralization was not observed.

INTRODUCTION

Tuberculosis of fish has been reported in 151 species and is world-wide in distribution.⁵ The classification of the causative organisms is not definite; however, several distinct species of mycobacterium, including *Mycobacterium fortuitum* and *M. marinum*, have been recognized,^{5,8} as well as non-speciated mycobacteria from Runyon Group III.⁶ The piscine tubercle is composed of mononuclear cells, mostly epithelioid macrophages, and small numbers of lymphocytes. Langhans' giant cells are not observed in spontaneous cases.² Lesions have been observed in most internal organs as well as on the fins and skin.⁵ In both freshwater and marine tropical fish, encapsulated tubercles often are observed in the liver, with miliary spread to other organs observed less often. In salmon, lesions are observed in the liver, kidney, spleen, intestine and pyloric caeca.⁷

We recently reported the occurrence of spontaneous tuberculosis in mountain whitefish taken from the Yakima River.¹ In the present communication, we report the morphologic appearance of the disease in whitefish.

METHODS AND MATERIALS

Whitefish were taken from the Yakima River near Richland, Washington in February and March, 1975 and 1976. Injured fish were killed by a blow on the head or by severing the spinal cord. The remaining fish were held in tanks of flowing water in the Battelle Freshwater Ecology Laboratory for periods of 1 week to 4 months. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 6 μ m sections, then stained with hematoxylin and eosin, Kinyoun's carbol Fuchsin-acid-fast, and Brown and Brenn Gram's stain.

RESULTS

Gross Lesions

A total of 115 fish were examined, with gross lesions observed in 10. In the most severely affected fish the kidneys were enlarged and swollen, and contained numerous soft white spots beneath the capsule, ranging in diameter from 1-5 mm (Fig. 1). The lesions were present throughout the thickness of the renal tissue. Kidneys were soft and friable. Off-white lesions in the liver (Fig. 2) were, on the average, larger (1-1.2 cm dia.) and

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FIGURE 1. Kidney of a whitefish with numerous small granulomas. In two areas the granulomas are nearly confluent (arrows).

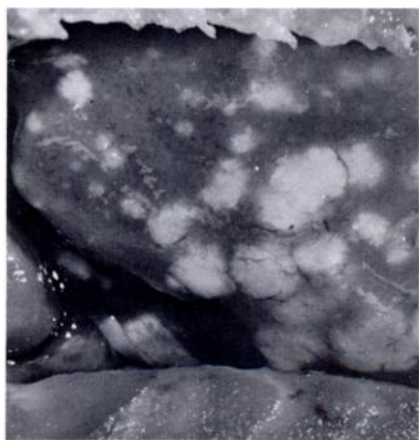


FIGURE 2. Large granulomas on the surface of the liver of a whitefish.

less numerous than those in the kidney. They were soft, and some were easily separated from the surrounding liver tissue. Gross lesions also were observed

in the stomach, pyloric caeca, intestine, pancreas, spleen, gill, ovary, and heart. In these organs, the lesions were similar in size to those observed in the kidney, but were fewer in number. The pyloric caeca were an exception, and in several fish contained many tubercles.

In less severely affected fish, the kidneys were not swollen. Occasional white spots were present in renal tissue, liver, pyloric cecum, or gills. In 2 fish, gross lesions consisted of 1 or 2 small (1-2 mm) white spots on the gills. One fish had small accumulations of acid-fast organisms in the kidney and gill that were not grossly visible.

Microscopic Lesions

Tissues from 18 whitefish were examined microscopically, excluding 2 fish with gross lesions. A summary of the lesions of 9 fish in which microscopic lesions were observed is presented in Table 1.

The tubercles consisted of accumulations of macrophages and a small to moderate number of lymphocytes (Fig. 3). The macrophages were round, with the nucleus located centrally or peripheral and ample cytoplasm which contained large numbers of acid-fast organisms (Figs. 4 and 5). Bacilli also were observed extracellularly. An occasional binucleated macrophage was observed; however, there were no multinucleated-foreign body giant cells in any of the lesions. Necrosis was not observed in the tubercles, but the macrophages in the central areas of the large lesions often had degenerative changes. No mineralization was observed.

The lesions were not encapsulated and most did not contain fibrous connective tissue, with the exception of those in the liver. In several fish, the hepatic lesions resembled fibromas. The center of the lesions consisted of spindle-shaped cells organized in a whorled pattern, with a small to moderate amount of intercellular collagen (Fig. 6). Occasional

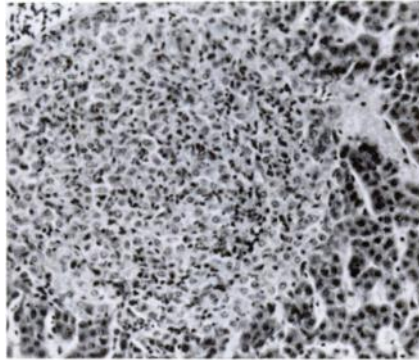


FIGURE 3. A small lesion in the liver of a whitefish consisting of a mixture of macrophages and lymphocytes with a small number of polymorphonuclear leukocytes. Fibrous tissue is not evident in or surrounding the lesion. Hematoxylin and eosin. 210X

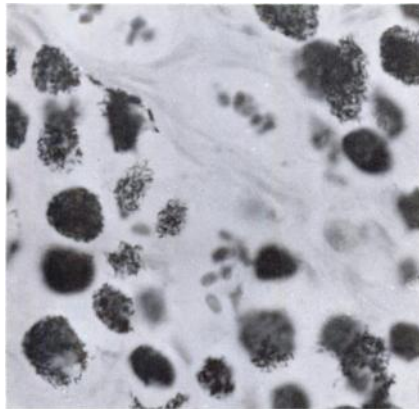


FIGURE 4. Macrophages in a pyloric cecum containing numerous Gram's positive bacteria. Kinyoun's carbol fuchsin-acid-fast. 1000X

inflammatory cells were present in the fibrous tissue. Macrophages containing acid-fast bacilli were present in the peripheral portion of the lesions. Some lesions were intermediate between those containing only inflammatory cells and

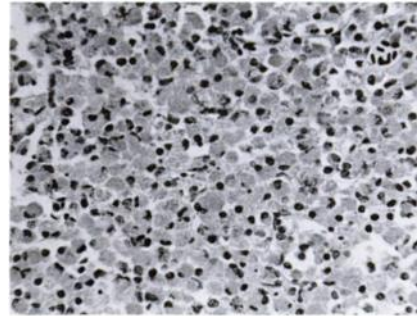


FIGURE 5. A higher magnification of epithelioid macrophages in a hepatic lesion. Note the ample cytoplasm, which contained large numbers of acid-fast bacilli. Hematoxylin and eosin. 540X

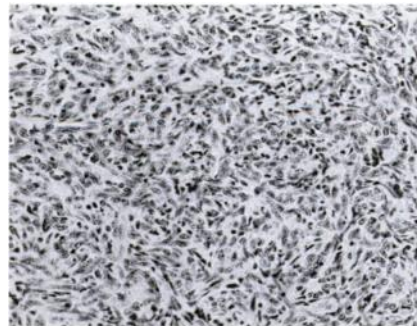


FIGURE 6. A photograph of the central area of a fibroma-like hepatic lesion. There is a moderate amount of mature intracellular collagen. Hematoxylin and eosin. 210X

those resembling fibromas. In these lesions the fibrous tissue appeared to proliferate around surviving blood vessels (Fig. 7). Associated with this proliferation of fibrous tissue were cells resembling multinucleated giant cells (Fig. 8). On ultrastructural examination, these were found to be proliferating bile ducts which contained a central lumen. Cell membranes containing junctional structures were present between the nuclei.

TABLE 1. Distribution of lesions in nine whitefish with mycobacterial infection.

Fish No.	Pyloric											
	Kidney	Liver	Pancreas	Spleen	Stomach	Cecum	Intestine	Muscle	Skin	Gill	Heart	Ovary
1	+	+	+	+	+	+	+	+	+	+	.	.
2	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	.	.	+	+	*	*	*	*	+
13	+	+	*	*	*	*	M
24	+	*	*	+	.	.
26	+	+	+	+	+	.	+	+
27	+	+	+	.	.	+	+	.
31	.	+	.	+	+	+	M
32	+	+	+	+	.	.	.	+	.	+	+	M
Total	8/9**	8/9	6/9	5/9	2/9	4/9	3/9	3/6	3/6	5/7	5/7	3/6

*Not examined microscopically

M = Male

** = No. infected/No. observed

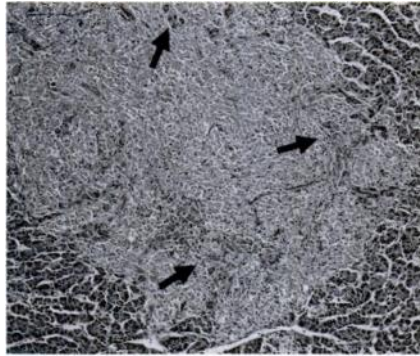


FIGURE 7. A hepatic lesion with areas of fibrosis and bile duct proliferation (arrows). Note the complete lack of fibrosis at the junction with normal hepatic tissue. Hematoxylin and eosin. 85×

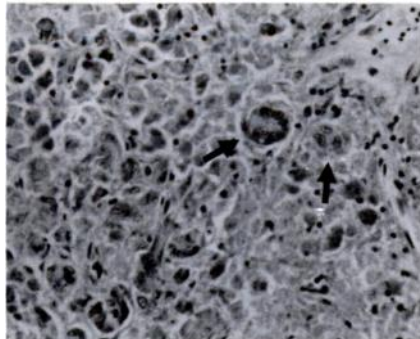


FIGURE 8. An area of bile duct proliferation in a large hepatic granuloma. Two regenerating bile ducts are evident (arrows). Note the similarity to Langhans'-type giant cells. Hematoxylin and eosin. 540×

The smaller lesions consisted of 1 or more macrophages containing large numbers of acid-fast bacilli along with extracellular organisms. Such small clusters of cells and bacilli were observed in the connective tissue of the muscle and subcutaneous connective tissue of severe-

ly affected fish. No gross lesions considered to be associated with tuberculosis were observed in the skin and muscle. Several fish in the holding tanks had large cutaneous ulcers extending into the underlying muscle. No inflammatory reaction or acid-fast bacilli were observed in these lesions. In all fish, macrophages containing acid-fast bacilli were present in blood vessels of some organs, including the chambers of the heart. These cells were more numerous in the severely affected fish.

A second type of granuloma was observed in the wall of the stomach, pyloric cecum and intestine of both mycobacteria-infected and noninfected fish (Fig. 9). These lesions differed from those associated with the acid-fast bacilli by the presence of a fibrous connective tissue capsule. Most contained cross sections of a nematode. The granulomas, with one exception, were found only in the wall of the gastrointestinal tract. In one fish (noninfected), a single granuloma was present in the liver.

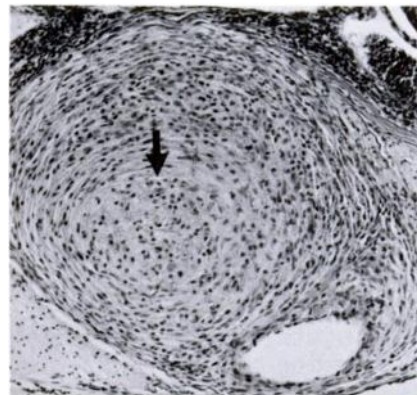


FIGURE 9. A granuloma in the muscle layer of a pyloric cecum from a whitefish with no evidence of mycobacterial infection. The central area (arrow) consists of macrophages and is surrounded by a fibrous connective tissue capsule. Hematoxylin and eosin. 85×

DISCUSSION

Several morphologic features of tuberculosis in whitefish differ from those reported in other piscine species. In contrast to the large numbers of epithelioid macrophages in the lesions in whitefish, salmonid mycobacterial infection is characterized by lack of inflammatory response.⁷ In tropical fish, the lesions are often encapsulated, with central necrosis and mineralization,⁸ none of which was observed in the whitefish. The most interesting difference was the fibroma-like areas of repair in the hepatic lesions, which have not been described in other piscine species. Gradation of fibrosis in liver lesions and the presence of macrophages containing acid-fast bacilli confirmed their inflammatory nature.

The lack of encapsulation of the tubercles apparently was not the result of the inability of the fish to respond to chronic stimuli, since well-encapsulated granulomas were elicited by nematode larvae. The absence of encapsulation could result from the lack of response by whitefish to the mycobacterium, or might indicate that the disease progressed very rapidly.³ Although there is no definitive evidence to exclude either of

these possibilities, the latter is perhaps the less plausible. The association of age and severity of the lesions suggest that the disease has a prolonged course.¹

Langhans'-type giant cells have been described in experimentally transmitted mycobacterial infection,³ in brook trout fed synthetic diets⁴ and in brook trout having a mycosis-like granuloma,¹¹ indicating that the lack of this cell in the mycobacterial lesions is not the result of the inability of fish to form such a cell. Its absence in primary tubercular lesions cannot be explained at present. The presence of binucleated cells in some of the lesions may indicate an abortive attempt to form giant cells.

Because of the distinct morphology and staining characteristics of the causative organism, the morphologic appearance of the mycobacterial disease in whitefish could not be confused with other agents which cause granulomas in fish: *Ichthyophonus hoferi*,⁹ *Nocardia*,¹⁰ *Flavobacterium* sp.,⁹ or migrating parasites. A slow-growing, acid-fast bacillus, which grows at room temperature, has been isolated from 1 fish¹ but has not been characterized as of this writing.

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