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POSTSPAWNING MORTALITY OF RAINBOW TROUT (*SALMO GAIRDNERI*) ASSOCIATED WITH *LACTOBACILLUS*

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ABSTRACT: A *Lactobacillus* sp. was consistently isolated from dead and moribund postspawning brood stock rainbow trout. Pathology was characterized by massive chronic inflammation throughout the abdominal cavity and formation of pseudomembranes. The bacteria were most abundant in spent testes. Repeated handling was considered to be the major stressor leading to this infection and subsequent high mortality.

INTRODUCTION

From December 1982 through April 1983 an unusually high and prolonged postspawning mortality of brood stock rainbow trout (*Salmo gairdneri*) was recorded at the Aquaculture Production/Demonstration Station, Kearneysville, West Virginia. Although monogenetic trematodes and the ciliate, *Ichthyophthirius* were sometimes found in sufficient number to require treatment, and bacterial gill disease was diagnosed in some cases, neither parasitism nor gill disease was considered to be the cause of the high mortality. We further examined moribund fish in an attempt to diagnose the cause of mortality.

MATERIALS AND METHODS

Between 20 and 30 fish were examined at necropsy during a period of several days. Wet mounts of gill tissue were examined for external parasites, and smears of gill tissue for bacterial infection. Smears were also made from kidneys and testes and examined after safranin or gram staining.

Kidneys were cultured on brain–heart infusion (BHI) agar and the plates incubated at 20 C. Cultures of a small, rapidly growing gram-positive rod that was consistently isolated, often in pure culture, were subjected to a series of 13 standard biochemical tests for identification. After tentative identification as *Lactobacillus* these cultures were sent to the University of Georgia for confirmation.

Tissues from 10 fish were fixed in Bouin's solution and examined histologically. Paraffin sections were cut at 4 μm and stained with hematoxylin and eosin (H&E), May-Grunwald Giemsa, or MacCallum-Goodpasture (Luna, 1968).

Five rainbow trout (average length 15 cm) were injected intraperitoneally and five were injected intramuscularly with 0.1 ml 24 hr BHI culture of the *Lactobacillus*. Fish were held in spring water (12–13 C) and observed for 2 wk.

RESULTS

Overall loss of winter spawning rainbow trout brood stock was 24%. Losses among twelve individual strains ranged from a high of 58% for the Donaldson strain to a low of 11% for the New Zealand strain. The epizootic began in December, peaked 3 mo later, and then declined during the following 2 mo.

External signs consisted primarily of hermorrhages at the bases of fins. Some males also had hyperemic skin lesions on the ventral abdominal wall that were circumscribed, eroded, and hemorrhagic. Internally, livers were pale and somewhat enlarged; the spleen and kidneys of some specimens were enlarged; and hemorrhages were present in the intestine and gonads (especially the testes). Gray membranes covered some organs, and visceral adhesions were present in some fish. Sev-

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eral specimens had clear to bloody ascites. Terminally ill fish refused food, were lethargic, and swam near the surface.

Most smears of kidneys and testes contained numerous short gram-positive rods. Colonies on BHI agar were small clear to white, round and entire at 20 C. The bacteria were gram-positive, nonmotile, catalase negative rods. The results of 12 additional biochemical tests were identical to those reported by Hui et al. (1984) for *L. piscicola* with the exception of being sorbitol positive, and Voges-Proskauer negative. Further study of these isolates at the University of Georgia showed them to be closely related, if not identical, to *L. piscicola* (Hiu et al., 1984).

Nearly all fish examined had fibrous or proliferative inflammation of the serosal membranes of the liver, spleen, pancreas, testes, and heart. Livers showed focal necrosis and perivascular and peribiliary inflammation, and hepatocytes were devoid of glycogen. Tubule cell degeneration was seen in all kidneys examined. It ranged from cytoplasmic vacuolation of proximal tubules to necrosis and sloughing of tubule cells and early formation of granulomas. In addition to epicarditis, the heart showed vacuolation and necrosis of muscle cells, as well as focal myocarditis. One fish also appeared to have several ventricular thrombi. Spleens were engorged with red blood cells, and macrophages containing melanin were abundant. Several testes and ovaries contained many macrophages in the process of phagocytizing spermatozoa or degenerating ova. In the testes, the resorption process included the formation of large granulomas. In the testes large numbers of free gram-positive bacteria were present, in addition to those phagocytized. Bacteria-laden macrophages were also present in the spleen, kidneys, and heart of some specimens.

A similar pattern of mortality occurred during the 1983–1984 spawning season. Again, gram-positive organisms were readily isolated and identified as *Lactobacillus*. Injected immature rainbow trout showed no sign of disease and no mortality occurred, but the *Lactobacillus* was reisolated from the kidneys of several of these fish.

DISCUSSION

Although some pathology can be expected in hatchery stocks of postspawning rainbow trout, the type and extent of pathology seen in the present case was considered to be a direct effect of the *Lactobacillus* infection. The infection was probably precipitated by the stress of the frequent and rough handling these fish received. Inasmuch as they represented several strains being evaluated by personnel at the Fish Genetics Station (Kearneysville, West Virginia), the fish were handled weekly to determine spawning condition throughout the period of mortality. Stress mitigating procedures (e.g., use of salt baths) were not used.

Postspawning fish were the source of bacteria described by Ross and Toth (1974) and Cone (1982). The isolates studied by Hiu et al. (1984) were from fish that were subject to stress conditions.

Although injection of the *Lactobacillus* did not produce signs of disease, the organism was recovered from kidneys of some test fish suggesting it can be considered a weak facultative pathogen. Likewise, Ross and Toth (1974) were unable to transmit disease by injection of *Lactobacillus* isolates into spent female or juvenile rainbow trout.

Takashima et al. (1975) reported degeneration of liver and kidney in rainbow trout that retained overripe eggs, but did not mention inflammation. The inflammation reported here is similar to that seen in cases of chronic infection with *Renibacterium salmoninarum*.

Previously reported opportunistic infection of postspawning rainbow trout by gram-positive organisms were apparently

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limited to females (Ajmal and Hobbs, 1967; Ross and Toth, 1974; Cone, 1982). In our case, the *Lactobacillus* was isolated from both sexes and mortality was equal. The pathologic changes in both sexes were similar, except that the organism seemed to have an affinity for testes undergoing sperm resorption.

This case underlines the necessity of minimizing stress when brood stock fish are maintained and handled.

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