MFR PAPER 1332

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Vibriosis in Maine and New Hampshire Salmonids

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Pen culture of coho salmon, *Oncorhynchus kisutch,* began in Maine and New Hampshire in 1972, and demonstrated both the potential of this new industry and the impact of bacterial disease on salmon entering Maine and New Hampshire coastal waters. Vibriosis proved to be a major problem, causing losses of 30-80 percent. Typically, the disease occurred several weeks after smolts were acclimatized to seawater, and at water temperatures above 15°C. Often, fish showed no macroscopic signs of disease before death. In 1973, we isolated *Vibrio anguillarum* from moribund coho salmon and found that this strain was serologically similar to the archetype west coast organism.

The following spring we made a heat-killed bacterin from this organism and tested it at two sites—a private salmon farm and the University of New Hampshire (UNH) seapens (Sawyer and Strout, 1977). At the private farm, 10,000 fish were injected intraperitoneally with 2 mg wet-packed cells per fish; another 10,000 fish were treated with Terramycin[1] when vibriosis occurred; and 10,000 controls received no treatment. Mortality was 3 percent in vaccinated fish, 7 percent in medicated fish, and 24 percent in controls. Also, twice as many vaccinated fish as controls reached market size in the following 6 months. Meanwhile, at the UNH seapens, 90 percent of both vaccinated and unvaccinated fish died from vibriosis. We isolated a second serotype of *V. anguillarum* from these fish.

In an effort to determine how many *Vibrio* serotypes were present in this area and which should be incorporated into a bacterin, we sampled cultured and feral fishes along the coast for the following 2 years. We obtained several hundred *Vibrio* isolates which we injected into coho salmon to test pathogenicity. These coho salmon had been reared in fresh water, and thus had no prior exposure to the organism. Thirty-seven *Vibrio* strains were pathogenic to coho salmon, and all these were similar biochemically to the archetype *V. anguillarum* (Evelyn, 1971). Nonpathogenic *Vibrio* strains differed from the archetype in arginine and lysine tests. Agglutinin titers were determined with the microtiter system using rabbit antisera and antigens prepared from each of the pathogenic strains. On the basis of these tests, it appears that we have three serotypes (Strout et al., 1978). Two of these are similar to the two serotypes found on the west coast (Harrell et al., 1976). We are now using three strains, representative of these three serotypes, to make a trivalent bacterin. This bacterin has proved effective in limited testing with coho salmon, and field tests are now in progress.

Many of the *V. anguillarum* isolates came from estuaries where Atlantic salmon, *Salmo salar,* smolts are released each spring. Since these fish are almost certainly exposed to the organism, we wondered if vibriosis might contribute to the relatively low percent returns of Atlantic salmon.

Our first step was to compare the susceptibility of Atlantic salmon and coho salmon. We found that susceptibility of the two fish species to each of our three *V. anguillarum* serotypes in both injection and water transmission tests was similar. At temperatures of 10° to 15°C, exposure levels of 1-2.5 $\times$ 10^5 organisms/ml in the water for 1 hour killed both coho salmon and Atlantic salmon smolts. These temperatures are found in Maine estuaries at the time Atlantic salmon are released, and numbers of bacteria in the water and natural exposure time may be similar.

Although we had only circumstantial evidence that the disease occurs in newly released Atlantic salmon, vaccination presented little risk, and the possibility of considerable benefit. In a cooperative effort with the Maine Atlantic Sea Run Salmon Commission and the U.S. Fish and Wildlife Service's Craig Brook National Fish Hatchery, we vaccinated 10,000 tagged Atlantic salmon smolts with the trivalent bacterin in the spring of 1977. The vaccination was done by intraperitoneal injection 5 weeks before the fish were released to seawater. During that time, we measured production of serum agglutinating antibody. Antibody titers at the time of release, ranging from 1:10 to 1:40, compared favorably with those found in vaccinated coho that withstood natural challenge from *V. anguillarum* (Antipa and Amend, 1977). However, we realize the limitations in using serum agglutinin levels as an index of protection against the disease. The real test of the vaccine's effectiveness will be the number of adult fish returning in 2 years.

[1]Mention of trade names or commercial products does not imply endorsement by the National Marine Fisheries Service, NOAA.

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MFR PAPER 1332. From Marine Fisheries Review, *Vol. 40, No. 10, October 1978. Copies of this paper, in limited numbers, are available from D822, User Services Branch, Environmental Science Information Center, NOAA, Rockville, MD 20852. Copies of* Marine Fisheries Review *are available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402 for $1.10 each.*

MFR PAPER 1333

Anaerobic Bacteria as Possible Disease Agents in Fish

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There are currently 23 recognized genera of heterotrophic, obligately anaerobic bacteria. Within this group are gram-positive rods and cocci and gram-negative rods and cocci. Many of the genera have representative species which are clinically important pathogens of man and animals. The most familiar genus, *Clostridium,* contains the species *C. botulinum, C. tetani,* and *C. perfringens,* causing botulism toxicity, tetanus, and gas gangrene, respectively. Other genera of anaerobes such as *Propionibacterium, Fusobacterium, Bacteroides,* and *Eubacterium* are less familiar, but also significantly involved in human disease.

Although anaerobic bacteria have been studied since the beginnings of microbiology, it has been only recently that they have been identified as a major group of pathogens in animals and man. A number of reasons can be cited for this lag. Probably the most important was that many people did not believe that strict anaerobes could survive and proliferate in "oxygenated" tissues of the body. Lungs, brain, liver, and other vital organs were good candidates for facultative bacteria, but not for anaerobes. Frequent coinfections with facultative organisms further complicated the picture.

The development of standardized procedures for working with anaerobic bacteria has helped to dispell some of these beliefs. The popularization of the roll-tube method (Hungates' method) by workers at the Virginia Polytechnic Institute's Anaerobe Laboratory and commercialization of the Gas-Pak[1] (BBL) anaerobic jar have both greatly aided in standardizing and facilitating procedures for cultivating anaerobic bacteria. Recent advances in the procedures used to classify anaerobes have made the task of identifying these organisms somewhat less tedious. In particular, the use of gas-liquid chromatography to identify the major metabolic by-products from fermentable substrates allows rapid identification to genus and sometimes to species. Although other confirmatory tests are required, much time can be saved by knowing which tests to run. Many of these advances have come about within the past 3-5 years, and their adoption by clinical microbiology laboratories is still underway. It seems to me that in the not too distant future a number of fish disease laboratories will also adopt these procedures and develop new ones particularly suited to fish disease studies.

At the Fish and Shellfish Pathology Laboratory at the University of Miami, we have been involved with the study of anaerobes from marine fish for the past 2 years. Our studies began following the occurrence of several large fish kills in Biscayne Bay. In addition to the kills, moribund fish were present which exhibited a "twirling" symptom not unlike that observed in trout with whirling disease. Analysis of toxicological and parasitological as well as aerobic bacteriological data revealed no consistent cause of this symptom. We began a search for anaerobic bacteria in the brains (because of the likelihood of neurologic involvement) of fish

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