1977

VIBRIOSIS IN FISH

G. L. Bullock
U.S. Fish and Wildlife Service

VIBRIOSIS IN FISH[1]

G. L. Bullock

U.S. Fish and Wildlife Service
Eastern Fish Disease Laboratory
Kearneysville, West Virginia 25430

FISH DISEASE LEAFLET 50 April 1977

UNITED STATES DEPARTMENT OF THE INTERIOR
U.S. Fish and Wildlife Service
Division of Fishery Research
Washington, D.C. 20240

---

[1] Revision of Fish Disease Leaflet 29 (1970), same title, by Avron J. Ross. Rerun August 1984.

INTRODUCTION

Fish vibriosis is a systemic disease of marine, estuarine, and some freshwater fishes, caused by bacteria of the genus *Vibrio* (Ross et al. 1968, Ghittino et al. 1972). The disease has been known for centuries; outbreaks along the Italian coast were recorded as early as the 1500's. Terms such as "red pest," "red boil," "red plague," or "saltwater furunculosis" have been applied to vibrio infections, but vibriosis is a more specific term and is now used by most fishery workers. With the rapid development of mariculture, vibriosis has become a major cause of fish loss--sometimes to the extent of being a limiting factor.

ETIOLOGY

Most reports on vibriosis implicate *Vibrio anguillarum* as the cause. Variously named isolants--*V. piscium*, *V. piscium* var. *japonicus*, and *V. ichthyodermis*--are now considered as biotypes of *V. anguillarum* (Muroga 1975; Evelyn 1971). On the basis of indole reaction and production of acid in sucrose and mannitol, Nybelin (1935) proposed that three biotypes of *V. anguillarum* be recognized, but his proposal is not now generally accepted (Evelyn 1971, McCarthy et al. 1974). However, several serotypes of *V. anguillarum* are known. Pacha and Kiehn (1969) found three distinct serotypes among 13 strains of *V. anguillarum:* one included salmonid strains from the Pacific Northwest, a second consisted of strains from Europe, and a third was from herring in the Pacific Northwest. Two serotypes of *V. anguillarum* are recognized from salmonids in salt water. A fast-growing strain predominates; the second strain, which grows slowly on initial isolation, has been isolated only infrequently. Both are typical *V. anguillarum* in biochemical reaction, but immunization experiments have shown that cross-protection is not afforded. E. Sawyer (personal communication) recently reported a third serotype from salmonids in salt water on the East Coast of the United States.

CLINICAL SIGNS AND PATHOLOGY

Vibriosis occurs in many species, and although pathologic changes vary somewhat, erythema, hemorrhaging, and anemia are common.

In salmonids, red, necrotic, or boil-like lesions occur in the musculature and erythema is present at the bases of fins and within the mouth. Petechiae may occur on the body

surface, and gills often hemorrhage (Fryer et al. 1972). Hemorrhages may also occur in the viscera, and the intestinal tract is inflamed. A rapidly developing septicemia characteristically occurs in eels, and there are hemorrhages in the fins and in the striated muscle of the abdominal region, and ulcerations on the body surfaces (Bullock et al. 1971). Dermal lesions accompanied by fin necrosis and hemorrhages are common in the flounder, *Pseudopleuronectes americanus* (Levin et al. 1972). Hacking and Budd (1971) noted that freshwater aquarium fishes mainly showed hemorrhages. Among cultured non-salmonid fishes in Japan, external and internal pathology is similar to that reported for salmonids (Tanaka 1975).

In diseased rainbow trout *(Salmo gairdneri)* histopathological changes consisted of muscle necrosis, accompanied by interfibrillar hemorrhages, congestion of interfibrillar vessels, and a lack of leucocytic response (McCarthy et al. 1974). In flounders, Levin et al. (1972) also noted muscle necrosis and focal interstitial and tubular necrosis of the kidneys.

Anemia, which commonly accompanies vibriosis, may be caused by the destruction of red blood cells by hemolysins or to blood loss from hemorrhaging. Umbreit and Tripp (1975) showed that *V. anguillarum* produced a substance that was toxic for goldfish *(Carassius auratus)* and that heating to 100 C increased the potency of the extracellular toxin.

DIAGNOSIS

Diagnosis of vibriosis caused by *Vibrio anguillarum* is based on the isolation (at 20-25 C) of a gram-negative, motile, asporogenous rod-shaped bacterium, which may be slightly curved; it is cytochrome oxidase positive, anaerogenically ferments glucose, and is sensitive to novobiocin and to the vibriostatic agent 0/129 (Calbiochem, La Jolla, California; 2,4, diamino 6,7-di-isopropyl pteridine). Serological confirmation of identification by rapid slide agglutination or fluorescent antibody technique is also desirable, provided a polyvalent antiserum (Conroy and Withnell 1974) is used or the isolate is the same serotype as the strain used to produce monovalent vibrio antiserum. *V. parahaemolyticus* can be separated from *V. anguillarum* by arginine dihydrolase and lysine decarboxylase reactions, sucrose fermentation, the Voges-Proskauer test, and sodium chloride tolerance (Muroga and Egusa 1975).

There is no reliable means of presumptive diagnosis of vibriosis because of its similarity to other septicemic diseases caused by gram-negative bacteria.

SOURCE AND RESERVOIR OF INFECTION

The organism is probably ubiquitous in marine and brackish waters and may be harbored in herring or other marine fishes.

MODE OF TRANSMISSION

Initial infection is probably water-borne. Once established in fishes, the disease is probably spread by contact. Use of infected marine fish in the feeds of healthy fish has also caused epizootics.

INCUBATION PERIOD

Salmonids usually die within 1 week after exposure. Injection of the bacterium produces death within 3-21 days, depending on species of fish, dosage, temperature, and virulence.

PERIOD OF COMMUNICABILITY

Fish of all ages are probably susceptible; the organism has been isolated from both young and adults.

GEOGRAPHIC AND HOST RANGE

Vibriosis occurs worldwide, but it is usually most severe in mariculture operations.

Virtually all species of marine and estuarine fishes are susceptible. Among salmonids, pink salmon *(Oncorhynchus gorbuscha)* and chum salmon *(O. keta)* are the most susceptible; however, serious epizootics have occurred in coho salmon *(O. kisutch)*, rainbow trout, and Atlantic salmon *(Salmo salar)*. In Japan the cultured species, eel (*Anguilla japonica*), yellowtail *(Seriola quinqueradiata)*, and ayu *(Plecoglossus altivelis)* are also highly susceptible. Stress from handling, low oxygen, and elevated temperature are important in predisposing fish to vibriosis.

OCCURRENCE

Outbreaks occur in salmonids at water temperatures higher than 10 C (50 F), and severity increases with temperature. Pathogenicity is markedly reduced at temperatures below 9 C (48 F).

METHODS OF CONTROL

Notice.--*In the United States, fish that are intended for human or animal food can be treated with drugs and chemicals only in accordance with current laws and regulations of the U.S. Food and Drug Administration and the Department of Agriculture. State and local agencies may impose additional regulations.*

Preventive Measures

Good sanitation and management procedures should be used, and care should be exercised to avoid crowding and minimize stress when fish must be handled.

During the last 10 years, there has been much interest in immunization as a means of preventing vibriosis. Rohovec et al. (1975) demonstrated that either wet-packed or lyophilized formalin-killed *Vibrio anguillarum* cells were effective as oral immunogens. A research group at the National Marine Fisheries Service Laboratory at Manchester, Washington, perfected an injection technique for mass immunization of salmonids. The hyperosmotic infiltration technique (Amend and Fender 1976), which involves changing osmotic gradients between fish and bacterin preparation, was used to immunize pink salmon with a bivalent bacterin prepared (by the Manchester group) from the two serotypes of *V. anguillarum* predominant in the Pacific Northwest.

Since both the size and numbers of fish which may be immunized vary, there is probably no single delivery route for vibrio bacterin suitable for all situations. An injection procedure may be preferable for large fingerling or yearling fish, whereas oral or hyperosmotic procedures are more suitable for large numbers of small fingerlings.

In theory, selection and breeding for resistance to *V. anguillarum* may be a means of control. Significant differences in resistance of salmon parr to *V. anguillarum* were found among populations from different rivers in Norway (Gjedrem and Aulstad 1974).

Therapy

Sulfamerazine used at the rate of 17 g/100 kg of fish per day for 10 days has controlled vibriosis. Oxytetracycline at 5.0 to 7.5 g/100 kg of fish per day for 10 days has also been successful.

Pearse et al. (1974) found that 17 strains of vibrios were inhibited by 1.6 to 3.1 µg/ml of furanace and suggested that this drug was promising for control of vibriosis in marine flatfish.

ANNOTATED BIBLIOGRAPHY

Amend, D. F., and D. C. Fender. 1976. Uptake of bovine serum albumin by rainbow trout from hyperosmotic solutions: A model for vaccinating fish. Science 192(4241): 793-794.

Immersion of rainbow trout in 5.32% sodium chloride for 3 min, followed by 3 min in 2% bovine serum albumin (BSA), resulted in uptake of BSA--primarily through the lateral line system and secondarily through the gills.

Anderson, J. I. W., and D. A. Conroy. 1970. Vibrio-disease in marine fish. Pages 266-272 in S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. Am. Fish. Soc., Spec. Publ. 5.

Consideration is given to the potential importance of vibriosis in fish-farming operations. A comprehensive review of the distribution of the disease by country and species of fish is included.

Antipa, R. 1976. Field testing of injected *V. anguillarum* bacterins in pen-reared Pacific salmon. J. Fish. Res. Board Can. 33(6): 1291-1296.

The author describes immunization tests with *Vibrio anguillarum* bacterin killed by heat or formalin, or a combination of the two, for prevention of vibriosis in chinook and coho salmon. Chinook salmon showed the highest survival when injected with the heat-killed preparation; a single intraperitoneal injection provided protection for 6 months against a natural challenge of *Vibrio anguillarum*. In coho salmon, however, both control and immunized groups showed less than 7% mortality, indicating that immunization was not beneficial to their survival.

Baross, J., and J. Liston. 1968. Isolation of *Vibrio parahaemolyticus* from the Northwest Pacific. Nature (Lond.) 217(5135): 1263-1264.

The authors characterized *Vibrio parahaemolyticus* and described features that differentiated this species from *V. anguillarum* and *V. alginolyticus*.

Braaten, B. A., and H. O. Hodgins. 1976. Protection of steelhead trout *(Salmo gairdneri)* against vibriosis with a living low-virulence strain of *Vibrio anguillarum*. J. Fish. Res. Board Can. 33(4, part 1): 845-847.

Steelhead trout that had been fed pellets soaked in low-virulence *Vibrio anguillarum* every other day for 2 weeks were protected against an intraperitoneal injection of 10^6 cells of high-virulence *V. anguillarum*.

Bullock, G. L., D. A. Conroy, and S. F. Snieszko. 1971. Bacterial diseases of fishes. Book 2A (151 pp.) in S. F. Snieszko and H. R. Axelrod, eds. Diseases of fishes. T.F.H. Publications, Inc., Neptune, N. J.

A comprehensive description of vibriosis, which includes historical aspects, pathologic changes and symptoms, etiology, diagnosis, and treatment.

Cisar, J. O., and J. L. Fryer. 1969. An epizootic of vibriosis in chinook salmon. Bull. Wildl. Dis. Assoc. 5(2): 73-76.

The authors describe the disease in juvenile chinook salmon in salt water. Biochemical characteristics of the isolates are discussed.

Conroy, D. A., and G. C. Withnell. 1974. The use of a slide agglutination test as an aid in the diagnosis of vibrio disease in fish. Riv. Ital. Piscic. Ittiopatologia 9(3): 69-74.

Description of a slide agglutination test in which a polyvalent *Vibrio anguillarum* antiserum is used for diagnosis of vibriosis.

Egusa. S. 1969. *Vibrio anguillarum*, a bacterium pathogenic to saltwater and freshwater fishes. Fish Pathol. 4(1): 31-44.

A comprehensive review of the disease, with comparisons of *Vibrio anguillarum* and other species causing vibriosis.

Evelyn, T. P. T. 1971. First records of vibriosis in Pacific salmon cultured in Canada and taxonomic status of the responsible bacterium, *Vibrio anguillarum*. J. Fish. Res. Board Can. 28(4): 517-525.

First proven outbreak of vibriosis in four species of salmonids in Nanaimo and West Vancouver, British Columbia. Affected salmonids showed redness at the base of paired fins, vent, and isthmus. Hemorrhages were present over body surface, and intestine showed inflammation and petechiation. The isolates were shown to be *Vibrio anguillarum*.

Fryer, J. L., J. S. Nelson, and R. L. Garrison. 1972. Vibriosis in fish. Prog. Fish Food Sci. 5: 129-133.

Reviews etiology, pathologic changes, and distribution of vibriosis and describes disease control in salmon by chemotherapy and peroral immunization with *Vibrio anguillarum* bacterin.

Fryer, J. L., J. S. Rohovec, G. L. Tebitt, and J. S. McMichael. 1976. Vaccination for control of infectious diseases in Pacific salmon. Fish Pathol. 10(2): 155-164.

Injectable and oral vaccines for control of furunculosis, vibriosis, and columnaris disease are discussed, as is live attenuated vaccine for infectious hematopoietic necrosis.

Ghittino, P., S. Andruetto, and E. Vigliani. 1972. "Red mouth" enzootic in hatchery rainbow trout caused by *Vibrio anguillarum*. (Enzoozia di "bocca rossa" in trote iridee di allevamento sostenuata da *Vibrio anguillarum*). Riv. Ital. Piscic. Ittiopatologia 7(2): 41-45.

Description of an outbreak of vibriosis in an Italian freshwater rainbow trout farm. Even though the authors describe the condition as red mouth, the etiologic agent was *Vibrio anguillarum*. Mortality was slight and was controlled by feeding chloramphenicol at 6 to 7 g/100 lb fish per day for 7 days.

Gjedrem, T., and D. Aulstad. 1974. Selection experiments with salmon. I. Differences in resistance to vibrio disease of salmon parr *(Salmo salar)*. Aquaculture 31(1):51-59

Analyses of the responses of strains of salmon parr to vibriosis *(Vibrio anguillarum)* are reported. Significant differences in resistance among various river runs were attributed at least partly to heritage.

Hacking, M. A., and J. Budd. 1971. Vibrio infections in tropical fish in a freshwater aquarium. J. Wildl. Dis. 7(4): 273-280.

Vibrio anguillarum was identified as the causative agent of an epizootic in tropical freshwater fishes. It was pathogenic for selected species of other freshwater fishes, and was isolated from inoculated gravid guinea pigs, and their

fetuses and dead young. Gross and microscopic lesions are described.

Hatai, K., Y. Iwahashi, and S. Egusa. 1975. *Vibrio parahaemolyticus* from diseased yellowtails. Fish Pathol. 10(1): 31-37.

In Japanese.

Levin, M. A., R. E. Wolke, and V. J. Cabelli. 1972. *Vibrio anguillarum* as a cause of disease in winter flounder *(Pseudopleuronectes americanus)*. Can. J. Microbiol. 18(10): 1585-1592.

Vibrio anguillarum was repeatedly isolated from skin and muscle lesions of winter flounder. In the acute phase dermal lesions were usually accompanied by fin necrosis, petechia, and ecchymoses; but frank ulceration was usual in more chronic cases. Anemia, as evidenced by lowered hematocrit values and increased renal hematopoiesis, was present. The disease was reproduced experimentally by intradermal injection of as few as 640 *V. anguillarum* cells. Differential identification of the *V. anguillarum* from aeromonads, plesiomonads, and other marine vibrios is described and discussed.

McCarthy, D. H., J. P. Stevenson, and M. S. Roberts. 1974. Vibriosis in rainbow trout. J. Wildl. Dis. 10(1): 2-7.

The report describes epizootic vibriosis among rainbow trout during their acclimation to seawater. Clinical signs and histopathological changes are described, as are the biochemical characteristics of the *Vibrio anguillarum* isolate. Source of infection was thought to be seawater used in acclimation.

Muroga, K. 1975. Studies on *Vibrio anguillarum* and *V. anguillarum* infection. J. Fac. Fish. Anim. Husb. Hiroshima Univ. 14(1): 101-215.

Vibrio anguillarum was found to be the cause of disease in ayu and eel. Epizootics occurred in both freshwater and saltwater lakes. On the basis of a study of fish-pathogenic vibrios, the author suggested that *V. piscium*, *V. piscium* var. *japonicus* and *V. ichthyodermis* be combined under *V. anguillarum*.

Muroga, K., and S. Egusa. 1975. Studies on *Vibrio anguillarum* isolated from salt-water and freshwater fishes. Fish Pathol. 8(1): 10-25.

Biochemical characteristics of 32 strains of *Vibrio anguillarum* from fish were studied. Methods are described for distinguishing between *Vibrio anguillarum*, *V. parahaemolyticus*, and *V. alginolyticus*.

Nybelin, O. 1935. Untersuchungen über den bei Fischen Krankheitserregenden Spaltpilz *Vibrio anguillarum*. Medd. unders.-Anst. Sotvattenfisk. Stockh. 8. 62 pp.

The author proposed that *Vibrio anguillarum* be separated into three biotypes based on fermentation of mannitol and sucrose and production of indole.

Novotny, A. J., L. W. Harrell, and C. W. Nyegaard. 1975. Vibriosis, a common disease of Pacific salmon cultured in marine waters. Wash. State Univ. Coop. Ext. Serv., Pullman, Wash. 8 pp.

Description of signs in Pacific salmon, methods of necropsy, isolation of bacteria, and disease treatment.

Pacha, R. E., and E. D. Kiehn. 1969. Characterization and relatedness of marine vibrios pathogenic to fish: physiology, serology, and epidemiology. J. Bacteriol. 100(3): 1242-1247.

Cultural characteristics and serological relationships of pathogenic marine vibrios isolated from fish in the Pacific Northwest were studied.

Pearse, L., R. S. V. Pullin, D. A. Conroy, and D. McGregor. 1974. Observations on the use of furanace for the control of vibrio disease in marine flatfish. Aquaculture 3(3): 295-302.

Seventeen strains of pathogenic vibrios showed minimum inhibitory concentrations of <1.6-3.1 µg/ml to furanace. Plaice and Dover sole absorbed furanace rapidly; 70% of the drug was eliminated from tissue within 2 h after exposure.

Rohovec, J., R. L. Garrison, and J. L. Fryer. 1975. Immunization of fish for the control of vibriosis. Pages 105-112 in Proceedings of the Third U.S.-Japan Meeting on Aquaculture. Tokyo, 15-16 October 1974.

Description of orally and parenterally administered vaccines. Fish injected with 2 X 10^8 formalin-killed cells mixed with Freunds complete adjuvant or fed a ration containing 0.5 mg formalin-killed whole cell bacterin for 15 days were protected against a natural challenge of *Vibrio anguillarum*. Oral immunization was accomplished at 4 to 21 C. Increasing the amount of vaccine fed or the period of feeding did not increase protection.

Ross, A. J., J. E. Martin, and V. Bressler. 1968. *Vibrio anguillarum* from an epizootic in rainbow trout *(Salmo gairdneri)* in the U.S.A. Bull. Off. int. Epiz. 69(7,8): 1139-1148.

The report characterizes *Vibrio anguillarum* isolated from a freshwater hatchery and compares the isolants with a known culture.

Rucker, R. R. 1959. Vibrio infections among marine and fresh-water fish. Prog. Fish-Cult. 21(1): 22-25.

A review of the literature and a report of the first documented cases in North American hatcheries.

Smith, I. W. 1961. A disease of finnock due to *Vibrio anguillarum*. J. Gen. Microbiol. 24(2): 247-252.

The author compares *Vibrio anguillarum* with other vibrios isolated from fish and discusses the disease in immature brown trout.

Tanaka, J. 1975. Vibrio infection of marine fishes. Pages 113-114 in Proceedings of the Third U.S.-Japan Meeting on Aquaculture. Tokyo, 15-16 October 1974.

The report describes vibriosis in marine fishes cultured in Japan. Characteristics of *Vibrio anguillarum* and control of vibriosis are also discussed.

Umbreit, T. H., and M. R. Tripp. 1975. Characterization of the factors responsible for the death of fish infected with *Vibrio anguillarum*. Can. J. Microbiol. 21(8): 1272-1274.

Authors describe an extracellular toxin produced by *Vibrio anguillarum* that affects goldfish. The substance is toxic after heating to 100 C.

Wood, J. W. 1974. Diseases of Pacific salmon: their prevention and treatment. Wash. State Dep. Fish. n.p.

The author describes signs, the etiological agent, prevention, and treatment of vibriosis in Pacific salmonids.