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An Epizootic of Vibriosis in Chinook Salmon*

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Abstract

Vibrio anguillarum was identified as the causal organism of an epizootic of vibriosis in juvenile chinook salmon *(Oncorhynchus tshawytscha)* reared in a salt-water impoundment on the Oregon coast.

Introduction

In recent years *Vibrio anguillarum* has received an increasing amount of attention. Originally associated with red disease of eels, this bacterium is now recognized as a pathogen in other species of fish. In two recent papers *V. anguillarum* was reported from diseased codling in Denmark[1] and from finnock (immature *Salmo trutta)* in Scotland[10]. Hoshina[3] described a vibrio pathogenic for rainbow trout *(Salmo gairdneri)* and designated it *V. piscium* var. *japonicus;* Smith[10] later studied this organism and concluded that it was a variant of *V. anguillarum.*

Vibrio spp. have been recognized as important pathogens in all species of Pacific salmon[8]. In studies with various Pacific Northwest isolates of marine vibrios pathogenic to fish, Pacha, Ordal, and Earp[7] found differences in cultural reactions but demonstrated definite serological relationships.

The purpose of this paper is to attribute an epizootic of vibriosis in chinook salmon *(Onchorhynchus tshawytscha)* to *V. anguillarum.*

Materials and Methods

Lint Slough, a salmonid rearing impoundment located near Waldport, Oregon, was developed by the Oregon Game Commission in 1963. The structure was designed to take advantage of the rich marine environment for rearing of Pacific salmon and steelhead trout *(Salmo gairdneri).* The impoundment receives controlled amounts of fresh-water from Lint Creek and salt-water from Alsea Bay.

On March 19, 1968 a total of 578,650 juvenile fall chinook salmon were placed in Lint Slough. During March and April water temperatures in the slough ranged from 10 to 16 C and a brackish environment existed. A sharp increase in the number of daily mortalities appeared on April 1, 1968. Daily losses rose to catas-

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trophic levels by the middle of April and then subsided toward the end of the month. Kidney tissue from 106 dead chinook collected at the beginning and peak of the epizootic, was streaked on Furunculosis Agar (Difco) and incubated at 18 C. From the cultures obtained three isolates of the bacterium were maintained for further study and designated LS-68-1, 2 and 3. To establish pathogenicity, a dilute saline suspension of the organism was prepared and 0.1 ml injected intraperitoneally into each of 16 juvenile salmon. The inoculum consisted of a one thousandfold dilution of bacterial cells which had been resuspended in saline and standardized against a McFarland No. 2 nephelometer tube. Kidney tissue from each fish that died during the experiment was streaked on Furunculosis Agar for reisolation of the bacterium.

The cultural reactions and morphological characteristics of the LS-68 isolates were established by standard bacteriological methods[11]. Purple Broth Base (Difco) containing 0.5% of the desired carbon source and the Hugh-Leifson method[3] were used to determine carbohydrate utilization (these media contained 0.5% NaCl). Two percent NaCl was added to the medium used to test for indole production.

The effect of the vibriostatic agent 0/129 (2,4-diamino-6,7-di-isopropyl pteridine)* was determined on: the LS-68 isolates, *Aeromonas salmonicida, A. punctata* and *Pseudomonas aeruginosa.*

Agglutination reactions with known antisera were conducted (rapid slide method) to detect common antigens between the LS-68 isolates and certain fish pathogens. The antisera employed were against *A. salmonicida, A. punctata,* an isolate of *Vibrio* sp. from salmon, and an isolate of *Vibrio* sp. from herring.

The method of Marmur[4] was used to isolate deoxyribonucleic acid (DNA) for thermal denaturation (T_m) studies. The T_m for each DNA sample was determined in triplicate with a Gilford multiple sample absorbance recorder. The mole percent guanine + cytosine (% GC) was calculated from T_m values[5].

Results

Only 9.8% (56,900) of the 578,650 juvenile chinook placed in Lint Slough survived until release in June, 1968. Mortality records indicated that a large portion of the total loss occurred in April. The pathology observed in the chinook mortalities was characterized by red necrotic lesions of the abdominal musculature and erythema at the bases of fins and within the mouth. Evidence of hemorrhaging was noted also in the gills and intestines of diseased fish. Pure cultures of the same bacterium, based on colony and cell morphology, were obtained from 83 of the 106 dead fish examined. Typical pathological lesions and death were produced in nine of 16 juvenile salmon injected with LS-68-2. The organism was reisolated in pure culture from each of the nine dead animals.

The infectious agent responsible for the Lint Slough epizootic was a small (2-3μ x 1μ) Gram negative, slightly curved rod which was motile by means of a single polar flagellum (Leifson flagella stain). Growth on potato slopes and Furunculosis Agar was light brown, and no diffusible pigment was produced. The organism caused coagulation, reduction, and proteolysis of Litmus Milk (Difco), did not grow in cellulose mineral salts medium, failed to produce hemolysis on sheep blood agar, and did not liquefy Loeffler's Blood Serum (Difco). Growth in Fluid Thioglycollate Medium (Difco) indicated the bacterium was a facultative anae-

*The vibriostatic agent 0/129 supplied by Allen & Hanburys Ltd., Ware, Hartfordshire, England.

robe. The organism grew slowly in broth at 4 and 37 C; good growth occurred at 20 and 27 C. Growth was obtained in media with NaCl concentrations from 0.5 to 5.0%, but no growth occurred in the absence of salt or with 10% salt in the medium. Catalase and cytochrome oxidase were produced on Nutrient Agar (Difco) containing 2% NaCl. Of the Gram negative organisms tested *(A. salmonicida, A. punctata, P. aeruginosa,* and the three LS-68 isolates), only the LS-68 isolates were sensitive to the vibriostatic agent 0/129. Results obtained by the Hugh-Leifson method for carbohydrate utilization indicated that the bacterium was an anaerogenic fermentor. Biochemical reactions of the three LS-68 isolates were identical and are summarized in Table 1.

TABLE 1. *Biochemical characteristics of the three LS-68 isolates*

TEST	RESULTS
Reaction on glucose, fructose, sucrose, maltose, cellobiose, mannose, galactose, trehalose, dextrin, glycogen, glycerol[2], and inulin[2].	Acid[1]
Reaction on lactose, rhaminose, raffinose, xylose, inositol, dulcitol, and salicin.	No Acid[1]
Starch, gelatin, and casein hydrolysis; indole and aceylmethylcarbinol production; nitrates reduced to nitrites.	Positive Reaction
Citrate utilization; urea hydrolysis; H_2S production; methyl red test.	Negative Reaction

[1]Carbohydrate reactions obtained in Purple Broth Base (Difco).
[2]Slight acid.

The three LS-68 isolates were agglutinated by antiserum which had been prepared against a *Vibrio* sp. obtained from Pacific salmon. No agglutination was observed with antisera against *A. salmonicida, A. punctata,* and an isolate of *Vibrio* sp. from Pacific Northwest herring.

Values of 43.2 and 44.4% GC (T_m = 87.0 and 87.5 C respectively) were obtained for DNA samples from LS-68-3. A value of 44.4% GC (T_m = 87.5 C) was determined for the DNA of LS-68-2.

Discussion

The organism isolated from Lint Slough juvenile chinook salmon was a Gram negative curved rod, possessed a single polar flagellum, and was an anaerogenic fermentor. The above criteria in addition to its sensitivity to the vibriostatic agent 0/129 were used to place the bacterium in the genus *Vibrio*[10]. Biochemical, physiological, and morphological characteristics of the LS-68 isolates appear very similar to *V. anguillarum* from finnock[10] and codling[1]. In addition to cultural similarities, the percent GC values obtained for the LS-68 isolates are in good agreement with the value of 44.5% GC determined by chromatographic methods for *V. anguillarum* from codling (National Collection of Marine Bacteria No. 6)[9].

Nybelin[6] studied a number of *V. anguillarum* isolates and suggested two types for the species. Type A produced acid but no gas from sucrose and mannitol and produced indole; type B failed to attack sucrose and mannitol and did not produce indole. Based on her studies, Smith[10] proposed a type C for those strains of *V. anguillarum* which ferment sucrose and mannitol but do not produce indole. Using the above scheme of typing, the LS-68 isolates are all *V. anguillarum* type A. Although biochemical comparisons were not made between the LS-68 isolates and other isolates of vibrios pathogenic to fish from the Pacific Northwest, serological evidence indicated that at least one common antigen was present.

Slightly less than ten percent of the fry placed in Lint Slough survived until release in June, 1968. Pure cultures of *V. anguillarum* were isolated from 78% of the dead fish examined. While *V. anguillarum* may not be the only marine vibrio pathogenic for salmonid fish in the Pacific Northwest, it was responsible for a major portion of the 1968 loss of chinook salmon in Lint Slough. To the authors' knowledge this is the first report specifically identifying *V. anguillarum* as the causative agent of vibriosis in a species of Pacific salmon.

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