PASTEURELLA-LIKE BACTERIA FROM AN EPIZOOTIC IN MENHADEN AND MULLET IN GALVESTON BAY

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Fish kills occurring sporadically in the estuarine environments of the Galveston Bay systems are attributed to a variety of causes. In one such kill that occurred in November, 1968, mortality appeared to be restricted to two species of fish, Atlantic menhaden *(Brevoortia typprannus)* and striped mullet *(Mugil cephalus)* and was observed in a widely separated pattern suggesting the possible involvement of an infectious agent.

Moribund fish were collected from several areas, examined for external evidence of disease and transported on ice to the laboratory for detailed examination. At the time of capture, the fish had considerable mucoid material on the gill surfaces and purulent material in the abdominal cavities. Water conditions, measured 2.5 cm below the surface with a galvanic cell oxygen analyzer, thermometer and conductivity meter (YSI model 50, Yellow Springs, Ohio) and a portable pH meter (Beckman Instruments, Palo Alto, California) indicated approximate concentrations of 10 - 11 ppm dissolved oxygen, salinity 28.6 o/oo, pH 8.0 and 18 C temperature.

At the laboratory (3-4 hrs. after capture), the fish were dipped in 10% benzalkonium chloride and necropsied using aseptic techniques. Giemsa stain and microscopic examination of the heart blood revealed an abundance of bipolar, thick, pleomorphic rods with rounded ends. Motile organisms in the heart blood were revealed by dark field examinations. Brain heart infusion agar and 5% bovine blood agar containing 3% NaCl were inoculated with a few drops of the heart blood. The media were incubated at 20 C and in 48 hrs. good bacterial growth was observed. Medium sized (2-5 mm) beta hemolytic, translucent, glistening, butyrous, raised colonies with entire margins developed on blood agar. Twenty apparently pure cultures of the same bacterium were obtained from the blood specimens of 12 menhaden and 8 mullet. Saline and heat tolerance tests of the bacterium were performed in nutrient broth. The microorganism did not grow in concentrations of saline below 0.5% nor above 10.0% but grew well in broths containing concentrations of saline between these values. Abundant growth was observed in nutrient broth supplemented with 1% NaCl, when incubated at 5 C, 10 C and 37 C. Turbidity developed in 24 hrs. in the cultures incubated at 20 C and 37 C but growth of the microorganism was not observed in this medium when incubated at 45 C. Motility appeared to be influenced by the temperature of incubation. When incubated at 37 C, the organism appeared to be non-motile, whereas at 20 C motility was apparent. Two polar flagellae were observed on cells incubated at both temperatures when Skerman's modification of Fontana's flagella staining method was used (Skerman, 1967, *A Guide to the Identification of the Genera of Bacteria*. p. 278. Williams and Wilkins Co., Baltimore). Detailed biochemical and physiological characterization tests of the organism are presented in Table 1.

White mice (average weight 20 g), Hartley strain guinea pigs (average weight 450 g), New Zealand white rabbits (average weight 750 g) and juvenile mullet (average length 3.8 cm) were exposed to a pure culture of the bacterium to study its pathogenicity. One tenth ml of an 18 hr. broth culture was administered intraperitoneally to the mice and 0.5 ml intraperitoneally to the guinea pigs and rabbits. The fish were exposed in tanks to which 10 ml of the broth culture had been mixed with 10 liters of artificial sea water. After an exposure period of 1 hr., the fish were returned to holding tanks and observed daily for evidence of disease. Death occurred within 48 hrs. in 5 of the 5 mice inoculated with the organism and within three days in all 20 of the fish exposed to the organisms. Small white areas of necrosis were observed in the liver parenchyma of the mice. Gross lesions were not apparent in the fish, but the fish exhibited signs of stress, i.e. gulping near the surface and sluggish motion. The bacterium was readily reisolated from speci-

TABLE 1. *Charcteristics of a bacterium associated with a fish kill in Galveston Bay.*

CHARACTERISTIC[a]	RESULTS
Cytochrome oxidase test	Positive
Carbohydrate metabolism	
Glucose	Acid, no gas, dissimilated fermentatively
Fructose, galactose mannose	Acid, no gas wthin 14 days
Maltose, lactose, sucrose, mannitol, sorbitol, dulctol, glycerol, inositol, raffinose, rhamnose, salicin, trehalose, xylose	No change after 21 days
2, 3 butanediol[b]	Not produced
Acetyl methyl carbinol	Produced
Methyl red test	Positive
Production of	
Ammonia from peptones	Negative
Hydrogen sulfide	Negative
Indole from tryptophane	Positive
Lysine decarboxylase	Negative
Ornithine decarboxylase	Positive
Arginine decarboxylase	Positive
Phenylalanine deaminase	Negative
Tributyrin lipase[c]	Positive
Urease, catalase	Positive
Litmus milk	No change
Hydrolysis of	
Starch	Negative
Casein[d]	Negative
Degradation of	
Chitin	Negative
Agar	Negative
Gelatin	Not liquefied
Nitrate to nitrite reduction	Positive
Substrates utilized as sole carbon sources	Citrate, malonate, and succinate
Pathogenicity tests	
Guinea pig	Negative
Rabbit	Negative
White mouse	Positive
Juvenile mullet	Positive

[a] Except where indicated otherwise, tests were performed according to Skerman (1967, A Guide to the Identification of the Genera of Bacteria, Williams and Wilkins Co., Baltimore).

[b] Performed according to Bullock (1961, Prog. Fish, Cult. 23: 147-151).

[c] Tested on spirit blue agar (DIFCO).

[d] Performed according to Cowan and Steel (1965, Manual for the Identification of Medical Bacteria, p. 159, Cambridge University Press, London).

TABLE 2. *Comparison between Galveston Bay isolate and similar pathogenic bacteria.*

	GBI*	CBI* *Past.* sp.	*Past. pseudo-tuberculosis*	*Past. hemolytica*	*Past. multocida*	*Aeromonas liquefaciens*	*Vibrio anguil-larium*
Bipolar Staining	+	+	+	+	+	—	±
Motility	+	—	+	—	—	+	+
Flagellation	polar	—	peritrichous	—	—	polar	polar
Hemolysis	Beta	—	—	Beta	—	—	Beta
Indole Formation	+	—	—	—	+	+	—
2, 3 Butanediol Formation	—	—	—	—	—	+	—
Mouse Path.	+	—	+	+	+	+	—

* GBI = Galveston Bay Isolate; CBI = Chesapeake Bay Isolate

mens of liver and heart blood of the mice and from the livers of the fish. The control mice (which had been injected with sterile broth), as well as the guinea pigs and white rabbits, appeared to be healthy two weeks after the challenge. Fish exposed to sterile broth and otherwise treated in a manner similar to the infected fish remained apparently healthy for one month after the exposure.

Bipolar staining and certain of the biochemical and physiologic reactions of the bacterium suggests that the organism belongs to the genus *Pasteurella*. Snieszko *et al.*, 1964, (J. Bacteriol. 88: 1814-1815) isolated *Pasteurella*-like bacteria from a fish epizootic in Chesapeake Bay. Results of a cultural comparison of the Galveston Bay isolate, the Chesapeake Bay isolate, a *Vibrio anguillarum* isolate and certain similar bacteria maintained in the Department of Veterinary Microbiology, Texas A&M University are presented in Table 2.

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