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Research Article

BACTERIAL HEMORRHAGIC SEPTICEMIA IN FISHES FOUND IN FISH MARKETS OF BHOPAL

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ABSTRACT

Aeromonas hydrophilla and other motile aeromonads are among the most common bacteria in freshwater habitats throughout the world, and are frequently associated with severe disease among cultured fishes. Determinations of the etiology of diseases involving aeromonad infections has been complicated by the genetic, biochemical, and antigenic heterogeneity of members of this group. Consequently, motile aeromonads consist of a complex of disease organisms that are associated with bacterial hemorrhagic septicemias in fishes. From descriptions of fish diseases in the early scientific literature, Otte (1963) speculated that septicemic infections in fish caused by motile aeromonads were common throughout Europe during the Middle Ages.

INTRODUCTION

Aeromonas hydrophila is a heterotrophic, gram-negative bacterium of the Aeromonadaceae family. This bacterium has a rod shape with rounded ends reaching 1 -3µm in length. Polar flagella allow motility. A. hydrophila can be considered as an opportunistic pathogen which is linked to infections in fish and humans. Infection most usually occurs and when an organism is dealing with environmental change. This could be a change in temperature or when infected with a different bacterium. In fish, the bacteria mainly infect the kidneys and liver resulting in disease that is thought to be the result of stress. Infection in humans can be caused by ingestion of infected food such as fish or meat. Once ingested the bacterium progresses to an organ via the bloodstream. Alternatively the bacterium can infect open wounds. The bacteria have pili which they utilise in attachment to host organism and invasion of the cells or organs. Colonisation occurs by asexual reproduction. Upon cell invasion, the enterotoxins are activated causing cell damage. Transmission in fish occurs when infected fish are moved into new fisheries without sanitation. The bacteria are largely antibiotic resistant, can survive refrigeration and are also able to grow at temperatures in a wide range up to 37°C. However, a sodium/calcium hypochlorite solution can kill this bacterium. Infection in fish may cause diseases including tail and fin rot, heamorrahagic septicemia and ulcer disease. Infection in humans can lead to a number of diseases including septicemia, meningitis, pneumonia and gastroenteritis. The bacteria can also infect open wounds causing cellulitis, myonecrosis and ecthyma gangrenosum. The latter two diseases occur more commonly in the immunocompromised, although infections do also occur in immunocompetent people

In many isolations of bacteria from hemorrhagic septicemias in fish, the putative bacterium was misidentified. Consequently, it is now recognized that certain isolations of bacteria ascribed to the genera Pseudomonas, Proteus, Bacillus, Aerobacter, and Achromobacter were actually in the genus Aeromonas. Although motile aeromonads have appropriately received much notoriety as pathogens of fish. these bacteria also compose part of the normal intestinal micro flora of healthy fish (Trust *et al*e 1974). Therefore, stress can be a contributing factor in outbreaks of disease caused by these bacteria. In the United States, A. hydrophila primarily causes disease in cultured warm water fishes: minnows, bait fishes, channel catfish (ictalurus punctatus), and striped bass (Morone saxatilis). However, the pathogen may also affect a variety of cool water and coldwater species.

MATERIALS AND METHODS

Fishes, from water bodies, with high microbial load reach the market. Following four major fish markets of Bhopal namely

Itwara, Bittan, Piplani and Govindpura fish markets were selected for present study

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Bacteriological examination For the purpose of bacteriological examination, the methodology given by Bullock *et al*. (1971), Austin and Austin (1987) and Plumb (1994) have been followed.

Observations

Aeromonas hydrophilla causes diverse pathologic conditions that include acute, chronic, and latent infections. Severity of disease is influenced by a number of interrelated factors, including bacterial virulence, the kind and degree of stress exerted on a population of fish, and the resistance and physiological condition of the host. Pathologic conditions attributed to members of the A. hydrophilla, complex include dermal ulceration, hemorrhagic septicemia, red sore disease, red rot disease, and scale protrusion disease. In the acute form, these conditions are characterized by a rapidly fatal septicemia with few gross signs of disease. When present, the following signs are most significant: exophthalmia, reddening of the skin, and accumulation of fluid in the scale pockets (Faktorovich 1969).

Table 1 Showing bacterial flora in different tissues of Channa striatus suffering from BHS

Bacteria	Skin	Gills	Muscles	Intestine
Aeromonas hydrophilla	$8.0x10^3$CFU/g	10.3 $x10^3$CFU/g	$2.0x10^3$CFU/g	$5.0x10^3$CFU/g
Pseudomonas fluorescence	$10.0x10^3$CFU/g	$6.0x10^3$CFU/g	$3.0x10^3$CFU/g	$6.0x10^3$CFU/g

Channa striatus suffering from BHS

mouth ulcers in channa stratius

Enlarged view of BHS

Exopthalmia

The abdomen may become distended and scales may bristle out from the skin. Internally, the liver and kidneys are target organs of an acute septicemia. The liver may become pale or green and the kidneys may become swollen and friable. These organs are apparently attacked by bacterial toxins and lose their structural integrity (Huizinga *et al*. 1979). Even when tissue damage in the liver and kidneys is extensive, the heart and spleen are not necessarily damaged. However, splenic ellipsoids are often centers of intense phagocytic activity by macrophages (Bach *et al*. 1978). Chronic stages of A. hydrophilla are primarily ulcerous forms of disease. Therefore, the predominant clinical signs include dermal ulceration, with focal hemorrhage and inflammation. Both the dermis and epidermis are eroded and the underlying musculature becomes severely necrotic (Huizinga *et al*. 1979). Inflammatory cells are usually lacking in the necrotic musculature, whereas the adjacent epidermis undergoes hyperplasia that results in a raised margin. At this stage, the infection has usually become systemic. Internally, tiny pinpoint hemorrhages (petechiae) sometimes occur throughout the peritoneum and musculature. The lower intestine and vent, which sometimes protrude from the body, are often swollen, inflamed, and hemorrhagic. Additionally, the intestine is devoid of food and filled with a yellow mucus-like material

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P. K. Chen, and G. B. Chapman. 1978. Changes in the spleen of channel catfish Icta Zurus punctatus Rafinesque induced by infection with Aeromonas hydrophilla. J. Fish Dis. 1(3):205-217. Pathological changes were apparent in the spleens of fish injected with virulent Aeromonas hydrophilla, whereas fish infected orally showed little or no splenic involvement. Bacteria were present within the reticular sheaths of the ellipsoids, where intense phagocytic activity by macrophages occurred. Phagocytized bacteria divided intracellularly and extracellularly and destroyed the endothelial and reticular cells of the ellipsoids.

Boulanger, Y., R. Lallier, and G. Cousineau. 1977. Isolation of enterotoxigenic Aeromonas from fish. Can. J. Microbiol. 23(9): 1161-1164. Strains of Aeromonas sobria were isolated only from healthy fish, whereas strains of A. hydrophilla were isolated from both healthy and diseased fish. Both species produced enterotoxins, as demonstrated by the rabbit ileal loop and suckling mouse tests. The enterotoxins were different but related antigenically.

Bullock, G. L. 1961. The identification and separation of Aeromonas Ziquefaciens from Pseudomonas fluorescence and related organisms occurring in diseased fish. J. Appl. Microbiol. 9(6):587-590. Biochemical criteria for identification of motile aeromonads and their separation from Pseudomonas fluorescence are described. Organisms were designated as Aeromonas Ziquefaciens if they fermented glucose, produced 2,3-butanediol, hydrogen sulfide from motility sulfide medium, were cytochrome oxidase positive, and hydrolyzed starch.

Bullock, G. L., and H. M. Stuckey. 1977. Ultraviolet treatment of water for destruction of five gram-negative bacteria pathogenic to fishes. J. Fish. Res. Board Can.

34(8):1244-1249. Discusses dosage rates of ultraviolet irradiation for filtered and unfiltered spring water to establish effective levels of killing five gram-negative bacterial pathogens, including Aeromonas hydrophilla.

Bullock, G. L., P. K. Chen, and H. M. Stuckey. 1972. Studies of motile aeromonads isolated from diseased warrnwater and coldwater fishes. Page 21 in Abstracts of the Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania, 23-28 April 1972. 11 Strains of aerogenic and anaerogenic motile aeromonads showed similar serological and fatty acid patterns when examined by gas liquid chromatography. Most strains formed a single precipitin band with antiserum against all free extract from an aerogenic strain. However, few strains were agglutinated by either the cellfree antiserum or a whole-cell antiserum. Electrophoretic serum patterns of infected fish were also studied.

Chang, M. C., and T. C. Huang. 1981. Effects of the predation of Tetrahymena pyriformis on the population of Aeromonas hydrophila. Natl. Sci. Counc. Mon. 9(7):552-556. Tetrahymena pyriformis was experimentally shown to graze on populations of Aeromonas hydrophila. The bacterium, at concentrations of 1 X 106 cells/mL co-existed with the protozoan. If a second bacterial species was introduced, predation by the protozoan increased.

Chodyniecki, A. 1965. Comparative antigens of Aeromonas punctata course of septicemia in trout. serological studies on somatic Zimmerman strains isolated in the Acta Hydrobiol. 7(2-3):269-278. During an outbreak of septicemia in common carp, the author noted a great diversity of somatic antigens of Aeromonas punctata in two different populations of carp, in the same population, in each fish, and in different organs from the same fish. After 6 months the somatic antigens isolated from the affected carp differed from a-antigens previously isolated from the same fish.

Calesante, R. T., R. Engstrom-Heg, N. Ehlinger, and N. Youmans. 1981. Cause and control of muskellunge fry mortality at Chautauqua Hatchery, New York. Prog. Fish-Cult. 43(1):17-20. Controlled laboratory experiments indicated that motile aeromonads caused muskellunge fry mortality during 1966-1967, 1970, and again from 1973 to 1976. Ultraviolet irradiation of lake and well water supplies during egg incubation and yolk absorption controlled fry mortality.

Colberg, P. J., and A. J. Lingg. 1978. Effect of ozonation on microbial fish pathogens, ammonia, nitrate, nitrite and biological oxygen demand in simulated reuse hatchery water. J. Fish. Res. Board Can. 35(10):1290-1296. The effectiveness of ozone as a disinfectant of makeup water and its potential for treatment of recycled water in commercial reuse hatcheries was examined. A specific microbial oxygen demand was exerted during batch ozonation, and more than 99% mortality of bacteria was noted within a 60-s contact during continuous flow exposure at 0.1-1.0 mg ozone per liter.

Davis, J. W., and R. K. Sizemore. 1981. Nonselectivity of RimlerShotts medium for Aeromonas hydrophilla in estuarine environments. J. Appl. Environ. Microbiol. 43(3):544-545. Presumptive Aeromonas hydrophilla isolates (as judged by characteristic growth on Rimler-Shotts medium) were obtained from estuarine sources. Many of the isolates, especially those from low-salanity sites, were also identified by the API 20E system as A. hydrophilla. However the DNA homology ratios of all of these isolates excluded them from this species.

Davis, W. A., J. G. Kane, V. F. Garagusi. 1978. Human Aeromonas infections: a review of the literature and a case report of endocarditis. J. Med. 57(3):267-277. A review of Aeromonas hydrophilla as a human pathogen. De Figueredo, J., and J. A. Plumb. 1977. Virulence of different isolates of Aeromonas hydrophilla in channel catfish. Aquaculture 11:349-354. Virulence of selected strains of Aeromonas hydrophilla was determined for channel catfish. As judged from LD50 values, bacteria isolated from the water column were significantly less virulent than isolates from diseased fish. All isolates were biochemically similar.

Esch, G. W., and T. C. Hazen. 1980. Stress and body condition in a population of largemouth bass: implications for red-sore disease. Trans. Am. Fish. Soc. 109(5):532-536. It was determined that hematocrit, lymphocyte fraction, and cortisol concentration accounted for 20.5% of the variation in body condition among largemouth bass (Micropterus salmoides) taken from Par Pond, South Carolina. The data collected supported a previous hypothesis that elevated water temperature promotes stress. This stress may be a critical factor that triggers outbreaks of red-sore disease caused by Aeromonas hydrophilla.

Eurell, T. E., D. H. Lewis, and L. C. Grumbles. 1978. Comparison of selected diagnostic tests for the detection of motile Aeromonas septicemia in fish. Am. J. Vet. Res. 39(8):1384-1386. Techniques of cultural isolation, slide agglutination, tube agglutination, micro agglutination, and fluorescent antibody were compared as methods to detect aeromonads in channel catfish. Slide agglutination proved to be an effective diagnostic test for field use. Under laboratory conditions, tube agglutination, micro agglutination, 13 and fluorescent antibody procedures were equally effective in detecting levels of serum agglutinins to Aeromonas hydrophilla. However, the fluorescent antibody technique was more sensitive and faster than the agglutination tests.

Ewing, W. H., R. Hugh, and J. G. Johnson. 1961. Studies on the Aeromonas group. u.S. Dep. Health Educ. Welfare, Public Health Serv., Communicable Disease Center, Atlanta, Georgia. 37 pp. Biochemical, morphological, and serological tests were performed on 88 cultures belonging to the genus Aeromonas. Serologically, 12 provisional O-antigen and 9 H-antigen groups were delineated. Preparations of O-antigen also indicated that antigenic similarities existed between the strains of A. hydrophilla and A. salmonella that were tested.

Faktorovich, K. A. 1969. Histological changes in the liver, kidneys, skin and brain of fish sick with red rot. Pages 83-101 in Infectious diseases of fish and their control. Div. Fish. Res., Bur. Sport Fish. Wildl., Washington, D. C. Translated from the Russian by R. M. Howland. Histopathology of liver, kidney, skin, and brains of sick common carp, wild carp, and crucian carp were not specific but varied widely, depending on the form of red rot disease. Histopathological changes could not be used as a reliable diagnostic sign.

Fliermans, C. B., and T. C. Hazen. 1980. Immunofluorescence of Aeromonas hydrophilla as

measured by fluorescence photometric microscopy. Can. J. Microbiol. 26(2):161-168. Only 27.5% of a total number of isolates studied reacted with three antisera to Aeromonas hydrophilla by fluorescence microscopy, indicating that other serotypes existed. Statistical analysis showed that isolates of A. hydrophilla obtained from aquatic environments were distinct from other isolates of A. hydrophilla as measured by immunofluorescence.

Hazen, T. C. 1979. Ecology of Aeromonas hydrophilla in a South Carolina cooling reservoir. Microb. Ecol. 5(3):179-195. High densities of Aeromonas hydrophilla were found in mats of decomposing Myriophyllum spicatum and, enterically, in largemouth bass, several other species of fish, turtles, alligators, and snails. Densities of A. hydrophilla in water were highest from March to June, and a second peak occurred in October. Mean monthly densities of A. hydrophilla were positively correlated with incidence of incubation in largemouth bass. Largemouth bass from thermally altered parts of the reservoir had a significantly higher incidence of infection.

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