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PASTEURELLOSIS OF FISHES

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INTRODUCTION

Septicemic infections of marine and estuarine fishes caused by *Pasteurella* have been known since the early 1960's. A *Pasteurella* caused a massive mortality of white perch *(Morone americanus)* and striped bass *(M. saxatilis)* in Chesapeake Bay in 1963 (Snieszko et al. 1964), and of cage-cultured yellowtails *(Seriola quinqueradiata)* in Japan in 1964 (Matsusato 1975). Pasteurellosis has spread to most areas in Japan where yellowtails are propagated and is one of the important bacterial diseases of the species. In 1972, 360 metric tons of yellowtails were lost. The terms bacterial tuberculoidosis and pseudotuberculosis (Kubota et al. 1970) have also been used to describe *Pasteurella* infections.

As additional marine species are propagated, pasteurellosis will be a potential disease problem. The purpose of this leaflet is to review what is now known about the disease.

ETIOLOGY

On the basis of cell morphology and biochemical reactions, Snieszko et al. (1964) suggested that the causative agent of the Chesapeake Bay white perch kill was a species of *Pasteurella*. Janssen and Surgalla (1968) agreed that the bacterium was a *Pasteurella*, and because it did not conform to any of the described species they named it *P. piscicida*. The Japanese investigators found that the bacteria from yellowtails and white perch were the same; however, there was divided opinion among them as to the classification. Kusuda and Yamaoka (1972) classified the organism as *P. piscicida*, whereas Kimura and Kitao (1971) placed the bacterium in the genus *Corynebacterium* and Simidu and Egusa (1972) placed it in *Arthrobacter* on the basis of its morphological and gram-staining characteristics. More recently, Koike et al. (1975) studied isolates from yellowtails and white perch and concluded that the organism should be classified as either a *Pasteurella* or *Yersinia*. Until it is shown that a move to *Yersinia* is justified, the name *P. piscicida* prevails.

The organism isolated from a *Pasteurella* infection found in cultured ayu *(Plecoglossus altivelis)* was named *P. plecoglosacida* n. sp. because it did not conform to *P. piscicida* or any other described species (Kusuda and Miura 1972).

CLINICAL SIGNS AND PATHOLOGY

Two forms of the disease have been described. In the acute form there are few gross pathological changes. Evidence of edema and darkening may occur in yellowtails just before death (Matsusato 1975). White perch showed only slight hemorrhages around the gill covers or bases of the fins. In the chronic form, yellowtails and striped bass show miliary lesions in the kidney and spleen. The lesions are 1 to 2 mm in diameter and are composed of masses of the causal bacterium, epithelial cells, and fibroblasts. In both chronic and acute forms, *Pasteurella piscicida* occurs throughout th internal organs.

DIAGNOSIS

Diagnosis is based on isolation and identification of the causal bacterium. Isolation is most reliable if brain heart infusion agar or tryptic soy agar with 1 to 2% NaCl is used and cultures are incubated at 20-25 C for 48 h.

1. Presumptive Diagnosis.

   For presumptive diagnosis, *Pasteurella piscicida* should be shown to be a gram-negative, nonpigmenting, nonmotile rod (1-2 µm long X 0.5-0.75 µm wide) which stains bipolarly. Gelatinase is not produced, and acid but no gas is produced in oxidation-fermentation (O/F) glucose medium.

2. Confirmatory Diagnosis.

   Strains of *P. piscicida* have been shown to possess common antigens, and a diagnosis can be confirmed by simple slide agglutination of isolated cultures or by a direct fluorescent antibody test on infected tissues (Kitao and Kimura 1974).

   For the present, confirmatory identification of *P. plecoglosacida* n. sp. would be based on fermentation of maltose, sucrose, dextrin, cellibiose, and salicin. Strains of *P. piscicida* do not ferment these carbohydrates.

Nonpigmenting strains of *Aeromonas salmonicida* that have been isolated from diseased fish closely resemble *P. piscicida* (Håstein and Bullock 1976; Ajmal and Hobbs 1967). However, the diagnostic tests described above and serological tests readily separate *P. piscicida* from the nonpigmenting *A. salmonicida*.

SOURCE AND RESERVOIR OF INFECTION

In the single occurrence of pasteurellosis in the United States, striped bass were chronically infected and were suspected of being the source of *Pasteurella piscicida*. Studies by Allen and Pelczar (1967) showed that this bacterium was not part of the normal flora of white perch. In Japan, feral fishes that live near cages containing yellowtails are suspected of harboring *P. piscicida*.

MODE OF TRANSMISSION

The exact mechanism of infection is still unknown. It is assumed that feral fishes may transmit the disease to yellowtails, and that once a population is infected the disease is spread by fish-to-fish contact. The disease is also spread by movement of yellowtails, which can be asymptomatic carriers.

INCUBATION PERIOD

Under experimental conditions, white perch died within 72 h after receiving an intraperitoneal injection of 10^7 bacteria (Allen and Pelczar 1967). The temperature (not stated) was probably in the range of 20-25 C.

PERIOD OF COMMUNICABILITY

What little is known suggests an age-resistance relationship. Young yellowtails and red sea bream *(Chrysophyrs major)* are more susceptible than are older fish.

GEOGRAPHIC AND HOST RANGE

Outbreaks of pasteurellosis were reported from the coastal regions of the United States and Japan. Therefore it may be assumed that this organism is widely distributed, but causes epizootics under environmental conditions that are as yet unknown.

Pasteurella piscicida has been isolated from white perch, striped bass, yellowtails, red sea bream, and black sea bream *(Mylio macrocephalus)*. *Pasteurella plecoglosacida* has been isolated only from the ayu.

OCCURRENCE

Outbreaks are most severe in autumn and spring, when water temperature is 23 to 26 C and salinity 30 to 33°/$_{oo}$ (Matsusato 1975).

METHODS OF CONTROL

Prevention

Good sanitation and management procedures should be used to avoid overcrowding and other stresses that may predispose fish to disease. Prophylactic chemotherapy with sulfonamides, nitrofurans, or antibiotics have been employed successfully, but dosages have not been published (Matsusato 1975).

Therapy

Sulfonamides at 200-400 mg per kilogram of body weight per day or chloramphenicol at 20-40 mg per kilogram of body weight per day, both fed for a minimum of 6 days, are used to control outbreaks (Matsusato 1975). Recent in vitro studies by Kusuda and Inoue (1976) showed that the antibacterial activity of ampicillin was 8 to 16 times that of chloramphenicol. They suggested that ampicillin would be useful in treatment of *Pasteurella* septicemia.

ANNOTATED BIBLIOGRAPHY

Ajmal, M., and B. Hobbs. 1967. Species of *Corynebacterium* and *Pasteurella* isolated from diseased salmon, trout and rudd. Nature (Lond.) 215:142-143.

The *Pasteurella* infections described were probably caused by nonpigmenting *Aeromonas salmonicida*.

Allen, N., and M. Pelczar. 1967. Bacteriological studies on the white perch *(Roccus americanus)*. Chesapeake Sci. 8(3):135-154.

A comprehensive study of the flora of the white perch in Chesapeake Bay. The *Pasteurella* from the 1963 white perch kill was not isolated, but was shown to be pathogenic when injected intraperitoneally into fish.

Buchanan, R.E., and N.E. Gibbons, editors. 1974. Bergey's manual of determinative bacteriology, 8th edition. Williams and Wilkins Co., Baltimore, Maryland. 1246 pp.

Most recent edition of the United States standard reference of bacterial taxonomy. Major taxonomic changes were made in groups containing fish pathogens. Some members of the genus *Pasteurella* were placed in *Yersinia*, and this change may affect the *Pasteurella* species pathogenic to fish.

Håstein, T., and G.L. Bullock. 1976. An acute septicemic disease of brown trout *(Salmo trutta)* and Atlantic salmon *(Salmo salar)* caused by a *Pasteurella*-like organism. J. Fish Biol. 8(1):23-26.

Description of a septicemic disease of salmonids caused by a bacterium that had characteristics of a *Pasteurella*. However, the bacterium has since been shown to be the nonpigmenting *Aeromonas salmonicida*.

Janssen, W.A., and M.J. Surgalla. 1968. Morphology, physiology, and serology of a *Pasteurella* species pathogenic for white perch. J. Bacteriol. 96(5):1606-1610.

Characterizes the organism from a 1963 epizootic of white perch in Chesapeake Bay and proposes that it be named *Pasteurella piscicida*.

Kimura, M., and T. Kitao. 1971. On the etiological agent of "bacterial tuberculoidosis" of *Seriola*. Fish Pathol. 6(1):8-14.

After examination of the bacterium causing pasteurellosis in yellowtails, the authors concluded that it should be classified in the genus *Corynebacterium*.

Kitao, T., and M. Kimura. 1974. Rapid diagnosis of pseudotuberculosis in yellowtail by means of the fluorescent antibody technique. Bull. Jpn. Soc. Sci. Fish. 40(9):889-893.

A direct fluorescent antibody test provided an accurate diagnosis of pseudotuberculosis within 3 h and could be used in place of conventional culture procedures.

Koike, Y., A. Kuwahara, and H. Fujiwara. 1975. Characterization of *"Pasteurella" piscicida* isolated from white perch and cultivated yellowtail. Jpn. J. Microbiol. 19(4):241-247.

The authors confirmed earlier findings that organisms from white perch and yellowtails were identical. They concluded that the correct taxonomic placement for the bacterium was either in the genus *Pasteurella* or *Yersinia*.

Kubota, S., M. Kimura, and S. Egusa. 1970. Studies of a bacterial tuberculoidosis of the yellowtail. I. Symptomatology and histopathology. Fish Pathol. 4(2):111-118.

First description of pasteurellosis in cultured yellowtails. The authors isolated the bacterium, determined its pathogenicity, and described external and internal pathology associated with the disease.

Kusuda, R., and K. Inoue. 1976. Studies on the application of ampicillin for pseudotuberculosis of cultured yellowtails. I. In vitro studies on sensitivity, development of drug-resistance, and reversion of acquired drug-resistance characteristics of *Pasteurella piscicida*. Bull. Jpn. Soc. Sci. Fish. 42(9):969-973.

The antibacterial activity of ampicillin was found to be 8-16 times greater than that of chloramphenicol, and although *Pasteurella piscicida* became resistant to ampicillin, this resistance was lost after five serial passages in a medium without ampicillin. The authors suggest ampicillin for treatment of *P. piscicida* infections.

Kusuda, R., and W. Miura. 1972. Characteristics of a *Pasteurella* sp. pathogenic for pond cultured ayu. Fish Pathol. 7(1):51-57.

A *Pasteurella* was found to cause an epizootic among pond-cultured ayu. Since the isolate differed in a number of characteristics from described species, the name *Pasteurella plecoglosacida* sp. nov. was suggested.

Kusuda, R., and M. Yamaoka. 1972. Etiological studies on bacterial pseudotuberculosis in cultured yellowtail with *Pasteurella piscicida* as the causative agent. I. On the morphological and biochemical properties. Bull. Jpn. Soc. Sci. Fish. 38(12):1325-1332.

The authors determined characteristics of the bacterium causing pseudotuberculosis and concluded that it was identical with *Pasteurella piscicida*.

Matsusato, T. 1975. Bacterial tuberculoidosis of culture yellow tail. Pages 115-118 in Proceedings of the Third U.S.-Japan Meeting on Aquaculture at Tokyo, Japan, 15-16 October 1974. Special Publication of Fishery Agency, Japanese Government and Japan Sea Regional Fisheries Research Laboratory.

Description of *Pasteurella piscicida* infections in cultured yellowtails, including history of the disease in Japan, causative agent, epizootiology, and treatment.

Muroga, K., T. Sugiyama, and N. Ueki. 1977. Pasteurellosis in cultured black sea bream *(Mylio macrocephalus)*. J. Fac. Fish. Anim. Husb. Hiroshima Univ. 16(1):17-21.

This is the first reported incidence of pasteurellosis in this cultured species. The authors concluded that the causative agent was *Pasteurella piscicida*.

Simidu, U., and S. Egusa. 1972. A re-examination of the fish-pathogenic bacterium that had been reported as a *Pasteurella* species. Bull. Jpn. Soc. Sci. Fish. 38(8):803-812.

On the basis of biochemical characterization, gram intermediate staining, and transformation of rods to cocci in the stationary growth phase, the authors suggest that the organisms from white perch and yellowtails belong in the genus *Arthrobacter* rather than in *Pasteurella*.

Snieszko, S. F., G. L. Bullock, E. Hollis, and J. G. Boone. 1964. *Pasteurella* species from an epizootic of white perch *(Roccus americanus)* in Chesapeake Bay tidewater areas. J. Bacteriol. 88(6):1814-1815.

A brief description of the 1963 epizootic and of the implicated pathogen.

Sugiyama, T., N. Ueki, and K. Muroga. 1977. Pasteurellosis occurring in cultured young black seabream, *Mylio macrocephalus*. Bull. Fish. Exp. Stn. Okayama Prefecture 1976. 51:152-158. (In Japanese).

A report of the first known occurrence of pasteurellosis in the black seabream.

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