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AEROMONAS HYDROPHILA AND MOTILE AEROMONAD SEPTICEMIAS OF FISH\(^1\)

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INTRODUCTION

*Aeromonas hydrophila* 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 and feral 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. The bacterial etiology of these early reports was often inconclusive; however, the pathology described was analogous to that of red leg disease in frogs, in which *A. hydrophila* was also implicated as the causal organism.

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 microflora of healthy fish (Trust et al. 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 warmwater fishes: minnows, baitfishes, channel catfish (*Ictalurus punctatus*), and striped bass (*Morone saxatilis*). However, the pathogen may also affect a variety of coolwater and coldwater species.

TAXONOMY AND CLASSIFICATION

Because the biochemistry, genetics, and serology of the motile *Aeromonas* taxon is heterogenous, the taxonomic position of this genus has been unstable. According to the scheme of Kluyver and van Niel (1936), many organisms such as *Bacillus*, *Pseudomonas*, *Proteus*, and *Aerobacter* that were associated with hemorrhagic septicemias in fish were transferred into the new genus *Aeromonas*. Aeromonads are short, gram-negative, motile bacilli with a single flagellum. They ferment glucose with or without the production of gas.

Snieszko (1957) divided the genus into three species: *A. hydrophila*, *A. punctata*, and *A. liquefaciens*. *Aeromonas liquefaciens* consisted primarily of fish pathogens. However, Schubert (1967) indicated that, although there was enough biochemical similarity to establish the genus, the species distinctions were invalid. Later, Popoff and Vernon (1976)
demonstrated that the motile aeromonads could be classified into two distinct species: *A. hydrophila* (organisms previously described as *A. punctata* and *A. liquefaciens*), and a new species, *A. sobria*. This classification is now generally accepted. Biochemically, *A. hydrophila* hydrolyzes esculin and ferments both salicin and arabinose, whereas *A. sobria* does not use these compounds (Lallier et al. 1981). The enzymatic profiles among members of the *Aeromonas* complex are otherwise similar (Waltman et al. 1982).

**PATHOLOGY**

*Aeromonas hydrophila* 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. hydrophila* 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). 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. hydrophila* 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.

*Aeromonas hydrophila* was generally considered to be a secondary invader in red sore disease, in which the primary etiological agent was believed to be the protozoan ciliate *Epistylis* (Rogers 1971). Recently, however, Hazen et al. (1978b), who reexamined the etiology of red sore disease, found that *A. hydrophila* was present in 96% of the initial lesions on fish, whereas *Epistylis* was present in only 35%. Furthermore, electron microscopy showed that *Epistylis* lacked structures that produced lytic enzymes. Because *Epistylis* could not produce these enzymes, they were considered to be incapable of initiating lesions. This study strongly suggested that *A. hydrophila* is indeed the primary etiological agent of red sore disease and that *Epistylis* is a secondary pathogen that rapidly colonizes the dermal lesions initiated by bacterial proteolytic enzymes.

In frogs and other amphibians, *A. hydrophila* infections cause distention of capillaries on the ventral surface of the legs and abdomen, giving them the red coloration that is the source of the name of the disease—red leg. Outbreaks of aeromonad septicemias in frogs and warmwater fishes usually occur in spring and coincide with an increase in water temperature. It is believed that disease resistance is low at this time because winter dormancy and starvation usually induce anemia and a decrease in serum proteins. Huizinga et al. (1979) also indicated that rising water temperatures increased metabolism, decreased overall condition, and stressed the fish. Stressed fish increased the production of corticosteroids, which in turn increased their susceptibility to infection.

Organisms recognized as *A. hydrophila* also cause disease in warm-blooded vertebrates. In immunocompromised human hosts, for example, *A. hydrophila* sometimes causes septic arthritis, diarrhea, corneal ulcers, skin and wound infections, meningitis, and fulminating septicemias (von Gravenitz and Mensch 1968; Davis 1978).

**VIRULENCE FACTORS**

Different strains of motile aeromonads differ in relative virulence. Under controlled laboratory conditions, De Figueredo and Plumb (1977) found that strains of motile aeromonads isolated from diseased fish were more virulent to channel catfish than were those isolated from pond water. After Popoff and Vernon (1976) taxonomically distinguished two distinct species of motile aeromonads, Lallier et al. (1981) performed additional studies on rainbow trout (*Salmo gairdneri*) to compare the relative virulence of the two species (*A. hydrophila* and *A. sobria*).
Their results indicated that strains of *A. hydrophila* isolated from either healthy or diseased fish were more virulent than strains of *A. sobria*. Additionally, *A. sobria* was not isolated from fish with clinical signs of motile aeromonad septicemia (Boulanger et al. 1977).

To determine a molecular basis for virulence in different strains of *A. hydrophila*, researchers have attempted to correlate subcellular bacterial components with functional activities. Kou (1973) found that each of the virulent, avirulent, and attenuated aeromonads that he studied possessed hemorrhagic factors and lethal toxins. However, virulent bacteria had quantitatively more toxins than did their avirulent or attenuated counterparts. Olivier et al. (1981) indicated that both *A. hydrophila* and *A. sobria* produced enterotoxins, dermonecrotic factors, and hemolysins. However, although both species produced hemolysis on blood agar plates at 30°C, only *A. hydrophila* did so at 10°C. The authors therefore suggested that the ability of *A. hydrophila* to hemolyze red blood cells at temperatures comparable to those of the water in which fish live may account for the difference in virulence between *A. hydrophila* and *A. sobria*.

A number of studies have concentrated on defining pathological effects induced by injecting purified subcellular components into experimental animals. Rigney et al. (1978) found that individual injections of either endotoxin or hemolysin did not affect frogs. However, when both endotoxin and hemolysin were injected, frogs showed pathology that mimicked clinical signs of red leg disease. Thune et al. (1982a) found that channel catfish were also tolerant of single injections of endotoxin at concentrations up to 400 μg endotoxin per 7.2 g fish. However, an extracellular cell-free extract of a spent culture medium had an LD50 value of 15.7 μg protein within 48 h after its injection into 7.2-g fish. Thune et al. (1982b) later showed that the cell-free extract did not contain hemolytic activity, but did have proteolytic activity. When the proteolytic activity was further refined, two proteases were identified. The LD50 of one, which was heat labile, was 18 μg protein per gram of fish, and that of the other, which was heat stable, was 3 μg protein per gram of fish.

Allan and Stevenson (1981) also found hemolytic and proteolytic activities in crude extracellular preparations from *A. hydrophila*; they noted that aeration increased growth rates, cell yield, and the amount of proteolytic activity. Proteolytic activity was reduced, however, when cultures were incubated at 37°C. The extracts from a protease deficient mutant were more toxic to fish than were similar extracts from isogenic wild strains. The extract from the protease deficient mutant did have a fivefold increase in hemolytic activity. Therefore, Allan and Stevenson concluded that hemolysin, not protease, was the principal virulence factor of *A. hydrophila*. 

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Besides the ability to produce certain toxins, the ability of virulent strains of *A. hydrophila* to locate and attach to a suitable host has been studied. Strains isolated from lesions on diseased fish had a greater chemotactic response to skin mucus than did strains obtained from pond water (Hazen et al. 1982). Additionally, Trust et al. (1980) indicated that *A. hydrophila* had adhesive agglutination characteristics that facilitated attachment to eukaryotic cells.

**IDENTIFICATION AND DIAGNOSIS**

Presumptive diagnosis of *A. hydrophila* may be based on the species of fish affected, the past disease status of those fish, and the presence of clinical signs of disease. However, bacteria must be isolated and identified biochemically to provide a definitive diagnosis. Either tryptic soy agar (TSA) or brain-heart infusion agar (BHIA) is a suitable medium for the primary isolation of *A. hydrophila* from diseased fish. However, because mixed bacterial infections are common in fish with hemorrhagic septicemia, it is often difficult to isolate pure cultures of *A. hydrophila* from clinically diseased tissue. Shotts and Rimler (1973) therefore designed a differential medium for selective isolation of motile aeromonads. This medium, termed R-S agar, is prepared by dissolving the following ingredients (grams) in distilled water to a volume of 1 liter: L-lysine hydrochloride (5.0), L-ornithine hydrochloride (6.5), L-cystine hydrochloride (0.3), maltose (3.5), sodium thiosulfate (6.8), bromothymol blue (0.03), ferric ammonium citrate (0.8), sodium deoxycholate (1.0), novobiocin (0.005), yeast extract (3.0), sodium chloride (5.0), and agar (13.5). The mixture is constantly stirred, heated to boiling for 1 min, and brought to pH 7.0. The medium is cooled to 45°C, dispensed into sterile petri dishes, and can be refrigerated in plastic sleeves until used.

After inoculation, R-S agar should be incubated at 37°C for 24 to 48 h, to ensure optimal differentiation of bacteria. Colonies of *A. hydrophila* are yellow; those of *Pseudomonas*, *Escherichia*, and *Enterobacter* are green; and those of *Edwardsiella* are green with black centers. Although *Proteus vulgaris* and some *Citrobacter* are also yellow on R-S agar, these colonies have black centers that are indicative of hydrogen sulfide gas. *Aeromonas salmonicida* also produces yellow colonies on R-S agar, but growth of this bacterium is inhibited at 37°C.

Rimler-Shotts agar has facilitated the primary isolation of *A. hydrophila*, but yellow colonies should not be accepted as a basis for definitive diagnosis. Davis and Sizemore (1981) indicated that R-S agar supported the growth of a limited number of yellow colonies whose DNA homology ratios indicated that they did not belong in the genus *Aeromonas*. Therefore, additional biochemical tests should be performed on isolated colonies from either TSA or BHIA. Reactions inconsistent for the
species can occur if one uses colonies picked from differential media. For example, Overman et al. (1979) found that 8% of the isolates they examined were cytochrome oxidase negative when taken from differential media.

Isolated bacteria should be short (0.5 X 1.0 μm), gram-negative bacilli that are oxidase positive, ferment glucose with or without the production of gas, and are insensitive to the vibriostatic agent 0/129.

Glucose fermentation is a critical reaction that differentiates *A. hydrophila* from species of *Pseudomonas* (Bullock 1961). Bacteria are inoculated into two tubes of oxidation fermentation (OF) basal medium (Difco) supplemented with 1% glucose. The medium in one tube is overlaid with a plug of sterile petrolatum and both tubes are incubated at 25°C for 24 to 48 h. Results are interpreted as follows: yellow coloration in both tubes indicates acidic fermentation of glucose typical for *Aeromonas*, whereas yellow coloration only in the tube without petrolatum indicates oxidation of glucose characteristic of *Pseudomonas*.

In a single-tube modification of this test described by Walters and Plumb (1978), a tube is prepared with twice the amount of OF glucose medium contained in the two-tube test. After incubation, a yellow coloration throughout the tube indicates fermentation, whereas a yellow-ish coloration only at the top of the medium indicates oxidation. Motility can also be determined by examination for diffused bacterial growth away from the origin of inoculation.

The production of gas is evidenced in either test by the formation of bubbles in the medium. Although most strains of *A. hydrophila* produce gas during the fermentation of glucose, some strains of this bacterium isolated from diseased fish are anaerogenic—that is, they do not generate gas (Ross 1962).

Because motile aeromonads are ubiquitous and have considerable antigenic diversity, serodiagnostic identification is not reliable. Within homologous antigen/antibody systems, agglutination procedures, fluorescent antibody tests (Eurell et al. 1978), and immunoenzyme microscopy procedures (Lewis 1981) were adapted to detect *A. hydrophila*. Although antibodies can detect homologous antigens, detection of heterologous antigens within this group makes serodiagnostic identification impractical. Fliermans and Hazen (1980) found that a total of three different antisera to *A. hydrophila* gave a positive fluorescent antibody reaction with only 27.5% of the *A. hydrophila* isolates tested. Therefore, the lack of an effective polyvalent antiserum specific for *A. hydrophila* still limits reliable serodiagnostic detection of this pathogen.
Motile aeromonads are probably the most antigenically diverse group of bacteria pathogenic to fish. The amount of antigenic diversity inherent within this group is especially expressed by the H and 0 somatic antigens. Ewing et al. (1961) described 12 O-antigen groups and 9 H-antigen groups. Each group was further divided into a number of serotypes. Chodyniecki (1965) also found a high degree of antigenic diversity among strains of *A. hydrophila* obtained from the same population of fish and even from different organs of the same fish. Although some strains of *A. hydrophila* have common somatic antigens (Rao and Foster 1977; Lallier et al. 1981), it has been consistently demonstrated that a monovalent antiserum could agglutinate only a small percentage of the total isolates examined. Kingma (1978) produced seven rabbit antisera to heat stable antigens of seven isolates. Collectively, these antisera agglutinated only 19.5% of the total number of *A. hydrophila* isolates studied.

Despite the existent serological diversity within this taxon, Mittal et al. (1980) found that strains of *A. hydrophila*, which were highly virulent for rainbow trout, had a common O-antigen; furthermore, these bacteria did not agglutinate in acriflavine, settled after boiling, and resisted the bactericidal activity of normal mammalian sera. In contrast, low-virulence strains of *A. sobria* did not share the common O-antigen; these bacteria settled after boiling and were lysed by normal mammalian sera.

Despite the heterogeneity of somatic antigens from *A. hydrophila*, Liu (1961) noted that the biological activity of extracellular toxins was neutralized by a single antiserum against *A. liquefaciens* (synonym for *A. hydrophila*). He therefore concluded that the motile aeromonads shared certain extracellular antigens. Bullock et al. (1972) also indicated that aerogenic and anaerogenic strains of *A. hydrophila* had common extracellular antigens.

Monovalent bacterins were initially prepared against *A. hydrophila*. Although these vaccines often provided acceptable levels of protection against challenge with a homologous bacterium, fish were not immune to infection by heterologous strains of *A. hydrophila* (Post 1966; Schäperclaus 1967). Because of antigenic diversity, additional vaccination strategies included the development of polyvalent vaccines, immunization against inactivated extracellular toxins (toxoids), and the development of vaccines consisting of cellular antigens plus toxoid.

Schäperclaus (1970) recognized that common carp (*Cyprinus carpio*) vaccinated against *A. hydrophila* developed both agglutinating serum antibodies against cellular antigens and antitoxic activity against extracellular antigens. In a later study, he found that common carp vaccinated by intraperitoneal injection of bacteria produced more
circulating antibodies than did carp vaccinated by the oral route (Schäperclaus 1972). Furthermore, vaccination with soluble extracellular antigens was more efficacious and provided a more widely based protection against heterologous serotypes than did vaccination with whole-cell antigen. Although oral immunization did not evoke a strong humoral antibody response, oral delivery was more protective than injection. Schachte (1978) also found that the delivery of vaccine by injection or immersion or per os stimulated differential kinetics of antibody production. However, no significant protection against natural exposure to heterologous A. hydrophila was afforded to channel catfish vaccinated by any of the methods.

Thune and Plumb (1982) found that both sac fry and swim-up fry vaccinated by immersion in sonicated polyvalent bacterin were protected against challenge with homologous bacteria, indicating an early onset of immunocompetence in channel catfish. This study was important because A. hydrophila causes a severe problem in channel catfish in spring and early summer, when fry are abundant. The early onset of immunocompetence among channel catfish fry indicates that immunization, when effective, could be used to reduce outbreaks of A. hydrophila infection. Regardless of whether whole cells, freeze-thawed cells, or cell sonicates were used, Thune and Plumb (1982) further indicated that injection was superior to either immersion or spray vaccination for developing humoral antibodies. However, cell sonicates evoked the best antibody response.

**OCCURRENCE AND RANGE**

Motile aeromonads cause diseases wherever baitfishes or warmwater or ornamental fishes are propagated. The bacterium can also cause disease in feral fish and is common in the intestinal flora of apparently healthy fish (Trust et al. 1974). The bacterium is ubiquitous; it occurs in most freshwater environments and can be found both in the water column and in the top centimeter of sediment (Hazen 1979). Aeromonas hydrophila occurs in environments having a wide range of conductivity, turbidity, pH, salinity, and temperature (Hazen et al. 1978a). Temperature optima extend from 25 to 35°C. Consequently, most epizootics among warmwater fishes in the southeastern United States are generally reported in spring and early summer (Meyer 1970).

Pond water, diseased fish, and diseased frogs, as well as convalescent frogs and fishes, serve as reservoirs of infection. Certain algae (Kawakami and Hashimoto 1978) and other protozoa (Chang and Huang 1981) that are grazed upon by fish, also harbor A. hydrophila. In contagion of these bacteria, the intestinal tract or epidermal abrasions are likely portals of entry. Under conditions of stress, it is likely that some strains of A. hydrophila that are part of the normal gut flora become pathogenic. It is believed that infection occurs in winter,
when fish are relatively inactive, and that the disease breaks out in spring. Aquarium fish, which are usually maintained at constant water temperature, can develop this disease at any time. Rainbow trout appear to be the most susceptible of the salmonids that develop motile aeromonad septicemias.

METHODS OF CONTROL

Prevention

Effective hatchery management is the best approach to avoid infections and subsequent epizootics caused by this bacterium. In water reuse hatcheries, both ozonation (Colberg and Lingg 1978) and filtration combined with ultraviolet irradiation (Bullock and Stuckey 1977) effectively eliminate the threat of *A. hydrophila*. Although ultraviolet irradiation may be prohibitively costly under some situations, it has been effectively used to control mortality among fry of muskellunge (*Esox masquinongy*) during outbreaks of *A. hydrophila* (Calesante et al. 1981).

Motile aeromonad septicemias are generally mediated by stress. Elevated water temperature (Esch and Hazen 1980), a decrease in dissolved oxygen concentration, or increases in ammonia and carbon dioxide concentrations (Walters and Plumb 1980) have been shown to promote stress in fish and trigger infections with *A. hydrophila*. The constant monitoring of major environmental variables can therefore enable one to forecast stressful situations and possibly avoid problems before they arise. Wherever this disease occurs frequently, pond fish should not be handled but transferred only after water temperature is high enough for fish to be active and feeding normally (Rychlicki and Zarnecki 1957). Mortalities were reduced dramatically (80-90%) when fish, at the time of spring transfer, were injected intraperitoneally with 10-20 mg of chloromycetin (not registered for use in the United States), or by dissolving 10-20 mg chloromycetin in water per kilogram of fish (5-10 mg/lb).

Managers should avoid introductions into their hatcheries of fish that have recently undergone infection. Shipments of new eggs should be disinfected. Wright and Snow (1975) found that either acriflavine (500-700 ppm for 15 min) or Betadine (100-150 ppm active ingredient for 15 min) successfully disinfected eggs of largemouth bass (*Micropterus salmoides*). Neither Roccal nor formalin were effective. When warmwater fishes are held in tanks or hauled in trucks or plastic bags, the value of adding disinfectants or antibiotics should be examined. The most promising compounds include chloramphenicol, oxytetracycline, chlortetracycline, and a mixture of penicillin and streptomycin added to water at a rate of 10-15 mg/L.
Treatment

Oxytetracycline (Terramycin) is the drug of choice for treating motile aeromonad septicemias in fishes. The drug is approved for use with pondfishes, channel catfish, and salmonids administered in feed at a daily rate of 50 to 75 mg/kg for 10 days. Fish must be withdrawn from treatment 3 weeks before they are stocked or eaten. This treatment sometimes produces dramatic results when it is administered for even 2 or 3 days, and is particularly effective when fish become infected after they have been handled, crowded, or held under stress for short periods of time (Meyer 1964; Meyer and Collar 1964).

Furanace, though not registered for use in the United States, is extremely effective against motile aeromonads if the affected fish are immersed for 5 to 10 minutes in water containing 1-2 ppm furanace, or by maintaining fish for 1 week in water containing 0.1 ppm drug. However, furanace can be toxic to fishes if used improperly (Mitchell and Plumb 1980).

Chloramphenicol (chloromycetin) was successfully used to treat frogs with red leg disease by gastric intubation of 3 to 5 mg per 100 g of frog for 5 days, twice daily. Chloromycetin, like oxytetracycline, was effective in treating fish when it was administered orally. However, its use is prohibited in food fishes and discouraged in other fishes because it is the drug of last resort in certain human diseases—e.g., typhoid fever. Indiscriminant use of chloramphenicol can result in drug resistance and thus reduce the value of the antibiotic in human medicine. Against strains of *A. hydrophila* that show multiple drug resistance, piromidic acid administered orally has been experimentally shown to be more effective than either chloromycetin or oxytetracycline (Katae et al. 1979). However, this drug is not registered for use on food fishes in the United States.

ANNOTATED BIBLIOGRAPHY


*Aeromonas hydrophila* produces heat labile proteolytic and hemolytic activities that are toxic to fish. A protease-deficient mutant was more toxic to salmonids than its analogous parent strain. This study indicated that hemolysin was a primary virulence factor of *A. hydrophila*. 

Pathological changes were apparent in the spleens of fish injected with virulent Aeromonas hydrophila, 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.


Strains of Aeromonas sobria were isolated only from healthy fish, whereas strains of A. hydrophila 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.


Biochemical criteria for identification of motile aeromonads and their separation from Pseudomonas fluorescens are described. Organisms were designated as Aeromonas liquefaciens if they fermented glucose, produced 2,3-butanediol, hydrogen sulfide from motility sulfide medium, were cytochrome oxidase positive, and hydrolyzed starch.


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 hydrophila.

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 cell-free antiserum or a whole-cell antiserum. Electrophoretic serum patterns of infected fish were also studied.


*Tetrahymena pyriformis* was experimentally shown to graze on populations of *Aeromonas hydrophila*. The bacterium, at concentrations of 1 X 10^6 cells/mL co-existed with the protozoan. If a second bacterial species was introduced, predation by the protozoan increased.


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 O-antigens previously isolated from the same fish.


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.
Presumptive Aeromonas hydrophila isolates (as judged by characteristic growth on Rimler-Shotts medium) were obtained from estuarine sources. Many of the isolates, especially those from low-salinity sites, were also identified by the API 20E system as A. hydrophila. However the DNA homology ratios of all of these isolates excluded them from this species.


A review of Aeromonas hydrophila as a human pathogen.


Virulence of selected strains of Aeromonas hydrophila 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.


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 hydrophila.


Techniques of cultural isolation, slide agglutination, tube agglutination, microagglutination, 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, microagglutination,
and fluorescent antibody procedures were equally effective in detecting levels of serum agglutinins to *Aeromonas hydrophila*. However, the fluorescent antibody technique was more sensitive and faster than the agglutination tests.


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. hydrophila* and *A. salmonicida* that were tested.


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 should not be used as a reliable diagnostic sign.


Only 27.5% of a total number of isolates studied reacted with three antisera to *Aeromonas hydrophila* by fluorescence microscopy, indicating that other serotypes existed. Statistical analysis showed that isolates of *A. hydrophila* obtained from aquatic environments were distinct from other isolates of *A. hydrophila* as measured by immunofluorescence.


High densities of *Aeromonas hydrophila* 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. hydrophila* in water were highest from March to June, and a second peak occurred in October. Mean monthly densities of *A. hydrophila* 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.
Isolates of *Aeromonas hydrophila* have different chemotactic responses to surface mucus of fish. Isolates of *A. hydrophila* from fish lesions were slightly more chemotactic than isolates from water. The chemotactic factor in fish mucus had a molecular weight of 100,000 and was heat stable at 56°C.

Abundance of *Aeromonas hydrophila* was studied in 147 aquatic environments in 30 states and Puerto Rico. *Aeromonas hydrophila* was not isolated from extremely saline, thermal, or polluted waters, even though it was found over a wide range of salinity, conductivity, temperature, pH, and turbidity.

Epistylis sp. was isolated from only 35% of 114 lesions on 114 fish, whereas *Aeromonas hydrophila* was found in 96% of the same lesions. Electron microscopy of these lesions indicated that neither the stalk nor attachment structure of *Epistylis* sp. has organelles that produce lytic enzymes. Because *A. hydrophila* produces strong lytic toxins, it was concluded that *Epistylis* sp. was a benign ectocommensal and that *A. hydrophila* is the primary etiologic agent of red sore disease.
Delivered orally, piromidic acid was more effective than chloramphenicol or oxytetracycline in controlling *Aeromonas hydrophila* and *Vibrio* sp. in goldfish (*Carassius auratus*) and eels (*Anguilla*).


Larger quantities of algae (up to 10^3 cells) were found in algae fed upon by ayu, than in the water itself. In surface water, the density of *Aeromonas hydrophila* was less than 10 cells/mL.


Summarizes serological analysis of 154 strains of *Aeromonas hydrophila* reacted with each of seven rabbit antisera to heat stable antigens. Of 93 isolates of *A. hydrophila* obtained from tropical fishes in Florida, and 61 isolates from the bacterial collection of the National Fish Health Research Laboratory, only 19.5% fluoresced collectively with the seven antisera.


Established the genus *Aeromonas* to classify short, gram-negative motile rods that have a single flagellum and ferment carbohydrates with gas. Established the type species *Aeromonas liquefaciens*, a synonym for *Aerobacter liquefaciens*.


All virulent, attenuated, and avirulent *Aeromonas hydrophila* have hemorrhagic, lethal, and destructive toxins, but pathogenicity is related to quantity of the toxins and not to this qualitative disposition.

A total of 195 strains of motile *Aeromonas* isolated from fish were characterized as either *A. hydrophila* or *A. sobria*. Serological classification on the basis of somatic antigen reactions was attempted.


Immunoenzyme techniques were developed to differentiate between subclinical infections of *Yersinia ruckeri* from motile *Aeromonas septicemia* in channel catfish. The sensitivity of immunoenzyme techniques was comparable to that of current fluorescent antibody methods and cultural isolation.


The serologic specificity of extracellular antigens from *Aeromonas hydrophila*, *A. punctata*, *A. liquefaciens*, *A. formicans*, and other Enterobacteriaceae was examined. The biological activity of extracellular antigens was neutralized by an antiserum prepared against a single strain of *A. liquefaciens*. The motile aeromonads shared a common extracellular antigen but were serologically distinct from *A. salmonicida*, *Serratia* sp., and other Enterobacteriaceae.


*Aeromonas liquefaciens* infections were the most frequent bacterial diseases in the South. Many species of fish and frogs were infected and diseased. In ponds, the disease may progress slowly or quickly. When fish are crowded in tanks, the infection is acute and progresses rapidly. It can start as a local dorsal lesion, or be systemic from the start. Isolated bacteria were sensitive to chloramphenicol and tetracycline antibiotics. Oxytetracycline (Terramycin) given at a rate of 2.5 g (of pure drug activity) per 45 kg (100 pounds) of fish for 10 days resulted in full control of the disease. Cost of treatment was 3.4% of gross income.


Seasonal occurrences were noted for several parasitic and bacterial diseases among warmwater fishes. Summer infections caused by
Aeromonas liquefaciens were shown to be related to oxygen depletion. Of all disease cases reported, 72% occurred during the period March to July. Relations between occurrences of a particular disease and water temperature, spawning season, and other stresses were noted.


Kanamycin injected intraperitoneally and oxytetracycline administered with feed showed satisfactory therapeutic effect.


Most effective treatment level for channel catfish infected experimentally with A. hydrophila was 2 mg furanace per liter for 6.5 h. In vitro bacterial growth was completely inhibited at this level and in vitro drug resistance was also noted.


Motile aeromonads isolated from fish were studied for their virulence relative to cell-surface characteristics. It is suggested that agglutination in acriflavine, stability after boiling, and sensitivity to normal fresh serum could be used to screen Aeromonas strains for virulence in fish.


All strains of Aeromonas hydrophila and A. sobria produced enterotoxins. However, only A. hydrophila produced a dermonecrotic factor and two zones of hemolysis on blood agar plates. Only A. hydrophila produced hemolysin at 10°C.


Descriptive pathology is reviewed and early evidence is given for the description of possible Aeromonas hydrophila infections dated to the Middle Ages.

Discussion of the variable nature of the oxidase reaction when these tests are performed on bacteria picked directly from selective or differential medium.


Two distinct species of motile aeromonads were identified. The first was assigned to the type species of the genus *Aeromonas hydrophila*. *Aeromonas punctata* was considered an illegitimate name for *A. hydrophila*. The second group was assigned to a new species, *A. sobria*.


Rainbow trout produced humoral antibodies against *Aeromonas hydrophila* after immunization by parenteral delivery. Higher antibody titers were produced in fish injected with whole cells emulsified in adjuvant than in fish immunized with vaccine lacking adjuvant. Fish immunized by parenteral vaccination were protected against experimental challenge with a homologous challenge strain of *A. hydrophila*.


Particulate and soluble heat stable antigens were prepared from all recognized species and subspecies of the genus *Aeromonas*. Antisera were prepared and cross adsorption was performed to identify specific and common antigenic relations between members of this genus.


Describes pathology induced by *Aeromonas hydrophila* with specific reference to the effects of hemolysin and endotoxin in frogs. Interaction between both hemolysin and endotoxin was necessary to produce death in frogs. However, the endotoxin source could be provided by a number of pathogenic and nonpathogenic bacteria. Other stress factors that also activate the serum histamine-heparin
releasing factor produced disease by way of a synergistic interaction between histamine and hemolysin.


Associates the protozoan *Epistylis* as the primary etiological agent of red sore disease and indicates that *Aeromonas hydrophila* is apparently a secondary pathogen.


This culture produced a brown, water-soluble pigment and showed anaerogenic fermentation of glucose similar to reactions observed with *Aeromonas salmonicida*. However, motility and other biochemical reactions indicated that this organism was *A. liquefaciens*.


In a new management method, yearling common carp were not transferred to winter ponds in fall and to production ponds in early spring. Instead the fish were overwintered and kept in nursery ponds until the water warmed in late spring. Also, carp from different nursery ponds were never mixed. Losses were reduced from 42% under the former method to only 10% under the new management method. In a special test, some carp handled in early spring developed dropsy, whereas fish handled in late spring remained free of it throughout the summer.


Differences in antibody levels from serum and gut mucus were noted in channel catfish immunized with a polyvalent vaccine that included both *Aeromonas hydrophila* and *Flexibacter columnaris*. Significant differences in antibody titers were noted among groups of fish vaccinated by oral, immersion, and injection deliveries.

Vaccination with an inactivated trivalent vaccine was successful under experimental conditions. Field tests were inconclusive.


As proven in test trials, common carp developed partial antibacterial and antitoxin immunity from Aeromonas punctata. The production of complex mixed vaccines that included bacteria and inactivated exotoxins was recommended.


Common carp were parenterally and orally vaccinated with whole cells and soluble antigens from Aeromonas punctata. Parenteral immunization resulted in better protection and higher antibody titers than observed by oral immunization. Soluble antigen produced better immunity than cells and also protected against more serotypes of the bacterium.


Three species, Aeromonas punctata, A. hydrophila, and A. salmonicida, are described. Special attention is placed on critical review of the nomenclature for the motile aerogenic aeromonads.


A new differential medium was described for the presumptive identification of Aeromonas hydrophila.


Proposed taxonomic separation of the genus Aeromonas into three species—hydrophila, punctata, and liquefaciens—on the basis of pathogenicity and limited biochemistry.
Therapeutic control of major fish diseases is discussed and the use and effectiveness of nitrofuran compounds against *Aeromonas hydrophila* are described.


Effects of extracellular products and endotoxin from *Aeromonas hydrophila* were determined in channel catfish. Endotoxin (up to 400 μg) was not lethal for fish, whereas the 48-h median lethal concentration for extracellular products was 15.7 μg per fish.


Extracellular products from *Aeromonas hydrophila* were lethal for channel catfish. Hemolysis was not detected but partial purification of the extracellular products indicated that the lethal factors were proteases. Median lethal dose (in micrograms of protein per gram of fish) was 18.0 for a heat labile protease and 3.0 for a heat stable protease.


Hyperosmotic infiltration appeared to be a practical alternative to injection vaccination of channel catfish.


A wide variety of facultative anaerobic bacteria were isolated from the gastrointestinal tract of healthy fishes. *Aeromonas hydrophila* was the predominant bacterium.

Hemagglutination patterns, which characterize the ability of bacteria to attach to eucaeryotic cells, are described for aeromonas hydrophila.


Thirty cases of human infections caused by aeromonas hydrophila or aeromonas shigelloides were reported.


Glucose motility deep medium was developed to determine motility, oxidation, fermentation, and gas production of gram-negative, cytochrome oxidase positive bacterial pathogens of fish.


Channel catfish stressed under conditions of limited dissolved oxygen and increased ammonia and carbon dioxide concentrations were more susceptible to infection by aeromonas hydrophila and edwardsiella tarda than were unstressed fish. aeromonas hydrophila was isolated from 67% of stressed catfish but only 9% of control fish, and e. tarda from 43% of stressed fish and 7% of control fish.


Enzyme characteristics were characterized for 48 isolates of the aeromonas hydrophila complex.


The effectiveness of six chemicals--formalin, acriflavine, Roccal, Merthiolate, Betadine, and Wescodyne--were compared for disinfection of largemouth bass eggs. Acriflavine (500-700 ppm for 15 min) and Betadine (100-150 ppm for 15 min) were particularly effective for disinfecting eggs from largemouth bass.