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Aeromonas hydrophila: A review with emphasis on its role in Fish Disease

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The group of bacteria referred to as the Aeromonas hydrophila complex is a phenotypically and genetically heterogenous taxon. Members of the genus are widespread and abundant throughout the aquatic environment. They are responsible for epizootic disease in fish and amphibians and cause severe disease problems in mammals including man. The current status of this taxon is reviewed from a historical perspective with emphasis on the role of A. hydrophila as a pathogen of cultured fish. Etiology, pathogenesis, treatment, prevention and control methods are discussed. A thorough large scale examination of this taxon is required in order to resolve the current chaotic status.

Aeromonas hydrophila is a phenotypically and genetically heterogeneous taxon, members of which are widely distributed throughout the aquatic environment. Strains of A. hydrophila have been responsible for massive mortalities of cultured and feral fish populations as well as epizootics in feral populations of amphibians. Outbreaks are often stress related making the disease difficult to control. A. hydrophila strains also pose a significant health hazard to human beings and potentially to other agricultural interests.

HISTORY

The first published reports of diseases in aquatic animals due to strains of what is presumed to be Aeromonas hydrophila were reported in the late 1800's (Sanarelli 1891), though the disease was probably common in Europe throughout the middle ages (Otte 1963). In 1924, a disease, coined Lublin disease, characterized by ulcerative skin lesions and septic hemorrhagic symptoms was reported in Poland. Spiczakow (1933) identified the etiologic agent as a bacterium and named the disease hemorrhagic septicemia. Several years prior to Spiczakow's identification, Schäperclaus (1930) had reported a similar disease in carp, though characterized largely by abdomial swelling due to ascites accumulation in the peritoneum. He identified the etiologic agent as Bacterium punctatum.

The role of a bacterium as the primary etiologic agent of hemorrhagic septicemia seemed well established by the time of these reports. However, a controversy existed as to whether or not the primary etiologic agent was viral and the bacteria merely secondary pathogens. The issue, as late as 1966 (Ghittino 1966) appeared unresolved. The consensus of opinion among fish pathologists in central Europe and in North America is that the disease is caused by A. hydrophila and not a virus. This will be discussed in further detail shortly.

Since the first reports of disease due to Aeromonas hydrophila were documented, the disease has been recorded with an increasing frequency in fish, reptiles, amphibians, invertebrates, and mammals including human beings (Table 1). Diseases due to A. hydrophila have been variously named hemorrhagic (red sore disease) septicemia, infectious dropsy, rubella, redmouth, red pest, or freshwater eel disease.

DISTRIBUTION

A. hydrophila has been isolated and identified in all countries where pond and ornamental fishes are cultured and

Table 1. A partial listing of fish species and other animals in which Aeromonas hydrophila infections have been documented

Species affected	Authors
Carp (Cyprinus carpia)	Schaperclaus 1954; Kozlowski et al. 19 Amlacher 1961; Kou 1972
Golden shiners (Notemigonus crysoleucas)	Lewis and Bender 1960
Channel catfish (Ictalurus punctatus)	Rock and Nelson 1965, Plumb 1975
Gizzard shad (Dorosoma cepedium)	Rock and Nelson 1965
Threadfinshad (Dorosoma petenense)	Haley et al. 1967
American shad (Alosa sapidissima)	
Bream (Abramis brama)	Goncharov 1965
Eel (Anguilla anguilla)	Kou 1972, Wakabayashi and Egusa 1979
Ayu (Plecoglossus altivelis)	Kou 1972
Perch (Perca florescens)	Vezina and Desrochers 1971
Bluegills (Lepomis macrochirus)	Shotts et al. 1972, Esch and Hazen 198
Bass (Morone spp)	
Trout (Salmo gairdneri)	Collins 1970, Wood 1968, Olivier 1980
Salmon (misc. species)	
Cod (Gadus morhua)	Larsen and Jensen 1977
Aquarium fish - many species	Shotts et al. 1976, van Duijn 1938 Axelrod 1962
Goldfish (Carassius auratus)	Kou 1972
Tilapia (Tilapia aurea)	Balarin 1979
Frogs (Rana pipiens, misc. species)	Gibbs 1963, Rigney et al. 1978
Alligators (Alligator mississipiensis)	Shotts et al. 1972, Gordon et al. 1979 McCoy and Seidler 1973
Turtles (several species)	
Snails (Achatina fulica)	Mead 1969
Shrimp (Machobrachium ohioni)	De Figueiredo and Plumb 1971
Humans	Davis et al. 1978
Cattle	Wohlgemuth et al. 1972

is perhaps the most important cause of severe outbreaks of disease in cultured and native freshwater fishes (Snieszko et al. 1976). A. hydrophila strains have been documented as causing disease in many species of cold blooded animals including fish, alligators, snakes and turtles as well as warm blooded animals including human beings (Table 1). Disease in many if not most instances is strongly associated with stress (Snieszko 1974; Meyer 1970; Haley et al. 1967). Though the first reports of A. hydrophila were associated exclusively with disease in fish, it is now well established that the organism is part of the intestinal flora of healthy fish (Trust et al. 1974; Wolke 1975; Bhotts et al. 1976; Boulanger et al. 1977; Heuschmann-Brunner 1978).

Strains of A. hydrophila are widely distributed in the aquatic environment (Hazen et al. 1978; Heuschmann-Brunner 1978; Kaper et al. 1981), in fresh (clean and polluted) and saline waters, though until recently they were not thought to be found in the marine environment (Gibson et al. 1977). However, Hazen et al. (1978), in a study of the abundance of A. hydrophila in 147 natural aquatic habitats, found strains of A. hydrophila in marine systems which interface with fresh water and at all salinities examined except the most extreme (100 parts per thousand). They found no correlation between distribution and pH though significant relationships between bacterial densities and conductivity were observed. Densities ranged from less than 1 cell per liter to several thousand per ml. Kaper et al. (1981), found similar densities in their studies of Chesapeake Bay.

In addition to being found in healthy and diseased fish and throughout the aquatic environment, A. hydrophila is abundant in sewage and waters contaminated with sewage (Geldreich 1973; Snieszko 1974). For this reason it has been suggested that A. hydrophila might serve as a water pollution indicator organism (Schubert 1976). However, since A. hydrophila is not considered a normal inhabitant of the human gastrointestinal tract, less than 1% of healthy adults carry aeromonads (von Graaventiz et al. 1968), the value as an indicator of human fecal contamination of water is doubtful.

The role of A. hydrophila in human disease is receiving a great deal of attention. Strains of A. hydrophila have been found to be responsible for septic infections, diarrhea, corneal ulcers, skin infections, wound infections, meningitis and fatal infections in compromised cancer patients (Dean et al. 1967; Ketover, et al. 1973; Saltonetal 1973; Quadri et al. 1976; Davis et al. 1978; Feaster et al. 1978; Fraire 1978; Ramsay et al. 1978; Cumberbatch et al. 1979; Joseph et al 1979).

It is quite evident that diseases due to strains of A. hydrophila are a hazard to healthy and stressed human beings and under stressful conditions, such as crowding, elevated water temperatures, on low dissolved oxygen levels, to the maintenance of healthy fish and other animal populations. The ubiquitous nature of A. hydrophila suggests an important if not crucial role in natural aquatic processes.

Table 2. Synonyms for *Aeromonas hydrophila* (Adapted from Snieszko and Axelrod 1971)

Achromobacter punctatum

Aeromonas liquifaciens

Aeromonas punctata

Bacillus punctatus

Bacillus rancida

Bacterium punctatum

Proteus hydrophilus

Pseudomonas granulata

Pseudomonas hirudinis

Pseudomonas punctata

ETIOLOGY

Classification

Aeromonas hydrophila species have been described under many synonyms (Table 2). As previously mentioned, the etiology of hemorrhagic septicemia has been and (to some extent apparently) is still disputed. Schaperclaus (1954, 1965) and Otte (1963) thoroughly reviewed the disparate views on etiology. There is evidence for both viral and bacterial etiology though definitive proof is lacking for either view. Nonetheless, most of the data supports a crucial role of strains of A. hydrophila (and in some cases Pseudomonas flourescens) in hemorrhagic septicemia (Schaperclaus 1965; Snieszko 1971, 1976). A role of a known or unknown viruses as secondary pathogens can not be precluded.

The A. hydrophila taxon is very heterogenous, biochemically, serologically and genetically. Kluvyer and van Niel (1936) proposed that the genus Aeromonas be used to encompass those microorganisms that are phenotypically similar to the enteric group but motile by means of a polar flagellum. Stanier (1943) and Miles and Miles (1951) noted that many organisms previously described as members of the genera Pseudomonas, Proteus, Bacillus and Aerobacter were really members of the genus Aeromonas. Snieszko (1957), in the 7th edition of Bergey's Manual, differentiated three species of motile aeromonads based upon pathogenicity and biochemical tests: Aeromonas hydrophila; A. punctata and A. liquefaciens. Subsequent studies by Eddy (1960, 1962), Ewing and Johnson (1960, 1961), Page (1962), and Schubert (1967a,b, 1968, 1969a,b) reiterated the existence of sufficient biochemical similarities to warrant the genus name but did not concur as to how the motile aeromonads should be divided into species.

In the 8th edition of Bergey's Manual (Schubert 1974), under the family Vibrionaceae, five subspecies, three of A. hydrophila; A. hydrophila hydrophila, A. hydrophila anaerogenes and A. hydrophila proteolytica and two of A. punctata; A. punctata punctata and A. punctata caviae are descibed. Both A. hydrophila hydrophila and A. hydrophila anaerogenes are further subdivided into two biotypes.

Popoff and Veron (1976) in a computerized numerical taxonomy study examined 68 strains of Aeromonas for 203 characters including morphological, biochemical and genetic properties. They concluded that A. punctata is an illegitimate synonym for A. hydrophila and suggested that the group of organisms belonging to the A. hydrophila-A. punctata taxons be classified as A. hydrophila and a new species, A. sobria. Though their taxonomic analysis failed to differentiate two strong biotypes in the A. hydrophila taxon, they; nonetheless, proposed that the species could be divided into two subspecies; A. hydrophila subs. hydrophila and A. hydrophila subs. anaerogenes (Table 4). They also suggested,

Table 3 Summary of taxonomic studies on the A. hydrophila taxon (Adapted from Popoff and Veron 1976)

Year	Author	
1957	Snieszko	A. hydrophila A. punctata A. liquifaciens
1961	Ewing et al.	A. hydrophila
1960, 1962	Eddy	A. punctata
1962	Leclerc	A. caviae
1964	Merkel et al.	A. dourgesi A. proteolytica
1969a,b, 1974	Schubert	A. hydrophila Susp. hydrophila (2 biotypes) Susp. anaerogenes (2 biotypes) Susp. proteolytica A. punctata Susp. punctata Susp. caviae
1976	Popoff and Veron	A. hydrophila (2 biotypes) A. sobria
1978	Shaw and Hodder	A. hydrophila A. sobria
1979	MacInnes et al.	A. hydrophila
1981	Holder-Franklin et al.	A. hydrophila (?)

in agreement with McCarthy (1975), that Schuberts A. hydrophila subs. proteolytica be removed from the genus. Supporting the classification of Popoff and Veron, Shaw and Hodder (1978) found that the carbohydrate moeities of the core lipopolysaccharides of A. hydrophila and A. sobria were sufficiently distinct to warrant separate species.

MacInnes et al. (1979), examining polynucleotide sequences among 24 motile aeromonads proposed that these organisms comprise a single species, A. hydrophila. The group of organisms variously described phenotypically as A. hydrophila, A. punctata and A. sobria were found to possess no internal homology groups and significant divergence in related sequences. Holder-Franklin et al. (1981) unwilling to state that the motile members of the taxon should be grouped into one species A. hydrophila, pointed out that a much more rigorous treatment of the classification of Aeromonas needs to be undertaken before any definitive conclusions can be made about dividing the species into subspecies.

In summary, it is quite apparent that phenotypic similarities do not necessarily coincide with genetic relationships. An exhaustive study of the taxon using genetic, serologic and physiologic characteristics is paramount before any consistent conclusions concerning the existence of biotypes can be made.

BIOCHEMICAL AND MORPHOLOGICAL PROPERTIES

A. hydrophila strains have the following characteristics; gram negative straight rods measuring approximately 0.5 X 1.0 micron, polar flagella usually monotrichous; facultative anaerobes, fermenting carbohydrates with formation of acid and/or gas; production of 2,3 butanediol; cytochrome oxidase positive; reducing nitrates; insensitive to the vibriostatic compound (2,4 diamino-6, 7-diisopropyl pteridine (0/129); G-C content of DNA, 57 to 63%. There is some variation in colonial morphology though colonies are generally circular, smooth and raised.

The taxon is phenotypically quite diverse with few other absolute phenotype consitencies though Popoff and Veron (1976) did find that 28 biochemical properties were found consistently in all of the strains they examined. Table 4 details the highlights of the biochemical analysis performed by Popoff and Veron (1976) on 68 strains in an attempt to differentiate the A. hydrophila-A. punctata taxon into subspecies. Popoff and Veron, as previously mentioned, divided the A. hydrophila-A. punctata group into two main strains: A. hydrophila and A. sobria. The phenotypic bases for this distinction is quite apparent (Table 4). Though it is quite evident from the literture that these phenotypic similarities allow an apparent division of the taxon into several subspecies, the genetic basis for this division is flimsy (MacInnes et.al. 1979).

Extracellular Factors

Strains of A. hydrophila produce many extracellular enzymes that may be virulence factors and possibly virulence

Table 4. Popoff and Verons proposed taxonomic scheme for the A. hydrophila-A. punctata group

Biochemical Property	Strain		
	A. hydrophila		A. sobria
	hydrophila	anaerogenes	
Growth on L-hist.	+	+	-
Esculin hydrolysis	+	+	-
Growth on KCN medium	+	+	-
Growth on L-arabinose	+	+	-
Fermentation of salicin	+	+	-
Growth on L-arginine	+	+	-
Growth on salicin	+	+	-
Elastase	+	-	-
Acetoin production	+	-	±
Gas from glucose	+	-	+
H_2S	+	-	+

determinants. These gelatinase(s), caseinase(s), elastase(s), lipase(s), lecithinase(s), staphylolyase(s), deoxyribonuclease and ribonuclease (Nord et al. 1975). Hemolysins, cytotoxins and enterotoxins are also produced (Caselitz et al. 1960; Wretlind et al. 1971; Boulanger et al 1977; MacIntyre et al. 1978; Donta et al. 1978; Rigney et al. 1978; Cumberbatch et al. 1979; Riddle et al. 1981).

The potential contributions of these factors to virulence is reviewed in another section.

Hemagglutination

One of the most critical steps of pathogenesis is attachment. Very little work has been done on the mechanisms of attachment of A. hydrophila. Atkinson et al. (1980) dmonstrated that many strains of A. hydrophila strongly agglutinate human blood cells. Hemagglutination can readily be inhibited by specific sugars. A role of pili in hemagglutination was also observed. Their work demonstrated that strains of A. hydrophila possess a variety of attachment mechanisms. The importance of these to classification and to the ability to produce disease remains to be established.

Plasmids

Roles of plasmid mediated products in the virulence of many diverse microorganisms, including the important fish pathogen Vibrio anguillarum are well documented. The existence of resistance factor plasmids in A. hydrophila is also well documented (Aoki et al. 1971b, 1972, 1973; Shotts et al. 1976). No published information is available on the occurrence of non R factor plasmids in A. hydrophila. Lewis (personal communication) has found multiple plasmids, three to six, in all of the strains examined. No clear cut pattern has emerged relating a phenotypic function to the presence or absence of a plasmid though preliminary evidence suggests that a colonization factor, probably pili, may be plasmid mediated.

Antigenic Structure

As if the taxonomic situation was already not complicated enough by the phenotypic and genotypic variations noted in this taxon, the antigenic diversity of A. hydrophila is potentially an even greater source of confusion. The antigenic studies to date have dealt primarily with the following areas: 1) serologic specificity of extracellular antigens and 2) diversity of somatic (O) and flagellar (H) antigens.

Liu (1961), examined the serologic specificity of extracellular material. He demonstrated that strains described as A. hydrophila, A. punctata, A. liquefaciens and A. formicans produced extracellular toxins, the biologic activities of which

were neutralized by antiserum to the extracellular toxins of a strain described as A. liqu faciens. One of the primary conclusions that can be drawn from this work is that many organisms previously considered sufficiently dissimilar to warrant being placed into separate species were found to elaborate extracellular materials that were quite similar. Furthermore, the extracellular antigens of A. hydrophila were quite distinct from those of A. salmonicida, Serratia species and other genera of Enterobacteriacae. Liu's preparations were crude extracts of extracellular antigens. The use of specific enzymes as antigens has shown that some extracellular antigens do cross react serologically with antigens from other microorganisms. One protease fraction has been shown to be serologically identical to a protease fraction of V. cholerae (Dahle et al. 1971). A casein hydrolyzing protease produced by A. salmonicida also appears to be produced by A. hydrophila. In contrast no cross reactivity was observed when lipases were extracted and studied (Gonzalez 1963, 1964). Thune (personal communication) has found that antiserum prepared against hemolysin from a single A. hydrophila strain neutralized the biologic activity of hemolysins from all other strains examined. He also noted lines of identity in Ouchterlony gels between heat labile proteases. The potential usefulness of extracellular antigens in diagnosis and immunoprophylaxis is an area that warrants further research.

Many serologic investigations have been done on the O antigens of A. hydrophila. The species has been found to have a heterogenous lipopolysaccharide antigenic structure (Eddy 1960; Liu 1961; Shaw et al. 1981). Snieszko et al. (1938) found very little cross agglutination among strains of A. liquifaciens isolated from carp. Guthrie and Hitchner (1943) examined eleven strains of A. hydrophila and found them antigenically heterogeneous. Miles and Miles (1951) also noted antigenic heterogeneity when twelve strains of A. hydrophila were examined for cross agglutinating properties. Ewing et al. (1961) found twelve provisional O antigen groups and nine H antigen groups among 71 A. hydrophila strains studied. They also found a number of serotypes within each group. Bullock (1966) found little cross agglutination among strains, but did note serologic cross reactivity of extracellular antigens, confirming the work of Liu (1961). Rao et al. (1977) found that strains of A. liquefaciens, A. hydrophila, A. punctata and A. punctata caviae possessed species specific and interrelated O-antigen complexes. Takahashi et al. (1977), examined thirteen strains of A. hydrophila by cross agglutination with adsorbed and non-adsorbed antiserum and found no antigenic homogeneity. Fliermans et al. (1977), using direct flourescent microscopy, examined 255 A. hydrophila strains from diverse environmental sources. They noted that strains associated with infections of bass and alligators were serologically distinct from American Type Culture Collection and water isolates, though they did cross react antigenically. Kingma (1978) prepared seven specific rabbit antisera against

Table 5. Summary of serologic cross reactivity of *Aeromonas hydrophila* strains from various environmental sources (adapted from Kingma 1978)

Source of Isolates	Number of Isolates	Total % Agglutinated by Antiserum
Florida Tropical Fish	93	18
Leetown Culture Collection	61	21
Totals	154	19.5

heat stable antigens of A. hydrophila strains from a variety of sources. Out of 154 A. hydrophila strains tested for their abilities to be agglutinated by his type specific antisera only 19.5% gave positive reactions (Table 5). Kingma's data and that of previous investigators repeatedly reiterate the serologic heterogeneity of the taxon A. hydrophila.

Though most of the papers discussed focus on the heterogeneity of this taxon, some of the work suggests that there are common antigenic determinants among strains from diverse sources (Fliermans 1978; Lewis, personal communication). A large scale examination of serologic relationships among motile aeromonads might reveal more common antigenic determinants.

Bacteriophages

Stevenson recent work

No work appears to have been published dealing specifically with bacteriophage typing of A. hydrophila. Bacteriophage typing has been used to identify A. salmonicida. The phages isolated display a high degree of specificity for A. salmonicida. Popoff (1971) found that thirteen bacteriophages were capable of infecting one hundred and one strains of A. salmonicida, but of forty A. hydrophila strains, only 9.5% were phage sensitive. Paterson (1974) found that bacteriophage specific for A. salmonicida formed no plaques with A. liquefaciens. Due to the complex serology of the taxon it is doubtful that bacteriophage typing, in the near future, will find much use. However in the long run, it may be extremely helpful in sorting out some of the serologic confusion. Unfortunately it appears that this is not being pursued.

Pathogenesis

As has been previously noted, strains of A. hydrophila have been isolated from a variety of environmental sources, including water, sediments, diseased and healthy animals. Strains of A. hydrophila have been associated with a wide range of infections affecting cold and warm blooded animals (Davis et al. 1978). Because of this, there has been a question concerning the degree of virulence of strains from various sources.

De Figueiredo et al. (1977) examined the virulence of nine A. hydrophila strains isolated from diseased fish, diseased freshwater shrimp and pond water to channel catfish (Ictalurus punctatus) fingerlings. The mean LD_{50}'s (the number of organisms required to kill 50% of the fish) ranged from 6.4×10^4 to 1.47×10^6 (Table 6). Significant differences in the ability to kill catfish were observed between strains isolated from water as compared to strains from diseased fish. It would appear that the source of the isolate may be related to its ability to produce disease.

Lallier et al. (1980) confirmed these observations when they found that strains of A. hydrophila isolated from healthy and diseased fish were more virulent for rainbow trout than strains of A. sobria isolated from healthy fish. This finding, though

Table 6. LD_{50}'s for channel catfish (Ictalurus punctatus) of Aeromonas hydrophila from fish and pond water (Adapted from De Figueiredo 1977)

Origin	LD_{50} (average of triplicate tests)
Fish kidney	1.08×10^5
Fish kidney	4.12×10^5
Fish kidney	4.2×10^5
Fish kidney	6.4×10^4
Pond Water	1.4×10^6
Pond Water	1.02×10^6

done with a relatively small number of isolates, further supports the classification scheme of Popoff and Veron (1976). Mittal et al. (1980) examined 25 strains of A. hydrophila in an attempt to correlate surface characteristics with virulence. They found that the most virulent strains (LD_{50} of $10^{4.5}$) shared a common O antigen, did not agglutinate in acriflavin, settled down after boiling and were resistant to the bactericidal action of mammalian serum. In contrast, the least virulent strains (LD_{50} greater than $10^{5.5}$) did not share a common O antigen, were sensitive to the bactericidal action of mammalian serum, and failed to settle down after boiling. The significance of their observed relationship between cell surface characteristics and virulence remains to be seen.

Olivier et al. (1981) examined the toxigenic properties of forty strains of motile aeromonads. Only those strains identified as A. hydrophila produced a dermonecrotic factor, as demonstrated by a rabbit skin test, and zones of hemolysis on blood agar plates at 10°C and 30°C. Those strains identified as A. sobria did not produce a dermonecrotic factor and only produced hemolysis at 30°C. All strains produced enterotoxins. They postulate that only A. hydrophila strains could be associated with hemorrhagic septicemia of fish.

Hsu et al. (1981) examined 127 shrains of A. hydrophila for the ability to utilize elastin, casein, gelatin, starch, esculin and to lyse staphylococcal cells. They observed a correlation between high levels of elastase and staphylolytic activity and high virulence for catfish.

Rigney et al. (1978) found that crude hemolysin failed to produce typical red leg disease symptoms in frogs, though when injected in combination with endotoxin did produce symptoms. Allan et al. (1981) demonstrated that crude extracellular products of A. hydrophila, possessing proteolytic and hemolytic activities, could produce pathology in trout. Their work suggests that hemolysin may be a virulence determinant. Thune et al. (1982b) also demonstrated that crude extracellular preparations from A. hydrophila, containing hemolysin, heat stable and heat labile proteases could produce gross clinical signs similar to those produced by the whole organism. Partially purified proteases were found to be lethal with the heat labile protease having an LD_{50} of 106.25 microgram/5.8g fish and the heat stable protease having an LD_{50} of 17.5 microgram/5.8g fish (Thune et al. 1982b).

It is apparent that much more work needs to be done before any definitive conclusions concerning the role of proteases and hemolysin(s) as virulence determinants can be drawn. There is strong evidence that they are virulence factors. The relationship of these extracellular products to cytotoxins and enterotoxins is not clear and warrants further investigation.

Transmission

Bacterial hemorrhagic septicemias due to strains of A. hydrophila may be transmitted through the water, diseased and

healthy fish and other affected vertebrates, external and internal parasites. A reservoir of potential pathogens probably exists in all natural and artificial bodies of water. Lewis and Bender (1961) demonstrated water borne infectivity by infecting golden shiners (Notemigonus crysoleucas) with several scales removed simply by exposing them to water containing approximately 1000 cells of A. liquefaciens per ml. This level of organisms approximates what could be expected in a natural aquatic environment (Hazen et al. 1978; Kaper et al. 1981). They also noted that stressful handling contributed to infection (Lewis et al. 1960).

It is apparent that healthy fish and fish recovered from epizootics often harbor and shed strains of A. hydrophila without being or becoming ill. These fish are a possible source of disease. Any type of stress such as crowding, excessive handling, high water temperatures, low dissolved oxygen levels, poor nutrition, etc. can cause healthy carrier fish to break out with the disease (Rock et al. 1965; Haley et al. 1967; Shotts et al. 1972). Failure to promptly remove diseased fish, allowing non-affected fish to cannabalize diseased mortalities, as well as elevating the levels of the causative microorganism in the water will no doubt contribute to the severity of a problem. Since frogs and fishes in the same area are often infected, it is probable that there may be intraspecies transmission of the disease. The role of external and internal parasites in transmission of the disease is probably much greater than is generally assumed to be the case. Dombrowski (1953) isolated A. liquefaciens from copepods (Argulus foliaceus) and from leeches (Piscicola geometra) that had been feeding on diseased carp. He also demonstrated that the disease was transmissable to healthy carp if they were parasitized by A. liquefaciens containing copepods or leeches.

Period of Communicability

There is no defined period of communicability. Due to the ubiquitous nature of A. hydrophila, fish can be at risk any time. However, the role of stress in increasing the potential for outbreaks of the disease cannot be understated (Snieszko 1974). As with many other diseases, healthy fish in a healthy environment are much less likely to contract the disease than fish in an unhealthy environment.

Carrier State

Since healthy fish harbor A. hydrophila, it would appear that many fish are carriers of the disease. No doubt fish recovered from the disease also serve as a reservoir for the disease. The carrier state, as in many other like fish diseases, is a lifelong phenomenon.

THE DISEASE

Symptoms, Histopathology

Since the disease caused by A. hydrophila is a bacterial hemorrhagic septicemia (BHS), the symptoms of the disease are similar in appearance to those found in other bacterial hemorrhagic septicemias. Symptoms and histopathology have been described by many workers (Schaperclaus 1930, 1954; Spiczakow 1933; Amlacher 1961; Gaines 1972; Wolke 1975; Bach et al., 1978; Huizinga et al. 1979), and can be differentiated into four general categories (Amlacher 1961, Otte 1963) (Table 7). This division is idealized since diseased animals may display a range of symptoms depending upon the state of the disease.

External symptoms are usually not present in acute outbreaks of the disease. Internal congestion of organs, hemorrhagic lower intestine, rectal prolapse and petechiae in the peritoneum and musculature may be present. Acute outbreaks are often associated with handling or crowding stress in waters with elevated water temperatures. Losses may be extensive. In 1973, 37,500 fish died over a thirteen day period in a North Carolina lake (Miller 1976).

According to Snieszko et al. (1971), the most frequent and severe epizootics occur in spring when water tempertures are increasing and the fish are stressed from overwintering under less than ideal conditions. Under these conditions, carp will often develop a dropsy-like condition resulting in mortality in a few days. Clear, yellow or bloody ascites fluid accumulates in the abdomen often accompanied by scale protrusion due to the pressure of fluid accumulation. Degenerative pathology of the liver, spleen, kidneys and intestinal tract are often observed. Eyes may protrude (exophthalmus) due to fluid accumulating behind the eye.

The less acute or chronic form of the disease is characterized by the appearance of shallow, thin blisters. Huizinga et al. (1979) described the histopathology of similar lesions and the transition from pinpoint lesions to chronic ulcerative lesions in naturally and artificially infected largemouth bass. These are described in Table 8. Visible, dark scars are often apparent in fish that have recovered from the disease.

In the "latent" form of the disease, there are no visible internal or external symptoms though bacteria can be isolated from internal organs, peritoneum, etc. These fish often have anti-A. hydrophila antibodies and quite likely serve as carriers and vectors of the disease.

DIAGNOSIS

Isolation and Identification

A presumptive diagnosis may be made based upon external symptoms. However, all of the bacterial hemorrhagic septicemias have similar external pathologies. For a definitive diagnosis, the

Table 7. Summary of disease symptoms of bacterial hemorrhagic septicemia due to Aeromonas hydrophila (from Snieszko and Axelrod 1971)

1. Acute, rapidly fatal septicemia with few gross symptoms.
2. An acute form with dropsy, blisters, abscesses, and scale protrusion.
3. Chronic ulcerous form with furuncles and abscesses.
4. Latent form with no symptoms.

Table 8. Detailed description of three stages of skin lesions (Huizinga et al. 1979)

Lesion		Histopathology
early pinpoint	macroscopic -	confined to one or two scales; hemorrhagic, edematous with peripheral capillary congestio
	microscopic -	epidermal necrosis, edema, inflammation
acute	macroscopic -	white to yellow in color; raise edematous areas diffusely sprea over a few to 30 scales; epiden and dermal necrosis in center lesion with hemorrhage, capilla congestion and central necroti exudate
	microscopic -	extensive hyperplasia of dermis fibrocyte proliferation and infiltration; dermis and under-lying muscle show early signs of necrosis and edema. Mono-nuclear cells and granulocytes are infiltrated throughout the lesion
chronic ulcerative	macroscopic -	crater shaped; dermis in center of lesion eroded with exposure of necrotic underlying muscle, epidermis thickened around margins of ulcer
	microscopic -	loss of epidermis and dermis, underlying muscles become severely necrotic; no inflam-matory cells present in necrotic muscle. Epidermis adjacent to lesion undergoes hyperplasia to form raised margin. At this phase, the infection becomes systemic.

etiologic agent must be isolated and identified. A number of workers have described methods for isolation and identification (Griffin et al. 1951; Ewing et al. 1961; Meeks 1963; Kozlowski et al. 1966; Bullock 1971, 1974; Shotts and et al. 1973. Standard method for isolation apply (Bullock 1971).

Lesions and target organs such as kidney, spleen and liver and ascites fluid are asceptically sampled and streaked onto either trypticase soy agar, if no other contaminating bacterial strains are anticipated or Rimler-Shotts (R-S) medium (Shotts et al. 1973) if mixed cultures are expected. R-S medium is a selective and differential medium that is useful for primary isolation of A. hydrophila from material where the probability of contamination with other types of bacteria, particularly of other genera of the family Enterobactericeae, is great. After inoculation, the plates are incubated at 37°C for 20-24 hours. Colonies of A. hydrophila are yellow; those of Escherichia, Enterobacter spp and Pseudomonas spp are greenish and those of Edwardsiella spp are green with black centers. Though this media has been extremely useful in increasing the ease of identification of A. hydrophila, it has been found to be useless in differentiating A. hydrophila from Group F or EF6 vibrios (Kaper et al. 1981). Because of this, Kaper et al. (1981), substituted MacConkey agar with trehalose in lieu of lactose for R-S in their ecological studies. In light of the drawbacks of R-S, care must be taken in attempting to identify A. hydrophila solely on the basis of this differential media. In general, a gram negative motile rod, cytochrome oxidase positive, fermentative, 0/129 resistant, isolated from fish displaying the symptoms of BHS is quite likely a strain of A. hydrophila.

Serological identification of A. hydrophila may never be feasible unless polyvalent antisera prepared against strains that cross react serologically with most other strains could be prepared. Bullock (1966) reported that an agglutination test with flagellar antigens was not reliable, presumably due to H antigen heterogeneity. However, he noted that an agar gel precipitin test, with soluble somatic antigens gave a more reliable identification (Bullock 1966). Most of the strains tested formed precipitin bands with antisera from A. liquefaciens cell extract material. It seems doubtful that in a taxon as heterogeneous as the A. hydrophila taxon that serological tools using H or O antigens will find a routine use in diagnosis. Though in light of the work of Liu (1961), Bullock (1966) and Thune (personal communication) some type of serologic test useful for rapid identification of A. hydrophila may eventually be devised using extracellular antigens.

TREATMENT

Chemotherapy

Several antibiotics have been used to treat A. hydrophila infections, with variable success (Table 9). Many of the documented reports lacked proper controls thus preventing determination of efficacy. Oxytetracycline, furanace and chloramphenicol have all

Table 9. Summary of treatment regimens

Date	Author	Drug	Comments
1940	Smith	Chloramphenicol	Treated frogs by gastric intubation of 3-5 mg/100g twice daily for five days- seemed successful.
1951	Seaman	sulfamerazine	200-300 mg/kg of fish per da apparently controlled disease in rainbow trout.
1963	Gibbs	tetracycline-HCL	Same as Smith 1950; 5 mg/30g frog twice daily for six days
1964	Meyer	oxytetracycline	Oral-5.5 mg/kg of golden ski per day for ten days; lost 95 of untreated fish.
1965	Chodyniecki	chloramphenicol	Mixed with feed; administered to carp-30 mg/kg/day - no therapeautic value.
1967	Fijan et al.	chloramphenicol furazolidone	Oral administration of 10-40 chloramphenicol; 160-320 mg furazolidone/50 g carp for five days - good results.
1976	Snieszko et al.	furanace	Immersion for 5-10 min. in water containing 1-2ppm or 1 week in water containing 0.1ppm.

1981 Plumb et al furanace

been used successfully. In the United States, only one of these, oxytetracycline, has been approved for use in fish used for food. The approved use is 50-75 mg/kg of fish for ten days mixed with the feed (Wood 1969). It is particularly useful when fish are handled, crowded, or held under stress for short periods of time. As with any other antibiotics, use should not substitute for good management practices. Abuse of antibiotics will lead to the development of drug resistant strains, and resistance factors have been found in A. hydrophila (Aoki et al. 1971, 1972, 1973; Shotts et al. 1976). The development of resistant strains may force the farmer to use less desirable antibiotics.

PREVENTION AND CONTROL

Management Procedures

It is well established that motile Aeromonas septicemia or bacterial hemorrhagic septicemia is strongly associated with waters containing heavy loads of organic material and fish parasites. Periodic disinfection and thorough cleaning or drying, of ponds or raceways if possible, can help to decrease the potential for serious problems. Mattheis (1966) found that quick lime or calcium cyanamide ($CaCn_2$) applied at a rate of 10 tons/hectare destroyed Pseudomonas-Aeromonas bacteria in the top three centimeters of the surface layers of ponds.

Since disease outbreaks due to A. hydrophila are often associated with stress, elimination of or minimizing the stress can effectively prevent and control the disease. Obviously, this is not always possible. Close monitoring and control of environmental parameters such as dissolved oxygen and temperature and avoiding handling, crowding and other stresses will reduce the incidence of the disease. In a classical example of the effect of avoiding stress, Rychlicki et al. (1975) observed that moving carp in the fall with handling early in the spring resulted in 43% mortality while carp overwintered in nursery ponds and not moved until late spring experienced only a 10% loss due to bacterial hemorrhagic septicemia. Apparently, at particular times during the growth cycle, the stress associated with handling can dramatically increase the susceptibility of fish to bacterial hemorrhagic septicemia. Boosting vitamins and trace element levels in feeds prior to and during overwintering of fish may help to decrease the depletion of crucial nutrients affecting the ability of the fish to defend itself.

Prophylactic Chemotherapy

The best way to prevent bacterial hemorrhagic septicemia is to improve the overall quality of the environment for the fish. Since this is often not economical nor feasible, the use of antibiotics to prevent the disease from occurring has been widely investigated and is a common practice in central Europe where millions of carp are treated prophylactically with antibiotics every year. In the mid 1950's, Schäperclaus (1956, 1958, 1959) examined

the affect of antibiotic prophylactic therapy upon the incidence of bacterial hemorrhagic septicemia. Of the antibiotics examined, chloramphenicol seemed to provide the best results in terms of duration and degree of protection. By a single intraperitoneal injection of carp, (20-50 mg/kg - transferred in early spring) mortality was reduced by 80-90%. Chodyniecki (1964) also reported significant reductions in mortality obtained by injecting carp with detreomycine. Two injections appeared to provide a higher level of prophylaxis than did one; only 2.8% of the fish treated died whereas 87.8% of an untreated control group died.

As with the use of any drug, a number of variables affect the success of a prophylactic regimen using injected antibiotics. Halvelka and Volf (1965) postulated that the failure of an injected antibiotic to protect a population of carp from mortality was due to the antibiotic levels diminishing before the water temperature rose and the carp had build up an immunity. Though the prophylactic use of antibiotics appears to have a great potential in minimizing the risks of bacterial hemorrhagic septicemia the use of antibiotics should never serve as a substitute for improving management practices. Antibiotics are a powerful weapon for dealing with disease problems that can not be solved in any other economical fashion. However, abuse of antibiotics, under the guise of prophylaxis or disease treatment, can lead to the emergence of drug resistant strains of the target pathogens and possibly other human pathogens (Aoki et al. 1971a,b, 1972, 1973; Shotts 1976).

Immunization

There are two major approaches to the production of bacterins or vaccines against bacterial hemorrhagic septicemia. One approach has been to prepare an inactivated whole cell bacterin composed of single or multiple strains. A polyvalent preparation would have to contain strains of A. hydrophila that share cell wall antigenic determinants with a sufficient percentage of known isolates to ensure protection. Most of the work has been done with monovalent bacterins. There has been no consistent success with this approach. The other approach involves the preparation of toxoids consisting of inactivated virulence factors or determinants. No data has yet been generated to support this as a viable means of immunization.

As previously discussed, A. hydrophila is an antigenically diverse group of organisms. Any bacterin dependent upon cell wall antigens for protection would either have to be very limited in its range or efficacy or consist of strains that cross react serologically with the majority of all known strains. The first reports of immunization against bacterial hemorrhagic septicemia using killed and living cultures were by Schäperclaus (1954). In field trials carried out from 1936 to 1951, the average losses of immunized yearling carp were 7.3% and in non-immunized carp 22.2%. Moreover, when carp were selected that had survived natural epizootics followed by an artificial challenge with the pathogens, a 10% mortality occurred contrasted with 60% for non-selected adults. Post (1966)

Table 10. Mortality in vaccinated catfish challenged with the homologous strain

	Challenge level 10^6 orgs/ml. % Mortality
Controls	44.9
Swim up fry	13.0
Sac fry	7.1

Table 11. Mortality in vaccinated catfish challenged with a heterologous isolate

		% Mortality	
		Control	Vaccinate
Expt.	1	37	31
	2	37	31
	3	64	66

demonstrated that a bacterin consisting of saline suspended heat killed A. hydrophila could protect fish against a challenge with the homologous organism. No challenge was done with a heterologous organism. He also found that injection of the bacterin provided at least eight months of protection.

All these reports have dealt with vaccination and challenge with a homologous organism. It was suspected by Schäperclaus and is now commonly felt that large scale immunization against A. hydrophila using heat stable cell wall antigens is not possible because of the serologic heterogeneity of the taxon. Schäperclaus (1954) apparently had his first insight into this by observing that when carp from two wintering ponds were transferred to separate summer ponds, the incidence of disease due to A. hydrophila was less than when the ponds received populations from several winter ponds. The most plausible explanation for this was that the carp in the wintering ponds had developed some immunity to the strain present in the pond but had no immunity to the strain present in fish from other ponds. In 1967, he generated further experimental evidence to substantiate this. Using a different type of bacterin, still monovalent, he found good protection against homologous challenge. However, in field trials, he obtained unsatisfactory results, presumbably due to challenge with a heterologous strain.

AcuiGrup (1980) field tested a monovalent A. hydrophila bacterin in rainbow trout. They immunized the fish by intraperitoneal injection and hypersmotic infiltration immersion. Their results were inconsistent though increased feed weight gain ratios were observed in some groups of vaccinates and substantial protection was apparent in some cases. They concluded that control of environmental factors is a significant factor in controlling the disease, independent of vaccination. One of the most interesting features of their work is a reiteration of the work of Schäperclaus, i.e. immunizing fish with a bacterin prepared against a species of A. hydrophila that is endemic in a particular area can result in significant protection.

Thune (1980) immunized channel catfish fry by immersion in a polyvalent sonicated bacterin of A. hydrophila. He found significant protection against challenge with homologous strains (Table 10) but poor protection against challenge with a heterologous, cross-reactive isolate (Table 11). It appears that a polyvalent bacterin might have to contain all of the isolates which the fish could encounter in order to provide adequate protection. This is obviously not feasible.

There is however some indication that this may not be necessary. Lewis (1978) found that autoclaving cell suspensions of A. hydrophila gave two types of antigenic preparations: 1) a soluble antigen which was specific to the isolate from which it was recovered and 2) a particulate component, polyvalent in nature. Solubilizing these cross-reactive core antigens such that their immunogenicity is retained might make immunization with a limited number of cross-reactive strains feasible.

Since lipopolysaccharide based bacterins have not shown much promise and may never lead to highly protective bacterins, a

number of workers have focused on the extracellular products (ECP) of A. hydrophila with the hope that ECP may be sufficiently serologically homogeneous to be useful in the development of a vaccine. As previously mentioned, ECP have been implicated in the pathogenesis of A. hydrophila by many workers (Schäperclaus 1939; Kou 1973; Boulanger et al. 1977; Bach et al. 1978; Allan et al. 1981; Olivier et al. 1981) though endotoxins may also be important (Bullock et al. 1971; Rigney et al. 1978). Bacteria free crude and partially purified preparations have been shown to produce mortality and pathology identical to that produced by the live microorganism (Allan et al. 1981; Thune et al. 1982). The serologic relatedness of some of the extracellular products is established and work is proceeding towards vaccinating against bacterial hemorrhagic septicemia using toxoid preparations. To date no successful immunization has been achieved.

Much more basic research needs to be done before a truly "universal" A. hydrophila bacterin, providing protection against most if not all strains of A. hydrophila, can be produced, if at all. The successful approach may utilize features of both of the approaches examined to date.