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# Infections by atypical strains of the bacterium *Aeromonas salmonicida*

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## SUMMARY

Infections due to atypical strains of the Gram-negative bacterium *Aeromonas salmonicida* cause atypical furunculosis and related diseases of both feral and cultivated fish stocks in freshwater and marine environment. More than 20 farmed and 30 wild fish species have been reported to harbor atypical *A. salmonicida*. The isolated strains are found to be a heterogeneous group as regards many phenotypic and genotypic characteristics. The clinical and pathological features of infection are different as many factors are involved, e.g. various hosts, strains and environment. Infections of fish in the temperate regions of the Northern hemisphere are most frequently reported, although disease problems have also occurred in other parts of the world as Australia, the Mediterranean and Chile. In Iceland atypical furunculosis has been the main bacterial disease in the salmonid farming industry. As diseases caused by atypical strains are of emerging importance worldwide, the prospects of their control by vaccination need to be considered. Currently all commercially available *A. salmonicida* vaccines are produced from typical *A. salmonicida* strains for prevention of classical furunculosis in salmonids. There is, however, evidence of cross protection against atypical furunculosis in Atlantic salmon vaccinated with commercial oil based furunculosis vaccine. Icelandic fish farmers have since 1992 vaccinated salmonids by injection with an autogenous bacterin produced by a commercial vaccine producer against atypical *A. salmonicida* and recently halibut farmers in Norway have started to use an autogenous injection vaccine against atypical furunculosis.

Key words: *Aeromonas salmonicida*, atypical strains, bacteria, fish, fish pathogens, furunculosis, infection, skin ulcers, vaccination.

## YFIRLIT

*Sýkingar atýpískra stofna bakteríunnar* Aeromonas salmonicida

Sýkingar atýpískra stofna Gram-neikvæðu bakteríunnar *Aeromonas salmonicida* valda kýlaveikibróður og hliðstæðum sjúkdómum í villtum og ræktuðum fiski, bæði í fersku vatni og sjó. Bakterían hefur verið einangruð úr yfir 20 tegundum eldisfisks og meira en 30 tegundum af villtum fiski. Rannsóknir á stofnasöfnum hafa leitt í ljós töluverðan breytileika á svipfari og erfðaeiginleikum atýpískra *A. salmonicida* stofna. Sjúkdómseinkenni eru breytileg og hafa ýmsir þættir þar áhrif, s.s. mismunandi hýslar, stofnar og umhverfi. Sjúkdómsvandamál eru algengust á norðlægum slóðum, en þó hafa komið upp vandamál í öðrum heimshlutum eins og í Ástralíu, Miðjarðarhafslöndum og Chile. Kýlaveikibróðir er sá bakteríusjúkdómur sem mestum skaða hefur valdið í íslensku fiskeldi. Þar sem vandamál vegna sýkinga af völdum atýpískra *A. salmonicida* stofna hafa aukist á veraldarvísu er nauðsynlegt að íhuga möguleika á notkun bóluefna sem sjúkdómsvarnar. Rannsóknir hafa leitt í ljós töluverðan mun á sýkiþáttum mismunandi *A. salmonicida* stofna. Týpískir stofnar eru einsleitur hópur hvað varðar framleiðslu ensíma sem eru virk í sýkingu bakteríunnar, en meðal atýpískra stofna er mikill breytileiki. Sem stendur eru öll markaðsett *A. salmonicida* bóluefni framleidd til varnar klassískri kýlaveiki í laxfiskum. Til eru niðurstöður sem sýna krossvörn gegn kýlaveikibróður í laxi sem var bólusettur gegn klassískri kýlaveiki. Íslenskir fiskeldis-

menn hafa frá 1992 bólusett laxfiska gegn kýlaveikibróður með sérlöguðu (autogenous) sprautubóluefni og lúðubændur í Noregi hófu nýlega bólusetningu með sérlöguðu sprautubóluefni gegn sýkingu atýpískra *A. salmonicida* stofna. Notkun sérlagaðra bóluefna takmarkast við það svæði eða þá stöð sem sá bakteríustofn sem notaður er við gerð bóluefnisins var einangraður á.

## INTRODUCTION

The first definitive isolation of the Gram-negative fish pathogenic bacterium *Aeromonas salmonicida* was from brown trout (*Salmo trutta*) in Germany by Emmerich and Weibel (1894) more than a century ago. As the bacterium has been a continious tread to the salmonid farming industry it has presumably been studied more than any other fish pathogen.

Currently the taxonomic status of *A. salmonicida* is within the family Aeromonadaceae, in the genus *Aeromonas* (Colwell *et al.*, 1986; Anonymous, 1992). Four subspecies of *A. salmonicida* have been described, i.e. ssp. *salmonicida, achromogenes, masoucida* and *smithia* (Holt *et al.*, 1994). *A. salmonicida* ssp. *salmonicida,* the causative agent of classical furunculosis of salmonids, is called typical, but other strains atypical. Although three subspecies within the atypicals have been described, some authors have suggested different delineation and new isolates that do not fit into the existing classification are frequently reported. Therefore, an atypical strain can only be defined as a strain that does not fit into the existing classification of *A. salmonicida* ssp. *salmonicida*.

*A. salmonicida* ssp. *salmonicida* has been described as a homogeneous taxon, with respect to biochemical and genotypic characteristics (Austin and Adams, 1996). On the contrary the group of atypical *A. salmonicida* strains consists of isolates showing a large variety of biochemical, molecular and virulence characteristics (Austin *et al.*, 1998).

One of the earliest indications of the existence of aberrant *A. salmonicida* strains dates back to 1963 when Smith described atypical non-pigmented strains (Smith, 1963). With the enormous expansion in aquaculture and cultivation of more fish species the economic importance of infections due to atypical *A. salmonicida* has concurrently increased. Epizootics occur in both cultivated and feral fish stocks and more than 20 farmed and 30 wild fish species have been reported to harbor atypical *A. salmonicida* (Wiklund and Dalsgaard, 1998).

### *Atypical* Aeromonas salmonicida

Like typical strains of *A. salmonicida*, atypical variants are described as a non-motile, oxidase-positive, fermentative, facultative anaerobic, Gram-negative rods. Coccoid forms occur frequently and staining has a tendency to be bipolar. The colonies on agar are after 2–5 days incubation circular, raised, friable and of a variable size. Many strains produce a water-soluble brown pigment when grown on tryptone-containing media (Popoff, 1984). The optimal growth temperature is 22–25°C and most strains do not grow at 37°C. However, there is a motile biogroup that does grow at this temperature. Furthermore, atypical as well as typical *A. salmonicida* that lack the oxidase reaction have been reported. This has made the identification of the bacteria more complicated. Serological differences have not been detected among fresh isolates of *A. salmonicida,* which makes serological methods feasible for identification to the species level (Bernoth, 1997).

Atypical isolates are characterized by biochemical properties differing from those described for *A. salmonicida* ssp. *salmonicida*. These most often include reduced or slow pigmentation, slow growth, nutritional fastidiousness (often requiring blood products), growth at elevated temperatures or oxidase-negativity. Although pigment production is usually associated with *A. salmonicida* ssp. *salmonicida*, there have been some reports of typical isolates that do not produce pigment (Wiklund and Dalsgaard, 1998). Phenotypic characteristics, which can be used for distinguishing subspecies and atypical strains of *A. sal-*

**Table 1**. Characteristics for differentiation between *Aeromonas salmonicida* ssp. *salmonicida* and atypical *A. salmonicida*. +, positive reaction (≥80%); –, negative reaction (≤20%); d, different results (21–79%); n.d, no data available; R, resistant; S, sensitive.
*1. tafla. Eiginleikar til að greina á milli* Aeromonas salmonicida ssp. salmonicida *og atýpiskra* A. salmonicida *baktería. +, jákvæð svörun (³80%); –, neikvæð svörun (£20%); d, mismunandi niðurstaða (21–79%); n.d, engar niðurstöður fundust; R, þolin; S, næm.*

| | Subspecies | | | | Atypical strains | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Austin *et al.*, 1998 Phenon | | | |
| Characteristic | *salmonicida* | *achromogenes* | *masoucida* | *smithia* | Nova | 1 | 2 | 7 | Fastidious flatfish isolates |
| Production of: | | | | | | | | | |
| Oxidase | + | + | + | + | + | + | + | + | d |
| Brown pigment | + | d | – | d | d | + | d | d | – |
| Indole | – | + | + | – | + | + | + | + | – |
| Metallo caseinase | – | + | – | – | – | d | d | d | – |
| Gas from glucose | + | – | + | + | – | n.d | n.d | n.d | – |
| Acid from sucrose | – | + | + | d | d | n.d | n.d | n.d | d |
| Degradation of: | | | | | | | | | |
| Blood | + | – | + | – | d | – | – | d | d |
| Esculin | + | – | + | – | d | d | d | – | + |
| Resistance to: | | | | | | | | | |
| Ampicillin 33 µg | S | R | d | S | d | d | d | R | S |
| Cephalothin 66 µg | S | R | d | d | d | R | d | d | d |

Data are from the following publications: Austin *et al.*, 1989; Olivier, 1992; Holt *et al.*, 1994; Wiklund *et al.*, 1994; Austin *et al.*, 1998; Wiklund and Dalsgaard, 1998. The data are obtained by different laboratories, methods and times of incubation (from 2–14 days at 20–25°C)—*Eftirfarandi heimildir eru fyrir niðurstöðum: Austin o.fl., 1989; Olivier, 1992; Holt o.fl., 1994; Wiklund o.fl., 1994; Austin o.fl., 1998; Wiklund og Dalsgaard, 1998. Niðurstöðurnar eru unnar af mismunandi rannsóknarhópum, sem notuðu mismunandi aðferðir og ræktunartíma (frá 2–14 dagar við 20–25°C).*

*monicida* are listed in Table 1. The data presented in Table 1 have, however, to be interpreted with caution, as it has been shown that large discrepancies occur in biochemical identification of atypical strains obtained in various laboratories (Dalsgaard *et al.*, 1998).

Atypical *A. salmonicida* strains have been isolated from a wide range of fish species in freshwater, brackish water as well as in seawater (Wiklund and Dalsgaard, 1998).

## GEOGRAPHICAL DISTRIBUTION

The geographical distribution of reported isolations indicate that atypical strains mainly infect in the temperate regions of the northern hemisphere, that is Canada, USA, Japan, and central and northern Europe, including the Nordic countries. However, atypical strains have also been isolated from fish in Australia and in the Mediterranean (Wiklund and Dalsgaard, 1998). Since 1995 infections by atypical *A. salmonicida* have also caused disease problems in cultivated salmon in the south of Chile, were the salmon production is very high. In this area fish farms have been using salmon eggs imported from countries where atypical *A. salmonicida* is endemic (Sandra Bravo, personal communication).

## ATYPICAL *A. SALMONICIDA* INFECTIONS

The term furunculosis is commonly used for all diseases of teleost fish caused by *A. salmonicida*. However, this is an inappropriate designation insofar as the classical necrotic lesions or ulcers that develop are not typical abscesses

as seen in mammals. In some regions infections by typical *A. salmonicida* strains may overshadow those of atypical strains, especially where pigment producing atypical strains are involved (Austin and Austin, 1993).

The clinical and pathological features of infections by atypical *A. salmonicida* strains can vary. Many factors are involved, e.g. environmental factors, virulence properties of the respective bacterium and different host responses. This makes the diagnosis difficult as the atypical strains are a heterogeneous group that infect various fish species both in freshwater and marine environment. Atypical *A. salmonicida* strains are the cause of ulcerative and systemic infections in a wide variety of fish including many economically important species as, salmonids, carp, goldfish, cod, eel, turbot, flounder, halibut and many others. The best known diseases include goldfish ulcerative disease, carp erythrodermatitis, ulcer disease of flounder, eel and salmonids and atypical furunculosis of salmonids and several other fish species. Apparently atypical strains are involved in more disease outbreaks in fish than was previously suspected (Wiklund and Dalsgaard, 1998).

Infections by atypical *A. salmonicida* have caused serious problems in the farming of salmonids in Canada and all the Nordic countries, with the exception of Denmark. In these countries problems have also occurred in the farming of non-salmonids as cod (*Gadus morhua*), halibut (*Hippoglossus hippoglossus*), common wolffish (*Anarhichas lupus*), spotted wolffish (*Anarhichas minor*), turbot (*Scophthalmus maximus*), wrasse (*Labrus berggylta*), European eel (*Anguilla anguilla* ) and goldfish (*Carrassius auratus*). Furthermore, atypical *A. salmonicida* has been detected in ulcerated wild fish of various marine and fresh water species. These include species such as sandeels (*Ammodytes lancea* and *Hyperoplus lanceolatus*), flounder (*Platichthys flesus*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), cod *(Gadus morhua*), haddock (*Melanogrammus aeglefinus*), whiting, (*Merlangius merlangus*), four bearded rockling (*Enchelyopus cimbrius),* bream (*Abramis brama*), dace (*Leuciscus cephalus*), minnows (*Phoxinus phoxinus*), perch (*Perca fluviatilis*), roach *(Rutilus rutilus*) and pike (*Esox lucius*) (Wiklund and Dalsgaard, 1998).

In Iceland atypical furunculosis caused by *A. salmonicida* ssp. *achromogenes* was first diagnosed in 1980, in the early days of the salmonid farming industry. Ever since it has been a threat to the fish farming industry. The disease problems have been most severe in fish reared in tanks with brackish water (salinity between 0.3 to 2%), where accumulative mortality as high as 30% has been recorded. The disease is frequently diagnosed in feral salmonids and outbreaks of the disease have also occurred in captive cod of wild origin reared in land based tanks. The susceptibility of halibut to the bacterium has been shown by an experimental infection. The bacterium has also been isolated from several wild fish species in the Icelandic waters (Gudmundsdóttir, 1997).

### *The clinical presentation of atypical* A. salmonicida *infection*

Atypical *A. salmonicida* infections associated with disease outbreaks in fish (e.g. atypical furunculosis of salmonids, cod and common wolffish) can be manifested, similar to furunculosis, as loss of appetite with darkening in colour and increased mortality. External clinical signs often include other features of an acute septicaemia like haemorrhage at fin bases and development of skin ulcers or lesions on the sides of the body. The gills are often pale with petechial haemorrhages. Internal features like hyperaemia of serosal surfaces, haemorrhages in internal organs and mucosa are frequently detected. The course of the disease can be peracute, acute, subacute or chronic as described for classical furunculosis. Pure cultures of the bacterium can be obtained from internal organs, for example the kidney, spleen and heart (Kimura, 1970; Paterson *et al.*, 1980; Morrison *et al.*, 1984; Groman *et al.*, 1992;

Olivier, 1992; Helleberg *et al.*, 1996; Gudmundsdóttir *et al.*, 1997; Helgason *et al.*, 1997).

In many cases, however, infections by atypical *A. salmonicida* (e.g. in salmonids, cyprinids, eels, wild flatfish species, etc.) cause an ulcerative disease with more superficial variety of pathological changes than furunculosis, starting as small haemorrhages in skin and progressing to multiple skin lesions. Other bacterial species often become involved, as secondary infections of the ulcers often occur, and the cause of death is not always clear. It has been observed that the fish can die although a bacteremia is not detected. In advanced stages of infection, however, bacteria are also found in the blood and internal organs. The ulcers can be located anywhere on the body surface, although they are most frequently found on the flanks. Infected fish may show inappetance, lethargy, loss of orientation, and abnormal swimming behavior. Bacterial isolation should be done from recently developed skin ulcerations as well as from internal organs (Mawdesley-Thomas, 1969; Fijan, 1972; Bootsma *et al.*, 1977; Nakai *et al*., 1989; Groman *et al.*, 1992; Austin and Austin, 1993; Wiklund and Bylund, 1993).

### *Comparison of* A. salmonicida *infections in Atlantic salmon and cod*

The variation in extracellular virulence factors produced by atypical and typical *A. salmonicida,* and within the heterogeneous group of atypical strains, may explain the different pathology caused by various *A. salmonicida* strains. Another reason is that the various hosts react differently.

Pathological changes have been compared in wild Atlantic salmons (2–3 kg) from an Icelandic river, that were naturally infected with typical or atypical *A. salmonicida,* respectively. The isolated organisms were classified as *A. salmonicida* ssp. *achromogenes* and *salmonicida,* respectively. A 20 kDa metallo-caseinase, AsaP1, was detected in the ECP of the ssp. *achromogenes* strain, but it did neither possess serine gelatinase nor a haemolytic activity. On the other hand the ECP of the ssp. *salmonicida* strain contained a 70 kDa serine protease, P1, and a glycerophospholipid: cholesterol acyltransferase, GCAT, which are the major exotoxins of typical *A. salmonicida* strains, but a metallo-caseinase was not detected in its ECP. The main difference of the gross pathology detected was that skin lesions caused by the ssp. *achromogenes* strains were usually shallower with haemorrhages at the edges, whereas lesions induced by ssp. *salmonicida* extended deeper into the muscle and haemorrhages were more extensive. Histopathological changes, in both cases, were similar to those typical for acute furunculosis, showing bacterial colonization in a variety of sites (skin, gills, spleen, pancreas, kidney, heart, brain and liver) with localized cellular necrosis. However, tissue damage induced by ssp. *salmonicida* was usually more severe and with more extensive necrosis (Gudmundsdóttir *et al.*, 1997).

Unlike the salmonids, cod shows a well-developed host reaction to atypical *A. salmonicida* including a marked leucocyte response with resultant encystment of the bacteria. Histopathological changes are uniform in the various tissues and characterized by granuloma formation. Centrally in the granuloma are colonies of bacteria usually with necrosis encircled by many layers of epitheloid cells, surrounded with a thin layer of fibroblasts. These changes are very different from those caused by atypical *A. salmonicida* strains in salmonids, even when the isolated strains are akin as regards biochemical characters and exotoxin production (Helgason *et al*., 1997).

## VIRULENCE FACTORS

Although *A. salmonicida* has been known as a fish pathogen and studied for over 100 years, its virulence mechanisms are still only partly understood. The application of sophisticated biochemical techniques in the recent years, however, continues to yield considerable new information. In a systemic infection like fu-

runculosis and related diseases, the successful pathogen must have properties that allow it to avoid, withstand, or overcome the nonspecific and immunospecific defense mechanisms of the host. Atypical and typical strains of *A. salmonicida* have been reported to share cell-associated antigens like lipopolysaccharide (LPS) and outer membrane proteins, but extracellular virulence factors produced by typical and atypical strains are more different (Austin *et al.*, 1998).

### *Cell-associated antigens*

The cell envelope of Gram-negative bacteria consists of a cell membrane, a peptidoglycan layer and an outer membrane. *A. salmonicida* has a surface protein layer (A-layer) external to the outer membrane which is interspersed between the repeated o-polysaccharide (O-antigen) subunits of the LPS (Udey and Fryer, 1978; Evenberg *et al.*, 1985). The A-layer plays an important role in the virulence of the organism as mutants lacking it have lost their pathogenic properties (Noonan and Trust, 1995a). Like many other bacterial surface layers the A-layer is composed of a single protein subunit, the A-protein (or VapA), which assembles on the surface to form a tetragonal array surrounding the entire cell (Udey and Fryer, 1978). The structural gene for the A-layer protein has been cloned (Chu *et al.*, 1991). The A-layer is multifunctional. It serves to protect *A. salmonicida* from serum effects (Munn *et al.*, 1982), the action of proteases (Chu *et al.*, 1991) and from phagocytosis (Trust *et al.*, 1996). Furthermore, it facilitates binding to certain porphyrins, immunoglobulins and a range of extracellular matrix proteins (Trust *et al.*, 1996). Results by Noonan and Trust (1995b) indicate that the primary requirement for the A-layer of *A. salmonicida* may be at the early stages of infection. The LPS endotoxin is an essential component of the outer membrane. It is composed of three moieties: lipid A, a core oligosaccharide and the O-antigen, which is exposed at the cell surface. The O-antigen carries the antigenic determinants that constitute the serological specificity. The characteristic endotoxic effects of Gram-negative bacteria are associated with the lipid A moiety. Fish have been shown to be extremely resistant to the lethal effects of LPS (Berczi *et al.*, 1966). The LPS of *A. salmonicida* have a structural function in assembly and maintenance of the A-layer (Noonan and Trust, 1995b) and are found to be species specific (Chart *et al.*, 1984).

The A-layer protein and LPS of typical and atypical strains are serologically cross reactive and the most potent *A. salmonicida* antigens in inducing antibody response in Atlantic salmon (Bjørnsdóttir *et al.*, 1992), although anti A-protein antibodies have not been shown to be protective (Gudmundsdóttir and Magnadóttir, 1997).

### *Iron uptake mechanisms*

One mechanism involved in host defense involves the binding of free iron to proteins such as transferrin to create iron-restricted conditions within the host. As iron is essential for bacterial growth, a successful pathogen must have mechanisms enabling it to compete with the iron binding proteins in the serum and extracellular fluids of the host. Such mechanisms include the production of siderophores and the induction of outer membrane proteins capable of binding iron containing host proteins. Typical and atypical strains of *A. salmonicida* possess an effective high-affinity iron-uptake mechanisms. However, atypical and typical *A. salmonicida* strains use some different mechanisms to acquire iron under conditions of iron-restriction. Hirst and Ellis (1994) identified four iron regulated outer membrane proteins (IROMPs) that are shared by typical and atypical strains. Furthermore, their results indicated that the IROMPs of *A. salmonicida* are important for induction of antibodies that are bactericidal for a virulent *A. salmonicida* strain *in vitro* and protective against furunculosis. Only typical *A. salmonicida* strains produce siderophore for iron uptake, but the atypical strains that have been tested so far, acquire

iron in the host by a siderophore-independent system (Hirst and Ellis, 1996).

### *Extracellular virulence factors*

It is well known that environmental conditions affect synthesis of certain bacterial components, a fact that must be kept in mind when results from different laboratories are compared. Studies of the virulence properties of *A. salmonicida* have mainly been performed using ssp. *salmonicida* strains grown *in vitro* and samonids as the host. Most of the available information has been obtained by biochemical characterization of secreted enzymes and toxins, but recently analysis at the genetic level have been carried out.

Extracellular products (ECP) of some atypical strains have been reported to be lethal for salmon and carp. The toxicity of the ECPs is of a proteinous nature and some virulence related factors have been identified (Gudmundsdóttir, 1997). It has been shown by genetic methods, that there are atypical strains that produce the P1 protease and/or the GCAT cytotoxin, which are the major exotoxins of typical strains, but some strains do not produce either of these enzymes (Austin *et al.*, 1998). A 20 kDa metallo-caseinase, AsaP1, has been isolated from the ECPs of some atypical strains and identified as the major exotoxin of a group of atypical strains including type strains for *A. salmonicida* ssp. *achromogenes* (Gudmundsdóttir and Magnadóttir, 1997; Gunnlaugsdóttir and Gudmundsdóttir, 1997). The AsaP1 toxin is a powerful mitogen of Atlantic salmon leukocytes but does not exert cytotoxic activity (Gudmundsdóttir, *et al.*, 1995). It has been shown that AsaP1 can induce protective immunity against atypical furunculosis in Atlantic salmon (Gudmundsdóttir and Magnadóttir, 1997). In a study where 52 fresh isolates of atypical *A. salmonicida* were investigated, the P1 protease was detected in the reference cultures of subspecies *salmonicida* and *smithia* and 10 of the fresh isolates, originating from 6 different fish species and 6 geographical locations. The AsaP1 protease was detected in the reference cultures for *A. salmonicida* ssp. *achromogenes* and 24 fresh isolates from 10 species of fish in the Nordic countries, Scotland and Canada. A total of 19 strains did not produce detectable amounts of either of the proteolytic exotoxins (Austin *et al.*, 1998). A lethal toxin that is neither caseinolytic nor haemolytic and several other proteases, with unknown pathogenic activity, have also been detected in the ECPs of atypical *A. salmonicida* strains (Gudmundsdóttir, 1997).

The exact roles played by the various virulence factors of *A. salmonicida* identified to date is unclear. When injected into fish, purified factors or combinations of factors can result in disease signs similar to those observed during infection with *A. salmonicida*. However, more research is needed before functions can be assigned to each of the virulence factors.

## PLASMID LINKAGE OF VIRULENCE FACTORS

Atypical *A. salmonicida* strains carry plasmids which are different from plasmids of typical strains. A great variability has been observed in plasmid content of different atypical strains (Austin *et al.*, 1998). None of the identified virulence factors of *A. salmonicida* have been linked to a specific plasmid (Noonan and Trust, 1995b).

## EPIDEMIOLOGY

Atypical *A. salmonicida* strains have occasionally been isolated from fish without any disease signs as well as diseased fish from nature, besides being associated with epizootics in wild fish populations. Losses in farmed salmonids and non-salmonids are increasingly associated with atypical *A. salmonicida* infections, presumably because of the intensive fish farming together with an increase in diagnostic awareness and capability (Wiklund and Dalsgaard, 1998). As with infections caused by typical *A. salmonicida* clinical disease outbreaks due to atypical strains usually occur

following stress-inducing events, like handling in the hatchery, overpopulation, rapid temperature or water flow changes, or following transfer of captured fish to cages. The explanation is presumably that covertly infected fish gets sick during stressful conditions.

The source of the infection usually remains uncertain. It is known that the organism can be transmitted horizontally both between and within fish populations, which includes contact with contaminated water and infected fish in addition to possible infection via the gastrointestinal tract (McCarthy, 1980). Transmission by diseased fish escaping from infected farm stocks held in both fresh- and seawater are a source of infection and in many cases infections have been traced to transport of fish. The transport of live fish, like ornamental fish and baithfish for angling, and fish eggs around the world has disseminated atypical strains and the pathogen may have been introduced to Australia (Whittington *et al.*, 1987) and Chile (Sandra Bravo, personal communication) by such transport.

Compared to typical *A. salmonicida* there is limited information available on transmission and survival of atypical strains in water. The available data indicate that the bacteria survive better in brackish and seawater than in freshwater. The data must, however, be interpreted with caution, as they are based only on laboratory results, and survival of various atypical strains in natural habitats may be distinct (Wiklund, 1995). In Norway, Sweden and Finland outbreaks of atypical furunculosis have occurred both in freshwater and seawater. In Iceland outbreaks of atypical furunculosis in farmed salmonids almost always occur in brackish environment. Furthermore, experimental infection of salmon by cohabitation with fish infected by an Icelandic isolate has been established in brackish but not in freshwater (Gudmundsdóttir and Gudmundsdóttir, 1997).

Although fish are still regarded as the main vector in transmission of *A. salmonicida*, parasites and marine plankton may also be involved (Enger, 1997) and recent results indicate that viable bacteria can also be spread by aerosols (Wooster and Bowser, 1996). Ribotyping is suggested as a valid method to study the epidemiology of infections caused by atypical *A. salmonicida* (Pedersen *et al.*, 1996).

## DIAGNOSIS

Diagnosis of *A. salmonicida* infection is based upon clinical signs of disease and isolation and identification of the causative agent. The diagnosis of atypical *A. salmonicida* infections is more difficult than the diagnosis of typical furunculosis as clinical and pathological signs vary and some of the strains are fastidious and slow growing on initial isolation. As many bacterial infections in fish can manifest with similar symptoms, the isolation and identification of the bacterium is crucial.

Sampling from more than one organ gives additional possibility of successful isolation of the bacterium. In addition to the anterior part of the kidney samples can be taken from liver, heart, spleen, intestine, gills, skin mucus and ulcers. For fish exhibiting skin lesions, material from the periphery of the lesions should be inoculated into the bacteriological medium (Bernoth, 1997). Often there are contamination problems on agar plates incubated with samples taken from the external organs and the intestine. It has, however, been stated that samples from skin-and gill mucus may be more reliable than those from internal organs and the advantage is also that samples can be taken without killing the fish (Benediktsdóttir and Helgason, 1990; Bernoth, 1997).

In order to detect covert infections in fish sampling from gills and intestine is recommended. Stress-testing is required to improve the sensitivity of detection. The fish is then stressed by an injection of corticosteroid (0.25 mg/kg prednisolon acetat) and kept for 14 days in 18°C before the bacterial examination is carried out (Bernoth, 1997; Hiney, 1997).

For the primary isolation of the bacterium cultivation on blood agar (BA), tryptic soy agar (TSA) or brain heart infusion agar (BHIA) supplemented with 15% serum, is recom-

mended. Eventually TSA or BHIA complemented with coomassie brilliant blue (CBB) (TSA-C or BHIA-C) can be used. CBB is a protein stain, which is absorbed by the A-layer-protein and gives *A. salmonicida* colonies a blue color after 2–7 days incubation. For the isolation of fastidious strains prolonged incubation (7–14 days) at 20–22°C is required. Morphological and biochemical characteristics, as described above for atypical *A. salmonicida*, are used to identify the bacterium. When the amount of bacteria in fish tissue is very low more sensitive methods, such as immunofluorescent antibody technique (IFAT enzyme-linked immunosorbent assay (ELISA), can be used (Bernoth, 1997; Wiklund and Dalsgaard, 1998).

## PROPHYLAXIS

The etiology of bacterial diseases is diverse. The presence of the relevant pathogen, a susceptible host and a conductive environment are the prerequisite for the development of a particular disease. Therefore, husbandry factors like hygiene, disinfection of transported material, nutrition, water quality, the stocking density, handling and disturbance are factors that are highly important for the fish health. The environment can affect the fish defense systems through a variety of mechanisms. Stress is known to impair the immune response significantly. The stress response in teleost fish is induced by environmental factors although factors inherent to the fish itself, like maturity, are also involved. Furunculosis and related diseases are one of the best examples of diseases strongly influenced by environmental factors. Good husbandry is therefore of great importance for preventing outbreaks of furunculosis (Pickering, 1997).

Vaccination is today the main preventive measure against furunculosis of salmonids. Although, the development of furunculosis vaccines has been one of the great challenges to researchers for many years, the first effective ones were not on the market until after 1992. These are injectable bacterins adjuvanted with oil emulsion. Currently all commercially available *A. salmonicida* vaccines are produced from typical *A. salmonicida* strains for prevention of classical furunculosis in salmonids. There is, however, evidence of cross protection against atypical furunculosis in Atlantic salmon vaccinated with commercial oil based furunculosis vaccine (Jones *et al*., 1996; Gudmundsdóttir and Gudmundsdóttir, 1997). As diseases caused by atypical strains are of emerging importance worldwide, the prospects of their control by vaccination need to be considered. Icelandic fish farmers have since 1992 successfully used an autogenous *A. salmonicida* ssp. *achromogenes* bacterin to vaccinate salmonids by injection and recently halibut farmers in Norway have started to use an autogenous injection vaccine against atypical furunculosis.

## TREATMENT

Chemotherapy has been the most used method for treatment of atypical *A. salmonicida* infections. The most commonly used antimicrobial agents are oxolinic acid, oxytetracycline and trimethoprim-sulfamethoxazole. Other antimicrobial agents that have been shown to be effective against these pathogens are i.e. chloramphenicol, neomycin, nitrofurantoin and ciprofloxacin (Wiklund and Dalsgaard, 1998; Heo and Seo, 1996). Atypical *A. salmonicida* strains are frequently resistant to amoxycillin, which in some places is used to treat classical furunculosis (Barnes *et al.*, 1991). Atypical strains with resistance to antibiotics have emerged following extensive use of chemotherapeutics in the aquaculture and it has been shown that a plasmid encoding for multiple resistance can be transferred from atypical *A. salmonicida* to some marine bacteria (Sandaa and Enger, 1996).

Depopulation and disinfection procedures have sometimes been used to control infections by atypical strains, especially at the first outbreak of the disease on a farm where the risk for reinfection from feral fish is considered to be low (Wichard *et al.*, 1989).

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