LITERATURE REVIEW
AEROMONAS SALMONICIDA,
THE CAUSATIVE AGENT OF FURUNCULOSIS

PELTON ROUND BUTTE HYDROELECTRIC PROJECT

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INTRODUCTION

Aeromonas salmonicida is a bacterial fish pathogen with a broad host range including cyprinids, salmon, and trout. The disease, furunculosis, caused by this gram negative bacterium has a widespread distribution and is endemic in North America, Europe, Australia and Asia. Isolation of a brown pigment producing non-motile coccobacillus was described in the first account of furunculosis in salmonids in 1894. The German investigators named this bacterium, *Bacterium salmonicida* (Emmereich and Weibel 1894). Six years later in 1902, American bacteriologists isolated a similar organism from cultured brown (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*). *Bacterium truttae* was the name given this bacterium (Marsh 1902). In 1939 *A. salmonicida* was first described in Oregon (Shaw and Seghetti 1940).

Furunculosis is one of the most commonly detected bacterial pathogens in cultured salmonids. Improved culture management and treatment have reduced the impacts of the disease. Although antibiotics have been effective in treating furunculosis, antibiotic resistance and stricter regulation of therapeutants for fish present difficulties. Vaccines to the bacterium have been developed by researchers that are effacacious under laboratory conditions. The vaccines may have practical applications to fish culture, with improvements in adjuvants and presentation of the antigens.

Clinical signs typical of *A. salmonicida* infections are liquefactive lesions in the musculature and systemic bacterial colonization. Furuncles or boil-like lesions were found in various tissues and led to the name, furunculosis. Bacteria can be found within a day of exposure in the spleen, heart, liver and kidney of rainbow trout (*Oncorhynchus mykiss*) (Tatner et al. 1984). In young susceptible fish mortality may reach 85%.

Virulence factors include both cell associated and secreted bacterial components. A single protein which covers the surface of the bacterial cell forms the A layer and is responsible forbacterial aggregation, tissue adhesiveness, and survival in serum. This is the major cell associated virulence factor. The function of the secreted enzymatic factors, proteases and cytolysins, is uncertain presently. However it has been suggested that the trout cell specific homolysin and the excreted serine protease are responsible for many of the clinical signs (Fyfe et al 1988).

Early attempts were made to vaccinate fish using bacterins (killed preparations of bacteria). Duff (1942) demonstrated effective protection in cutthroat trout (*O. clarki*) by long term oral administration of chloroform killed *A. salmonicida*. Other attempts to use oral vaccines were inconsistent in efficacy and most researchers failed to obtain significant protection from disease (Udey and Fryer 1978; McCarthy et al. 1983). The ineffectiveness of vaccination was

true no matter what delivery system was used. Some successful vaccinations were reported using extracellular proteins of *A. salmonicida*. Several workers have shown that the extra cellular protease is a potential protective antigen (Shieh 1985, Sakai 1985, Ellis et al. 1988).

Salmonids are capable of producing high levels of specific antibodies to *A. salmonicida*, which are never fully protective (Paterson and Fryer 1974a, 1974b). Unlike rabbits, salmonids do not produce antibodies to several protective antigens (Rockey 1988). This lack of recognition of protective antigens allows the generalized susceptibility of salmonids to furunculosis. Greater susceptibility to infection of some species such as brook trout appears to be related to non-specific resistance mechanisms (Ellis and Stapleton 1987). These mechanisms seem to inactivate or inhibit the extracellular proteins elaborated by bacterium (Adams et al. 1988).

Furunculosis may result in serious negative consequences for populations if a significant number of the brood stock die before spawning (Johnson and Jensen 1994).

ETIOLOGICAL AGENT

Aeromonas salmonicida is a gram negative short to oval rod shaped bacterium (Bullock 1971). Occasionally short rods appear to be coccus or round shaped (1.3 -2.0 X 0.8 - 1.3 µm). The genus *Aeromonas* ("gas producing") is in the family Vibronaceae. The species name *salmonicida* means "salmon killer". The bacterium is non-motile, non-spore forming and non acid fast. Respiration is generally oxidative but the bacterium is a facultative anaerobe. Unlike all other members of the genus, little or no gas is produced. The temperature growth range is 6 to 34.5°C, with optimum growth in the range of 20 to 22°C. It shows a similarly broad pH growth range of pH 5.3 to 9.0. Almost all strains are chromogenic and produce a water soluble pigment of brown to red brown in color when cultured in the presence of the amino acids, tyrosine, or phenylalanine. This pigment apparently is not produced within infected fish. The production of this pigment is often used in presumptive diagnosis. Certain strains of *A. salmonicida* are achromogenic (*A. salmonicida achromogenes*). The biochemical characteristics of the organism are well known. It is cytochrome oxidase positive and produces little or no gas fermentatively from glucose. It is catalase positive. Achromogenic species differ in some biochemical characteristics from the pigment producing type strain. The DNA homology between strains is very high indicating the strain differences are based on minor genetic differences.

Virulence of *A. salmonicida* has been associated in part with proteolytic enzymes which destroy tissues. *Aeromonas salmonicida* also produces leucocytolytic factors which repress the inflammatory response early in infection. Effects of endotoxins that are produced include a complete loss of hematopoiesis in the kidney and spleen. The bacterium also protects itself from host defense mechanisms with an array of proteins which form the A-layer in the cell wall. These

characteristics allow *A. salmonicida* to increase its invasiveness and defeat host protective responses (Udey and Fryer 1978).

GEOGRAPHIC DISTRIBUTION

Furunculosis as noted above has almost a world wide distribution. It occurs in wild fish in many areas of the United States. In Canada it was reported in hatchery populations and wild fishes as early as 1933 (Bullock 1971). It has not been reported in New Zealand or Tasmania. Australia had *A. salmonicida* introduced with shipments of goldfish. Recently it has been increasingly reported in the marine environment, often in association with net pen rearing of salmon in sea water. In 1985, furunculosis was discovered in marine fish farms in Nord-Troedelag, Norway, following importation of salmon smolts from Scotland (Johnson and Jensen 1994). It has been reported in South Africa. Both pathogenic and non-pathogenic bacteria were isolated from fish, both salmonid and non-salmonid, from selected river systems in Natal. The isolation of *Yersinia ruckeri , A. salmonicida*, and *Edwardsiella tarda* were recorded for the first time from fish in Natal, South Africa (Bragg 1991). Furunculosis was first discovered in a fish farm in Norway in 1964, following the importation of rainbow trout from Denmark (Johnson and Jensen 1994). The presence of *Aeromonas salmonicida* was examined using polymerase chain reaction (PCR) and DNA probe methodology in wild Atlantic salmon, *Salmo salar*, taken from three Irish river systems in Ireland. Blood samples from 61 individual fish were examined and the presence of pathogen was determined in 87% of the fish examined. However, the level of pathogen was extremely low, calculated at less than 100 *A. salmonicida* genome equivalents per fish. These data imply a widespread low level infection of *A. salmonicida* amongst wild salmon in Irish rivers (Mooney et al. 1995).

SUSCEPTIBLE SPECIES

Furunculosis has been found in trout, salmon, chars, grayling and whitefish. Cutthroat trout appear to be the most susceptible salmonid and rainbow trout among the most resistant to *A. salmonicida* infection. Epizootic losses of salmon and wild fish have occurred. American eels have also been victims to the disease. A number of other fishes are also susceptible to furunculosis. Chubs, dace, tench, carp, catfish, pike, sculpins, perch and others have had clinical or latent infections with *A. salmonicida*. It is also known to occur in the marine environment. Eels appear to be resistant to the bacterium. Goldfish are severely afflicted with *A. salmonicida*. Among the susceptible marine fishes are the goldsinny wrasse, which are used to control sea lice

in net pen reared salmon. Suckers in Washington state and lamprey at Siletz Hatchery in Oregon have been found infected with the organism.

In general, young fish are more susceptible than adult fish. The occurrence of disease is temperature related and rarely occurs at temperatures less than 8°C. However the organism is still viable and can be found in feces or water holding latently infected fish at low water temperatures. Culturing the intestinal tract or other organs may reveal the presence of *A. salmonicid*a.

TRANSMISSION

The disease is transmitted horizontally through the water and either orally or from direct contact. Contact transmission may be more proficient if there is injury or abrasion to the skin. However *A. salmonicida* is highly invasive and can infect a healthy, uninjured, or stressed susceptible host. Ingestion of the organism causes disease. It is not clear if the gills provide a normal portal of entry. The incubation period varies with water temperature varying from four to twelve days at 20°C. At water temperatures below 8°C, the infection may never progress to clinical disease stages.

Furuncles do not always appear; acutely infected fish do not produce furuncles. Furuncles appear most often in chronically or subacutely infected fish. Rupture of furuncles to the outside may not always occur; but when it does, infectious bacteria are released into the water. Water contaminated with material from furuncles, or from feces of infected fish, may infect fish within the water or downstream (Plehn, 1911).

Control of furunculosis is often made difficult because of the development of a carrier state in fish with chronic or latent infections. In one report, there was a six year period between the stocking of latently infected fish until outbreak of disease several miles from the original liberation site. High water temperatures created a favorable environment for the acute disease state to be expressed (Post 1959). Another consideration in the transmission of furunculosis is the migratory nature of many fish. Movement of fish either during stocking procedures or as part of the natural life history of certain species may move infected fish into previously uncontaminated watersheds. Anadromous fish often become infected upon re-entry into fresh water (McGraw 1952). Other species have been shown to carry *A. salmonicida* including amphibians, reptiles and invertebrates. These animals serve as an alternative host reservoir for the disease (Nese and Enger 1993). Transmission to hatchery fish often occurs from release of bacteria from carrier fish upstream of a hatchery (Wood 1974). Finally, transmission has also occurred from the bacterium on the surface of trout eggs. Infected female trout may carry *A. salmonicida* in the ovaries and contaminate the exterior of eggs during spawning.

It is an obligate pathogen of fish and is not found in environments free of diseased or carrier fish. Survival of *A. salmonicida* in fresh water, sediment, and salt water may be as long as several weeks. The organism apparently does not reproduce outside of the host, and eventually, it is no longer possible to isolate it from the environment without hosts (Allen-Austin et al. 1984, Wedemeyer and Nelson 1977).

DISEASE SIGNS

Four types of infection have been noted: acute, sub-acute, chronic, and latent. Rapid death of large numbers of fish occurs from an acute infection. Very few external signs may appear because of the speed with which the infection proceeds. Sub-acute or chronic infections are less rapid and a variety of signs noted below are often exhibited. Latently infected fish show no signs of disease and low mortality is found in such groups of fish (Ferguson and McCarthy 1978; Bullocket al. 1976).

Fish often are lethargic, dark in color, and will not eat. They swim poorly and seek slow moving or static water. Erythema is noted externally on the base of fins, in the mouth, around the vent and opercula, and along the lower jaw (brachiostegals). The erythema develops from areas of focal necrosis in the muscle. On the surface a crateriform lesion may appear at sites of abscess formation. Bleeding of the gills occurs in net pen reared fish in salt water. Blue eyes have often been noted and used as presumptive identification. Hemorrhaging, or erythema, is noted internally throughout the body cavity including; the body wall, organs, and adipose tissue. Petechial hemorrhages are often noted in the adipose tissue, gonads, stomach wall pericardium, swim bladder, and muscles. Furuncles may be found internally on the liver, spleen, kidney and muscles. The kidney and liver may have gray necrotic areas in fish with latent infections. Intestinal contents may be bloody and only partly digested (Post 1987). Experiments have been able to enhance the virulence of the organism by continued passage in fish. As few as ten bacteria have produced a lethal dose to fifty per cent of the fish. In non salmonid species different forms of disease appear. In carp a subacute chronic contagious skin lesion occurs, carp erytthrodermatitis. This form of infection occurs at all water temperatures. In cyprinids the disease is characterized by deep, extensive ulcers and involvement of the liver and spleen.

The humoral antibody response of healthy Atlantic salmon and of two groups of salmon, naturally infected with *A. salmonicida* ssp. *achromogenes*, was examined in some detail. One diseased group was chronically infected and the other recently infected. It was found that the humoral response of these two infected groups was quite different. The chronically infected fish showed poor specific response to the causative agent, whereas the recently infected salmon produced strong specific antibody response. The chronically infected fish showed evidence of

increased non-specific response including an elevated level of natural antibodies. The specific humoral response of the recently infected fish was primarily directed against two cell-associated antigens of the *A. salmonicida* ssp. *achromogenes* bacterium, the A-layer protein, and the o-polysaccharide component of LPS. In the chronically infected fish, the humoral response was primarily directed against the A-layer protein (Magnadottir et al. 1995).

PATHOGENIC MECHANISMS

There are cell associated and secreted factors of the bacterium that are associated with virulence. The A layer of the bacterium is an outer membrane component made of a single repeated protein array (Rockey et al. 1988). This cell associated structure allows bacterial aggregation, adhesion to tissues and survival in the presence of blood serum. Virulence has been associated with presence of the A layer. However autoaggregating strains from clinical isolations, that do not have the A layer, have been detected (Johnson et al. 1985). The A layer is also implicated with other proteins in the sequestering of iron from the host and protection from complement-mediated lysis.

Another cell associated factor is the lipopolysaccharide (LPS) of the cell wall structure. This component may function to prevent lysis from non-specific serum factors in the host blood (Munn et al. 1982).

A variety of proteins are released from the bacterium and are important in the disease process. These enzymes include proteases and cytolysins of unknown functions. They may function to provide entry into the host. These extracellular products have been shown to be proteolytic, hemolytic and leukocytolytic (Munro et al. 1980). Lesions, spleenomegaly, and tissue destruction occur from the injection of fish with these components (Ellis et al. 1981). It is suggested that these products are major factors in the establishment of disease. A number of these proteins have been characterized (Rockey et al. 1988).

DIAGNOSIS

Diagnosis is accomplished by a review of the disease signs, bacterial identification and occasionally, histopathology. Definitive diagnosis requires isolation and identification of the causative agent, *A. salmonicida*. Presumptive identification can be made quickly based on disease signs and observations of short to oval, gram negative, rod shaped bacteria from kidney squash preparations. Growth of the bacteria is most rapid between 20 and 22°C on plate cultures. Four factors are used in presumptive diagnosis: morphology, staining, motility, and pigment production. Culture characteristics of *A. salmonicida* include round, complete, convex, and

semitranslucent colonies on agar medium containing tyrosine or phenylalanine (e.g. Tryptic Soy Agar, TSA). A brown water soluble pigment develops within 24 to 48 hours of inoculation in chromogenic strains. Certain other bacteria, such as *A. hydrophila* strains, also produce pigment that can confuse results. Two colony types occur: a rough type if the A layer is present or smooth type if it is absent. The bacterium is a short or oval shaped rod and does not form spores. It stains gram negative and is non acid fast. The colonies are oxidase positive. Further biochemical testing is required for definitive identification. The following tests may be used for this purpose, but require up to three days incubation. In glucose, maltose, or mannitol broth, acid and gas are produced. No metabolism is noted in lactose, sucrose, xylose, or sorbitol broth medium. Nitrite is produced in nitrate broth. The bacterium is oxidative and fermentative positive and completely liquefies nutrient gelatin with prolonged incubation. *Aeromonas salmonicida* is non motile. On Comassie Brilliant Blue agar, the bacterium produces deep blue colonies from uptake of the blue dye by the A-layer (Teska and Cipriano 1993).

Diagnosis of achromogenic (non-pigment producing) strains of *A. salmonicida* requires more effort. Often these strains tend to be fastidious and grow more slowly. Identification is completed using specific antiserum or with molecular methods. Fortunately these organisms are less common and occur most often in carp or goldfish. Presumptive identification is often based on morphology, staining, disease signs and the host of origin (Elliot and Shotts 1980).

The slide agglutination test is a rapid presumptive method for identification of *A. salmonicida*. Specific agglutination occurs when one or two colonies are mixed with saline and antiserum to *A. salmonicida.* Agglutination of the bacterial cells occurs within a few minutes and can be observed visually or with the aid of a dissecting scope. Problems do arise from auto agglutination of the virulent strains. Several methods developed to avoid this problem include the mini-passive agglutination, latex agglutination, and coagglutination tests. The fluorescent antibody test is used by some diagnostic laboratories, if the proper microscope is available. Molecular techniques including ELISA, DNA probe hybridization and polymerase chain reaction (PCR) have been reported in identifying *A. salmonicida*, but are not routinely used (Hennigan et al 1989). Culturing the organism is important not only for diagnosis but also in revealing drug sensitivities for treatments.

Finally the distinctive pathology produced by this pathogen can be observed in histological preparations. Lesions are often small in acute cases, but as noted above, chronic cases often have large areas of tissue damage in the internal organs. The necrotic areas have little leucocytic infiltration, and large numbers of bacteria are present. A leucocidin is produce which destroys macrophages, monocytes, and lymphocytes. Necrosis develops from the proteases elaborated by *A. salmonicida,* which destroys muscle and connective tissue. The parenchymal area exhibits foci of bacteria.

A DNA fragment that is specific to *Aeromonas salmonicida* has been isolated from a genomic DNA library by differential hybridization. The specificity of this fragment as a DNA probe for *A. salmonicida* was shown by hybridization against reference strains and clinical isolates of *A. salmonicida* , related aeromonads, and species from several other bacterial genera. The sensitivity of detection by a PCR test, based on this fragment, was approximately two *A. salmonicida cells* (Hinney et al. 1992).

Mucus of salmonids was evaluated as a source for nonlethal detection of the pathogen *Aeromonas salmonicida* in fish. The bacterium was readily isolated from mucus on dilution plates when Coomassie Brilliant Blue agar was the primary plating medium. Kidney samples from the fish that served as sources of mucus were similarly processed. Infection was detected in 56% of mucus samples from lake trout, *S. namaycush* that were undergoing an epizootic of furunculosis, but only 6% of the kidneys from these fish were positive for the pathogen. Only 1% of asymptomatic brown trout, *S. trutta* , sampled at another fish hatchery had *A. salmonicida* in their mucus, and none had a kidney infection. Combined results from the examination of two pools of Atlantic salmon, *Salmo salar*, reared at a third hatchery indicated that 37% of these fish had mucus infections caused by *A. salmonicida* , but only 4% of the kidneys were infected (Cipriano et al. 1992).

TREATMENT –THERAPY AND CONTROL

Drug Therapy

Standard treatment for many years was sulfamethazine (Sulmet) given orally. In the early 1960s sulfa-resistant forms of *A. salmonicida* were found, and the treatment of infected fish was ineffective. Terramycin (TM -50 or TM-100, oxytetracycline hydrochloride) replaced the sulfa treatment, but by the late 1960s, this treatment also became less effective. A return to sulfamethazine treatment protocol occurred as resistance to it declined in the *A. salmonicida* population (Wood 1974). Romet -30 (sulfadimethoxine enhanced with ormetoprim), a potentiated sulfonamide, is an available antibiotic that is in current use and effective in treatment. Extended use of sulfonamides can cause toxicity at the maximum allowable doses in sensitive fish. Kidney damage may result from drug crystals forming in the renal tubules. Sulmet is no longer commercially available. Chloramphenicol, oxolinic acid and furoxone are chemotherapuetants that were once used effectively but are no longer allowed. Current restrictions by the United States Food and Drug Administration now only allow fish with *A. salmonicid*a to only be treated with oxytetracycline and Romet - 80 under these conditions.

Antibiotic sensitivity patterns of 304 isolates of *A. salmonicida* from 229 outbreaks of furunculosis among salmon in Scotland between 1988 and 1990 were investigated. Fifty-five percent were resistant to oxytetracycline and 37% resistant to oxolinic acid. Multiple resistance was common (52%), and 18 out of 19 antibiograms which were found in the first year recurred in the succeeding year. More than a quarter of the outbreaks were associated with two or more *A. salmonicida* variants distinguishable by their antibiotic sensitivity patterns (Lee and Ellis 1991). Resistance to a wide range of antimicrobials including 4-quinolones, ß-lactams and tetracycline in *A. salmonicida* is associated with increased expression of a 37 kDa protein and decreased expression of a 43-kDa protein. These proteins were shown to be non-covalently associated with peptidoglycan and thus, may function as porins. In a previous study, the authors have shown that selecting for oxytetracycline resistance could select for resistance to the 4-quinolones including oxolinic acid. This is a cause for concern because these are the two major classes of antimicrobial used to treat furunculosis. However, it is unclear if this mechanism of resistance occurs in nature because the alterations in outer membrane proteins (OMPs) were identified in resistant mutants from *in vitro* experiments (Barnes et al. 1992).

Temperature of the water when fish are treated can effect the outcome of treatment. The *in vitro* antimicrobial activities of oxolinic acid, flumequine, sarafloxacin, enrofloxacin, and oxytetracycline against strains *A. salmonicida* subsp. *salmonicida* , atypical *A. salmonicida* were determined at two different incubation temperatures, 4 and 15°C, by a drug microdilution method. The main objective of the study was to examine the effect of incubation temperature on the *in vitr*o activities of 4-quinolones and oxytetracycline against these bacteria. When tested against *A. salmonicida* subsp. salmonicida, all of the quinolones examined had Minimum Inhibitory Concentrations, MICs two- to threefold higher at 4°C than at 15°C. In contrast to those of the quinolones, the MICs of oxytetracycline were two- to eightfold lower at 4°C than at 15°C against all of the bacterial species tested (Martinsen et al. 1992).

Vaccines

The earliest reported protection by vaccination to *A. salmonicid*a was Duff in 1942. A chloroform killed *A. salmonicida* vaccine protected cutthroat trout when given orally for long periods (Duff 1942). Although successful vaccines to other bacterial pathogens of fish such as vibriosis have been developed (Rohovec 1974). Oral vaccination for furunculosis has been given mixed reviews as to efficacy (Klontz and Anderson 1970; Udey and Fryer 1978). In general protection is low or absent no matter the delivery method with whole cell type vaccines (Hastings 1988). Extracellular component vaccines have in general been more successful. The evidence suggests that proteases of the bacteria are the most significant protective antigenic portion of

these vaccines (Ellis et al. 1988). Commercial vaccines still remain of debatable efficacy. Injectable vaccines have been much more effective than immersion bacterins.

A body of research has demonstrated that fish can mount a specific and high level antibody response to the bacteria by vaccination. Increased survival of these immunized fish has not been demonstrated (Michel 1985; Cipriano and Heartwell 1986). The antisera made in response to vaccination is not fully protective to *A. salmonicida*. Other work has shown that fish do not respond immunologically to certain protective antigens of the bacteria (Rockey et al. 1988). Genetically engineered vaccines may allow for better presentation of these antigens. Control measures have included the use of disease-resistant strains of fish, vaccines, nonspecific immunostimulants such as glucans, and probiotics.

A genetically attenuated strain of *A. salmonicida*has been developed that has a complete deletion of the aroA gene (Brivax II), making it suitable for development as a commercial vaccine. Brivax II was effectively cleared from Atlantic salmon, *S. salar*, a species highly susceptible to furnculosis, confirming that it is attenuated. Clearance rate was dependent on the vaccine dose administered, being longer with higher doses. Immunological studies using Brivax II injected in rainbow trout, *O. mykiss*, confirmed that live vaccines stimulate a greater response in terms of generating leucocytes able to proliferate to a subsequent encounter with antigen, relative to killed vaccines. Development of strains of Brivax II as carriers of heterologous antigens was also investigated. *Escherichia coli* beta-galactosidase was chosen as the model antigen, and three strains containing plasmids with the LacZ gene were constructed (Brivax 12, Brivax 61 and Brivax 107). All three strains were shown to express beta -galactosidase *in vivo* in rainbow trout and to be cleared effectively. Interestingly, Brivax 107 was cleared faster than the other two lac super (+) strains and had the highest level of beta -galactosidase activity. The two strains expressing lower levels of activity also behaved differently *in vivo*, in that Brivax 12 accumulated derivatives expressing lower levels of beta -galactosidase activity, suggesting that mutants are being selected *in vivo*. (Marsden et al.1996).

A study was conducted to evaluate the efficacy of 7 substances at potentiating a formalin-killed *Aeromonas salmonicida* bacterin in juvenile coho salmon, *O. kisutch.* The substances were injected into the fish along with the bacterin. The fish were challenged 27 d later with viable *A. salmonicida* cells by two methods (cohabitation and immersion). The cumulative mortalities in each of the experimental groups was then determined. A significant and consistent increase in protection over the groups receiving only the *A. salmonicida* bacterin was observed with three of the substances tested. These were VitaStim-Taito, lentinan, and formalin-killed *Renibacterium salmoninarum* cells. One of these materials, VitaStim-Taito (a beta -1,3 glucan), showed particular promise for further studies (Nikl et al. 1991).

Bathing rainbow trout for 30 minutes in immunostimulant solutions before a two minute bath in *A. salmonicida* O antigen bacterin elevated both the non-specific defense mechanisms and the specific immune response. Levamisole, a known T lymphocyte stimulator in mammals; QAC, a quaternary ammonium compound, and ISK a short-chain polypeptide, heightened the neutrophil oxidative activity as measured by nitroblue tetrazolium assay and increased the phagocytic uptake of glutaraldehyde-fixed sheep red blood cells. The heightened activity of the specific immune response was monitored by counting numbers of plaque-forming cells, and by demonstrating elevated circulatory antibody titres. Protection levels against the virulent pathogen were also increased when the fish were challenged 14 days later. Giving an immunostimulatory bath before the antigen bath is a relatively easy method of increasing efficacy and potency of immunogens for the prevention of diseases in fishes (Jenney and Anderson 1993).

The data suggest that the immune response generated by the use of live vaccine strains is different from that generated by a bacterin, and useful mutations may be incorporated into existing furunculosis live vaccines for further attenuation. Significant protection resulted from all strains of live vaccines to challenge with a heterologous virulent strain of *A. salmonicida* five weeks later. The levels of protection conferred were all greater than or equal to that provided by an injected bacterin using the same vaccination schedule (Thorton et al. 1994).

A dual vaccine that showed protection against both viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV) utilized an attenuated strain of *A. salmonicida* as an expression vector. Fragments of the glycoprotein genes of VHSV and IHNV were cloned into a bacterial broad-host-range expression vector under the control of the plac promoter. Western blot (immunoblot) analysis with monoclonal antibodies specific to the glycoproteins demonstrated the inducible expression of the fusion proteins in *Escherichia coli.* It was confirmed that an avirulent strain of *A. salmonicida,* A440, which contains a deletion in the structural gene for the paracrystalline surface protein array, will provide protective immunity against furunculosis when used as a live attenuated vaccine. The plasmid-encoded viral epitopes were then mobilized into A440 for use as a shuttle system for the expression of fragments of the glycoprotein genes of IHNV and VHSV. Vaccination of rainbow trout with A440 containing the viral epitopes resulted in the development of protective immunity against both VHSV and IHNV. This indicates that the use of cloned fragments of the glycoproteins and the use of *A. salmonicida* as a shuttle system constitute a feasible approach to fish vaccine development (Noonan et al. 1995).

DETECTION OF *AEROMONAS SALMONICIDA* IN THE DESCHUTES WATERSHED

Oak Springs Fish Hatchery

There have been no recorded losses or detections of *A. salmonicida* in fish stocks at this location.

Round Butte Fish Hatchery

Aeromonas salmonicida has been found in adult chinook salmon and steelhead trout in a few fish, especially prespawning losses.

Wizard Falls Fish Hatchery

There have been no recorded losses or detections of *A. salmonicida* in fish stocks at this location since 1984. Information before this time has not been investigated

Fall River Hatchery

There have been no recorded losses or detections of *A. salmonicida* in fish stocks at this location since 1984. Information before this time has not been investigated.

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