Infectious Salmon Anemia Virus

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Hosts and Geographic Range

Infectious Salmon Anemia Virus (ISAV) is a highly infectious disease of Atlantic salmon (*Salmo salar*) that was first reported within Norwegian aquaculture facilities. The disease has since been described among pre-market Atlantic salmon, *Salmo salar* in Scotland (Bricknell et. al. 1998), New Brunswick, Canada (Lovely et al. 1999), the United Kingdom (Rodger et al. 1999), and the Cobscook Bay region of the United States (Bouchard et al. 2001) and from coho salmon (*Oncorhynchus kisutch)* in Chile and the Faroe Islands (Kibenge et al. 2001). The rapid invasion of ISAV into three bays within New Brunswick affecting 21 farms (Bouchard et al. 1998) is one indicator of the severe threat that ISAV represents for Atlantic salmon aquaculture. Furthermore, the annual cost of infectious salmon anemia outbreaks among farmed fish in 1999 was reported to be $11million (U.S. dollars) in Norway, $14 million in Canada, and the 1998 – 1999 epidemics in Scotland were valued at a cost of $32 million (Hastings et al. 1999). Although epizootics of ISAV have been specifically associated with cultured salmon, salmon, Department of Fisheries and Oceans (DFO) - Canada biologists also detected the presence of ISAV among Atlantic salmon populations that are wild or have escaped from aquaculture operations collected at the Magaguadavic River fish trap (Bay of Fundy, New Brunswick). In addition to Atlantic and Chinook salmon, the pathogen infects but has not

produced disease in freshwater brown trout, *Salmo trutta* (Nylund et al. 1995); sea trout, *S. trutta* (Nyland and Jakobsen 1995); and rainbow trout, *Onchorhynchys mykiss* (Nyland et al. 1997).

Figure 1: Clinical signs of ISAV include a paling of the gills and a slight swelling of the liver, which may turn a dark brown to black coloration. There may also be some swelling of the spleen and there are generally extensive petechial hemorrhages on the pyloric caecae, mesenteric fat, and swimbladder. Picture courtesy of Dr. David Bruno.

Etiology

The cause of ISA is an enveloped virus of 45-140 nm in diameter (Dannevid et al. 1995b) with a buoyant density 1.18 g/ml in sucrose and CsCl gradients and showed maximum replication at 15°C, but no replication at 25°C (Falk et al. 1997). The virus may be cultured in the Atlantic salmon head kidney (SHK-1) cell line and produces CPE between 3 and 12 days after inoculation (Dannevig et al. 1995a; Kibenge et al. 2000). Some strains also replicate in Chinook salmon embryo (CHSE-214) cells and produce CPE between 4 and 17 days post inoculation (Kibenge et al. 2000). The virus may also be cultured and produce CPE within 9 days in the TO cell line derived from Atlantic salmon head kidney leukocytes at 20°C in EMEM under 5% CO_2 or in HMEM without CO_2 supplementation (Wergeland and Jakobsen 2001). Growth is inhibited by actinomycin D but not by 5-bromo-2-deoxyuridine (Sommer and Mennen 1997) and is most closely related to other orthomyxoviruses (Mjaaland et al. 1997; Krossoy et al. 1999; Sandvik et al. 2000). Four major polypeptides are evident with estimated molecular sizes of 71, 53, 43 and 24 kDa (Falk et al. 1997). Cunningham and Snow (2000) found nucleotide and amino acid variations in a Scottish ISAV isolate that differentiated it from isolates of Norwegian or North American origin. The Scottish isolate was more closely related to the Norwegian strain than to the North American strain. The virus possesses both hemagglutinating as well as fusion and receptor-destroying activity; - the latter suggested to be an acetylesterase (Aspehaug et al. 2001). DeVold et al. (2001) have shown that the hemagglutinin gene contains a highly polymorphic region (HPR), which shows sequence variation in time and space, with certain geographical areas being dominated by distinct groups of isolates.

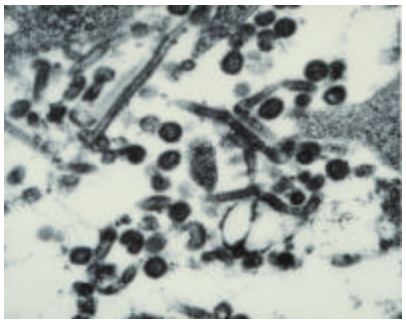

Figure 2: Electron micrograph revealing pleomorphic variation of the ISAV enveloped virus from cell cultures. The spherical or oval virus particles are approximately 100-150 nm in diameter and have a fringed surface projection from 10 – 12 nm long (Rowley et al. 1999). Larger particles, that may be up to 700 nm long, are occasionally

observed in unpurified preparations (Dannevig et al. 1995). Picture courtesy of Dr. Frederick Kibenge.

Virulence and Pathology

Clinical signs may be evident 2 – 4 weeks post infection and commonly include pale gills, an accumulation of ascites, liver congestion, enlarged spleen, petechiae in the visceral fat, congestion of the gut, and severe anaemia (Hovland et al. 1994; Throud and Djupvik 1988; Evensen et. al. 1991). A severe leucopenia often develops concomitantly with anemia (Throud 1991). However, fish injected with the ISA virus may display a suppressed leukocyte response that does not necessarily correlate with the development of anemia ISA (Dannevig et al. 1993). This suggests that suppression of immune function and development of anemia are independent events. Considerable viral replication occurs within infected fish and the virus is widely disseminated throughout most tissues including mid-kidney, head kidney, liver, spleen, intestine, gills, muscle and heart (Jones et al. 1999; Rimstad et al. 1999). Microscopic pathological changes are commonly characterized by renal interstitial hemorrhage and tubular necrosis, branchial lamellar and filamental congestion, congestion of the intestine and pyloric caecae, and perivascular inflammation in the livers (Mullins et al. 1998b, Rimstad et al. 1999). Decreases in hepatic glutathione (GSH) of up to 70% observed in diseased fish may affect the capability of the liver to transform and excrete xenobiotics from the body (Hjeltnes et al. 1992). Experimentally, elevated plasma cortisol concentrations have been correlated to the severity of anemia, as measured by haematocrit values (Olsen et al. 1992). Plasma lactate may also be elevated in diseased fish (Olsen et al. 1992).

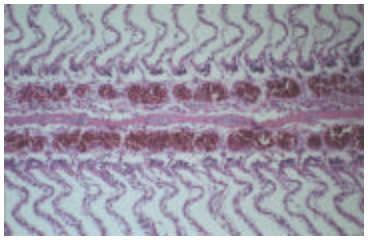

Figure 3: Severe **v**ascular congestion may be evident in the filamentous sinus of gill tissue. Picture courtesy of Dr. Fred Kibenge.

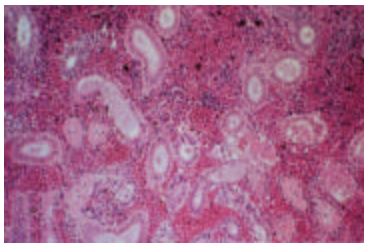

Figure 4: Multiple foci of interstitial hemorrhage may appear confluent throught the kidney. Renal tubules may display different stages of degeneration ranging from tubular epithelial swelling to complete tubular collapse (Bouchard et al. 1999). .Picture courtesy of Dr. Frederick Kibenge.

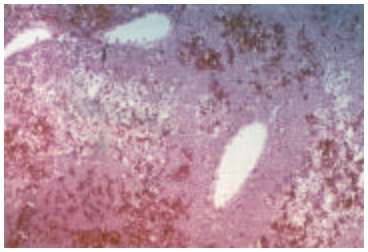

Figure 5: Multiple necrotic areas may be present throughout the liver. In some cases, central hepatic vessels may look quite normal producing a distinctive zonal appearance to liver pathology . Picture courtesy of Dr. Frederick Kibenge.

Transmission

The disease is pronounced in the marine environment, where it is transmitted by cohabitation with infected live salmon or infected biological materials such as animal wastes or discharges from normal culture operations, slaughter facilities (Vagsholm et al. 1994) and contaminated well boats (Shannon 1998; Murray et al. 2002). Infected fish may transmit the disease weeks before they show apparent signs of infection. The virus may spread horizontally, from fish to fish, by shedding of virons from the blood, gut contents, urine, and epidermal mucus of infected salmon (Totland et al. 1996). Moreover, fsh that survive epizootics may shed viral particles for more then one month into the surrounding water (Hjeltnes et al. 1994). Blood and mucus contain large amounts of virus and more effectively transmit the disease than feces, plankton and salmon lice (Rolland and Nylund 1998).

Sea lice of the species *Caligulus elongatus* and *Lepeophterius salmonis* may also play an important role as vectors that can enhance contagion during epidemics (Nyland et. al. 1994). Furthermore, there is no evidence that scallops cultured with Atlantic salmon either accumulate the pathogen or transmit the disease (Bjoershol et al. 1999). The pathogen can be transmitted to but has not produced disease in freshwater brown trout *Salmo trutta* (Nylund et al. 1995) and sea trout *S. trutta* (Nyland and Jakobsen 1995), sea run brown trout; Rolland and Nyund 1999), and rainbow trout (*Onchorhynchys mykiss)* suggesting that these fish may become carriers and serve as potential reservoirs of infection (Nyland et al. 1997). Clearance of the virus following experimental infection progresses at a greater rate in Arctic char (*Salvelinus alpinus*) than in rainbow trout and brown trout. Thus, the potential for this species to act as a long-term carrier of ISAV may be less than that of other salmonids, all of which apparently clear viable virus by 40 days post injection (Snow et al. 2001). Horizontal transmission of the ISA virus in fresh water has been achieved experimentally (Brown et. al. 1998) and occurs rapidly between infected and naive smolts in freshwater. Even under these conditions, asymptomatic smolts may remain infective to naive parr for 18 months after the original challenge (Melville and Griffiths 2000). Transmission via vertical passage of the virus from parent to offspring via intra-ovum infection has not been demonstrated (Melville and Griffiths 1999).

Diagnostics and Detection

Culture remains the definitive assay against which other diagnostic tests are compared. As previously mentioned, the virus can be cultured in Atlantic salmon head kidney (SHK-1) cells (Dannevig et al. 1995), Chinook salmon embryo (CHSE-214) cells and Atlantic salmon head kidney leukocyte (TO). Bouchard et. al. (1999) have suggested that the focal nature of the CPE by ISAV grown in CHSE-214 cells provides a better foundation for a culture-based diagnostic than the use of SHK-1 cells, in which the virus may not produce a definitive CPE. However, unlike the SHK-1 cells which support the growth of ISAV isolates from a broad geographic area, the CHSE-214 line does not support the

growth of all ISAV isolates (Frederick et. al. 2000). This lack of growth of some ISAV isolates on CHSE-214 cells is indicative of at least one type of problem that must be addressed in a diagnostic. In controlled experiments, the parallel use of both cell lines was more sensitive than the use of either SHK-1 or CHSE 214 cells alone (Opitz et al. 2000). High yields of ISAV and production of CPE in TO cells (Wergeland and Jakobsen 2001) may also make this cell line a valuable diagnostic tool.

Non-culture based diagnostics have been developed to detect ISAV that include an indirect fluorescent antibody test (Falk and Dannevig 1995a) and a reverse transcriptase-poylmerase chain reaction (RT-PCR) procedure (Mjaaland et. al. 1997). Further confirmation has been effected by the use of a DNA probe in PCR products (McBeath et al. 2000). Devold et al. (2000) found RT-PCR to be more sensitive for detection of ISAV among carrier sea trout, than either culture or injection of suspect blood samples into naïve fish. Furthermore, RT-PCR screens of gill mucus presents an accurate and sensitive non-lethal alternative for detection of the virus from other tissues that require necropsy (Giffiths and Melville (2000). Production of a monoclonal antibody against ISA enabled Falk et al. (1998) to conduct several serodiagnostically based assays including ELISA, immunofluorescent staining of virus infected cell cultures, immunoelectron microscopy of negatively stained virus preparations, virus neutralization assay and haemagglutination inhibition assay. Because Atlantic salmon that survive infections with ISA virus develop a specific antibody-dependent immune response against the pathogen (Falk and Dannevig 1995b), An indirect ELISA assay has also been used to detect antibodies to ISAV in Atlantic salmon sera, which theoretically denotes previous exposure to the pathogen among non-vaccinated fish. In general, the current methods for routine detection and confirmation of ISAV, in decreasing order of sensitivity and specificity, are RT-PCR, Antibody ELISA, Immunofluoescence, and histopathological examination (Groman et al. 2001). Phenotypic (Frederick et. al. 2000) and genomic (Blake et al. 1999) differences that are known to exist among ISAV isolates may influence the use of specific assays.

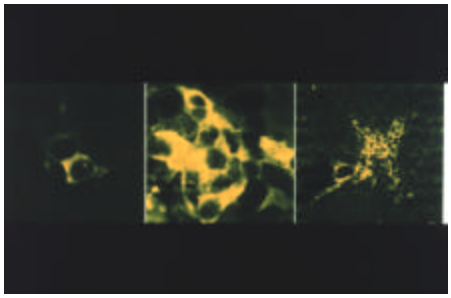

Figure 6: Indirect immunofluorescence of cell cultures infected with ISAV showing perinuclear fluoirescent staining throughout the cytoplasm. Picture courtesy of Dr. Frederick Kibenge.

Management

Because of the acute nature of the disease and an inability to control mortality, European Economic Community-countries require compulsory slaughter of infect stocks (Hill 1994). Similar eradication programs have been effected in Canada (Mullins 1998a). It

has already been discussed that the virus is readily transmitted in seawater. Such dissemination may readily contaminate culture facilities within 5-6 km of an infected site (Eide 1992). It is recommended, therefore, that culture sites be spaced no less than 5-6 km apart and that waste water from slaughter and processing facilities should be thoroughly disinfected (Jarp and Karlsen 1997). Further contagion may be managed by control of ship and personnel movements among sites, destruction of infected lots, and the closure and fallowing of contaminated sites (Murray 2001). Iodophor, chloramine-T and chlorine dioxide have nbeen shown to be effective topical disinfectants against ISAV, when used for a minimum of five minutes according to manufacturer's instructions (Smail et al. 2001).

Vaccination

Although autologous vaccines against ISAV are used in certain areas, no product is currently licensed for general use. Jones and MacKinnon (1999) have shown that vaccination via intraperitoneal injection of inactivated virus elicited the best protection if at least 734 degree days had elapsed between vaccination and challenge. They also noted that protection was significantly improved if the viral antigen was delivered in an oil emulsion. Christie et al. (2001) also indicated that vaccination may produce relative percent survival values of 90 or higher (54% mortality among controls) for 6 months post vaccination without significant risk of viral transmission by vaccinated fish that may have become asymptomatic carriers.

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