Bull. Eur. Ass. Fish Pathol. 19(2),70, 1999.

OUTBREAK OF ISA DURING FIRST FEEDING OF SALMON FRY (*SALMO SALAR*).

A. NYLUND, B. KROSSØY, M. DEVOLD, V. ASPEHAUG, N.O. STEINE* AND T. HOVLAND*
Department of Fisheries and Marine Biology, University of Bergen, N-5020 Bergen, Norway
*Næringsmiddeltilsynet i Salten N-8017 Bodø, Norway

Abstract

The first case of ISA during first feeding in Atlantic salmon fry is described. The presence of ISA virus is shown with the help of cell cultures (ASK-cells), IFAT, RT-PCR and transmission electron microscopy. The possibility that this may be a case of vertical transmission is discussed, but it is argued that the ISA virus may have entered the hatchery through an as yet unidentified route.

Introduction

The first officially reported outbreak of Infectious Salmon Anaemia (ISA) in Norway started in November 1984, and during the 80´s and early 90´s there was a build up of the disease. The maximum was reached in 1990 with over 80 affected farms. However, a program for eradication of the disease and new strategies for management of the salmon farming industry reduced the number of new cases of ISA to one in 1994. The eradication program used high mortality rates, clinical signs and histopathology of the liver as diagnostic criteria for the disease. These were the only official diagnostic criteria used until 1995 when the first cell culture, SHK-1 cells (Dannevig *et al.*, 1995, 1997), that were susceptible to the ISA virus, became available. This means that during the first 10 years with ISA in Norway there has been a selection for eradication of "visible" ISA, while less virulent strains of the ISA virus (if they exist) may have escaped attention and contributed to the spread of this orthomyxovirus (Falk *et al.*, 1997; Krossøy *et al.*, 1999) along the Norwegian coast.

In the years since 1994 there has been a slow but steady increase of new cases of ISA in Norway in addition to outbreaks of the disease in Canada (Mullins *et al.*, 1998), Scotland (Rodger *et al.*, 1998) and the Shetland Islands. In the same period, new specific and sensitive methods like IFAT (Falk *et al.*, 1998) and RT-PCR (Devold *et al.*, submitted) have become available. This means that it is now possible to show the presence of the ISA virus in salmon farms even in absence of mortality and clinical signs of the disease. This raises new and important questions for the management of the disease since it is now possible to screen brood-fish, smolt, salmon farms in high risk areas, and wild populations of salmonids for presence of the ISA virus.

The possibility of vertical transmission is always an issue when a virus is the causal agent of a disease. It has been an accepted, but an undocumented truth, that the ISA virus is not spread vertically. The present case-report shows that this dogma should be given more attention, maybe even challenged.

Materials and Methods

In the beginning of February 1998 we received, at the Fish Disease Laboratory, University of Bergen, a large number of salmon fry (in the first feeding phase) from a hatchery with high mortalities. Infectious pancreatic necrosis (IPN) was suspected, but the proper diagnostic authorities were not able to show the presence of IPN virus in moribund larvae. A definitive diagnosis was not given.

Most of the salmon larvae that we received (mean weight = 0.16 gram) were alive at arrival and a sub-sample was screened for parasites. In addition, samples were taken for cell-culture (ASK cells which are susceptible to IPN and ISA virus), IFAT, RT-PCR and transmission electron microscopy (TEM). The conditions for the cell cultures, IFAT and RT-PCR are described in Devold *et al.*, (submitted). A modified Karnovsky fixative was used for the TEM examination (Nylund *et al.*, 1995a).

A primer set corresponding to segment eight (Accession no: AJ012285) of the ISA virus

were used for the RT-PCR; Forward FA-3: 5´- GAA GAG TCA GGA TGC CAA GAC G – 3´, Reverse RA-3: 5´- GAA GTC GAT GAA CTG CAG CGA – 3´. The DNA product resulting from amplification gives a 211 bp long segment (cf. Devold et al., submitted).

History of the salmon frye

Two batches of eggs (20 and 180 litre, group A and B) that were delivered from the broodstock plant arrived at the smolt hatchery at 330 and 365 degree days, and hatched at about 483 degree days at 11 °C. The larvae were kept at 14 °C and first feeding began the 12th of January 1998. Seven days later larvae from group B started to die. Reduction of the temperature to 8 °C and increased water flow reduced the mortality a little. Still, 56 % (500 000 / 900 000) of the salmon larvae died. At this time there was no mortality in the larvae tanks that came from group A which were kept separate from the other larvae. Mortality started in this group when the surviving larvae from group B was moved into the same room as group A.

Mortalities in the hatchery reached close to 80 %, but with the exception of sporadic presence of bacteria in a few larvae, no disease agent was found. The bacteria were most likely a result of contamination during sampling due to the small size of the larvae. Histology showed slight changes of the gills and in the pancreatic tissue. The hatchery did not use addition of seawater since the UV-"filters" were down. Water analysis showed a slight increase in bacteria just before the outbreak and the veterinary authorities concluded that the mortality could be a result of some environmental factors. Water analyses at the end of the outbreak showed normal values.

The hatchery received two additional batches of eggs (40 and 160 litre, groups C and D) the 20th and 27th January 1998 to compensate for the loss of larvae. These two batches ended up in the same room as the dying larvae, i.e. no protective measures were made to prevent a possible disease agent to be transmitted from group B to the two new groups (C and D). During first feeding group D experienced similar mortality rates to groups B and A, while there were no mortality in group C. This time there were petechiae in the liver and on the mid-gut, haemorrhages at the base of the dorsal and tail fins, and slight changes of the gills.

The brood fish were screened for IPNV but

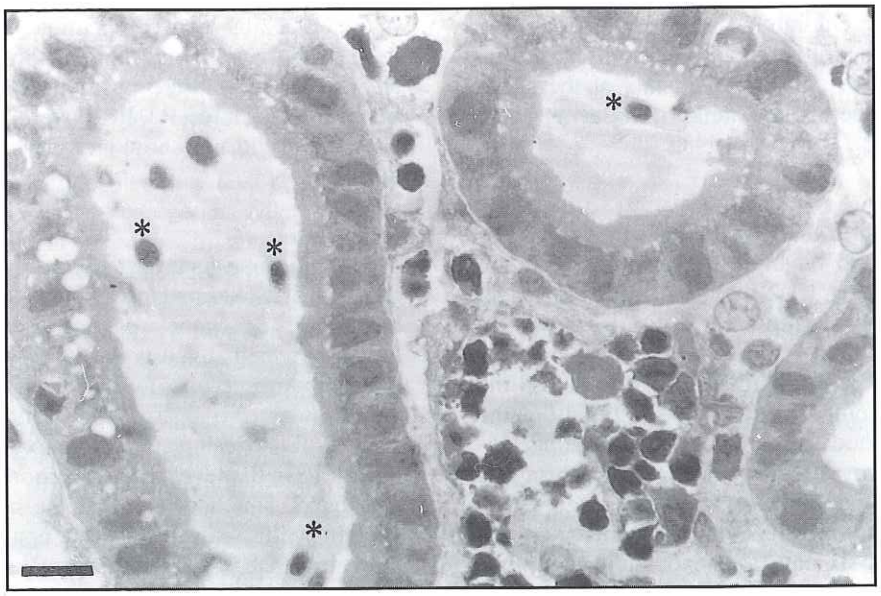

Figure 1. Kidney. Renal tubules with erythrocytes (asters) in the lumen. Bar = 10 µm.

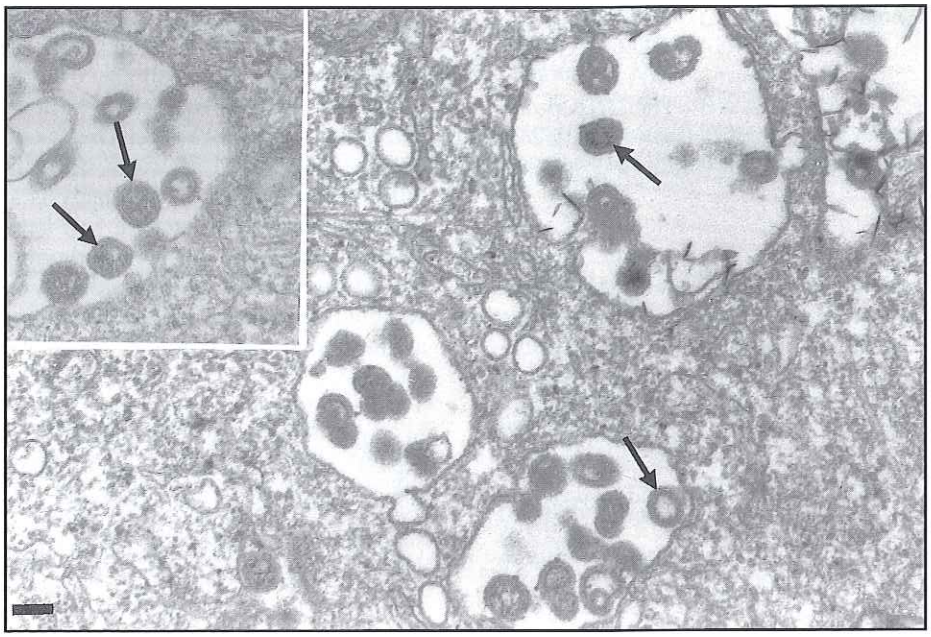

Figure 2. Micrograph of the cell-cultures revealing enveloped viruses (arrows) of the same size and morphology as the ISA virus. Bar = 125 nm.

not for ISA. It has not been possible to obtain any tissue samples from brood fish. However, the eggs were, according to the responsible veterinarian, disinfected according to the guide-lines. After the outbreak started some of the larvae were treated with formalin, chloramine, and up to 7 ‰ sea water for a short period. None of the treatments gave any improvements of the situation in the treated tanks. All larvae were killed the 23rd March 1998.

Results

No parasites that could explain the mortality were found on or in the examined salmon larvae. Nor were any bacteria shown on semi- and ultra-thin section of tissues from the moribund larvae. A few specimens of sessiline peritrich ciliates were found on the skin of some of the larvae. Histology of the kidney showed some necrosis of the haematopoetic tissue and presence of erythrocytes in the lumen of the renal tubules (Figure 1).

A cytopathic effect on the ASK-cells was observed six days after inoculation with homogenised and sterile filtered (0.2 µm) tissues from the moribund larvae. The cells stained positive for ISA virus using a monoclonal antibody against the virus. TEM examination of the cell-cultures revealed an enveloped virus of the same size and morphology as the ISA virus (Fig. 2). IPN virus was not found in the cell cultures.

A total of 22 larvae were examined for presence of ISA virus with the help of RT-PCR and six of these were positive. The larvae that were used for RT-PCR were alive on arrival and did not show any clear signs of disease.

Discussion

This is the first documented case of ISA affecting first-feeding larvae in Norwegian hatcheries. Seawater was not used in the hatchery which suggests that there may also be natural reservoirs for the virus in fresh water, unless this is a case of vertical transmission. The fresh water intake to the hatchery is above the level where anadromous fish are found, which means that the only connection with seawater is the egg batches taken from the brood fish in seawater.

Virus survival in nature requires a continuous infection of susceptible individuals or

establishment of long-term persistence (or latency). Viruses may also posses structural features that permit them to survive the rigors of an extra-organismal environment until contact with a susceptible host occurs. Orthomyxoviruses such as influenza A survive by circulating in more than one species and this may also be the case for the ISA virus since several species may serve as hosts for the virus (Nylund *et al.,* 1994, 1995b, 1997; Nylund and Jakobsen 1995; Rolland and Nylund 1998). It has also been shown that the ISA virus may persist in trout (Nylund *et al.*, 1995b; Devold *et al.*, submitted). Hence, there are several ways the ISA virus may survive in nature, even if the populations of salmon, trout and rainbow trout occur at low density. It is also possible that the "original" ISA virus occurring in natural populations of salmonids may have a lower virulence compared to the strains causing ISA in salmon farms. The high number of susceptible hosts in a salmon farm and a steady introduction of new and naïve hosts might have selected for increased virulence of the ISA virus.

Persistence of a virus over a long term in a host requires that the virus must be able to infect host cells without being cytopathic, there must be mechanisms for long-term maintenance of the viral genome in host cells, and the virus has to avoid detection and elimination by the host´s immune system (cf. Fields *et al.*, 1996). In contrast to DNA and Retroviruses, there are no known mechanisms for long-term propagation of virus RNA molecules in a host cell. Therefore, RNA viruses can persist only by a low grade, chronic infection in which the viral RNA is replicated continuously at a low rate producing infective particles. Even if ISA virus particles have been found in ovaries of infected salmon (Nylund *et al.*, 1995a) to date there exists no support for believing that egg cells may serve as host cells for the virus. So far, the only known target cells for the ISA virus are endothelial cells lining blood vessels, endocardial cells and some polymorphonuclear leukocytes (Koren and Nylund 1997; Nylund *et al.*, 1995a, 1996, 1997). In addition, the requirement of a continuous viral RNA replication for the ISA virus to survive in an egg-cell through a vertical transmission makes this scenario even less likely to occur. There is, however, a possible explanation for vertical transmission. The ISA virus may have survived in the ovarian fluids or entered the egg during fertilisation. Since the eggs were treated according to the regulations it is not very likely that the virus survived in the ovarian fluids. ISA virus entering the egg-cells during fertilisation may be a bit far fetched. The most plausible "explanation" is that the ISA virus may have entered the hatchery through an hitherto unknown route. Still, the occurrence if ISA during start-feeding of salmon larvae shows that screening of brood-stock should be carried out on a routine basis

References

Dannevig, B.H., Falk, K. and Namork, E. (1995). Isolation of the causal virus of infectious anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. *Journal of General Virology* **76**, 1353-1359.

Dannevig, B.H., Brudeseth, B. E., Gjøen, T., Rode, M., Wergeland, H. I., Evensen, Ø. and Press, C. McL. (1997). Characterisation of a long-term cell line (SHK-1) developed from the head kidney of Atlantic salmon (*Salmo salar* L.). *Fish & Shellfish Immunology* **7**, 213 – 226.

Devold, M., Krossøy, B., Aspehaug, V. and Nylund, A. Use of RT-PCR for diagnosis of Infectious Salmon Anaemia virus (ISAV) in carrier sea trout *(Salmo trutta). Dis. Aquat. Org.* (submitted).

Falk, K., Namork, E., Rimstad, E., Mjaaland, S., and Dannevig, B.H. (1997). Characterisation of infectious salmon anemia virus, an Orthomyxo-like virus isolated from Atlantic salmon (*Salmo salar* L.). *Journal of Virology* **71**, 9016-9023.

Falk, K., Namork, E. and Dannevig, B.H. (1998). Characterization and application of a monoclonal antibody against infectious salmon anaemia virus. *Dis. Aquat. Org.* **34**, 77-85.

Fields, B.N., Knipe, D.M. and Howley, P.M. (1996). *Virology*. Lippincott-Raven Publishers, Philadelphia, U.S.A.

Koren, C.W.R. and Nylund, A. (1997). Morphology and morphogenesis of infectious salmon anaemia virus replicating in the endothelium of Atlantic salmon *Salmo salar* L. *Dis. Aquat. Org.* **29**, 99 - 109.

Krossøy, B., Hordvik, I., Nilsen, F., Nylund, A. and Endresen, C. (1999) Cloning and sequence analysis of the predicted polymerase gene of infectious salmon anaemia virus. *Journal of Virology* **73**(3), 2136-2142.

Mullins, J.E., Groman, D. and Wadowska, D. (1998). Infectious salmon anaemia in salt water Atlantic

salmon (*Salmo salar* L.) in New Brunswick, Canada. *Bull. Eur. Ass. Fish Pathol.* **18(4)**,110-114.

Nylund, A., Hovland, T., Watanabe, K. and Endresen, C. (1995a). Presence of infectious salmon anaemia virus (ISAV) in different organs of *Salmo salar* L. collected from three fish farms. *J. Fish Dis.* **18**, 135-145.

Nylund, A. and Jakobsen, P. (1995). Sea trout as a carrier of infectious salmon anaemia virus. *J. Fish Biol.* **47**, 174 - 176.

Nylund, A., Alexandersen, S., Jakobsen, P. and Rolland, J.B. (1995b). Infectious salmon anaemia (ISA) in brown trout. *J. Aquat. Anim. Health* **7**, 236 - 240.

Nylund, A., Alexandersen, S., Løvik, P. and Jakobsen, P. (1994). The respons of brown trout (*Salmo trutta* L.) to repeated challenge with infectious salmon anaemia (ISA). *Bull. Eur. Ass. Fish Pathol.* **14(5),** 167 - 170.

Nylund,,A., Krossøy, B., Watanabe, K. and Holm, J. A. (1996). Target cells for the ISA virus in Atlantic salmon (*Salmo salar* L.). *Bull. Eur. Ass. Fish Pathol.* **16(2)**,68-72.

Nylund, A., Kvenseth, A.M., Krossøy, B. and Hodneland,, K. (1997). Rainbow trout (*Onchorhynchus mykiss*, Walbaum, 1792): A carrier of infectious salmon anaemia (ISAV). *J. Fish Dis.* **20**, 275-279.

Rodger, H.D., Turnbull, T., Muir, F., Millar, S. and Richards, R.H. (1998). Infectious salmon anaemia (ISA) in the United Kingdom. *Bull. Eur. Ass. Fish Pathol.* **18(4)**, 115 - 116.

Rolland, J.B. and Nylund, A. (1998). Sea running trout: Carrier and transmitter of the infectious salmon anemia virus (ISAV). *Bull. Eur. Ass. Fish. Pathol.* **18(2),** 1 - 6.