Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety
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Which risk factors relating to spread of Infectious Salmon Anaemia (ISA) require development of management strategies?

SUMMARY

Infectious salmon anaemia (ISA) is a viral disease that was first recorded in 1984 in fish from an Atlantic salmon (*Salmo salar*) hatchery. The original disease spread in a pattern consistent with a contagious disease and the contagious nature of the disease was also confirmed experimentally.

The ISA epidemic in Norway peaked in 1990 when ISA was detected in 80 fish farms. ISA was placed on the list B of *notifiable* diseases in Norway in 1988. During 1988-1991, various biosecurity actions were implemented. The essence of these regulatory actions was to interrupt the transmission of infection and to reduce infection pressure. The result of these actions, together with significant improvements in husbandry practices, was a remarkable and a rapid reduction in the number of ISA outbreaks to only 2 outbreaks in both 1994 and 1995. The present accumulated number of ISA disease outbreaks registered in Norway is 437, of which 3 (0.7 %) have occurred in the fresh water phase. Today, the apparent annual prevalence of ISA is about 2 %.

To ensure that the Norwegian Food Safety Authority bases its management on internationally accepted knowledge, the Norwegian Scientific Committee for Food Safety was asked to consider a number of specific questions.

To prepare scientific background documents necessary to answer the questions, the Norwegian Scientific Committee for Food Safety, Panel on Animal Health and Welfare, established an *ad hoc* group consisting of 9 national and international experts. The international experts came from Scotland, Canada and the USA. The group was chaired by Professor Espen Rimstad from the Scientific Committee.

The Panel on Animal Health and Welfare discussed a preliminary version of the report in a meeting on the 12th of December and the full report in a meeting on the the 15th of January, and gave its support to the conclusions drawn by the *ad hoc* group. Concerning Question 6, the Panel agrees with the opinion of the majority of the *ad hoc* group.

Conclusions of the risk assessment from the *ad hoc* group

1A) Can ISA virus transmit vertically?

The *ad hoc* group is of the opinion that vertical transmission of ISA virus cannot be excluded. However, available data are inconsistent, and there was disagreement within the *ad hoc* group on the interpretation of the available data.

1B) How high is the probability of spread of the agent and/or development of disease, as a result of vertical transmission?

The probability of spread of the ISA virus as a result of vertical transmission may depend on individual characteristics, such as clinical status and virus titre in the parent fish, intracellular or extracellular transmission, which is regarded as the dominant route; and/or strain characteristics of the virus.

The probability of further spread via eggs, fry or smolt as a result of vertical transmission will depend on the efficacy of intervening management procedures, such as disinfection and prophylactic treatment post-stripping.

Present relevant knowledge is scarce on these variables. It is not possible from the available information to estimate the probability of spreading of the agent through vertical transmission.

Spread of the *disease* as a result of vertical transmission may be regarded as a consequence of ISA virus being vertically transmitted. The low number of outbreaks in the fresh water stage (0.7 %) and lack of ISA disease in some countries that over the years have imported substantial numbers of eggs from Norway, suggest that the probability of disease emergence following vertical transmission of virus is low.

2) Do present levels of knowledge relating to the virus and disease provide a good enough basis for tracing the source of infection and routes of transmission of ISA?

The *ad hoc* group is of the opinion that epidemiological information together with phylogenetic information are both necessary to resolve the source of infection and routes of transmission. Present levels of knowledge relating to the virus and disease do not provide a good enough basis for tracing of the source of infection and routes of transmission. Each case has to be handled based on its own merits.

3A) Of what importance are the different production stages, including transport, slaughter and other related activities for spread of infectious materials?

Concerning the importance of different production stages and other related activities for spread of virus and disease, the *ad hoc* group is of the opinion that the risk of viral transmission between *freshwater production sites* is minimal.

The risk of spread of ISA disease between *marine production sites* is in general not possible to estimate. Epidemiological studies and general field observations point to clustering of outbreaks in space and time indicating horizontal transmission of the virus in seawater. The strength of the associations estimated in some early epidemiological studies may have

changed following the implementation of biosecurity measures. However, phylogenetic analyses of virus from some of the claimed clusters have not always supported that horizontal transmission has occurred between the sites. These diverging conclusions need to be followed up by more detailed studies.

Concerning *transportation*, the *ad hoc* group is of the opinion that well boat transportation is an important risk factor for the spread of ISA virus. Well boats operate in a national and international market and interchangeably carry smolt and fish for slaughter.

The number of *slaughterhouses* has been reduced, thus giving longer transportation routes. An increased number of fish is delivered for slaughter to each slaughterhouse, and these slaughterhouses are becoming centres in a large network, that may increase the risk of spreading infectious agents including ISA virus. The common use of waiting pens in the slaughterhouses instead of on-shore waiting tanks from which the effluent water is disinfected, is a possible risk factor.

3B) How long does ISA-virus remain infective under different conditions?

Concerning the persistence of infectivity of ISA virus under different conditions, the *ad hoc* group emphasizes that the ISA virus is relatively sensitive to disinfectants. However, the virus is able to survive for weeks in offal and carcasses. Although the information regarding survival of ISA virus infectivity varies, there are several findings that indicate long-term survival in water. Assessment of survival rates of viruses belonging to the same virus family indicates that ISA virus is likely to remain infective in water under field conditions for weeks.

4) Does infection with different variants of the virus pose greater or lesser risk of disease outbreak?

The outcome of any virus infection is dependent upon properties of the virus strain, infective dose, environmental factors including management practises, and the host. These factors have to be considered when assessing the virulence of the virus in any ISA outbreak.

Properties such as receptor-binding and release, fusion and interferon antagonism, which all are important factors of virulence in influenza viruses, have been identified for the ISA virus. However, it is still not known to what extent variations in these properties influence the outcome of an ISA virus infection.

The highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) most likely represents an important virulence marker, and it is so far the only genetic marker that has been associated with virulence in ISA virus. The HPR is characterised as having differential deletions compared to a theoretical full-length precursor gene named HPR0.

There are no reports of detection of HPR0 from ISA-diseased fish with classical clinical and pathological changes consistent with ISA. The majority of HPR0 detections have been from healthy fish. However, based on the lack of adequate experimental infection models with HPR0 virus, caution should be exercised in stating that HPR0 is avirulent. A gene sequence corresponding to HPR0 was identified in gill samples in a case of suspicion of ISA in a marine fish farm in Scotland, and it was also found in a group of smolts with proliferative gill disease in Norway.

The circumstantial evidence as of today indicates that infection with HPR0 virus in itself poses a lesser risk of disease outbreak than infection with ISA virus with other HPRs.

5A) What are the reservoirs for ISA virus?

The *ad hoc* group is of the opinion that the most likely reservoirs for ISA virus are farmed salmon themselves and wild salmonids, of which the most important are brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*).

5B) What is the importance of various reservoirs for outbreak of disease?

Considering the importance of various reservoirs for outbreaks of the disease, the *ad hoc* group assumes that the reservoir for ISA virus causing approximately 30 % of ISA outbreaks in Norway is other farms in the proximity of and within the network of locations with recorded ISA disease. The model from which the *ad hoc* group based its opinion could not explain the remaining 70 %. Alternative reservoirs for the remaining outbreaks of disease could be:

a. Potential disease-causing ISA virus is circulating in the industry, but is not regularly detected. This reservoir is documented; however, the relative importance is unknown. Wellboats commonly used for both transport of smolts and fish to slaughter may be an important risk factor for such circulation.

b. Putative avirulent ISA virus is transmitted from wild fish to farmed salmon, followed by a conversion from an avirulent to a virulent form, which occurs with an unknown frequency. This reservoir is present; however, the relative importance is unknown.

c. ISA virus is prevalent in farmed salmon and is transmitted vertically. The virus then passes through the different life stages and may cause disease in the marine stage. The virus has been detected in several life stages, but the relative importance for disease outbreaks is unknown.

6) Is screening for ISA virus in apparently healthy fish appropriate for limiting ISA distribution?

When evaluating the ability of targets for screening for ISA virus one should consider not only the target itself but also the ability to intervene after a positive finding. The basis for giving advice on screening could be clearer if the prevalence of ISA virus in different life stages in Norwegian aquaculture was known to some extent.

The majority of the *ad hoc* group recommends that screening of broodfish in the “breeding nucleus” should be encouraged. However, this recommendation should be combined with strict biosecurity procedures for handling of broodfish and sexual products. After spawning, the eggs from the breeding nucleus must be kept in separate cylinders, one for each family. This means that progeny from individual parents that have tested positive can be discarded. The group majority is also of the opinion that testing of fish ready for slaughter, prior to transport should be encouraged. A cost-benefit analysis to evaluate the testing should be performed after a sufficient time period has elapsed. Because the possible effect of the proposed screening on ISA outbreaks is currently not possible to assess, screening should be voluntary.

In the answer to question 6 of the assessment, the *ad hoc* group did not achieve consensus. One member of the *ad hoc* group disagreed with the majority. To the answer whether screening is appropriate, the conclusion of the dissent is that screening should not be applied unless it is known to be cost-efficient with regard to disease control or eradication.

CONTENTS

GLOSSARY

Brown trout: *Salmo trutta* (includes sea running trout)

Clade: A group of organisms consisting of a single common ancestor and all the descendants of that ancestor.

Cut-off-value: The cut-off value (k) is a pre-defined value that determines the test outcome for a diagnostic test with a continuous output, e.g. the test is positive if the result of the test is on one side of the k-value and negative otherwise.

Ct-value: The number of cycles run in a real-time RT-PCR when the fluorescence in the sample crosses a threshold value and enters a log-linear phase, i.e. when a sample is considered positive (cycle threshold).

Egg incubation: The time after fertilization before the eyed egg stage. At this time, the eggs are extremely fragile and handling is not possible.

Eyed eggs: The stage after the eyes of the embryo has appeared as two dark spots on the egg. The eggs are then robust and can be transported.

Farm site: The geographical location of a production unit.

Gold standard: A sample or an animal with known true infection status. The term has also been used to describe a diagnostic test that gives the true status of an animal.

Grow-out farm: Farms at sea that receive smolts and grow the fish to market size.

Hatcheries/smolt producers: Locations that receive eyed eggs from egg producers in order to hatch them and produce juveniles. Most hatcheries are also smolt producers, but there are some independent smolt locations.

HE: Haemagglutinin-esterase

HPR: Highly polymorphic region, that is located near the stem of the HE molecule, adjacent to the transmembrane region.

HPR0: The full-length HPR. HPR0 is, theoretically, considered to be a precursor of other HPRs.

Juvenile stage: The time between hatching and smoltification, which includes the yolk sac, start feeding and parr stages.

Molecular clock: A technique in genetics, which is used to date when two species (in this report virus) diverged.

Molecular phylogeny: The use of the structure of molecules to gain information on a virus' evolutionary relationships to other viruses.

Pathogenesis: The mechanism by which a certain aetiological factor (in this report virus) causes disease. The term is also used to describe pathological changes during disease development.

Population: A collection of animals living within geographical boundaries, - a given area or space.

Predictive value: The probability of a positive or a negative test result being a true positive or negative, respectively. Estimation of this value is based on diagnostic sensitivity, diagnostic specificity and prevalence of the pathogen in the tested population. This predictive value is of interest especially when prevalence is low.

Prevalence: The fraction of events (e.g. number of ISA outbreaks) registered in a population at a particular point in time. For example, if n animals are randomly sampled at a given time, and y animals are classified as positive for a particular disease, the prevalence (p) of that disease at that point in time is estimated to be y/n. If the entire population has been sampled, then the prevalence, p, is exactly y/n. Apparent prevalence (AP) is the fraction most often estimated since only a subset of the population is normally tested. AP may be different from p due to improper sampling strategies, registration routines or test characteristics.

Quasispecies: A viral population composed of a spectrum of mutants in which the most fit genotype dominates.

Real-time RT-PCR: A laboratory technique used to quantify and amplify simultaneously a specific part of a given DNA molecule. The DNA molecule arises from reverse transcription of RNA molecules. Diagnostically real-time RT-PCR is applied to detect rapidly the presence of genes involved in infectious diseases.

RT-PCR: PCR preceded by reverse transcription of RNA into DNA.

Screening: Systematic examination of animals or groups of animals in a population to ascertain the apparent prevalence of a specific infection or disease. The results will depend on test characteristics and sampling procedures.

Sensitivity: Diagnostic or epidemiological sensitivity is the probability that a test returns a positive result, given that the true status of the animal tested, is positive for the disease. The probability of the test to return a positive result in a positive herd (herd level sensitivity) is in general higher than the probability at individual level. Laboratory or analytic sensitivity is the test's ability to detect an agent in a sample in the laboratory (detection limit).

Smolt stage: Fish that has gone through the significant physiological changes that enable it to be transferred to sea. Smolts are silvery in appearance, as opposed to the brownish parr.

Specificity: Diagnostic or epidemiologic specificity is the probability that a test returns a negative result, given that the true status of the animal tested is negative for the disease. The probability of the test to return a negative result in a negative herd (herd level specificity) is in general lower than the probability at individual level. Laboratory or analytic specificity is the test's ability to identify a specific agent correctly.

TCID: Tissues culture infected dose. This term is used for quantification of virus. Usually the quantity of virus is expressed as the number when 50 % of the cultures are infective, $TCID_{50.}$

True negative: A negative test result for an animal that is truly negative for a particular infection or disease.
True positive: A positive test result for an animal that is truly positive for a particular infection or disease.

Validation: The evaluation of a test to determine its fitness for a particular use. This includes optimisation and demonstration of its performance characteristics.

Virulence: The relative ability of a virus to damage the host.

BACKGROUND

Infectious salmon anaemia (ISA) is a viral disease that was first recorded in 1984 in fish from an Atlantic salmon hatchery (Thorud and Djupvik, 1988). Affected fish were severely anaemic, hence the name of the disease, and the fish showed typical macroscopic lesions including ascites, petechiae on internal organs and haemorrhagic liver necrosis (Thorud and Djupvik, 1988). The original disease spread in a pattern consistent with a contagious disease and the contagious nature of the disease was also confirmed experimentally. Even though some farms that raise both rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon have had ISA, disease outbreaks have only been found in Atlantic salmon, whereas the rainbow trout have remained unaffected.

The epidemic of ISA increased steadily in Norwegian salmon farming and peaked in 1990 when ISA was detected in 80 fish farms. During 1988-1991, regulatory actions were implemented to control the disease. The essence of these regulatory actions was to interrupt the transmission of infection and to reduce infection pressure. The result of these actions together with significant improvements in husbandry practices was a remarkable and rapid reduction of ISA outbreaks to only 2 outbreaks in both 1994 and 1995. The present accumulated number of ISA disease outbreaks registered in Norway is 437, of which 3 (0.7 %) have occurred in the fresh water phase.

Outside Norway, ISA was reported in the period from 1997 to 2000 in salmon farms on the Atlantic coast of Canada, and USA, in Scotland and the Faeroes. Isolation or detection of the ISA virus has also been reported from Pacific Coho salmon (*O. kisutch*) in Chile and from rainbow trout in Ireland.

PREVENTION AND CONTROL USED FOR ISA IN NORWAY

The ISA epidemic in Norway peaked in 1990 when outbreaks were diagnosed in 80 fish farms. ISA was placed on list B of *notifiable* diseases in Norway in 1988. During 1988-1991, biosecurity actions were implemented. The essence of these regulatory actions was to interrupt the transmission of infection and to reduce infection pressure. Generally, the measures were based on experience from decades of successful combating of terrestrial animal diseases, accumulating aquaculture field experience, and epidemiological information and knowledge together with ISA-specific preliminary risk analysis results generated during the epidemic (Vågsholm et al., 1994).

From the beginning of the ISA history, the disease tended not to disappear from affected sites until each site had been emptied. This experience was implemented early in the combat strategy as part of a stamping out policy. The strategy was later expanded to include fallowing of whole areas for a designated period of time, usually 6 months (Thorud and Håstein, 2002). An outbreak was defined to be present when the daily cage mortality exceeded 0.05 %.

Slaughterhouses receiving fish from an ISA site needed special approval from the Food Safety Authorities. Such approval was also needed for the chosen transportation route between the actual site and the slaughterhouse. Due to the assumed risk of horizontal transmission of the agent, an area around the affected farm was declared a combat zone and was closely monitored. All sites within this zone were controlled by restrictions on production, fallowing procedures and transportation in and out of the zone. Outside this zone, an observation zone was established with intensified surveillance and reporting duties, but

usually no restrictions were imposed. Strict segregation of generations was encouraged, although this measure was not formally incorporated in the legal acts until 2005. General hygienic measures such as frequent (daily) removal and sanitary handling of dead fish became common practice. All these biosecurity measures were quickly implemented in agreement between the authorities and the industry through a common campaign called "Stop ISA" that was initiated in 1990.

In 1996, existing experience and scientific knowledge were summed up in the first official contingency plan for combating ISA, and these guidelines became the basis of an ISA policy within the EU. The principle of a stamping out procedure was maintained in the present contingency plan released in 2004, which required all fish to be removed from infected sites within 80 days. This specific time limit was later modified "to be decided" in each case (www.mattilsynet.no).

Based on the Norwegian experience, a general approach to the control of ISA is summarised in the Diagnostic Manual for Aquatic Animal Disease published by the Office International des Epizooties (OIE):"*The incidence of ISA may be greatly reduced by implementation of general legislatory measures regarding the movement of fish, mandatory health control, and slaughterhouse and transport regulations, as well as specific measures including restrictions on affected, suspected and neighbouring farms, epizootiological studies, enforced sanitary slaughtering, generation segregation ('all in/all out'), and disinfection of offal and wastewater, etc., from slaughterhouses and fish processing plants."*

The ISA specific legislation in Norway is described in detail in "Contingency plan for control of infectious salmon anaemia in Norway" by the Norwegian Food Safety Authority (www. mattilsynet.no). Norway has a general prohibition on vaccination against serious infectious diseases. However, the Norwegian Food Safety Authority-Head Office may give permission to vaccinate under specific conditions. "*Vaccination may, however, under certain circumstances be a useful measure to avoid multiplication of the infectious agent and spread of disease following an outbreak. This is especially applicable in areas with several disease outbreaks over relatively short periods of time and where there is extensive aquaculture activity*."

Table 1. Chronological list of measures implemented to control ISA in Norway

1988	ISA was placed on list B of *notifiable* diseases
1989	Obligatory health certificate - health control in hatcheries - 12 regulatory health assessments a year - Disinfection of eggs Ban on use of sea water in hatcheries Ban on moving fish already put to sea
1990	Regulation on transport - disinfection of well boats Segregation of generations ('all in/all out') encouraged on a voluntarily basis

	Stop ISA campaign
1991	Regulation of disinfection of waste water from slaughter houses and processing plants* Regulation for disinfecting sea water for use in hatcheries Control and containment of dead fish in farms
1992	Introduction of zones to combat outbreaks
1996	Official guidelines for dealing with outbreaks
2001	Contingency plan
2004	Contingency plan revised
2005	Segregation of generations ('all in/all out') made mandatory

*The slaughterhouse regulations also regulated santitation of dead fish and organic waste in fish farms (i.e. acid ensilage)

OCCURRENCE OF ISA OUTBREAKS

The number of ISA outbreaks in Norway and the production of Atlantic salmon during the last twenty years are presented in Fig. 1. From 1988 to 1991 some of the most important measures for controlling the disease were implemented. Due to the combined effort of industry and authorities, these measures were immediately put into action.

In addition to ISA, furunculosis, cold-water vibriosis and vibriosis were frequently diagnosed in the late 1980's and early 1990's. New bacterial vaccines proved very efficient, and almost the entire Atlantic salmon smolt population was vaccinated against the three bacterial diseases from 1991/1992 onwards. A marked decline in the number of ISA outbreaks was seen simultaneously with the introduction of efficient bacterial vaccines (Fig. 2). This favourable situation may have been due to a pure vaccination effect; however, sanitary measures and the focus on biosecurity were almost simultaneously brought into effect. The combined sets of actions may therefore have created a synergistically favourable situation as a decrease in infectious load and risk of transmission may have underpinned the effect of the vaccines and vice versa.

Obviously, the improved health status regarding bacterial diseases and the stimulation of the innate immune response through vaccines might also have had beneficial effects on the clinical occurrence of ISA.

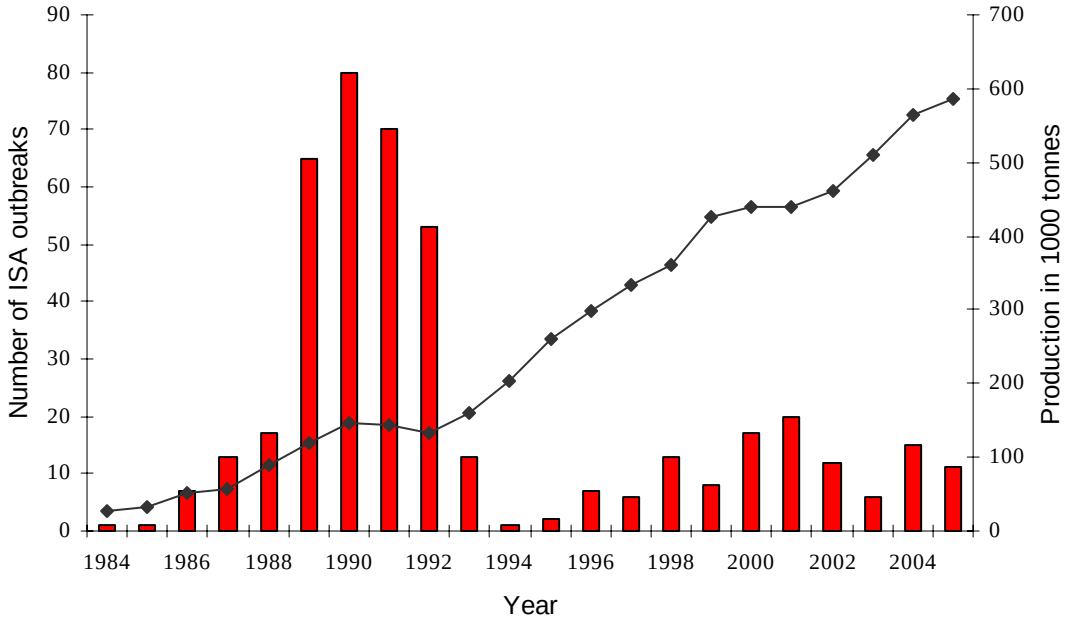


Figure 1. Number of confirmed ISA outbreaks (red columns) and salmon production in 1000 tonnes (black line) in Norway from 1984 until 2005.

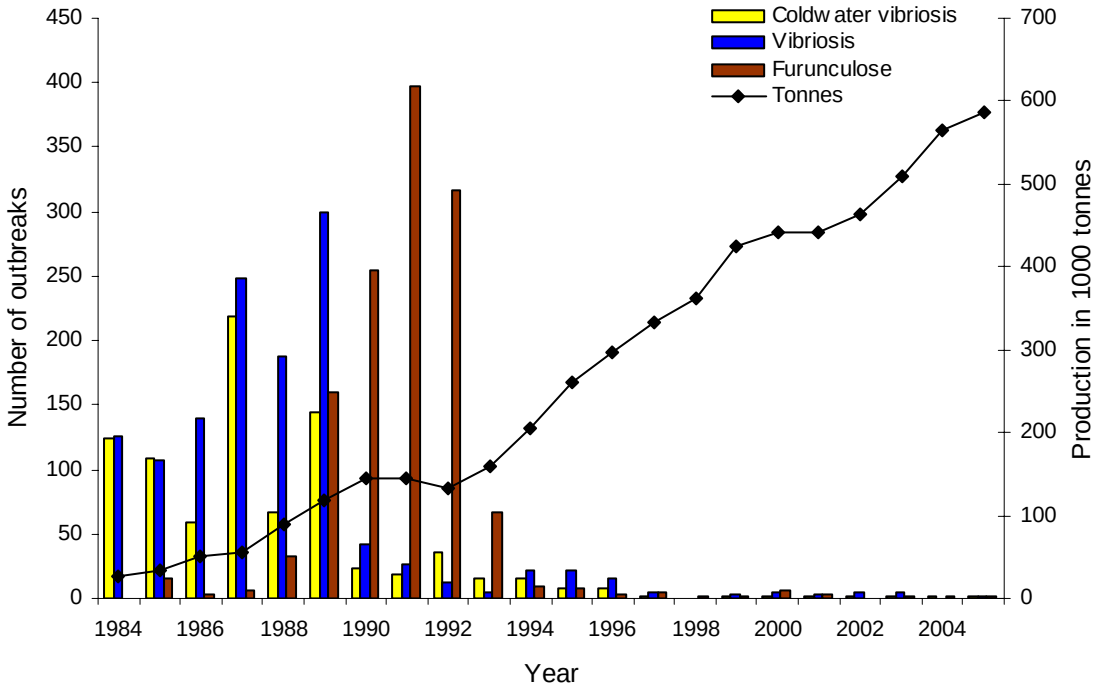


Figure 2 Number of confirmed outbreaks with cold water vibriosis, vibriosis or furunculosis together with salmon production (black line) in Norway from 1984 until 2005.

CHARACTERISTICS OF THE ISA DISEASE

ISA outbreaks are mainly seen in seawater farms, though a few outbreaks have occurred in hatcheries. Experimental infections have shown that ISA can also occur in fresh-water. The disease usually starts in one net pen, and it may take many months before disease develops in neighbouring net pens. Daily mortality generally stays low, but often increases in early summer and winter to more significant levels (0.5–1 %). Without intervention the cumulative mortality may become very high. Acute episodes of extremely high mortality may occur.

ISA is a systemic disease affecting the circulatory system, and the major target cells for the infection are endothelial cells lining blood vessels of all organs including sinusoids, endocardium and endothelial macrophages. Final stages of the disease are characterised by hemorrhage, a circulatory collapse and an extreme anaemia (haematocrit <10). Though virus infected endothelial cells are observed in all organs by immunohistochemistry (IHC), a striking observation is the lack of an inflammatory cellular response as observed by histopathological examinations.

On necropsy, the key finding is a variable set of circulatory disturbances due to endothelial injury in peripheral blood vessels. Changes indicating a systemic infection such as oedema and small bleedings of eyes, skin and serosal surfaces are present along with the changes more characteristic of ISA. Characteristic pathological changes that may be seen to a variable degree include dark livers due to haemorrhagic liver necrosis, moderately swollen kidneys with interstitial haemorrhaging and tubular necrosis, dark red guts due to bleeding within the intestinal wall, and blood accumulation in the gill filaments. It is noteworthy that hemorrhagic organ lesion can be absent or very rare in the initial stages of an ISA outbreak, leaving only the anaemia and the more subtle circulatory disturbances as a clue to the aetiology.

CHARACTERISTICS OF ISA VIRUS

Isolation in cell culture

There are no available cell lines that support propagation of all ISA virus strains. The virus may be propagated in the Atlantic salmon derived cell lines SHK, ASK and TO, but also in the Pacific salmon derived cell line CHSE-214. The CHSE-214 cell line does not or does only poorly support growth of the European variants of ISA virus.

ISA virus – physical and chemical properties

ISA virus is a pleiomorphic, enveloped virus, with surface projections associated with haemagglutination (receptor-binding), receptor-destroying and fusion activity. The genome consists of eight single-stranded RNA segments with negative polarity ranging in length from 2.4 to 1.0 kb and with a total size of approximately 14.3 kb. The properties of ISA virus are consistent with those of the *Orthomyxoviridae*, and ISA virus is classified as the type species of the genus *Isavirus* within this virus family (Fauquet et al., 2005). The organization of ISA virus genes and gene products are however unique.

The nucleotide sequences of all eight genome segments have been described (see Appendix, Table 10). The viral genome encodes at least ten proteins, where nine are known to be present in the mature virus particle. Four major structural proteins have been classified, including the nucleoprotein (NP), the fusion (F) glycoprotein responsible for virus entry, the haemaglutinin-

esterase glycoprotein (HE) responsible for receptor binding and release, and the matrix (M) protein.

Survival of ISA virus infectivity outside the host

The infectivity of ISA virus may be retained for a long time outside the host (see Appendix, Tables 11-13). The virus is stable in the pH range 5-9 (Falk et al., 1997). At pH 4, the virus is completely inactivated after 30 min, and after 30 min at pH 11 the infectivity is reduced by 90 %. Five cycles of freezing (-80 ºC) and thawing (20 ºC) do not reduce infectivity (Falk et al., 1997).
Since ISA virus is a water-borne virus, attempts have been made to estimate the viral survival time in an aquatic environment. However, several factors present in a natural environment such as temperature fluctuations, UV-radiation, heavy metals, binding to non-living particles causing sedimentation and ocean currents may affect viral infectivity and are factors difficult to reproduce in the laboratory. Consequently, the limited information available on ISA virus survival in aquatic environments comes from laboratory experiments incubating ISA virus either in sterile seawater (MacLeod et al., 2003, Rimstad and Mjaaland, 2002), in natural seawater (MacLeod et al., 2003, Nylund, 2004), and in sterile freshwater (MacLeod et al., 2003). The samples were kept at different temperatures (4-6 ºC and 15 ºC), and infectivity was measured over time (Table 11).

For comparison, some data regarding survival of influenza A virus could be considered. This virus is prevalent in water birds and is transmitted partly through water. An interactive effect between salinity and pH on persistence of avian influenza A viruses in water has been demonstrated, and differences in the response to these variables are apparent, even between different types of avian influenza viruses. Duration of infectivity decreases with increasing temperature, salinity and pH; $1x10^6$ $TCID_{50}$ was maintained for 100 d at 17 ºC/0 ppt/pH 8.2, and for 9 d at 28 ºC/20 ppt/pH 8.2. Persistence of infectivity of avian influenza A virus should be regarded as good outside the host (Stallknecht et al., 1990 a, b).

Available data suggest that ISA virus may remain infective for extended periods of time outside its host.

VIRULENCE OF ISA VIRUS

Virulence is not a quality of the virion alone, but a result of interactions between virions, hosts and environment. ISA disease is complex, as observed by differences in disease development.

For comparative reasons, and to illustrate the complexity of viral virulence, a few points important for the virulence of influenza virus are presented. Virulence of influenza viruses is usually the result of optimal gene constellations, although genetic changes located to a single gene or between two gene products may confer changes in virulence. Some of the genes are, however, regarded as being more central than others i.e. the surface glycoproteins responsible for receptor binding, viral entry, including fusion, and viral release from the cell. The haemagglutinin (HA) gene of influenza A virus, which is responsible for receptor binding and virus entry into the cell, plays a pivotal role in determining the severity of infection, at least in avian strains. The development of highly pathogenic viruses is associated with alteration (insertions of basic amino acids) near the cleavage sites of subtypes H5 and H7. On the other hand, the pandemic influenza virus of 1918 did not have any obvious alteration in its cleavage

site. Recently, a central role in the virulence of a human influenza A virus isolate was demonstrated for neuraminidase (NA), which is responsible for viral release. Moreover, domains on the NP, PB2, NS1 and M2 proteins may also be associated with virulence.

Functional properties associated with ISA virus virulence

ISA virus has been demonstrated to possess the major functional characteristics of the orthomyxovirus family associated with virulence and pathogenesis. These characteristics include both haemagglutinating (i.e. receptor binding) and receptor destroying (RDE) and fusion activities. In orthomyxoviruses, these functions are essential for pathogenesis, virulence and host tropism. Variation in these properties between different isolates and types of ISA virus and the relative importance of the factors for virulence are unknown.

In orthomyxoviruses, reassortment of gene segments occurs frequently and is a major contributor to the evolution of these viruses and the emergence of new virulent strains. An extensive molecular and phylogenetic sequence analysis of Norwegian ISA virus isolates provides strong evidence for the occurrence of genetic reassortment involving several ISAV gene segments (S. Mjaaland, personal communication).

The receptor binding of orthomyxoviruses are two sialic acids, which are acidic monosaccharides typically found at the outermost ends of the sugar chains of glycoproteins or glycolipids. This binding is very specific, and ISA virus has been found to bind to 4-*O*-acetylated sialic acids. Occurrence of this type of sialic acid on the cell surface is thus a prerequisite for a cell to be infected. Receptor recognition properties are of general importance for tissue and species recognition.

The function of the RDE is to cleave the receptor binding, and its major function is associated with the release of new virus particles from infected cells and to prevent agglutination of these virus particles. ISA virus haemagglutinates erythrocytes from several fish species including Atlantic salmon. RDE activity dissolved the haemagglutination for all erythrocytes that were agglutinated by ISA virus, except for Atlantic salmon erythrocytes. The inability of RDE to dissolve the hemagglutination of salmon erythrocytes may be of importance for ISA virulence and pathogenesis since virus isolates that show RDE activity with salmon erythrocytes seem to be less virulent, as demonstrated by experimental infections (K. Falk, personal communication).

The fusion activity is associated with uptake of virus into the cell during infection, and is thus essential for virulence. Analysis of the gene encoding the fusion protein has revealed a 30 nucleotide long insertion in several virus isolates, immediately upstream of the protein's putative cleavage site. The same recombination was found in two unrelated ISAV isolates which suggests the presence of a recombinational hot spot. It could be speculated that this could lead to alterations in the cleavage specificity of the fusion protein (S. Mjaaland, personal communication). However, there has so far not been revealed motifs that may be markers of variation in virulence similar to those markers seen in highly virulent avian influenza viruses.

ISA virus, as with influenza virus, encodes for a non-structural protein with an interferon antagonistic effect. This interferon antagonism may be of importance in reducing the innate response of the host, and thus is a potential virulence factor.

Genetic markers of virulence

In contrast to influenza A HA, where most of the variability is located in distal parts of the molecule, most of the variability within the ISA virus HE is concentrated to a small highly polymorphic region (HPR) near the transmembrane domain. This region is characterized by the presence of gaps, rather than single-nucleotide substitutions. The polymorphism in this region has been suggested to result from deletions, or possibly recombination, of a full-length precursor gene (HPR0). The presence of a long HPR0 gene has been confirmed in healthy wild (Cunningham et al., 2002) and farmed Atlantic salmon (Cook-Versloot et al., 2004). The number of different HPR groups is, so far, 26 and 4 among European and North American genotypes of ISA virus, respectively. The pattern of variation is constrained to the 35 amino acids defined as the HPR (Mjaaland et al., 2002, Devold et al., 2001, Nylund et al., 2003, 2007, F. Kibenge personal communication).

Recently, an alternative theory for the occurrence of HPR was presented, suggesting that insertion, rather than deletions, takes place. This suggestion is based on *in silico* estimates of the time of origin of the different viral isolates (F. Kibenge et al., 2006b poster presentation).

Virus isolated from ISA diseased fish contains gaps in the HPR region, as compared to the HPR0. However, viruses with HPR0 have been found in fish with gill disease that has not been characterised as ISA (Anonymous, 2005, Nylund et al., 2007). A gene sequence corresponding to HPR0 was identified in gill samples in a case of suspicion of ISA in a marine fish farm in Scotland in 2004 (Anonymous, 2005). There were significant mortalities, but the clinical signs and post mortem findings (mainly gill inflammation and proliferation) were not consistent with classical ISA. Similarly, Nylund et al. (2007) found HPR0 in a group of smolts with proliferative gill disease, where 6/6 smolt were real-time RT-PCR positive. No further study of a correlation between the virus and the gill inflammation was pursued.

In summary, the HE-HPR therefore most likely represents an important virulence marker. Whether there is a connection between the size or type of the gap and virulence and how deletions in the HPR influence viral virulence remains to be determined. The virulence of ISA virus, however, cannot be attributed to the HE-HPR region alone, as isolates with identical HPR vary in virulence in experimental infections using standardized experimental fish (Mjaaland et al., 2005).

Phylogenetic analysis of ISA virus

For several viruses important to fish farming, a molecular phylogenetic analysis has proven efficient in resolving epidemiological problems (Kurath et al., 2003, Einer-Jensen et al., 2004, Snow et al., 2004, Thiery et al., 2004). Such sequence analysis may provide important information on the spread of pathogens, and hence, can be used as a tool in fish disease management.

The basis for phylogenetic analysis is information on the rate of nucleotide substitutions in the genome of viruses. Substitution rates for the ISA virus are essential to the understanding of the evolution of the ISA virus in farmed populations of Atlantic salmon. The degree of changes constitutes the basis for genotyping.

Several publications demonstrate the highly conserved nature of ISA virus (Devold et al., 2006, Nylund et al., 2007, Jenkins et al., 2002). The two genomic segments with the highest variability are the two surface glycoproteins – the HE and the F. These segments may therefore be phylogenetically informative in contrast to several of the other genomic segments where very little or no phylogenetic information can be extracted.

Most of the phylogenetic analysis of ISA viruses is based on the information obtained from the 5'-end of the HE gene. The HPR region is excluded because this region varies through deletion and/or recombination between related isolates, and is therefore not useful as an indicator of relatedness. Based on the differences in 5'-end of the HE gene, ISA virus has been divided into two major clusters; the North American and the European (Devold, 2001). Analysis of the genomic segment 5 has supported this division (Devold, 2006). ISA virus isolates within the same outbreak cluster together in phylogenetic analysis, although they demonstrate some sequence variation. This finding indicates a common origin for isolates from the same outbreak (Lyngstad et al., 2005).

The European cluster has further been sub-divided into three groups (EU-G1-G3) (Nylund et al., 2007). Some ISA virus isolates from North-America are European-like and are often referred to as "European-in-North America". There are conflicting interpretations as to whether they represent as a separate group or can be placed within existing European groups (EU-G2) (Nylund, 2007, F. Kibenge, personal communications).

Molecular clock

Molecular clock is a technique in genetics, which is used to date when two viruses diverged.

For some viruses or viral genes, a molecular clock can be calculated based on the rate of nucleotide substitutions in the viral genomes.

Due to their error prone RNA-dependent RNA polymerase, RNA viruses mutate quickly and hence may evolve quickly. If viral RNA is not evolving neutrally due to undetermined effects such as codon usage, RNA secondary structures, fluctuations in adaptive environments and population size, then the observed substitution rate and its constancy over time will be affected (Jenkins et al., 2002). Hanada et al. (2004) found that the main source of the rate variation was due to differences in the replication frequency because the rates of replication error were roughly constant for different RNA viruses.

Molecular evolution will only follow a molecular clock if mutation and replication rates are constant and most substitutions neutral. It should not be expected that the molecular evolution of the ISA virus HE gene would follow a molecular clock. Recent studies have confirmed that the substitution rates of segments 5 (F) and 6 (HE) are not constant over time (Devold et al., 2006, Nylund et al., 2007). Hence, it can be concluded that mutation rates, replication rates, or undefined selective constraints vary to some extent. Moreover, identical HE sequences found in ISA virus isolates separated by at least 9 years further support the conclusion that a molecular clock cannot be assumed to be constant for ISA virus (Devold, 2006, Nylund, 2007).

Despite the lack of a molecular clock in ISA virus, there is a relationship between genetic divergence and time.

Important constraints on the evolution of ISA virus are caused by different measures implemented to combat the disease, including segregation of generations and slaughtering of infected fish. The ISA virus infecting salmon is lost, unless there is a significant horizontal transmission of the ISA virus between farms and from farms to wild fish in the marine phase.

A possible vertical transmission of ISA virus isolates could constitute a bottleneck for transmission of genetic variation accumulated in ISA virus populations.

HOST FACTORS

The outcome of a virus infection will not only depend upon the properties of the virus, but also on different host factors. These factors might be of genetic origin, and it has been shown that susceptibility towards ISA virus is linked to certain MHC class I and class II types in Atlantic salmon. Family testing performed in association with Norwegian breeding programmes indicates significant variability in ISA susceptibility between family groups (Gjøen et al., 1997).

Many researchers have experienced seasonal variability when performing controlled challenge experiments with ISA virus on Atlantic salmon. During the autumn, a chronic progression of the disease, often with low cumulative mortality, is observed. However, during the spring, an acute progression is normal. This topic is discussed by Rolland and Winton (2003). In their study, Atlantic salmon challenged during the spring experienced significantly higher mortality compared with fish challenged during the autumn. In a recent paper, Glover and co-workers (2006) show that the susceptibility of Atlantic salmon towards ISA virus infection increases markedly when the fish is in the process of smoltification. These investigators also argue that the association with smoltification might explain the observations of Rolland and Winton. Thus, it could be the physiological status of the fish, and not the season of the year, that is linked to disease progression. In the same study, Glover et al. (2006) could not find any differences between farmed, wild or hybrid fish regarding susceptibility to ISA virus infection. This finding is in contrast to a study by Nylund and co-workers (1995b), which demonstrated that farmed fish are more susceptible than wild fish.

HOST RANGE

The host range of ISA virus can be divided into species in which disease occurs naturally, which is restricted to Atlantic salmon; and secondly, into species in which virus can replicate but no disease is found. The latter may be of importance as carriers of virus and as reservoirs.

In experimental infections injecting intrperitoneally large dose of virus and also several different virus isolates, disease/histopathological changes have been described in rainbow trout inoculated with some of the isolates (Kibenge et al., 2006a).

Replication of ISA virus occurs in experimentally infected Atlantic salmon, brown trout, rainbow trout, Arctic char *(Salvelinus alpinus)*, chum salmon (*O. keta)*, coho salmon, herring (*Clupea harengus)* and Atlantic cod (*Gadus morhua).* No replication has been found in bivalves.

In a survey of about 50,000 non-salmonid marine fish that were tested in Maine by cell culture and RT-PCR over a period of three years, only one positive sample was found and that was in the species alewife (*Alosa pseudoharengus*)(Rolland, 2004). Other species included in that study were American eel (*Anguilla rostrata*), herring, Atlantic mackerel (*Scomber scombrus*), Atlantic cod, haddock (*Melanogrammus aeglefinus*), Atlantic halibut (*Hippoglossus hippoglossus*), Pollock (*Pollachius virens*), American shad (*Alosa sapidissima*) and winter flounder (*Pseudopleuronectes americanus*).

However, the fact that surveys have not yet revealed a non-salmonid reservoir does not prove that a reservoir does not exist. For example, there are molecular indications from the west coast of North America that IHN virus is maintained in some marine species, but extensive surveys have not yet revealed the species (J. Winton, personal communication).

Based on the detection of ISA virus by RT-PCR methods in wild fish, both *S. salar* and *S. trutta* are the most likely candidates as the natural hosts. Hence, it can be worthwhile to

consider the biology of *S. salar* and *S. trutta* when making assumptions on the epidemiology of the ISA virus in wild fish.

ISA VIRUS IN WILD SALMONID POPULATION

Norwegian lakes and river systems constitute a natural environment for three sea running salmonid species (*S. salar, S. trutta* and *Salvelinus alpinus*). The ISA virus and the populations of brown trout and salmon may have co-evolved.

The number of host species and the density, size and distribution pattern of host populations are important factors for the maintenance and spread of viruses.

The population structure of sea running salmonids is strongly influenced by migration behaviour. The highest population densities are found in rivers and lakes. The most frequent interactions between salmonids occur during spawning. The rivers are the rearing areas for salmon fry, parr and smolts, i.e. individuals that can be expected to lack acquired immunity towards viral diseases. The population densities in open seas (salmon) and in fjords (brown trout and salmon) will in most cases be significantly lower than in the rivers. Based on these facts, it is expected that a virus that has co-evolved with salmon/ brown trout should have the best opportunities to transmit in the freshwater phase, i.e. in the rivers. The interactions between individuals are highest during spawning.

Most Atlantic salmon follow an anadromous fish migration pattern, spending a large part of the life span in the ocean, while this is more variable for the different morphs of brown trout. If exposure to ISA virus for wild Atlantic salmon and brown trout mainly occurs in rivers, such exposure will possibly occur at a lower frequency for Atlantic salmon than for brown trout. There are indications that ISA virus may be more closely adapted to brown trout than Atlantic salmon (Nylund et al., 2003, Nylund and Jakobsen, 1995, Rolland and Nylund, 1998a, Devold et al., 2000).

However, co-evolution between virus and host implies adaptation of host factors such as behaviour-related (staying away from the breeding grounds) or innate immune response or factors related to the agent (i.e. low-virulent variants, cf: HPR0 discussion).

Co-evolution may explain the presence of the ISA virus, which is assumed to be avirulent, in wild salmon populations in both fresh and sea water (Nylund et al., 2003, Plarre et al., 2005). Such viruses could represent the “wild type” ISA virus, from which virulent isolates connected to clinical outbreaks of ISA in farmed populations of salmon have originated.

DIAGNOSIS OF ISA

Generally, disease diagnosis is a process involving 3 major steps: 1) description of the disease, i.e. clinical observations and pathomorphological examinations; 2) identification of putative causes/aetiological agents i.e. analyses for detection of infectious agents; 3) establishment of a causative association between 1 and 2.

A general evaluation of some aspects about diagnostic tests is presented in the Appendix.

The diagnosis of ISA (as a disease) was initially based on clinical and pathological findings only. Following the isolation of the causative agent, direct methods for the detection of virus and confirmation of the diagnosis have been established. These methods include isolation of the virus in cell culture followed by immunological identification, antibody-based demonstration of ISA virus antigen in tissues and RT-PCR techniques.

Patho-morphological evaluation

A cornerstone of this evaluation is a histopathological evaluation of formalin-fixed paraffin-embedded tissue sections. The patho-morphological evaluations are routinely supplied by an evaluation of IHC-stained tissue sections.

Cell culture isolation of ISA virus

Diagnostic cell culture isolation of ISA virus from infected fish is usually performed using either SHK-1 and/or ASK-II cell lines. Recent experiences indicate that ASK-II cells should be the first choice for primary isolation (Rolland et al., 2005). ISA virus in cell culture is usually identified by an immunefluorescent antibody technique (IFAT) test using anti-ISA virus monoclonal antibodies.

Demonstration of ISA virus antigens

These methods include detection of ISA virus using anti-ISA virus antibodies on tissue cryosections (IFAT), formalin-fixed paraffin-embedded tissue sections (IHC) and tissue imprints (IFAT). The methods are rapid, relatively cheap, robust and suitable for detection of ISA virus in fish with clinical ISA. Detection of ISA virus in subclinical infected fish is less reliable (or unsuitable) due to restricted sensitivity. IHC is currently the first choice for detection of ISA virus in diseased fish and has a major advantage of being able to associate virus detection with known target cells and pathological lesions.

RT-PCR

This method detects virus genetic material and is rapid, specific and sensitive. Different applications of this method have been described including applications using the improved real-time RT-PCR methods. The main advantages of these methods are the sensitivity and speed, and it is currently the method of choice for detection of ISA virus in subclinically infected fish (i.e. in ISA virus infected fish showing no disease symptoms). In disease diagnostic work, RT-PCR is used for confirmation of virus findings in diseased fish.

VALIDATION OF DIAGNOSTIC TESTS

Diagnostic tests need to be properly validated. In particular, performance and operating characteristics in field situations are important when healthy populations with expected low prevalence are tested. In this situation, false positive tests may have importance as an infection-free population can be classified as infected. Verification of such test results must be performed by another, independent test. In essence, validation determines whether a true finding in the laboratory is also true in the field. This requirement is not encountered in the initial optimization of the tests in laboratory simulations. General and special guidelines for validation are given in OIE's Manual of diagnostic tests for aquatic animals 2006 (http://www.oie.int/eng/normes/fmanual/A_summry.htm) and include both test optimization and documentation of performance characteristics. All screening tests that are used should be standardized, and quality control procedures should be implemented before they are used as a diagnostic test for prevention and control of ISA (Nerette et al. 2005, a, b). Cut-off values must be determined for tests with quantitative output and the analytical sensitivity and

specificity of the test should be known. Then good repeatability (i.e. within-laboratory consistency) and reproducibility (i.e. between-laboratory consistency) should be assured. A test that is used for testing of healthy fish to make regulatory decisions should also be able to distinguish between virulent and avirulent forms of the virus. However, today this requirement is restricted to determining whether the virus has HPR0 or not. The eventual pitfalls of using HPR as a virulence marker are discussed under Question 4.

The most important consideration for test validation is to measure the capacity of a positive or negative test result to predict accurately the infection status of the fish or fish population. This capacity is not only dependent on a highly sensitive, precise and accurate assay using well characterised and standardised reagents (see analytical validation above), but carefully derived estimates of diagnostic (also often termed epidemiological) sensitivity and specificity. These parameters are cumbersome and difficult to determine, but are the most important parameters to establish during a validation process. They determine - together with the infection prevalence in the population - the probability that a given test result reflects the true status of the fish (Dohoo et al., 2003).

NORWEGIAN EGG EXPORT

Norway pioneered Atlantic salmon farming, which also included a systematic breeding programme. This programme made Norwegian breeding material (i.e. fertilized eggs) attractive for other countries. Fertilized eggs have been exported during the last thirty years to most salmon producing countries. Norway is traditionally the largest exporter of Atlantic salmon eggs and probably is also the only country that currently does not import eggs. Unfortunately, official records of Norwegian egg export are not available and details concerning the volume and extent of this export have to be based on information from the exporting companies, importing countries and anecdotal information.

Official records from Chile (www.sernapesca.cl) indicate that there has been a significant yearly export from Norway to Chile since 1998. Based on information from two of the major egg producers in Norway (i.e. Aquagen and Salmo Breed), significant amounts of eggs have also been annually exported to Scotland and the Faroe Islands since 2003. In the early 1980's, there were also minor exports to Ireland and Scotland (T. Håstein personal communication). Official records from Iceland show that eggs were imported from Norway in 1984, 1986 and 1987 (G. Jónsson, personal communication). Interestingly, the 1986 and 1987 import came from the company Bolaks (location Eikelandsosen), which at that time had significant ISA problems.

EPIDEMIOLOGICAL ASSESSMENT OF VERTICAL TRANSMISSION

To assess the relative importance of vertical transmission for the spread of ISA virus and/or ISA outbreaks, detailed information on virus characteristics, company history, epidemiological information, and production procedures need to be included.

The risk of producing smolts carrying ISA virus due to vertical transmission may be quantified by a risk assessment starting at the brood stock – hatchery level. Some of the data requirements for such an assessment are listed in Table 2. For assessing the risk of outbreaks due to vertically transmitted virus, further knowledge on virus, biosecurity measures and the production system is needed, as currently most of this information is not available.

Table 2. Data required for assessing the risk of producing ISA virus infected fry resulting from vertical transmission

Probabilities	Comments
Prevalence in brood stock (male and females)	
Probability of internally- externally infected eggs	Infective stage (clinical, sub clinical), abundance of virus, strain of virus
Probability of externally contaminated milt	
Probability of eggs becoming internally infected after fertilisation	Virus load, time to water hardening
Effect of disinfection on external and internal virus	Virus load, type of disinfection, disinfection procedures
Probability of internal/external virus surviving to hatching	Disinfection procedures during incubation

The probability of ISA outbreak

In Table 3 the production volume of eggs and smolts since 1998/99 is shown. The number of production units has decreased through the years, but in 2005, 273 hatcheries were registered (Source: The Directorate of Fisheries). The annual average smolt production capacity may be estimated to 8-900 000 smolts per hatchery. Since 2000, approximately 1.4 billion eggs have been produced for the Norwegian market. There have been no reports of ISA outbreaks in hatcheries in this period. With this huge number of potentially infected eggs going through the production cycle, the probability for ISA outbreaks in hatcheries must therefore be regarded as very low.

Table 3. Egg and smolt production in Norwegian farmed salmon industry

Year	Eggs for incubation (in millions)	Year	Smolts to sea (in millions)
1998/99	192	1999	123
1999/00	183	2000	133
2000/01	224	2001	134
2001/02	248	2002	135
2002/03	260	2003	128
2003/04	215	2004	134
2004/05	225	2005	153
2005/06	245	2006	

Source: The Directorate of Fisheries

Roughly calculated, each generation occupies about 275 sea sites and a total of approximately 600 sites are actively in use at any given time every year (assuming three generations present). According to Lyngstad et al. (2005), the time from sea transfer to the diagnosis of an ISA outbreak is from 7 to 21 months. This time period indicates that the entire number of active marine sites is at risk of experiencing an ISA-outbreak. With an average of 10 official outbreaks annually in the time period 2003-2005, the apparent annual incidence of ISA outbreaks has varied from 0.8 % to 2.5 %, which may be regarded as the annual site probability of experiencing an ISA outbreak. The true prevalence may be higher assuming under-reporting of the disease.

Egg production in the Norwegian salmon industry

For the production year 2004/2005, 225 million eggs were incubated, and in the 2005 season 153 million smolts were stocked to sea (www.laksefakta.no). The corresponding numbers for rainbow trout were 35 million eggs and 19 million smolts. This production mainly originates from four commercial egg producers with 23 brood fish locations. The eggs are then distributed among 273 hatcheries/smolt producers and finally to approximately 275 grow-out locations at sea.

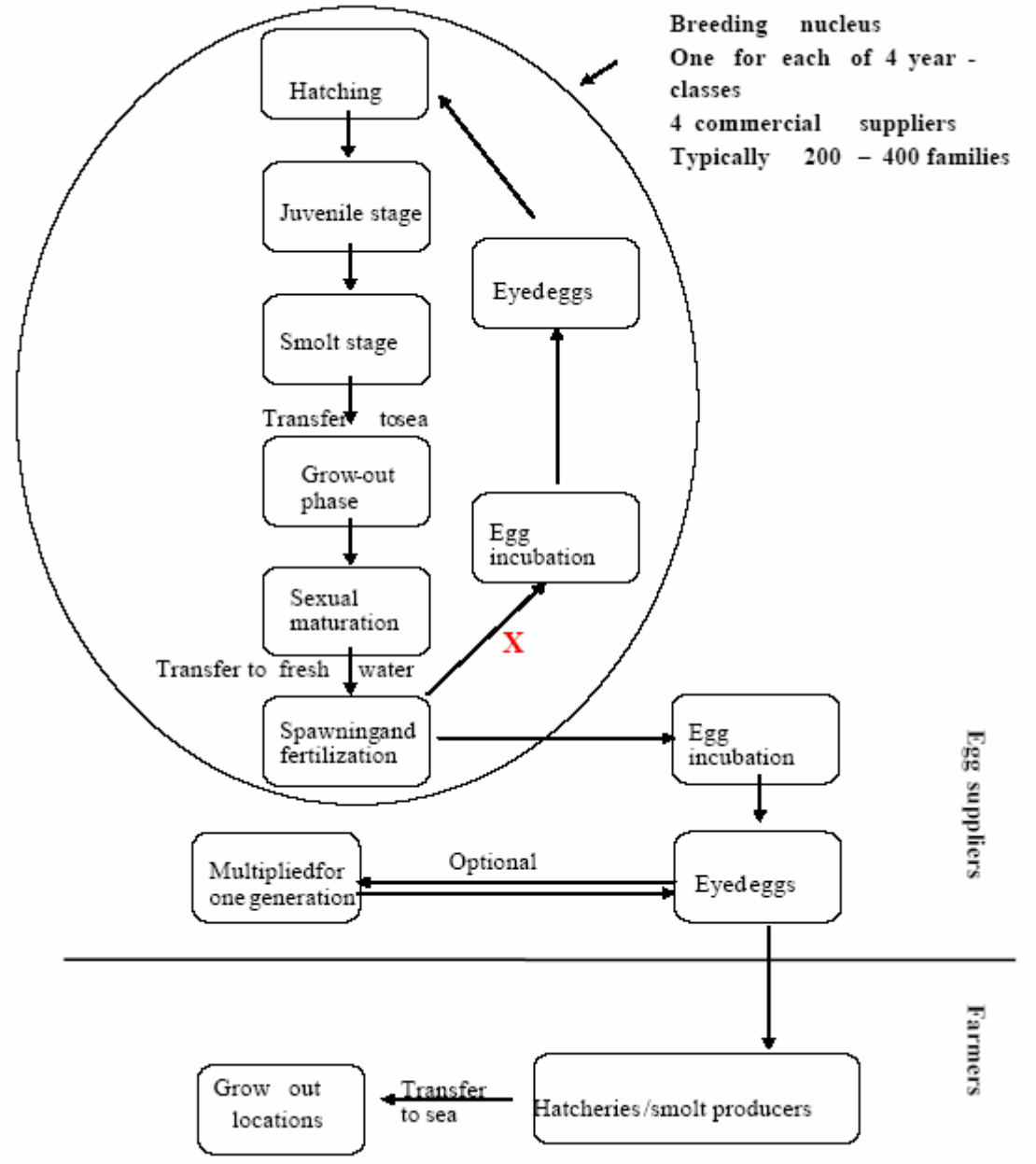
Because of the continuously changing infrastructure of the industry, detailed insight is vital when using historic information in epidemiological studies. Since the turn of the century, only a few companies have been involved in egg production of which SalmoBreed and Aqua Gen are the two main companies. These two companies act as umbrellas under which five different egg producers belong to SalmoBreed and four belong to Aqua Gen. The egg production units are located in different parts of the west-northwest coast of the country, and are clearly hydro-geographically separated. The genetic family core both for SalmoBreed and Aqua Gen dates back to about year 2000, which means that the coordinated breeding system under these umbrellas of today represents about 6-8 generations. The different sub-contractors may therefore have produced different genetic material before and just after 2000/2001. An example is shown at http://www.sevareid.no/avl,rogn.htm where one of the egg producers that currently belong to SalmoBreed, produced brood stock in 2001 and smolt in 2001/2002 based on eggs from Aqua Gen in 1998.

In a typical breeding system, there is 4 year-classes of salmon in production at any given time. These individuals will be the source of eggs for production, but also of eggs for the next generation of brood fish. This part of the population is termed the “breeding nucleus”. The brood stock is kept at sea, usually in separate locations for each generation, and transferred to freshwater before maturation is complete. After spawning, the eggs from the breeding nucleus are kept in separate cylinders, one for each family, whereas the eggs intended for production are usually pooled before incubation. In this setup, most of the production eggs will not be from the families that are chosen for the new breeding nucleus. It is also possible to use full siblings of the new breeding nucleus for production. In this case, it is necessary to raise these individuals to maturity for another spawning that will provide production eggs. For a schematic overview of the production, see Fig. 3.

After fertilization, the eggs are disinfected by use of iodophore (100 ppm, 10 min). This disinfection is also repeated at the eyed egg stage, before the eggs are shipped to hatcheries. In the hatcheries, normally the eggs are routinely disinfected by iodophore upon arrival. At the egg stage, it is routine to use of formalin (0.01 %, 10-30 min) to prevent fungal infections, typically every second day, depending on the water quality.

If vertical infection is a significant mode of transmission for ISA virus, the line marked with an X in Fig. 3 is a point where the cycle can be interrupted. The individuals chosen for the new breeding nucleus will be the parents of all salmon in the next generation. If each of four commercial producers has 300 families, the total number of samples to be screened would be approximately 2400 each year (300 families X 2 (female and male) X 4. Since the males normally are used for several females the male/female ratio is lower than one. The total number presented is therefore a maximum number). Also, at this stage the eggs from each family are kept in separate cylinders, making it possible to discard eggs from positive parents. All other possible control points would necessarily mean a much higher number of samples.

Figure 3. Schematic drawing of the flow of biological material within salmon breeding



ISA outbreaks versus genotyping and brood stock

A total of 46 different hatchery locations delivered smolts to the 32 sites experiencing an ISA outbreak during 2003-2005. All except one outbreak site contained smolts from two or more hatcheries. Nine hatcheries were involved with two or more ISA outbreak sites during this period. The geographical locations of these hatcheries are given in Table 4 together with year of outbreak and the virus group isolated from diseased fish. From the table, one may see that two of the hatcheries were associated with outbreaks, from which viruses belonging to EU-G2 group were found. Seven hatcheries (9 cases) were associated with two or more outbreaks within the same year. Of these, three were associated with the same group while six were associated with outbreaks of different groups. The information available is not coherent enough to conclude whether any significant association exists between hatcheries and a specific group of virus isolate.

Table 4. Hatcheries delivering smolts to two or more ISA outbreaks during 2003-2005

Hatchery (county)/group	EU-G1[a]	EU-G2	EU-G3
1 (N-Trøndelag)	2003	2003	
2 (Sogn and Fjordane)		2004	2003
3 (Møre and Romsdal)	2004		2004
4 (N-Trøndelag)	2003	2003	
5 (N-Trøndelag)		2004, 2005, 2005	
6 (S-Trøndelag)	2003	2003	
7 (Hordaland)		2004, 2005	
8 (Hordaland)	2003		2003, 2004, 2004, 2004, 2005
9 (Finnmark)		2004, 2005, 2005	2004

[a] Nomenclature according to Nylund et al., 2006.

Nylund et al. (2007) combined the information on brood stock companies (denoted A-E) with ISA virus isolates from outbreaks registered in the period 1997-2005 using information on clades (here used as the term for the phylogenetic level below the groups EU-G2; EU-G1 is treated as a clade by itself). Results are shown in Table 5. All the five clades are associated with company A, four with B, two with D, and and a single clade was common to both C and E. Company B is clearly associated with EU-H2 –isolates, but any overall pattern associating clade and broodstock company, is not obvious from the data. The data are however, fragmented especially when viewed in relation to the complex infrastructure of the industry, and the low number of genotyped isolates. Also, the relative frequencies in the Table have to be considered in relation to the market shares of the different companies.

Table 5. Brood stock companies (A-E) associated with ISA virus clades isolated from ISA outbreaks during 1997-2005[1]

Year/clades	EU-G1	EU-NN (G2)	EU-NW (G2)	EU-H2 (G2)	EU-MN (G3)
1997	A				
1998				B, B	

1999	A, AD			B	A
2000	A				
2001					
2002		ABD	A		
2003	AB, AB	CD, CD		B	
2004	AB, AB, AB,	A, ABE		AB, B, B, B, B, B, B, B	A, A, ABD
2005	AB, AB	AE, A, E			A

[1] Information is not available for all outbreaks in the period

Outbreaks related to contact network

In a stochastic time-space model (Scheel et al., submitted), the risk of an ISA outbreak due to proximity to an infectious site and/or contact network was estimated. Cases in this model were outbreaks from 2000-2005. The model concluded that roughly 30 % of the ISA outbreaks were related to the contact network variables in the model and distance to infectious site, assuming an ISAV infected site has an infectious period of nine months. The relative share of these two risk factors was approximately the same. The model concluded however, that the relative risk of proximity to infectious site was higher than contact network when sites were less than 5 km apart but that separation by more than five km may be of less importance if the contact network was maintained. The remaining 70 % could not be explained by the model. The model is based on the data available and the defined assumptions, which may be a subject for discussion. The small number of ISA cases in the dataset limits the precision of the results. The relatively high percentage of risk not explained by the model emphasizes the need for more data to evaluate more fully the assumed risk factors and identify others.