Infectious Salmon Anaemia

Frederick S.B. Kibenge, BVM, PhD, DACVM

OIE Reference Laboratory for ISA,
Department of Pathology and Microbiology,
Atlantic Veterinary College,
University of Prince Edward Island, Charlottetown, P.E.I., Canada.
Atlantic Veterinary College, University of Prince Edward Island
Outline

• Introduction to ISA

• Typical clinical manifestation

• Significance to industry/wildlife

• Transmission and current distribution of the disease

• ISA Diagnostics (sampling and approved diagnostic methods)

• List of reference laboratories for ISA as well as approved labs to test for ISAV for international export within the United States

• Control and prevention (vaccination and chemotherapy)
Infectious salmon anaemia (ISA)

- OIE-listed viral disease of marine-farmed Atlantic salmon
  - First reported in 1984 in Norway.
  - OIE recognized the disease in 1990 & named it ISA.
  - ISA virus was characterized by Falk et al., in 1997 (now classified in virus family Orthomyxoviridae, genus Isavirus).
  - First reported outbreak outside Norway was in Canada (New Brunswick) in 1996.
  - First reported outbreak in USA was in Maine in 2001.
  - First reported outbreak in the Southern Hemisphere was in Chile in 2007.
  - This year (as of April 2012), only Norway and Canada (Nova Scotia) have reported ISA outbreaks.
Infectious salmon anaemia (ISA)

- Mortality in a fish cage rises slowly and can vary from 0 to 90%.
  - Disease course is prolonged with low daily mortality (0.05-0.1%) typically only in a few cages
  - Virus may be present in fish in a cage up to 6 months before significant mortality is noted.
Infectious salmon anaemia (ISA) virus (ISAV)

- Virions are enveloped & highly pleiomorphic: may be filamentous, spherical, oval, donut-shaped, etc.

- Genome is segmented negative sense single-stranded RNA; eight RNA segments, ranging in length from 1 to 2.4 kb; total is \(~14.3\) kb.
### Isavirus genes and proteins

<table>
<thead>
<tr>
<th>Genome segment</th>
<th>Gene products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PB2 (84kDa)</td>
</tr>
<tr>
<td>2</td>
<td>PB1 (84kDa)</td>
</tr>
<tr>
<td>3</td>
<td>NP (77kDa)</td>
</tr>
<tr>
<td>4</td>
<td>PA (71kDa)</td>
</tr>
<tr>
<td>5</td>
<td>F (50kDa)</td>
</tr>
<tr>
<td>6</td>
<td>HE (42kDa)</td>
</tr>
<tr>
<td>7</td>
<td>p32 (35kDa), NEP (18kDa), p11 (10.6kDa)</td>
</tr>
<tr>
<td>8</td>
<td>M1 (24kDa), p16</td>
</tr>
</tbody>
</table>

ISAV Strains

2 basic genotypes/serotypes

North American

European

real-European

European-in-North America

2-to-3 genogroups

HPR20

HPR21

EU-G1

EU-G2

EU-G3

EU-G2

Kibenge et al., 2001  Kibenge et al., 2007  Nylund et al., 2007
ISAV Strain identification

• ISAV strain designation is mostly based on sequence deletions/insertions in a 35-amino acid highly polymorphic region (HPR) of the HE protein (Nylund et al., 2003, 2007; Plarre et al., 2005)

• ISAV without any deletion/insertion in HPR is designated HPR0 to indicate “full-length HPR”

• All ISAV isolated to date from clinical disease have deletions in HPR relative to HPR0
  – are categorized numerically from HPR1 to >30 presently.
ISAV HPR0 viruses

- ISAV HPR0 sequences have been found in:
  - fish with proliferative gill inflammation
  - healthy wild Atlantic salmon
  - healthy farmed Atlantic salmon
  - ISA-affected farmed Atlantic salmon, often in presence with HPR-Ds (Chile)
  - ubiquitous in regions with previous experience with ISA.

- All ISAV isolated to date from clinical disease have deletions in HPR relative to HPR0
  - HPR is important in ISAV virulence
  - Presence of HPR0 could represent a risk factor in the re-emergence of ISA.

- HPR0 viruses:
  - non-cultivable; detected solely by RT-PCR (high Ct values)
  - limited tissue tropism (best sample is gill tissue)
  - examples of “frag-viruses” since they are known only through genomic sequence fragments.
ISAV HPR0 viruses

- In Chile, since 2010, ISAV HPR0 has been found in different production stages of Atlantic salmon [fry, pre-smolt, growout in marine farms, & brood stock] without associated clinical signs of ISA.

- OIE Aquatic Animal Health Commission recently amended the listed disease name for ISA as “Infectious salmon anaemia (infection with HPR-deleted or HPR0 forms of ISAV)”. i.e., for reporting purposes, ISA means infection with ISAV including HPR-deleted and HPR0.
Comparison of amino acid sequences around the proteolytic cleavage site $^{267}$RA/G$^{268}$ of the F protein of various ISAV strains

<table>
<thead>
<tr>
<th>ISAV isolate (GenBank Acc. No.)</th>
<th>Predicted amino acid sequence</th>
<th>aa inserted at cleavage site &amp; in-vivo/in-vitro virulence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norway HR60/01 (AY853944)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>8 (IN1) *</td>
</tr>
<tr>
<td>Norway H46/99 (AY853962)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>11 (IN2) *</td>
</tr>
<tr>
<td>Norway SP70/02 (AY853938)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>10 (IN3) *</td>
</tr>
<tr>
<td>Norway SP75/00 (AY853939)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>10 (IN3) *</td>
</tr>
<tr>
<td>Norway MR62/01 (AY853937)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>10 (IN3) *</td>
</tr>
<tr>
<td>Norway SP71/02 (AY853936)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>10 (IN3) *</td>
</tr>
<tr>
<td>Norway MR62/01 (AY853935)</td>
<td>RANLANQHWSGYFPGEMIKCQTGI</td>
<td>10 (IN3) *</td>
</tr>
<tr>
<td>Chile 1508-6 (Apr 2008)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 1508-7 (Apr 2008)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile U24636 (BAU130923)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 26415-3 (BAU149791)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 26572-6 (BAU149795)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 26572-6 (BAU149766)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 26590-1 (BAU130923)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Chile 26590-5 (BAU130923)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>11 (IN4)</td>
</tr>
<tr>
<td>Norway SK770/06 (BAU130923)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0 (ISAV HPV0) **</td>
</tr>
<tr>
<td>Norway SP83/04 (AY744392)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0 (low virul. ISAV)***</td>
</tr>
<tr>
<td>Can RPC/ND 04-085-1 (BF432567)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0 (low virul. ISAV)</td>
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<tr>
<td>Chile 26536-1 (Nov 2007)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Chile 26536-2 (Nov 2007)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Norway SP4/98 (AY853925)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0*</td>
</tr>
<tr>
<td>Norway N32/98 (AY853921)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0*</td>
</tr>
<tr>
<td>Can Nova Scotia NS2003 (AY853919)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Norway 810/9/99 (BF213713)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Scotland 390/98 (AF429988)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Norway 485/9/97 (BF213715)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
<td>0</td>
</tr>
<tr>
<td>Can Nova Scotia US575-1 (BF213714)</td>
<td>RAGLANQHWSKYNFNGGKSAIISQRA</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465043)</td>
<td>KANFVKRHSEAYFPGMKCSSGTLIGGAWFQAYN</td>
<td>0 (high virul. ISAV)</td>
</tr>
<tr>
<td>Can RPC/ND 02-0775-14 (DQ465045)</td>
<td>KANFVKRHSEAYFPGMKCSSGTLIGGAWFQAYN</td>
<td>0 (high virul. ISAV)</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465046)</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465047)</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465048)</td>
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<td>Can RPC/ND 02-0775-14 (DQ465049)</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465050)</td>
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<td>0 (high virul. ISAV)</td>
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<tr>
<td>Can RPC/ND 02-0775-14 (DQ465051)</td>
<td>KANFVKRHSEAYFPGMKCSSGTLIGGAWFQAYN</td>
<td>0 (high virul. ISAV)</td>
</tr>
<tr>
<td>Can RPC/ND 02-0775-14 (DQ465052)</td>
<td>KANFVKRHSEAYFPGMKCSSGTLIGGAWFQAYN</td>
<td>0 (high virul. ISAV)</td>
</tr>
<tr>
<td>Can RPC/ND 02-0775-14 (DQ465053)</td>
<td>KANFVKRHSEAYFPGMKCSSGTLIGGAWFQAYN</td>
<td>0 (high virul. ISAV)</td>
</tr>
</tbody>
</table>

The Fusion protein of the Chile 2007-2008 ISAV strains has an 11-amino acid insert derived from RNA segment 2, which encodes the PB1 polymerase. The insertion is at the $^{265}$YP$^{266}$ motif (Kibenge et al., 2007) associated with reduced virus virulence. To date insertion of specific peptides has also been reported to occur at this site in the F protein of 8 Norwegian ISAV strains (Devold et al., 2006; Markussen et al., 2008); in 7 strains the insert was from different parts of segment 5, & in 1 strain the insert came from RNA segment 3 which encodes the nucleoprotein.
ISA - Typical clinical manifestation

- **ISAV stability in the environment**
  - Virus in seawater can be detected by RT-PCR.
  - Cell-culture virus is stable at 15°C for 10 days or 4°C for 14 days.
  - Virus is inactivated by:
    - UV irradiation (72 Jm⁻²) & ozone (4 min with 8 mg ml⁻¹, 600-750 mV redox).
    - Heating at 56°C for 30 min.
    - pH 4 or pH 12 for 24 hr.
    - Chlorine treatment (100 mg ml⁻¹) for 15 min.
ISA - Typical clinical manifestation

• Host range
  • Susceptible host species:
    • Natural clinical disease - Farmed Atlantic salmon (*Salmo salar*)
    • Experimental clinical disease – Rainbow trout (*Oncorhynchus mykiss*)
    • Natural asymptomatic infection (detected by RT-PCR):
      • Rainbow trout (Ireland)
      • Coho salmon (*O. kisutch*) (Chile)
      • Feral Atlantic salmon
      • Feral brown trout (*S. trutta morpha fario & S. trutta morpha lacustris*)
      • Feral sea trout (*S. trutta morpha trutta*)
      • Feral Pollock (*Pollachius virens*)
      • Feral Atlantic cod (*Gadus morhua*).
    • Experimental asymptomatic infection (detected by RT-PCR):
      • Arctic char (*Salvelinus alpinus*)
      • Herring (*Clupea harengus*)
ISA - Typical clinical manifestation

- ISA clinical signs
  - Anorexia
  - Lethargy
  - Anaemia
  - Mortality (slow increase; variable)

- Gross lesions
  - Pale gills
  - Ascites
  - Exophthalmos
  - Petechial haemorrhages on abdomen, visceral adipose tissue & eyes
  - Congestion and enlargement of liver & spleen

Godoy et al., 2008
ISA Histopathology

- Intestinal mucosal ulceration and haemorrhage
- Liver haemorrhage and hepatocyte necrosis
- Erythrophagocytosis in spleen
- Interstitial haemorrhage with tubular necrosis in kidney

Godoy et al., 2008
ISA - Typical clinical manifestation

• Pathogenesis
  • Primary target cells are endothelial cells; virus replication occurs in several organs.
  • Most extensive & prolonged replication is in heart tissue.
ISA – Significance to industry

• ISA is arguably the most important viral disease of marine-farmed Atlantic salmon
  • OIE Listed disease (trade implications)
  • Reportable disease
    • Therefore expensive eradication/control efforts
    • Quarantine of suspect populations until definitive diagnosis is obtained.
Global economic losses due to ISA

- Norway: 1 billion KR/yr.
- New Brunswick: Can$80 million in 2006.
- Scotland: £37-100 million in 1999.
- Chile:
  - 2007: 9% reduction in US$2.24 billion industry (i.e., $20 million) & ~3.0% reduction in workforce;
  - 2009: ~60% drop in Atlantic salmon production (from peak of ~400,000 tons before ISA crisis)
  - Projected losses for 2007-2011 will be ~$1 billion (i.e., 50% of the economic value of the industry);
  - Full recovery of industry not before 2013.
ISA – Significance to wildlife

• ISA is arguably the most important viral disease of marine-farmed Atlantic salmon

• ISAV-infected farmed fish occur in same water column with wild fish
  • environmental concerns for aquaculture
    • farmed fish may incubate and transmit ISAV to already diminishing stocks of wild fish.
    • prevent use of attenuated/modified live virus vaccines.
  • aquaculture concerns for wild fish reservoir of HPR0 viruses.
## ISA – Significance to wildlife

### Timeline (chronological history) of the detection of ISAV in wild fish

<table>
<thead>
<tr>
<th>Year of sample &amp; Test used</th>
<th>Country (location)</th>
<th>Wild fish species with ISAV</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000, RT-PCR</td>
<td>Canada (New Brunswick)</td>
<td>salmonids</td>
<td>Olivier 2002</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>UK (Scotland)</td>
<td>Atlantic salmon</td>
<td>Cunningham et al., 2002</td>
</tr>
<tr>
<td>2000, RT-PCR</td>
<td>UK (Scotland)</td>
<td>Sea trout, Brown trout, Atlantic salmon</td>
<td>Raynard et al., 2002</td>
</tr>
<tr>
<td>2001, RT-PCR</td>
<td>West Greenland fishery</td>
<td>Atlantic salmon</td>
<td>MacLean and Brown 2002</td>
</tr>
<tr>
<td>2001, RT-PCR</td>
<td>USA (Maine)</td>
<td>Atlantic salmon</td>
<td>P. Barbash (cited by MacLean et al., 2003)</td>
</tr>
<tr>
<td>2000-2002, Virus Isolation &amp; RT-PCR</td>
<td>USA (Maine)</td>
<td>Pollock*, Atlantic cod**</td>
<td>MacLean et al., 2003</td>
</tr>
<tr>
<td>1998; 2001-2003, RT-PCR</td>
<td>Norway (western Norway)</td>
<td>Salmonids (wild trout, Atlantic salmon)</td>
<td>Plarre et al., 2005</td>
</tr>
<tr>
<td>2010, RT-PCR</td>
<td>Denmark</td>
<td>Atlantic salmon⁵</td>
<td>Skall 2010</td>
</tr>
<tr>
<td>2010, RT-PCR</td>
<td>Chile (an estuary in southern Chile)</td>
<td>free-living <em>Salmo salar</em> (escapees)</td>
<td>González et al., 2011</td>
</tr>
</tbody>
</table>

*Pollock taken from inside a marine cage with ISA-disease salmon was weak RT-PCR positive; **Atlantic cod taken from a well boat holding salmon from a marine cage with clinically diseased fish was CPE positive on SHK cell culture.  
⁵Danish salmon produced for restocking purposes.
ISA – Transmission & current distribution

• Disease pattern
  • Disease outbreaks are mainly reported in seawater cages.
  
  • Stressful events may initiate disease outbreaks on infected farms, e.g., handling of fish (sorting, treatment, splitting, moving of cages).
ISA – Transmission & current distribution

• Transmission
  • Disease is spread **horizontally** by water-borne transmission
  • Main infection route is most likely through gills and/or intestinal tract
  • Virus shedding by infected fish may be through natural excretions/secretions.
  • Sea lice (*Lepeophtheirus salmonis*) may serve as mechanical vectors.
  • Reservoir for ISAV:
    • Recovered farmed Atlantic salmon can become carriers
      • 30% of ISA outbreaks in Norway were attributed to other farms in proximity
      • 70% could be
        • Virus circulating in the industry, e.g., in smolts by well boats
        • Wild salmonids (Atlantic salmon, rainbow trout, brown trout)
        • Vertical transmission (as evidenced by virus in brood fish or pre-smolts)
        • Other wild fish?
        • Some wild aquatic animal?
ISA – Transmission & current distribution

- Risk factors
  - Linked to husbandry practices in aquaculture & horizontal transmission.
  - Geographical or hydrological proximity (<5-10 kms) to farms with ISA outbreaks or slaughterhouse processing plants
  - Sharing of staff & equipment.
The Atlantic salmon is a world traveller. It is an anadromous fish - one that spawns in fresh water but spends much of its life at sea (travelling up to 4,000 km in the open sea).

http://www.asf.ca/about_salmon.php
OIE Reported First-time Outbreaks of ISA
Prevalence of ISA outbreaks in Norway (1984 to August 2010)

- Norway is the only country that reported ISA in 2011

Disease control measures were set in place:
- Ban use of sea water in hatcheries
- Ban movement of fish between seawater sites
- Introduced compulsory health certificates for aquaculture farms
- Disinfection of waste water from slaughterhouses, processing plants & smolt transport
- Year class separation
- Fallowing
ISA situation in Scotland

- First ISA outbreak in 1998. Disease was eradicated in 1999

- ISAV from different sites was 100% identical on segment 8, suggesting a single point source
  - ISAV HPR7b

- Suspected case in November 2004 (ISAV HPR0)
  - no instructions to withdraw fish
  - controls lifted after 6 months

- Second ISA outbreak in southwest Shetland in January 2009
  - Infection started after June 2008
  - ISAV HPR10; from unknown source
ISA situation in Faroe Islands

Disease control measures introduced in 2006 eradicated ISA:
- Synchronized fallowing of 24/25 farming areas
- Re-establishment of salmon farming industry
- Adoption of practical management strategies:
  ---tighter biosecurity procedures
  ---reduced production intensity
  ---scheduled fallowing
  ---year class separation
  ---ISAV vaccination
  ---comprehensive screening program for ISAV.
The Chilean ISA crisis

Source: Cesar Barros, SalmonChile 2011

Poor management:
- High concentrations of farms; high fish densities; poor smolt quality
- No zone management
- No fallowing
- Frequent movement of fish between farms
- No comprehensive Government regulations and control
Prevalence of ISAV (HPR-D and HPR0) positive cases in Chile (July 2007 to April 2011)

Top 7 disease control measures introduced in 2009 & responsible for the recovery:
(1) All-in all-out farming; fallowing; zone management. (2) Restriction of fish movements.
(3) Coordination of sea lice control; (4) ISAV vaccination. (5) Use of good quality smolts.
(6) Reduction of farm stocking numbers. (6) Better surveillance and better diagnostic capacities.
ISA situation in Eastern Canada and USA

- First ISA outbreak outside of Norway was in New Brunswick, Canada, in 1996; virus might have been present by 1995.


- ISA first confirmed in Maine, USA, in 2001.

- ISAV HPR0 has now completely replaced the virulent ISAV in both New Brunswick and Maine.

- A single ISA outbreak occurred in Prince Edward Island, Canada, in 2009.

- ISA outbreaks occurred in Nova Scotia, Canada, in 2012.
ISA – Diagnostics

• Sampling
  • To verify suspected cases based on clinical signs and gross pathology or positive RT-PCR.

  • Pooling of samples is not recommended (pooling of samples can be done for surveillance purposes; number of fish pooled depends on suggested prevalence of ISAV in population and test method to be used).
## ISA – Diagnostics

<table>
<thead>
<tr>
<th>Test method</th>
<th>Preservative</th>
<th>Fish tissue sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus isolation</td>
<td>viral transport medium*</td>
<td>heart, mid-kidney, (liver &amp; spleen)</td>
</tr>
<tr>
<td><strong>RT-PCR &amp; DNA sequencing</strong></td>
<td>RNALater®*</td>
<td>heart, mid-kidney, (liver &amp; spleen), gills**</td>
</tr>
<tr>
<td>IFAT</td>
<td>dried; dried &amp; fixed in 100% acetone (smears); or frozen (tissue)</td>
<td>smears (imprints) of mid-kidney or frozen tissue</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>dried; dried &amp; fixed in 100% acetone</td>
<td>smears (imprints) of mid-kidney</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>10% buffered formalin</td>
<td>mid-kidney, heart (include valves, bulbus arteriosus)</td>
</tr>
<tr>
<td>Histology</td>
<td>10% buffered formalin</td>
<td>mid-kidney, liver, heart, pancreas/intestine, spleen, gills, skin/muscle</td>
</tr>
</tbody>
</table>

*ship samples frozen (if not possible, ship cold).

**Not recommended for virus isolation; should be included for surveillance purposes using RT-PCR.
ISA – Diagnostics

• Diagnostic methods
  • ISA diagnosis used to be based on clinical signs and pathological findings only.

  • Following isolation of ISAV, several direct methods for detection and confirmation of diagnosis have been established.
ISA – Diagnostics

- Approved diagnostic methods
  - Indirect fluorescent antibody test (IFAT) using validated monoclonal antibodies on kidney smears/imprints of frozen tissue sections
  - Immunohistochemistry (IHC) using polyclonal antibody to ISAV nucleoprotein on paraffin sections from formalin-fixed tissues.
  - Virus isolation and identification
    - Using susceptible fish cell lines (SHK-1, ASK-2, TO, CHSE-214) [note virus strain variability; cell line susceptibility in development of CPE]
ISA – Diagnostics

• Approved diagnostic methods

• RT-PCR
  • Purity and integrity of RNA must be verified [by OD 260/280; internal control/house keeping gene(s)]
    • Atlantic salmon elongation factor 1 alpha (EF1α)
  • 2-step RT-PCR or 1-step RT-PCR; several primer sets in use
    • Conventional RT-PCR targeting segment 8 (ILA1/ILA2 – Mjaaland et al., 2002; FA3/RA3 – Devold et al., 2000); segment 6 (Seg6U/Seg6L)OIE
      • test for both segments 8 and 6 or sequence PCR product.
    • Real-time RT-PCR (TaqMan assays) targeting segments 7 & 8 (Plarre et al., 2005; Snow et al., 2006).
Rating of tests for targeted surveillance & diagnosis of ISA (Table 5.1)

<table>
<thead>
<tr>
<th>Method</th>
<th>Targeted surveillance for ISAV</th>
<th>Presumptive ISA diagnosis</th>
<th>Confirmatory ISA diagnosis</th>
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<tbody>
<tr>
<td></td>
<td>Larvae</td>
<td>PLs</td>
<td>Juveniles</td>
</tr>
<tr>
<td>Gross signs</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Histopathology</td>
<td>d</td>
<td>d</td>
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</tr>
<tr>
<td>IFAT on kidney imprints</td>
<td>c</td>
<td>c</td>
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<tr>
<td>Immunohistochemistry</td>
<td>c</td>
<td>c</td>
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<tr>
<td>Transmission EM</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Isolation in cell culture with virus identification</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>RT-PCR or real-time RT-PCR (Sequencing for genotyping)</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

PLs = postlarvae; IFAT = indirect fluorescent antibody test; EM = electron microscopy; RT-PCR = reverse-transcription polymerase chain reaction.

From Manual of Diagnostic Tests for Aquatic animals 2009, Ch. 2.3.5. – ISA, pg 232.
ISA – Diagnostics

• Definition of cases of ISA or infection with ISAV

  • **Suspect case;** any one of the following criteria:
    • Clinical signs consistent with ISA or pathological changes consistent with ISA whether or not associated with clinical signs of disease.
    • Isolation and identification of ISAV in cell culture from a single sample from any fish on the farm.
    • Evidence for presence of ISAV from two independent laboratory tests such as RT-PCR and IFAT.
    • Detection of antibodies to ISAV.
ISA – Diagnostics

- Definition of cases of ISA or infection with ISAV
  - Confirmed ISA case:
    - Mortality, clinical signs and pathological changes consistent with ISA, and detection of ISAV in tissue by IFAT or IHC, in addition to either
      - Isolation and identification of ISAV in cell culture from a single sample from any fish on the farm OR
      - Detection of ISAV by RT-PCR.
ISA – Diagnostics

• Definition of cases of ISA or infection with ISAV

  • **Confirmed ISAV infection;** one of the following criteria:
    • Isolation and identification of ISAV in cell culture from at least two independent samples from any fish on the farm tested on separate occasions.
    
    • Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm with corroborating evidence of ISAV in tissue preparations using either RT-PCR or IFAT.
OIE Reference Labs for ISA

- Two OIE Reference Laboratories for ISA:

  Dr. Frederick Kibenge
  Atlantic Veterinary College
  Department of Pathology and Microbiology
  Faculty of Veterinary Medicine
  University of Prince Edward Island
  550 University Avenue
  Charlottetown, Prince Edward Island C1A 4P3
  CANADA
  Tel: +1-902 566 09 67   Fax: +1-902 566 08 51
  Email: kibenge@upei.ca

  Dr. Birgit Dannevig
  National Veterinary Institute
  P.O. Box 750
  Sentrum
  0106 Oslo
  NORWAY
  Tel: +47-23 21 64 04   Fax: +47-23 21 63 01
  Email: birgit.dannevig@vetinst.no

From: http://www.oie.int/our-scientific-expertise/reference-laboratories/list-of-laboratories/
ISA – Approved Labs within USA

• Three Laboratories approved to conduct diagnostic testing in support of Export Health Certification of Aquaculture Species:

  Idaho Fish Health Center, Orofino, ID
  Tel: (208) 476-9500
  Testing method: virus isolation; RT-PCR for virus identification.

  Kennebec River Biosciences, Richmond, ME
  Tel: (208) 476-9500
  Testing method: virus isolation; IFAT, RT-PCR.

  Washington Animal Disease Diagnostic Laboratory, WSU, Pullman, WA
  Tel: (509) 335-9696
  Testing method: virus isolation.

ISA – Control and prevention

• Vaccination
  • Vaccination is used in North America (New Brunswick & Maine) since 1999, and in Faroe Islands since 2004 with questionable results
    • inactivated whole virus vaccines do not give sterile immunity, and vaccinated fish may become virus carriers.
    • level of protection is correlated to amount of ISAV antigen in vaccine (RPS of 86% has been achieved).
  • Vaccination was allowed in most parts of Norway in 2010
  • Vaccination is used in Chile since 2010
    • high demand for ISA vaccines has resulted in improved vaccine products (at least 6 different ISA vaccine products are currently marketed in Chile).

• Efficacy of ISA vaccines in presence of the widespread HPR0 infections is not known.
QUESTIONS?