Atlantic cod virus infections and viruses

David Smail

Introduction: objectives

- To summarize the virus infections in farmed and wild Atlantic cod
- To discuss the susceptibility or resistance of farmed Atlantic cod to the major fish disease viruses
- To discuss the significance of wild cod virus infections
- To outline future work needs

Introduction:scope

- The susceptibility or resistance of cod to different viruses will be emphasized.
- The impact of high virulence infections will be stated.
- The need for more virulence information will be outlined.
- Wild cod virus infections will be discussed.

Cod nodavirus infection:details

- Starkey et.al (2001) reported cod fingerling mortalities in a UK nodavirus outbreak
- Fish size: 1.5 -3.5g
- Clinical signs: whirling behaviour.
- Mortality. 2 per cent. over 3 months
- Histopathology: Retinal and brain vacuolation.
- Virus isolation on SSN-1 cells.
- Positive RT-PCR: cold-water virus clade (type isolate Barfin Flounder Nervous Necrosis coldwater nodavirus)

Cod nodavirus infection:impact

- Cod nodavirus is clearly highly virulent to developing cod fry
- Cod broodstock were not found positive in the UK outbreak
- Nodavirus of warmwater cultured species e.g seabass, is vertically transmitted via the gametes
- Therefore, research is needed on the possible risks associated with the use of wild broodstock

Nodavirus diagnostics

- Cell culture isolation is made using striped snakehead (SSN-1) cells or cloned E11 cells (obtainable from the European Animal Cell Culture Collection)
- Virus is identified by an immunofluorescent antibody test (IFAT) using virus-infected E11 cells
- RT-PCR has been developed
- Tissue imprints on nervous tissue stained by IFAT can identify virus

Infectious Pancreatic Necrosis (IPN) Virus

- IPNV is a major pathogenic virus of salmonids
- IPNV is a non-enveloped RNA virus
- There are 4 serogroups A,B,C&D: A & B serogroups are prevalent in Euorpean waters
- There is no published information on IPN outbreaks in cod or experimental IPNV cod infection

IPN Infection in cod

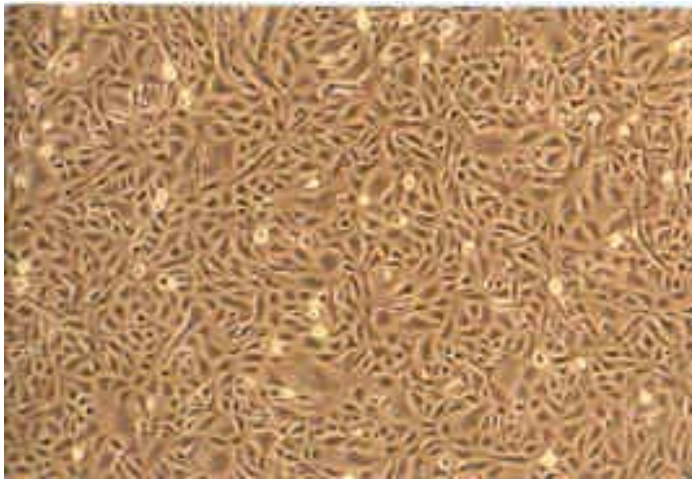
- Ellen Lorenzen (EAFP Int. Conf. 1995, Abstract)
- Danish west coast, 1991, 100% mortality in fry recorded
- Faroes, 1992-94, high mortality found in 2-3g fry and also in 8-10g fingerlings
- High titres of IPNV Sp, at $3x10^{9}TCID_{50}/g$ assayed
- Specific immunostaining in exocrine pancreas described

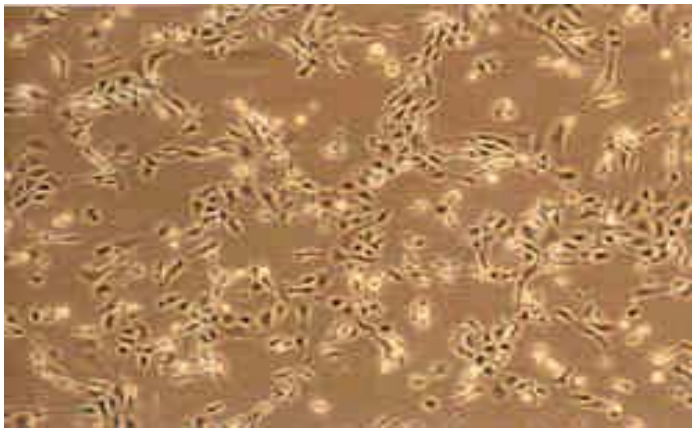
IPN experimental infection in cod

- Katy Adamson (FRS Marine Laboratory, Aberdeen, Scotland)
- Cod fingerlings infected with an IPNV isolate from sea trout
- Cod infected by injection (10^6 $TCID_{50}$ per fish) and cohabitation at 1/1 fish ratio
- No mortalities found
- Virus detected at low levels by culture and IPNV ELISA

IPNV Isolation: CHSE cells

Cytopathic effect (CPE) of IPNV
Top - normal Bottom - 48h CPE

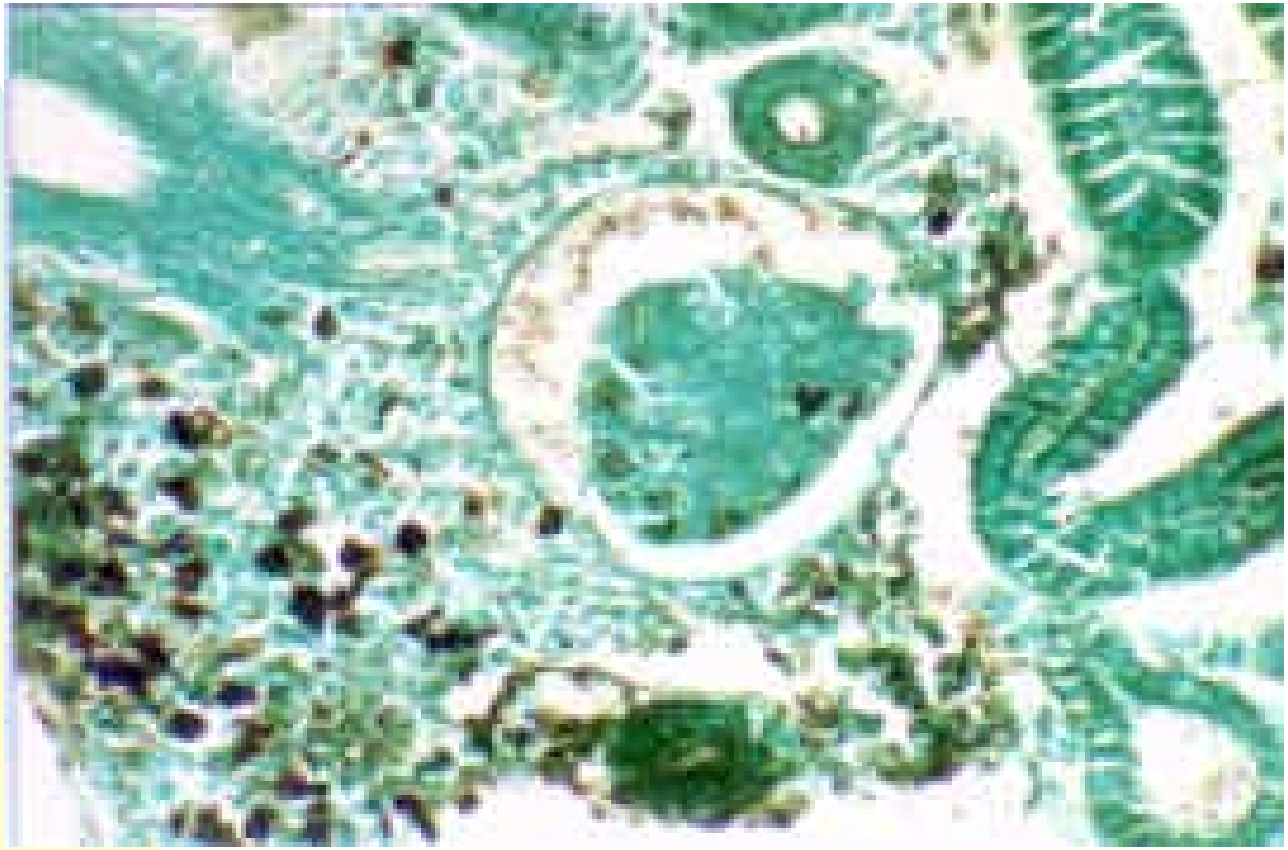




IPN Field kit

- A coagglutination test kit based on *Staph.aureus* coated with IPNV antibody is available (T.Taksdal)
- IPNV at >10^5 $TCID_{50}$ per g. is detectable in this kit
- The kit is useful for *clinical* infection in fry/parr/post-smolts

IPNV immunostaining: Atlantic salmon kidney, immunoperoxidase/light green



Viral Haemorrhagic Septicaemia (VHS) virus

- VHS is an enveloped RNA rhabdovirus with pathogenicity for rainbow trout and marine species
- Genogroups have been established (Snow):-
- Genogroup 1: freshwater, pathogenic to rainbow trout
- Genogroups 2-4: marine, Baltic Sea and Atlantic Ocean, infect marine species.& rainbow trout (Baltic)
- Genogroup 5: USA, Pacific coast, infects Pacific herring & Atlantic salmon

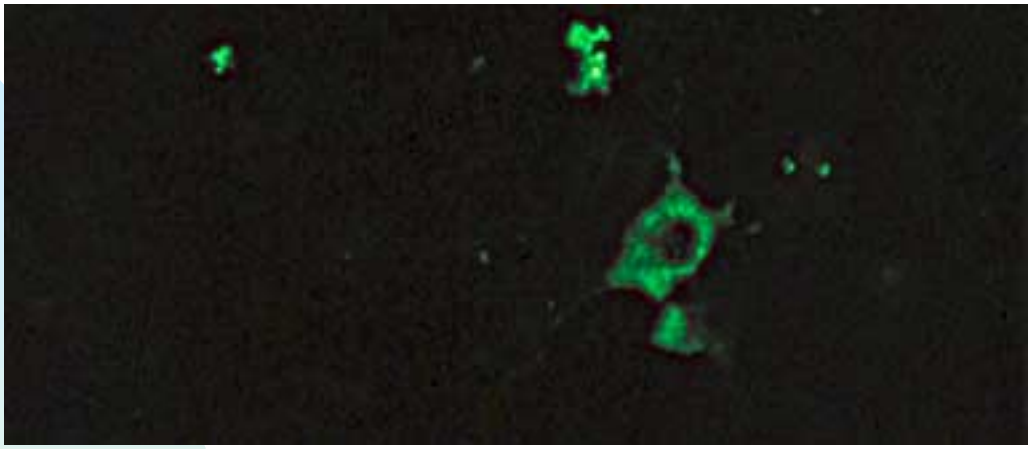
VHS experimental infection of juvenile cod

- Snow et al.(2000) reported intra-peritoneal infection of 14g cod
- A wild cod virus isolate was used from a skin lesion, H16/7/95 (Smail,2000)
- Virus-associated mortality of 80% was found
- No virus was recovered from fish cohabiting with the injected fish
- No infection was established by bathing at 10^5 $TCID_{50}$ per ml water for 4hours
- Conclude: Virus virulence was shown by injection only but not via the water route

VHSV bath infection: testing virulence of genogroups 1-4

- Rob Raynard's group (Aberdeen)
- 12g fingerling
- Bath challenge, 5h, 10^5 $TCID_{50}$ml
- 17 isolates from genogroups 1-4
- Only 2 fish with virus-associated mortality, both genogroup 4
- Conclude: Genogroups 1-3 were not virulent to cod fingerlings but genogroup 4 was of very low virulence to cod fingerlings

VHSV Diagnosis



- Cell culture isolation: BF-2 or RTG-2 cells are recommended producing cytopathic effect (CPE). EPC cells have also been used in research.
- Confirmation of virus by ELISA, IFAT or RT-PCR
- Antibody assay by ELISA for surveillance of VHSV infection at a population level is valuable

Infectious salmon anaemia (ISA) virus

- ISA virus is an enveloped RNA orthomyxovirus causal for ISA in Atlantic salmon
- ISAV is a segmented genome virus which permits recombination mutation events, similar to influenza virus
- Such mutations could lead to the emergence of new virulent strains of ISA virus

ISA virus and cod

- Rob Raynard's group (Aberdeen)
- Fish: 46g cod juveniles
- Dose:Injection infection, 3 x 10^7 $TCID_{50}$ml, reference isolate 390/98
- Route:10 injection/10 cohab./ tank, 3 replicates
- Outcome; No mortalities
- No ISAV detected by RT-PCR or cell culture 8 weeks after challenge
- Salmon positive controls gave mortalities
- Conclude: Cod juveniles were resistant to ISAV and appear not to replicate ISAV.

Detection of ISA virus

- Cell culture isolation on SHK-1, ASK or TO cells with or without CPE
- Haemadsorption on cell cultures is important to detect ISAV within cells without CPE
- Virus confirmation is made by cell culture IFAT or RT-PCR
- Tissue imprint IFAT for ISAV-specific haemagglutinin antigen is useful

Viruses in aquaculture of unknown virulence

- Halibut aquareovirus: isolated in Canadian halibut outbreaks
- Research needed to investigate cod susceptibility as halibut aquareovirus distribution may overlap with cod culture

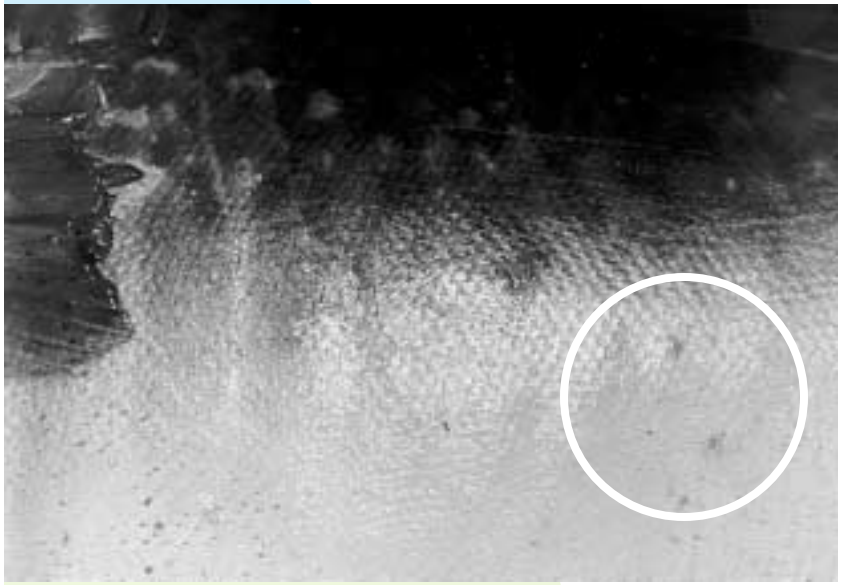
Viruses of unknown virulence (continued)

- Salmon pancreas disease alphavirus (togavirus)
- The causal agent of Pancreas Disease of Atlantic salmon in seawater
- Wasting disease of salmon growers
- Research on cod susceptibility needed because the distribution of the virus may overlap with cod culture

Viruses of unknown virulence (continued)

- Infectious Haematopoietic Necrosis (IHN) virus
- Type novirhabdovirus, pathogenic to salmonids as fry
- Clinical disease in Atlantic salmon post-smolts in the Pacific Ocean due to marine strains of IHNV suggests a marine distribution of the virus
- Research on Atlantic cod susceptibility needed

VHSV of wild cod

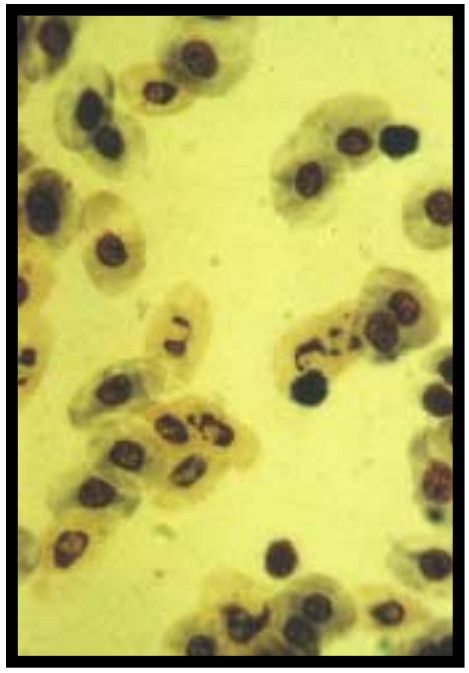


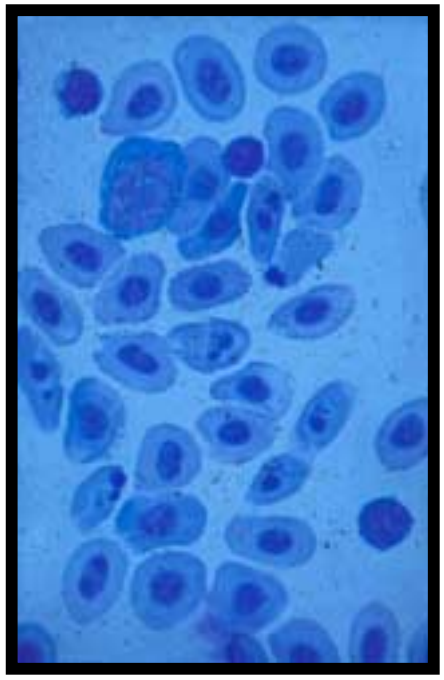
- VHS virus is associated with the cod ulcus lesion of wild cod
- Smail (2000) reported 3/35 (8.6%) lesions VHSV-positive, out of 691 cod examined for lesions
- King et al.(2001) reported 1/23 (4.3%) lesions VHSV-positive, out of 506 examined for lesions

Viral Erythrocytic Necrosis (VEN)

- An DNA iridovirus infection of cod erythrocytes
- Nuclear fragmentation is apparent
- Basophilic inclusion bodies are seen
- Prevalence: 1-28% at stations in the North Sea (Smail & Egglestone, 1980)
- Age distribution: 32% (O+group) but not detected in 4+group cod in the North Sea

VEN cytology





- The degree of anaemia caused by VEN has not been investigated
- The importance of VEN in the health of farmed and wild cod has not been investigated

Atlantic cod adenovirus

- Epidermal warts observed on the surface of cod from the Baltic Sea (Jensen & Bloch,1980)
- Size: 3-20 mm diameter
- Appearance:transparent
- Location:mainly over the tail
- Histology: epidermis thickened four times normal thickness
- Adenovirus-like icosahedral particles observed by electron microscopy: 77nm diameter with 20-25nm attached fibres

Summary points

- Virulent infections of farmed cod are nodavirus, IPN and VHS in order of importance.
- Unpublished studies indicate juvenile Atlantic cod are resistant to ISAV.
- The significance of the wild cod virus infections VEN and adenovirus to the health of farmed cod is unknown.
- The significance of virus infections of farmed cod to the health of wild cod has not been investigated.
- Research on cod virus infections of unknown virulence is needed.