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TOPIC SHEET NUMBER 59 V3
Scottish Government
Riaghaltas na h-Alba
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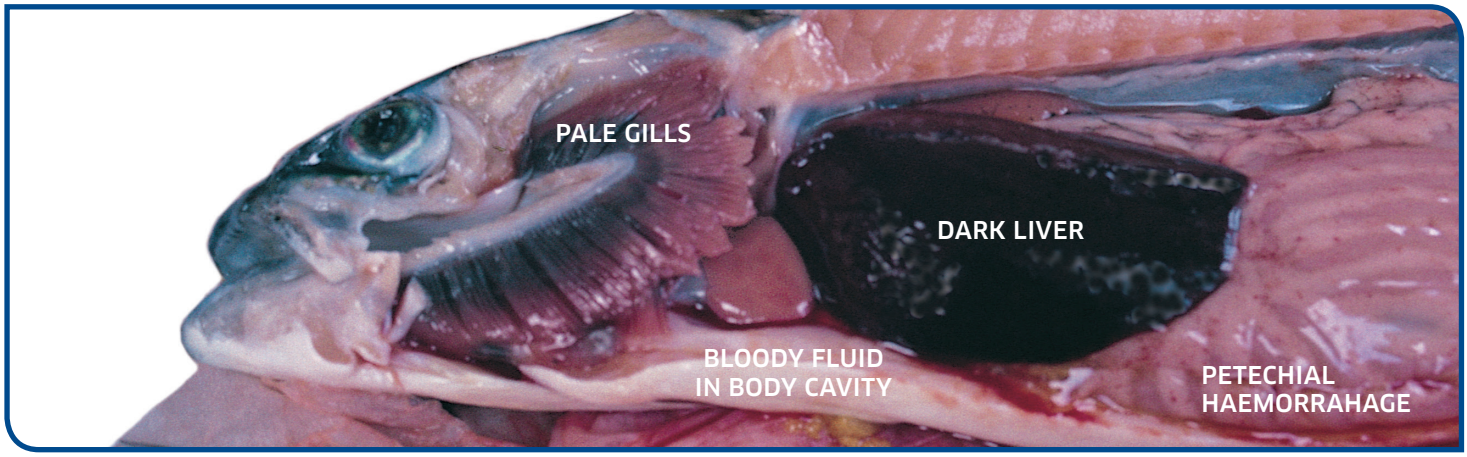
DIAGNOSIS OF INFECTIOUS SALMON ANAEMIA (ISA)



What is ISA?

ISA is a contagious viral disease of Atlantic salmon (Salmo salar) in sea water. The virus primarily affects endothelial cells lining the blood vessels of the fish, resulting in haemorrhage and severe anaemia. The disease can cause significant mortality, although within a farm this may spread slowly so that not all cages are affected simultaneously. The disease has been found in Norway, Canada, the USA, Faroe Islands, Scotland and Chile. ISA is a notifable disease under UK and European legislation. If the presence of ISA is suspected on a farm, strict controls are put in place. If the disease is confirmed, affected stocks may have to be immediately withdrawn.

A reputedly avirulent variant of infectious salmon anaemia virus (ISAV), termed HPR0, appears not to cause disease. The disease causing variants are termed HPR-deleted.

To date, isolates from all ISA disease outbreaks possess deletions in the highly polymorphic region (HPR) with respect to HPR0.

How is ISA diagnosed?

Diagnosis of ISA is achieved using a combination of clinical signs, histopathology and laboratory tests for evidence of viral infection.

Signs may include:

- Lethargy
- Loss of appetite
- Gasping at water surface
- Pale gills (anaemia)
- Dark liver
- Accumulation of fluid in the body cavity
- Haemorrhage in internal organs
- High levels of mortality

Histopathology

Histopathology involves microscopic examination of fish tissues for signs of disease. In the case of ISA there can be serious damage to liver tissue (Fig. 1)

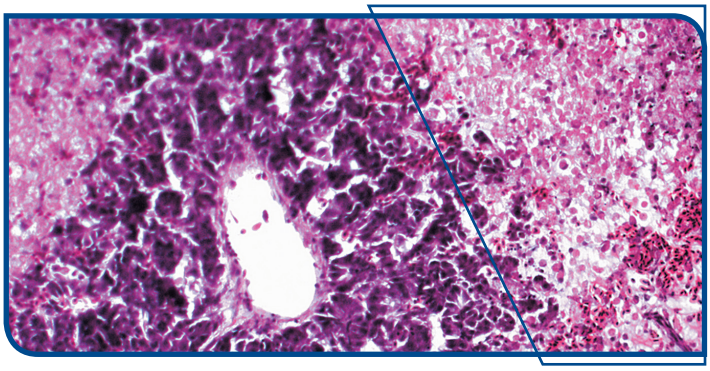


FIGURE 1.
HISTOLOGICAL SECTION OF LIVER OF AN ATLANTIC SALMON SHOWING DAMAGE ASSOCIATED WITH ISA.

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IHC

If histopathological changes consistent with ISA are observed, an immunohistochemistry test (IHC) can be performed to detect an ISA virus (ISAV) protein in fish tissues.

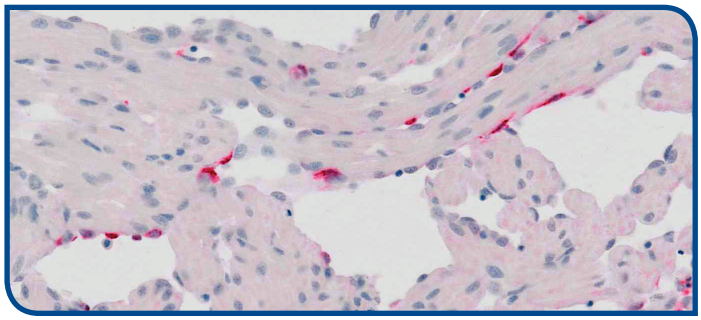

FIGURE 2.
FOLLOWING A SPECIFIC STAINING PROCEDURE, CELLS OF AN INFECTED SALMON SHOW A PINK/RED COLOURATION WHEN VIEWED UNDER THE MICROSCOPE.

qRT-PCR

A real-time reverse transcription polymerase chain reaction (qRT-PCR) detects small quantities of RNA, the genetic material of the ISAV, in fish tissues. The analysis is sensitive and specific and highly controlled to ensure confidence in the results obtained. qRT-PCR provides information on the relative amount of ISAV RNA present in the original samples (Figure 3).

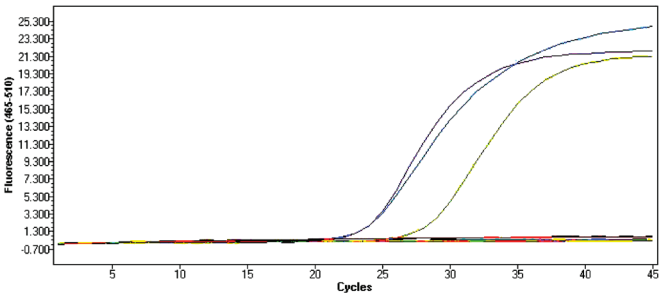

FIGURE 3
SPECIFIC DETECTION OF ISAV USING REAL-TIME REVERSE TRANSCRIPTION PCR (QRT-PCR). AN INTERNAL CONTROL REACTION CONDUCTED ON EACH SAMPLE (ELONGATION FACTOR) VERIFIES A HIGH QUALITY AND QUANTITY OF RNA USED IN EACH ASSAY. APPROPRIATE NEGATIVE AND POSITIVE CONTROLS ALLOW RESULTS TO BE INTERPRETED WITH CONFIDENCE. (FOR ILLUSTRATIVE PURPOSES ONLY)

Sequence analysis

The putitative virulence of qRT-PCR positive samples are determined by nucleotide sequence analysis (Figure 4). Sequencing of segment 6 of ISAV determines the HPR status of the virus. Different HPR types indicate virulent or avirulent strains of the virus.

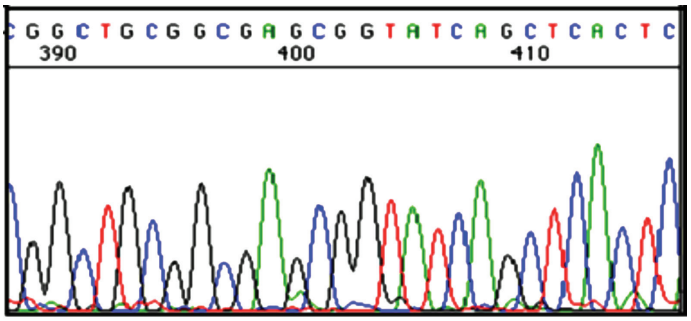

FIGURE 4
SEQUENCE CHROMATOGRAM. EACH PEAK REPRESENTS A DIFFERENT NUCLEOTIDE BASE (A, T, C OR G). (FOR ILLUSTRATIVE PURPOSES ONLY)

Virus isolation

It is possible to isolate ISAV from the internal organs of an infected fish. The technique used is to place samples of heart, liver, kidney and spleen from the fish into sterile tissue cultures grown in the laboratory. ISAV, if present, will grow in the tissue cultures and cause a cytopathic effect indicated by cell rounding and death (Fig. 5). ISAV associated CPE is confirmed by a specific antibody test.

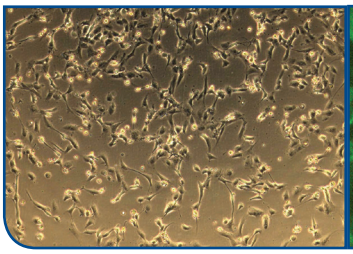

FIGURE 5.
ISAV ASSOCIATED CPE IN A TO CELL CULTURE.

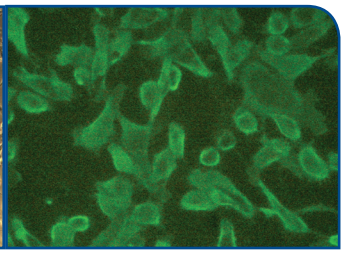

FIGURE 6.
ALL CPE POSITIVE CULTURES ARE CONFIRMED AS ISAV BY USING AN INDIRECT FLUORESCENT ANTIBODY TEST, A SPECIFIC ANTIBODY-BASED ANTIGEN DETECTION METHOD.

Accreditation

The IHC, qRT-PCR and virus isolation testing methods are accredited to ISO/IEC 17025 standard. To maintain this accreditation, Marine Scotland Science (MSS) participates in external proficiency exercises.