Australian Government
Department of Agriculture

Australian aquatic veterinary emergency plan (AQUAVETPLAN) for infectious salmon anaemia

Version 2.0, 2019

AQUAVETPLAN is a series of manuals that outline Australia's approach to national disease preparedness and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency.

National Biosecurity Committee

Cataloguing data

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This strategy will be reviewed regularly. Forward suggestions and recommendations for amendments to:

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Being a guide only, outbreaks or suspected outbreaks must be assessed case by case and expert advice should be obtained to determine the most appropriate management plan in response to the risk.

NOTE: Important regulatory information for infectious salmon anaemia is contained in the World Organisation for Animal Health Aquatic Animal Health Code, which is updated annually.

Disease watch hotline 1 800 675 888

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency animal disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance.

Preface

This disease strategy for the control and eradication of infectious salmon anaemia is an integral part of the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN).

AQUAVETPLAN disease strategy manuals are response manuals and do not include information about preventing the introduction of disease.

The Department of Agriculture provides biosecurity inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Biosecurity controls at Australia's borders minimise the risk of entry of exotic pests and diseases, and protect Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture BICON website.

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of infectious salmon anaemia in Australia. The strategy was scientifically reviewed by the Sub Committee for Aquatic Animal Health of the Animal Health Committee, before being endorsed by the Animal Health Committee of the National Biosecurity Committee in September 2018.

Infectious salmon anaemia is listed by the OIE in the Aquatic Animal Health Code. Infectious salmon anaemia is listed on Australia's National List of Reportable Diseases of Aquatic Animals (Agriculture 2019).

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the enterprise manual. The full list of AQUAVETPLAN manuals that may need to be accessed in an emergency are:

- disease strategies
  - individual strategies for each disease
- operational procedures manuals
  - disposal
  - destruction
  - decontamination
- enterprise manual, including sections on
  - open systems
  - semi-open systems
  - semi-closed systems
- management manuals
  - control centres manual
  - enterprise manual.

Aquatic Animal Diseases Significant to Australia: Identification Field Guide (Department of Agriculture 2012) is a source of information about the aetiology, diagnosis and epidemiology of infection with infectious salmon anaemia and should be read in conjunction with this strategy.

The first edition of this manual was prepared by Dr Mark Crane, AAHL Fish Diseases Laboratory, CSIRO Australian Animal Health Laboratory, Geelong. The revision was prepared by Dr Brendan Cowled and Dr Charles Caraguel and completed in 2015. The authors were responsible for

drafting the strategy, in consultation with a wide range of stakeholders from aquaculture, recreational fishing and government sectors throughout Australia. However, the text was amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not necessarily reflect the views of the authors. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual was adapted from similar manuals in AUSVETPLAN (the Australian veterinary emergency plan for terrestrial animal diseases) and from the AQUAVETPLAN enterprise manual. The format and content have been kept as similar as possible to these documents, so animal health professionals trained in AUSVETPLAN procedures can work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents are gratefully acknowledged.

The revised manual has been reviewed and approved by representatives of government and industry:

- **Government**
  - CSIRO Australian Animal Health Laboratory
  - Department of Primary Industries, New South Wales
  - Department of Primary Industry and Fisheries, Northern Territory
  - Department of Agriculture and Fisheries, Queensland
  - Department of Primary Industries, Parks, Water and Environment, Tasmania
  - Department of Fisheries, Western Australia
  - Department of Economic Development, Jobs, Transport and Resources, Victoria
  - Department of Primary Industries and Regions, South Australia
  - Biosecurity Animal Division, Department of Agriculture, Australian Government
  - Department of the Environment, Australian Government
- **Industry**
  - Dr Christine Huynh (Tassal)
  - Dr Steve Percival (Huon Aquaculture)
  - Dr Phillipa Sims (Petuna)

The complete series of AQUAVETPLAN documents is available on the Department of Agriculture website.

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1 Nature of the disease

Infectious salmon anaemia virus (ISAV), the cause of infectious salmon anaemia (ISA), is exotic to Australia. It has the potential to cause large-scale mortality in farmed Atlantic salmon (*Salmo salar*) populations if introduced to Australia. Therefore, state and territory governments, and the salmonid aquaculture industries, must be adequately prepared to manage a disease outbreak. An incursion of the disease could devastate the Atlantic salmon aquaculture industry in Australia, centred in Tasmania, which contributed AUS$626 million to the Tasmanian gross state product in 2014–15 (Anonymous 2015b).

1.1 Aetiology

ISA was first reported from an Atlantic salmon hatchery in Norway in 1984 and is caused by the infectious salmon anaemia virus (ISAV). ISAV is an enveloped virus, 100–130 nanometres in diameter, with a genome made up of 8 single-stranded, negative-sense RNA segments. Its morphological, biochemical and genomic properties place it within the family *Orthomyxoviridae* (Krossoy et al. 1999) and it has been classified as the type species of the genus *Isavirus.*

The ISAV genome has been fully sequenced (Clouthier et al. 2002) and is highly conserved. Analysis of various gene sequences (particularly the Haemaglutinin esterase (HE) and F segments (Rimstad et al. 2011) has revealed two major clusters or genotypes from Europe and North America. The European genotype is divided into two genogroups, European and European-in-North-America genogroups (Kibenge et al. 2007). Chilean viruses are descended from European (Norwegian) isolates (Castro-Nallar et al. 2011; Kibenge et al. 2009).

Isolates can also be typed according to variations within a small, highly polymorphic region (HPR) of the HE gene (Christiansen et al. 2011; Cook-Versloot et al. 2004; Devold et al. 2001; Lyngstad et al. 2012). The ancestral form of ISAV is designated HPR0 and has been detected in healthy farmed and wild fish in all salmon producing areas (Snow 2011) that have previously suffered ISA outbreaks. Australia, New Zealand and western North America have not experienced any ISA outbreaks and HPR0 has not been reported.

Numerous observational studies suggest that HPR region mutations (deletions) of the ancestral form HPR0 may be linked to the development of pathogenic ISAV and ISA outbreaks (Christiansen et al. 2011; Godoy et al. 2013; Godoy et al. 2014; Lyngstad et al. 2011; Lyngstad et al. 2012; Markussen et al. 2013). Additionally, it is thought that a single amino acid insertion or substitution on the F protein gene (surface fusion protein) also contributes to virulence because all HPR0 reported deduced amino acid sequences have a glutamine in position 266 compared with a leucine in virulent ISAV isolates (Markussen et al. 2013).

It is thought that certain management practices can reduce the probability of mutation of HPR0 to virulence (Christiansen et al. 2011). The presence of HPR0 and ability to reduce the probability of transition to virulence has important implications for planning responses to incursions of ISAV.

1.2 Susceptible species

1.2.1 Atlantic salmon

Natural outbreaks of ISA have only been recorded in Atlantic salmon (Rimstad et al. 2011), other than a single report of ISA disease outbreaks in farmed Coho salmon in Chile (Kibenge et al. 2001). Farmed Atlantic salmon only show clinical signs when infected with a pathogenic strain of ISAV (HPR-deleted). In contrast, disease has not been observed or reported in wild salmon in the field (Raynard et al. 2001a). However, wild salmon will develop the disease when challenged experimentally (Glover et al. 2006; Raynard et al. 2001b). An absence of observed disease in

wild salmon may be due to difficulties in detecting clinical disease in wild populations, the absence of important co-determinant factors present under farming conditions, genetic differences between farmed and wild stock, or a true absence in wild populations.

1.2.2 Subclinical infection in other species

Subclinical infection with ISAV has been demonstrated in experimentally infected brown trout (*S. trutta*) (Nylund & Jakobsen 1995; Snow et al. 2001), rainbow trout (*Oncorhynchus mykiss*) (Nylund et al. 1997; Snow et al. 2001), chum salmon (*O. keta*), herring (*Clupea harengus*) (Nylund et al. 2002).

Subclinical infection with ISAV has also been demonstrated in wild (feral) sea trout (*S. trutta*) (Plarre et al. 2005), and trout have been putatively identified as a reservoir species for infection in Atlantic salmon (Nylund et al. 2003).

1.2.3 Disease in other species

Clinical disease (mortality and pathology) has been described in rainbow trout following experimental infection but this has not been observed in wild populations (Biacchesi et al. 2007; MacWilliams et al. 2007).

ISAV was reportedly associated with disease outbreaks in farmed Coho salmon in Chile (Kibenge et al. 2001), but this has not been reported elsewhere. Challenge trials in Pacific salmon (including Coho, *Oncorhynchus kisutch*) were able to recover virus from some infected fish but did not detect any disease attributable to infection with ISAV (Rolland & Winton 2003). Infection with ISAV resulting in clinical disease in Coho salmon may be multifactorial in nature, requiring, for example, co-infection with other pathogenic organisms to cause ISA.

1.2.4 ISAV and non-salmonids

Studies have not discovered any non-salmonid reservoirs (Rimstad et al. 2011). Non-salmonid species that have tested negative with RT-PCR (MacLean & Bouchard 2003) are:

- Alewife (*Alosa pseudoharengus*)
- American eel (*Anguilla rostrata*)
- Atlantic mackerel (*Scomber scombrus*)
- Haddock (*Melanogrammus aeglefinus*)
- Atlantic halibut (*Hippoglossus hippoglossus*)
- American shad (*Alosa sapidissima*)
- Winter flounder (*Pseudopleuronectus americanus*).

For a more comprehensive assessment see Rimstad et al. (2011).

Table 1 presents a summary of species susceptible to infection with ISAV.

Table 1 Fish species known to be susceptible[a] to infectious salmon anaemia virus

Species	Subclinical infection	Clinical disease	Comments
Atlantic salmon (*Salmo salar*) (Evensen et al. 1991; Nylund et al. 1995)	Yes	Yes	–
Sea/brown trout (*Salmo trutta*) (Raynard et al. 2001a)	Yes	No	–

a The OIE Aquatic Animal Health Code defines susceptible species as a species of aquatic animal in which infection has been demonstrated by the occurrence of natural cases or by experimental exposure to the pathogenic agent that mimics natural transmission pathways. See Rimstad et al. (2011) for a comprehensive summary.

Table 2 Fish species known to be infected with salmon anaemia virus via intraperitoneal injection of ISAV homogenates

Species	Subclinical infection	Clinical disease	Comments
Atlantic salmon (*Salmo salar*) (Glover et al. 2006; Rimstad 2011; Rimstad et al. 1999; Simko et al. 2000)	Yes	Yes	–
Herring (*Clupea harengus*) (MacLean & Bouchard 2003; Nylund et al. 2002; Rimstad et al. 2011)	Yes	No	Experimentally herring have been infected but field studies have failed to detect infection.
Pacific (chum) salmon (*Oncorhynchus keta*) (Rolland & Winton 2003)	Yes	No	Experimental studies with intraperitoneal injection.
Sea/brown trout (*Salmo trutta*) (Nylund & Jakobsen 1995; Rodger et al. 1998; Snow et al. 2001)	Yes	No	–
Steelhead/rainbow trout (*Oncorhynchus mykiss*) (Biacchesi et al. 2007; Kibenge et al. 2006; MacWilliams et al. 2007; Nylund et al. 1997; Rolland & Winton 2003; Snow et al. 2001)	Yes	Yes	Some disease evident during experimental studies but not observed in wild.

1.3 World distribution

ISA has been reported from Norway since the 1980s (Thorud & Djupvik 1988) and subsequently from Canada (Bouchard et al. 1999; Mullins et al. 1998), Chile (Kibenge et al. 2001), the Faroe Islands (OIE 2016b), Ireland (OIE 2015), the United Kingdom (Scotland) (Rodger et al. 1998; Rowley et al. 1999) and the United States (Bouchard et al. 2001). Phylogenetically distinct orthomyxoviruses have been isolated from both wild pilchards (*Sardinops sagax neopilchardus*) and farmed Atlantic salmon in Australia (Crane & Williams 2008), but neither ISA nor ISAV are present in Australia.

In 2015 the world sanitary situation as reported by the World Organisation for Animal Health (OIE) for HPR-deleted and HPR0 ISAV (OIE 2015) for disease or infection was:

- present in country or zone(s)—Canada, Chile, Norway and Iceland

- absent in most recent reporting period (last outbreak): United Kingdom (2009), Ireland (2010), United States (2006), Denmark (2010)
- has never occurred: New Zealand, Australia and many other countries.

1.4 Diagnosis of infection with infectious salmon anaemia

Detailed diagnostic procedures for ISA and for the detection and isolation of ISAV are detailed in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE Aquatic Manual) (OIE 2016c). A brief summary is provided here.

Diagnosis of ISAV infection is based on a range of procedures. Presumptive diagnosis is made following clinical and pathological observations. ISA is confirmed following histopathological examination, demonstration of ISAV antigen in tissues by immunoassay and virus isolation in tissue culture combined with virus identification by either immunofluorescence or immunoperoxidase staining. RT-PCR techniques are also available. Section 1.4.3 provides further details of confirmatory diagnosis.

1.4.1 Field methods: clinical signs and gross pathology

As with many infectious agents of fish, ISAV-infected fish may not exhibit clinical signs, especially when infected with the HPR0 variant of ISAV. Where infection occurs with a pathogenic variant (HPR deleted ISAV) onset of disease can be precipitated by any of a number of host (immune status) or adverse environmental conditions, such as increased temperature or poor water quality (Dannevig et al. 1994; OIE 2016b). Disease may be acute or chronic (Rimstad et al. 2011).

Diseased fish are usually in normal nutritional condition but swim sluggishly at the water surface or hang listlessly at the net pen wall (Rimstad et al. 2011). Although no clinical signs are considered specifically diagnostic (pathognomonic) for ISA, signs are generally consistent with anaemia, haemorrhage or circulatory disturbances (OIE 2016b; Rimstad et al. 2011). Clinical signs and gross pathology consistent with ISA (Evensen et al. 1991; Rimstad et al. 2011; Rimstad et al. 1999; Speilberg et al. 1995; Thorud & Djupvik 1988) are:

- pale gills
- exophthalmia (protrusion of the eyeballs)
- ascites (distended abdomen due to fluid in peritoneal and pericardial cavities)
- skin and eye haemorrhages
- scale oedema (swollen scales)
- progressive anaemia that may result in ‘watery blood’ (haematocrit<10)
- petechiae (pinpoint haemorrhages) in the peritoneum and skeletal muscle
- congestion and enlargement of the liver and spleen
- congestion of the foregut.

During an outbreak, daily mortalities typically range between 0.5% and 1% in sea cages, and total mortalities may exceed 90% over several months (OIE 2016b).

A significant fall in haematocrit value was detected by day 18 post-experimental infection. This coincided with the first appearance of macroscopic lesions—congestion and darkening of the liver, congestion of the spleen and foregut, and ascites formation. At later stages, peritoneal petechiae, exophthalmia and gill pallor were present (Speilberg et al. 1995).

Fall in haematocrit level (< 10 in end stages and 25–30 in less severe cases) can also occur with other conditions (ulcerations and erythrocytic inclusion body syndrome).

1.4.2 Laboratory methods

Sample submission

In the first instance, the relevant state laboratory—most likely to be the Department of Primary Industries, Parks, Water and Environment, Tasmania, since the vast majority of Atlantic salmon is farmed in Tasmania—should be contacted directly to ensure that samples are collected using appropriate techniques. These include:

- *Sample submission for virological examination*: Tissue samples suitable for virological examination at the CSIRO Australian Animal Health Laboratory (CSIRO–AAHL) include kidney, spleen, heart, fresh gills and liver placed into virus transport medium. These samples should be collected aseptically and placed in sterile containers. Samples should not be frozen before processing but should be maintained between 4°C and 10°C. To maximise sensitivity, samples should be processed and assayed within 24 hours of sampling, but if this is not possible they must be processed within 72 hours of sampling.
- *Sample submission for histology, immunohistochemistry and electron microscopy*: Samples submitted for histopathology and immunohistochemistry should be fixed in neutral 10% phosphate buffered formalin; samples for electron microscopy should be fixed in glutaraldehyde.
- *Sample submission for molecular analysis,* for example RT-PCR and sequencing: Samples submitted for analysis by PCR should be tissues preserved in an appropriate medium for preservation of RNA, such as 80% ethanol or RNAlater® (if this is not possible, tissues should be frozen).
- *Sample submission for IFAT*: Kidney imprints are useful for indirect fluorescent antibody test (IFAT). Imprints are prepared by taking a small piece of the mid-kidney and blotting the cut surface with absorbent paper to remove excess fluid and then making several imprints within a 2-square centimetre area on poly-L-lysine-coated microscope slides. The imprints are air-dried and fixed in cold 100% acetone (on ice) for 10 minutes and stored at 4°C or, if not used immediately, at –80°C until use.
- *Sample submission for haematology:* Heparin or EDTA (ethylene diamine tetra-acetic acid) preserved blood samples.

Microscopy

Histopathology

Histological lesions are present in the liver and kidney in typical cases (Evensen et al. 1991; Speilberg et al. 1995). In the liver there is multifocal branching haemorrhagic coagulative necrosis of the parenchyma with necrotic areas often coalescing. Blood-filled spaces in the necrotic hepatic parenchyma (peliosis) may be present in later stages of the disease (Simko et al. 2000; Speilberg et al. 1995).

In the kidney, multifocal interstitial congestion and haemorrhage may be found in both the anterior (head) and posterior regions (Simko et al. 2000). This may be accompanied by multifocal necrosis of haematopoietic tissue in the anterior kidney, and tubular epithelium in the posterior kidney. Splenic congestion with erythrophagocytosis (ingestion of red blood cells by macrophages and other phagocytic cells) may be seen in the late stages of disease, while less

consistent changes may affect the heart muscle (myocardium), gills and intestines (Simko et al. 2000).

Electron microscopy

Virions can be observed in endothelial cells and leukocytes (OIE 2016b; Speilberg et al. 1995). Also, secondary lesions caused by the destruction of the endothelial lining of blood capillaries and leakage of blood into the liver tissue may be observed; however, no virus particles were observed in endothelial cells in this study. Endothelial cells appear to be the primary target cells for ISAV (Hovland et al. 1994; Koren & Nylund 1997). Electron microscopy has not been used for diagnostic purposes (OIE 2016b).

Culture methods

Several cell lines—SHK-1 (Dannevig et al. 1995), ASK (Devold et al. 2000), TO (Wergeland & Jakobsen 2001) and CHSE214 (Kibenge et al. 2000)—are susceptible to infection with various ISAV strains, with ASK then SHK-1 cell lines being preferred (OIE 2016b). HPR0 has not been successfully isolated in cell culture (OIE 2016b).

Spleen, heart, gill and kidney tissue should be sampled from affected fish and processed for inoculation onto cell cultures using standard procedures (Crane & Williams 2008; OIE 2016b). Inoculated cell cultures are incubated at 15°C and examined by light microscopy for the development of a cytopathic effect (CPE) typical for ISAV (Dannevig et al. 1995). At 14 days post-inoculation, or earlier if CPE develops, cell culture supernatants are inoculated onto fresh cell cultures and incubated for at least a further 14 days. When CPE develops, the cultures should be processed for ISAV identification by either IFAT or PCR (OIE 2016b). If CPE has not developed, the cultures should always be processed for detection of ISAV by either IFAT or PCR because virus replication may occur without CPE development (OIE 2016b).

Molecular Techniques

In experimentally HPR-deleted ISAV infected fish, for the first 8 days post-infection, positive PCR results were found predominantly in the head kidney and mid-kidney. Subsequently, fish yielding positive results at 13 days post-infection and beyond, were positive in most organs sampled—mid-kidney, head kidney, liver, spleen, intestine, gills, muscle, and heart (Rimstad et al. 1999). Since these initial studies, several PCR primer sets have been developed for detection of ISAV based on segments 6, 7 and 8 (OIE 2016b). In addition, full length sequences have been reported and maybe useful for diagnosis (Merour et al. 2011).

RT-PCR and real time RT-PCR will detect European and North American HPR-deleted ISAV and HPRO ISAV (OIE 2016b).

Positive results to primers based on Segment 7 or 8 should be followed by sequencing of the HPR of Segment 6 to distinguish between HPR0 and HPR-deleted variants.

For further detail on application of these diagnostic tools see the OIE Aquatic Manual (OIE 2016b).

Other methods

Haematology and biochemistry (OIE 2016b)

Haematocrit is reduced and values of <10 should be investigated for ISA. Blood smears contain degenerate and vacuolated erythrocytes and erythroblasts. Leucocytes are reduced in number relative to erythrocytes and liver enzyme levels are elevated.

Indirect fluorescent antibody test

IFAT on kidney imprints or frozen tissue sections using anti-ISAV monoclonal antibodies can be used to confirm suspected cases of ISA. Similarly, polyclonal anti-ISAV nucleoprotein antiserum can be used in immunohistochemical tests on formalin fixed tissues (mid-kidney and heart sections) to confirm cases suspected on the basis of pathological signs (OIE 2016b).

Immunohistochemistry (IHC)

As stated in the OIE Aquatic Manual:

Polyclonal antibody against ISAV nucleoprotein is used on paraffin sections from formalin-fixed tissue. This IHC staining has given positive reactions in both experimentally and naturally infected Atlantic salmon. Preferred organs are mid-kidney and heart (transitional area including all three chambers and valves). Suspected cases due to pathological signs are verified with a positive IHC. Histological sections are prepared according to standard methods. (OIE 2016b)

1.4.3 Confirmation of infection

Possible disease investigation outcomes
It is important to realise there are several possible outcomes when investigating a suspect case of infection with ISAV. These include:

1) Diagnosis of HPR0 ISAV

Diagnostic techniques suitable for HPR0 ISAV are limited as virus isolation is not successful and infection is transient and subclinical. Detection requires random sampling of individual fish over time. Fresh gill tissue is the primary sample required.

2) Diagnosis of HPR-deleted (pathogenic) ISAV

HPR-deleted ISAV can be isolated from infected fish. HPR-deleted ISAV infected fish generally carry high viral loads during clinical stages for a period of time. Although prevalence can be variable, targeting of moribund or fresh dead fish can enhance the sensitivity of surveillance to detect ISAV.

3) Diagnosis of ISA

Diagnosis of disease caused by ISAV (ISA) requires clinical signs and gross pathology (see section 1.4.1) and confirmation of the presence of HPR-deleted ISAV in relevant tissue.

Suspect and confirmed case definitions
Suspect and confirmed case definitions have been developed by the OIE for ISA HPR0 ISAV and HPR-deleted ISAV (OIE 2016b).

1) Suspect case

ISA or infection with HPR-deleted ISAV is suspected if at least one of these criteria is met:

- clinical signs (gross pathology) consistent with ISA (see section 1.4.1)
- pathological changes consistent with ISA, with or without clinical signs (see Histopathology in section 1.4.2)
- isolation and identification of ISAV in cell culture from tissues from a single fish (see Agent isolation and identification in section 1.4.2)
- evidence of ISAV from any ISAV-specific laboratory test, for example RT-qPCR, RT-PCR, IFAT on tissue imprints, immunohistochemistry.

2) Confirmed ISA (clinical disease)

Confirmation of ISA will be made if these criteria are met:

- detection of ISAV in tissue from at least one fish from a suspect case by means of specific antibodies against ISAV (immunohistochemistry on fixed sections or immunofluorescent antibody test on tissue imprints or fixed sections), and either
- isolation and identification of ISAV in cell culture from at least one sample from any affected fish on the farm (see Agent isolation and identification in section 1.4.2), or

- detection of ISAV by RT-PCR from at least one sample from any affected fish on the farm (see Molecular techniques in section 1.4.2).

3) Confirmed HPR-deleted ISAV sub-clinical infection

Confirmation of HPR-deleted ISAV sub-clinical infection will be made if any of these three criteria are met:

- detection of ISAV by two RT-qPCR tests targeting different regions of the genome from at least two fish on a farm
- detection of ISAV by RT-qPCR followed by RT-PCR and sequence analysis demonstrating ISAV-specific sequence from at least two fish on a farm
- isolation and identification of ISAV in cell culture from tissue samples from at least two affected fish on the farm (see Agent isolation and identification in section 1.4.2)

It should be noted that in sub-clinical infections these tests may not be sufficiently sensitive.

4) Confirmed infection with HPR0 ISAV

- Detection of ISAV by RT-qPCR followed by independent amplification by RT-PCR and sequencing of the HPR region of Segment 6 to confirm the presence of HPR0 only (see Molecular Techniques in section 1.4.2).

Relevant tools for diagnosis

The OIE has recently reviewed the current clinical and laboratory diagnostic techniques that are available and detailed how useful they are for detection of HPR-deleted ISAV. This is captured in Table 3. HPR0 ISAV detection is more limited and requires RT-PCR and sequencing (not included in the table).

Table 3 Methods for targeted surveillance and diagnosis of HPR-deleted ISAV

Method	Targeted surveillance for infection with HPR-deleted ISAV				Presumptive diagnosis	Confirmatory diagnosis
	Fry	Parr	Smolt	Adults		
Gross signs	d	d	d	d	c	b
Histopathology	d	d	d	b	b	b
IFAT on kidney imprints	d	d	d	d	b	a
Immunohistochemistry	d	d	d	d	b	a
Isolation in cell culture with virus identification	a	a	a	a	a	a
Real-time RT-PCR or RT-PCR followed by sequencing	a	a	a	a	b	a

a The method is recommended for reasons of availability, utility, sensitivity and specificity. **b** The method is standard for reasons of sensitivity and specificity. **c** The method has application in some circumstances but for reasons cost, accuracy or other factors application is limited. **d** The method is not presently recommended.

Note: The diagnosis of ISA is not based on a single test and therefore the information in the table should be used with care using criteria for ISA diagnosis.

Source: (OIE 2016b)

1.4.4 Differential diagnosis

None of the clinical signs documented are specifically diagnostic for ISA. However, the signs can be observed singly or in any combination in Atlantic salmon suffering from disease caused by infection with any one of the these pathogens:

- *Aeromonas salmonicida*
- *Renibacterium salmoninarum*
- rickettsia-like organisms such as *Piscirickettsia salmonis*
- viral haemorrhagic septicaemia virus
- infectious haematopoietic necrosis virus
- infectious pancreatic necrosis virus
- pilchard orthomyxovirus (not ISAV).

1.5 Resistance and immunity

It is important to recognise that there are two main variants of ISAV. HPR0 ISAV induces subclinical gill infections and has never been associated with disease in Atlantic salmon (Christiansen et al. 2011; Lyngstad et al. 2011; OIE 2016b). Deletions to the highly polymorphic region (HPR-deleted variants) reduce the resistance of Atlantic salmon to ISAV by influencing fusion of the virus and target cells (Fourrier et al. 2014). The rest of this section refers to HPR-deleted ISAV.

Innate and adaptive cellular and humoral immune responses to pathogenic ISAV have been experimentally demonstrated in Atlantic salmon (Falk 2014; Falk & Dannevig 1995; Jorgensen et al. 2007; Lauscher et al. 2011; LeBlanc et al. 2010; Ritchie et al. 2009). Also, it has been demonstrated that convalescent antiserum has ISAV-neutralising activity (Falk & Dannevig 1995). Vaccines have been developed to stimulate immunity and allow salmon to continue to be produced in the face of ISA.

There are several vaccines commercially available, in two main forms (Falk 2014). These are registered overseas, especially in Chile, but not in Australia. Use in Australia would require registration or application for an emergency use permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA).

The first vaccine type is a traditional oil-adjuvanted, inactivated ISAV cell culture antigen-based vaccine. For example, these are produced by Novartis Animal Health (Birnagen Forte® 3 Plus) and Pharmaq (ALPHA JECT® micro 1 ISA). They are injectable vaccines. There is only modest detail in the literature but it appears that this type of vaccine can achieve relative percent survival of 85–95% in experimental situations (Jones et al. 1999; Lauscher et al. 2011).

Another type of commercial vaccine is one based on ISAV HE-Protein expressed in yeast which is available in an injectable (oil-adjuvanted) or oral (food delivered) form (Falk 2014; Tobar et al. 2015). Tobar et al. (2015) demonstrated in Chile that immunity in the field following vaccination is short lived (peaking at 600–800 degree days) regardless of vaccine type. Immunity declined to negligible levels by 1300 degree days. Re-vaccination with an oral vaccine rapidly increased immunity again. They concluded vaccination for ISAV in commercial Atlantic salmon typically leads to a variable and limited duration antibody response requiring regular re-vaccination. The existence of a food-based oral vaccine makes this practically possible.

Some caution is required when using vaccines. Inactivated viral vaccines are not fully protective (Kibenge et al. 2003); immunised fish do not completely eliminate virus and may become carriers. In addition, the existence of different ISAV strains indicates that efficacy of any vaccine against local or introduced ISAV strains needs to be demonstrated.

However, vaccination has formed an important part of a disease response in several countries or regions including Canada, Faroe Islands and Chile (Falk 2014).

1.6 Epidemiology

Infection with virulent strains of ISAV (HPR-deleted ISAV) can lead to ISA which is a highly contagious and lethal disease of Atlantic salmon. Natural outbreaks of ISA have only been described in Atlantic salmon. Other fish species, such as herring (*Clupea harengus*), rainbow trout (*Oncorhynchus mykiss)*, sea and brown trout (*Salmo trutta*), can be experimentally infected and are considered potential carriers and reservoirs for ISAV (see Table 1).

Infection with the ancestral variant of ISAV, HPR0 leads to subclinical infection. In recent years much progress has been made in understanding HPR0. It is now understood that HPR0 is found in subclinically-infected wild and cultured salmonids in all countries that have previously suffered ISA epidemics or endemic disease (Christiansen et al. 2011; Cook-Versloot et al. 2004; Cunningham et al. 2002; Kibenge et al. 2009; Nylund et al. 2007). It is believed that mutation of HPR0, namely in the haemagglutinin esterase gene (HPR) and then the F gene, leads to potential development of ISA outbreaks.

1.6.1 Incubation period

The incubation period of ISA is dependent on viral (strain and dose), environmental (water temperature), and host (host age, immune status) factors. A number of experimental infectivity trials have been undertaken using various types of inoculum, different routes of inoculation and under different environmental conditions (temperature, salt water, freshwater) (Dannevig et al. 1994; Rimstad et al. 1999; Simko et al. 2000; Totland et al. 1996). In these studies the first fish deaths attributable to ISA occurred around 15 days after intra peritoneal injection of the virus and, in some studies, continued for several weeks. The earliest demonstration of viral replication in all tissues examined occurred at around 13 days after inoculation. The incubation period appears to be approximately 1.5 weeks in non-injected cohabitants in experimental studies (Rimstad et al. 2011).

The incubation period can be a few weeks to several months in natural outbreaks (Jarp & Karlsen 1997; Rimstad et al. 2011; Vågsholm et al. 1994).

1.6.2 Persistence of the pathogen

Persistence of infectious particles in the environment is dependent on local conditions, for example temperature, presence of substances that bind and/or inactivate the virus. However, available data suggest that ISAV may remain infective for extended periods of time outside the host (Rimstad et al. 2011). Infectious virus particles could be obtained from the heart tissue of dead fish for 4–5 days post-mortem (Vike et al. 2014).

The virus is stable in a pH range from 5–9 but inactivated at extremes outside this range (Falk et al. 1997). The virus remains infective even after freezing and thawing at –80°C (Smail & Grant 2012) and also remains infective in cell culture at cooler temperatures (after 14 days at 4°C or 10 days at 15°C). In contrast ISAV is highly susceptible to heat inactivation. ISAV can be completely inactivated after 5 minutes incubation at 56°C, while 6 hours at 37°C resulted in an 80% reduction in infectivity (Falk et al. 1997).

Torgersen (1997) showed that ISAV can be inactivated by treating ISA-infected tissue homogenates with physical and chemical disinfectants such as:

- temperatures of 50°C or higher for 2 minutes
- pH less than 4.0 for 8 hours, greater than 11.5 for 48 hours, or greater than 12.0 for 24 hours
- 100 ppm sodium hypochlorite for 15 minutes
- UV doses of 4 millijoules per square centimetre or higher.

The presence of organic matter reduces the effectiveness of UV and hypochlorite.

Smail et al. (2004) tested three different iodophor products at 100 ppm: 1% (w/v) chloramine T, chlorine dioxide, and a peracetic acid (0.02–0.06%)/hydrogen peroxide (0.08–0.25%)/acetic acid (0.04–0.13%) mixture. In each case, a 5-minute contact time reduced ISAV infectivity for SHK-1 cell cultures by greater than 4 $\log_{10}$. A summary of these and other data is provided Rimstad et al. (2011).

More details on decontamination are provided in Section 2.2.8 and in the AQUAVETPLAN Decontamination Manual.

1.6.3 Modes of transmission

Most evidence demonstrates that the predominant form of transmission is horizontal (Gustafson et al. 2005; Gustafson et al. 2007b; Jarp & Karlsen 1997; Mardones et al. 2013; Mardones et al. 2014; Mardones et al. 2009; Murray et al. 2002; Vågsholm et al. 1994). The general mechanism is that virus is shed into water (virus originates from fish skin, mucus, faeces, urine, blood or waste from dead fish), and is then absorbed through gills to infect susceptible fish (Rimstad et al. 2011).

Specifically, there are three main mechanisms of horizontal transmission: passive waterborne spread, for example virus particles distributed locally by sea or fresh water movements, fomite spread (objects spread virus such as contaminated equipment or fish material) or movement of live but infected fish. Salmon lice (*Caligus elongatus* and *Lepeophtheirus salmonis*) have also been implicated as vectors (Nylund et al. 1994; Nylund et al. 1993) with *Caligulus rogercresseyi* demonstrated as a mechanical vector (Oelckers et al. 2014). However, their importance in disease spread compared to other modes of transmission is not clear.

The role of vertical transmission (from parents to offspring) as a key transmission pathway is controversial (Rimstad et al. 2011). There is evidence that vertical transmission is possible (Marshall et al. 2014) and may have occurred—for example, introduction of ISAV to Chile (Vike et al. 2009). However, the importance of vertical transmission in the maintenance of infection in endemically infected countries is debatable. Some authors argue that vertical transmission is the predominant mode of transmission in Norway (Nylund et al. 2007), while other authors argue that horizontal transmission is most important, with little evidence of vertical transmission (Lyngstad et al. 2008).

1.6.4 Factors influencing transmission and expression of disease

Several authors have conducted epidemiological studies (generally observational) on outbreaks or endemically infected areas to infer which factors affect the risk of ISA at a site (Gustafson et al. 2014; Gustafson et al. 2007a; Gustafson et al. 2005; Gustafson et al. 2007b; Jarp & Karlsen 1997; Mardones et al. 2014; Mardones et al. 2009; McClure et al. 2005; Murray et al. 2010; Murray et al. 2002).

Factors associated with the risk of disease are:

- proximity to infected farms or processing plants (Aldrin et al. 2011; Gustafson et al. 2014; Gustafson et al. 2005; Gustafson et al. 2007b; Jarp & Karlsen 1997; Mardones et al. 2014; Mardones et al. 2009; Murray et al. 2010)
- marine water hydrography (Gustafson et al. 2014; Gustafson et al. 2007b; Murray et al. 2010)
- movement of live fish, harvested fish or by-products (Gustafson et al. 2014; Mardones et al. 2014; Murray et al. 2002)
- well-boat and processing plant hygiene (Gustafson et al. 2014; Murray et al. 2002)

- common company ownership of sites (Vanderstichel et al. 2015)
- co-infection with other viruses (Cortez-San Martin et al. 2012)
- husbandry
  - multiple generations at a site/absence of fallowing (Gustafson et al. 2014; Gustafson et al. 2005; Mardones et al. 2014)
  - absence of coordinated production by farms within a contiguous water body (Murray et al. 2002)
  - smolt weight at stocking (Mardones et al. 2014; McClure et al. 2005)
  - farm area (Mardones et al. 2014)
  - fish and site density (Gustafson et al. 2014)
  - sea lice status (Gustafson et al. 2014) and number of treatments for sea lice (McClure et al. 2005)
  - broodstock HPR0 status (Gustafson et al. 2014)
  - net pen depth and depth of water under net pen (McClure et al. 2005)
  - presence of large populations of wild pollock in cages (McClure et al. 2005)
  - excessive post transfer mortalities (McClure et al. 2005)
  - stressful situations for fish (Hammell & Dohoo 2005).

Of the many risk factors listed for ISA, several are also likely to be important for infection with HPR0 ISAV—for example, fallowing and spatial proximity. However, this is an assumed conclusion as the listed risk factors were mostly identified during observational studies on ISA.

Importantly, several recent molecular epidemiological studies have indicated that circulation of endemic HPR0 often precedes outbreaks of ISA (Christiansen et al. 2011; Godoy et al. 2013; Lyngstad et al. 2011; Lyngstad et al. 2012; McBeath et al. 2009; Vanderstichel et al. 2015), and may be a risk factor for the disease (Godoy et al. 2013). HPR0 ISAV is found in subclinically infected wild and cultured salmonids in all countries that have previously suffered ISA epidemics or endemic disease (Christiansen et al. 2011; Cook-Versloot et al. 2004; Cunningham et al. 2002; Kibenge et al. 2009; Nylund et al. 2007), and it is hypothesised that the continual presence of HPR0 ISAV provides an opportunity for mutation of ISAV to become virulent.

1.7 Impact

ISAV is exotic to Australia. The countries and regions where it has had most impact through ISA outbreaks are addressed in this section.

Chile

Chile has a long history of ISA after importing ISAV-infected products from Norway. Chile experienced an isolated epidemic in Coho salmon in 1999 (Kibenge et al. 2001), suffered a major outbreak that decimated the industry from 2007–2010 (Alvial et al. 2012) and again experienced an outbreak in 2013 (Godoy et al. 2013). The Chilean outbreak in 2007 decreased production by 230 000 tonnes and resulted in the loss of 25,000 direct jobs.

Canada

Canada has suffered from recurrent outbreaks starting in New Brunswick in 1996, Nova Scotia in 2012, and Newfoundland and Labrador in 2012. There was also a false positive test result to ISAV in west Canada in 2011, which resulted in a large and expensive surveillance program (Amos et al. 2014).

Norway

In Norway, ISA is endemic and has been present since 1984 causing ongoing production impacts. In 2014 there were 10 outbreaks of ISA (Anonymous 2015a).

Scotland

Scotland has had two ISA outbreaks, in 1998–99 and in 2008–09. In the first Scottish outbreak, infection was confirmed on 11 farms and suspected in a further 18 farms, out of a total of 340 salmon farms. The outbreak affected 25% of Scottish production, resulting in approximately 15% loss in turnover, job losses of more than 10%, and further impacts upstream, for example smolt producers, and downstream, for example feed companies, processors, service providers (Scottish Parliament 1999). The second outbreak was much smaller because of better biosecurity practices implemented as a response to the first outbreak (Murray et al. 2010).

United States

An ISA outbreak occurred in 2001 (Gustafson et al. 2007a) and continued for many years, being last identified in 2006 (OIE 2015).

Faroe Islands

There was an outbreak of ISA in 2000 which was eradicated. Comprehensive management strategies, such as fallowing, are in place to reduce the probability of mutation (Christiansen et al. 2011). However, HPR0 is endemic and may have led to a re-emergence of ISA, which was reported to the OIE in March 2017.

Apart from the direct impact of lost production because of death caused by both disease and enforced depopulation, control measures during and after the outbreak have had further impacts on the industry (Stagg 2003a, b, c):

- withdrawal of fish from the farm
- disposal or marketing of all fish
- disinfection and cleaning
- fallowing
- surveillance of adjacent farms in established zones
- improving disease awareness
- further development of slaughter, processing plant and disinfection protocols
- consolidation of industry participants
- increased government/industry expenditure on surveillance, and research and development.

Threats to Australia

Impacts of HPR-deleted ISAV in Australia are likely to be severe as has occurred elsewhere in the world, but would depend upon the scale of the epidemic. In contrast, an incursion of avirulent ISAV (HPR0) would not be as severe. For example, ongoing infection with HPR0 is common in many productive salmon producing areas. The main impact of HPR0 infection is likely to be dependent upon the response to detection of HPR0. If eradication is chosen, impacts could be high in the short term, and it may be difficult to justify this approach on cost benefit grounds. If control and mitigation are chosen as a management option, impacts are likely to be less and associated with increased biosecurity and management measures adopted to reduce the probability of mutation to HPR-deleted ISAV. These measures focus on vigilant bay area

fallowing/coordination of production (single year class) (see section 2.2.21). In addition, the risk of an outbreak of ISA is greater in the future as there will be ongoing presence of ISAV in the salmonid industry.

2 Principles of control and eradication

2.1 Introduction

Infection with ISAV in Australian salmon could result in two main outcomes:

1) Subclinical infection that does not cause disease (HPR0 ISAV).
2) An outbreak of ISA that causes mass mortality of farmed Atlantic salmon with significant economic loss to those Atlantic salmon aquaculture sectors affected (HPR-deleted ISAV preceded or not by HPR0).

An outbreak of ISA in Australia would be a serious threat to the Atlantic salmon farming industry centred in Tasmania, with sales of approximately $620 million annually (Anonymous 2015b). Detection of ISA virus (ISAV), even in the absence of clinical signs, for example HPR0 ISAV, would also be cause for serious concern, but may not warrant eradication. This section provides background information to enable the choice of the most appropriate response option following detection of ISA/ISAV in Australia.

Three disease control strategies could be adopted if ISAV is isolated in Australia:

- Eradication—the scale of eradication may be national, state-wide (eradicate ISAV from Tasmania), or local (eradicate from a production area within Tasmania, for example Macquarie Harbour)
- Containment and control via zoning/compartmentalisation—includes measures to exclude ISAV from defined geographic areas and unaffected populations, for example by quarantine and biosecurity, and contain the virus to areas with established infection
- Control and mitigation—measures aimed at managing the frequency and severity of disease episodes in infected populations and keeping them within acceptable levels.

The basic principles of eradication and other response options are described in the AQUAVETPLAN Enterprise Manual (Department of Agriculture 2015) and the AQUAVETPLAN Control Centres Management Manual (DAFF 2001). The AQUAVETPLAN Enterprise Manual lists the state and territory legislation relating to disease control and eradication.

The nature of any outbreak of disease or the strain of the virus—for example, HPR0 versus HPR-deleted ISAV—will affect which option is considered most viable. Outbreaks that are widely disseminated or in both wild and aquaculture populations may be more difficult to eradicate. In contrast, an outbreak at a single aquaculture facility may be relatively easily eradicated. There may be less desire to immediately eradicate an outbreak of HPR0 ISAV as this will not be causing any disease (ISA) and a cost-benefit analysis may favour control and mitigation.

The general strategies that should be used in the control of this disease include:

- rapidly delineate the infection
  - time—for example, age lesions and increased start of mortality to estimate introduction date
  - fish—for example, aquaculture versus wild salmonids or species
  - place (geographical extent of epidemic)
- rapidly prevent further dissemination of the infection
  - immediate zoning and movement restrictions (between and within farms) of stock, water and fomites

  - enhanced biocontainment (reduce fomite spread)
  - education
- consider whether reduction of infection or eradication is required
  - cost-benefit analyses—for example, is it warranted to eradicate HPR0
  - if eradication is desired: depopulation, disinfection and disposal of infected aquaculture populations (consider impracticality or practicality for wild populations)
  - if control and mitigation are desired: vaccination, coordinated production in contiguous water—for example, coordinated fallowing; excellent management practices and hygiene standards.

In any disease outbreak, a number of management tools are immediately available:

- destruction of diseased fish—rapid removal and appropriate disposal of diseased fish are considered high priorities for controlling the spread of the disease and causative agent if immediate eradication is desired
- quarantine—restrictions on movement of animals, materials, waste, personnel, vehicles and equipment. This option requires rapid identification of the affected geographical area, disinfection of personnel and equipment leaving affected sites, and disinfection of waste water and waste products from processing plants.
- emergency harvest—grow-out of fish to harvest size (possibly in a quarantined area) may be an option, as is the slaughter and processing of commercial-sized, clinically normal but infected fish for marketing and human consumption.
- treatment of affected populations—research on a number of chemotherapeutic products is ongoing, but no viable products have yet been registered in Australia. Some products that may have potential in the future include
  - Ribavirin: a broad spectrum antiviral drug which has proved effective at reducing ISAV replication *in vitro* and *in vivo* (Rivas-Aravena et al. 2011)
  - 7-O-methyleriodictyol: a natural flavonoid which has inhibited the infectivity of ISAV in vitro assay and protected fish in vivo against death due to infection with ISAV (Modak et al. 2012)
  - double-stranded RNA: to induce RNA interference and silence targeted ISAV genes (Garcia et al. 2015; Papic et al. 2015). This method is experimental and has a number of limitations.

Not all of these options will be appropriate in all given circumstances. For example, there are currently no vaccines registered in Australia and no chemotherapeutics that have been developed to a marketable product.

Salmonids are farmed in two phases—both phases using semi-open systems. In Tasmania, the hatcheries (freshwater phase) are located on rivers isolated from the seawater or grow-out phase, which occurs in sea cages at various locations around the state (Huon Estuary, D'Entrecasteaux Channel, Macquarie Harbour, Tasman Peninsula, Tamar River, Port Esperence). The main production areas are the Huon Estuary, D'Entrecasteaux Channel and Macquarie Harbour.

While control of fish, personnel and equipment movement can be achieved, there can be little, if any, control of water movement. Therefore response options need to be aimed at rapid control of spread or modifying production processes to ensure coordinated bay area management approaches that ensure coordinated stocking of single year classes, fallowing and depopulation

to control infection (see section 2.2.21) (Jones et al. 2015; Murray et al. 2010; Olivares et al. 2015; Werkman et al. 2011). For example, experience from overseas indicates that restricting the large-scale shedding of virus, for example from diseased, dying or dead fish, into the water column plays an important role in limiting transmission. Additionally, coordinating production to a single age class in a bay that shares common water dynamics has formed a major focus in overseas ISA control or eradication programs, for example, in Scotland, the United States and Canada and Chile.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

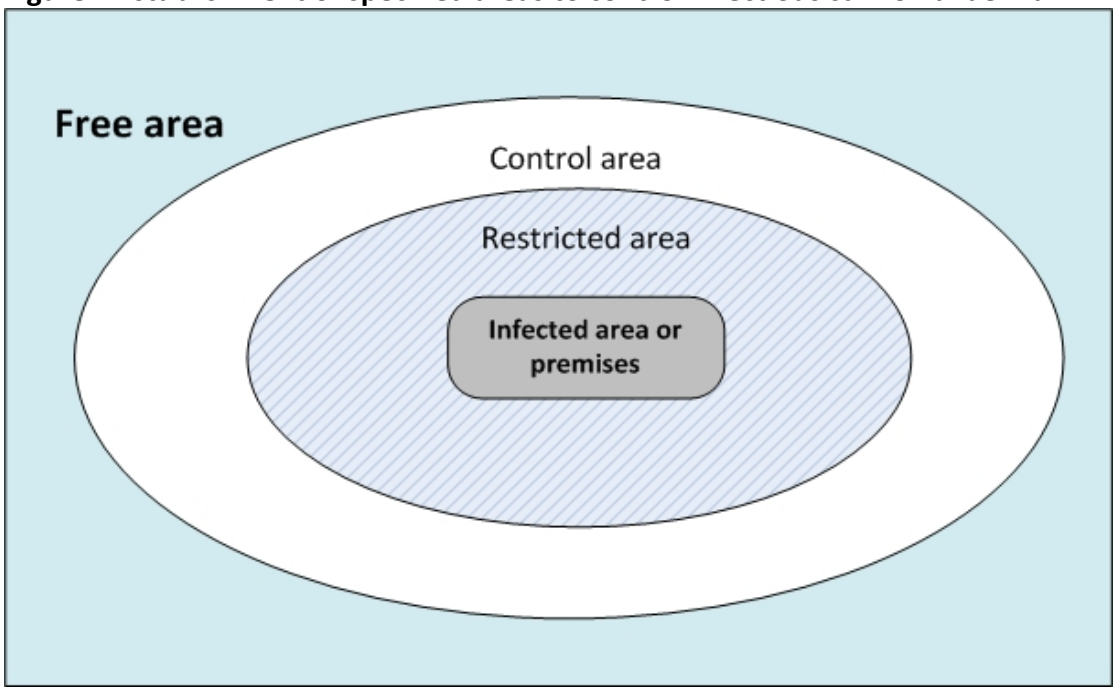
Quarantine and movement restrictions should be implemented immediately upon suspicion of ISA or infection with ISAV. A decision can then be made about the desire for future eradication or management while the disease is at least temporarily contained.

2.2.2 Establishment of Quarantine areas

Establishment of specified areas (see AQUAVETPLAN Enterprise Manual Section A for more details), including:

- declared area—infected, restricted and control areas
  - infected area or premises—the premises (for example, farm) or area where the infection is present and the immediate vicinity
  - restricted area—area around infected premises or area
  - control area—a buffer between the restricted area and free areas
- free area—non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of ISAV remains unassessed).

Figure 1 Establishment of specified areas to control infectious salmon anaemia



In the declaration of quarantine areas, the factors listed in this section need to be taken into account.

Epidemiological and surveillance information
While epidemiological and surveillance data on infection distribution and susceptible populations is the best means of establishing zones, this is unlikely to be comprehensively available, especially early in an epidemic. Consideration of the factors in this section may allow prediction of likely transmission/dispersal and early establishment of zones in the absence of good surveillance data.

Natural factors that could facilitate or hinder transmission:

- the contiguous distribution of naturalised salmonid populations
- movements of salmonid reservoirs
- water movements that can disperse virus (use sea distances and current directions instead of Euclidean distance between farms to set declared areas)
- natural catchment divisions that may contain infection because water or salmonids do not move between catchments.

Anthropogenic factors (industry)

- connectedness of aquaculture facilities and transmission of infection between facilities, for example company structure and movement of stock or equipment/fomites and fish to processing, shared jetty
- presence of other aquaculture facilities with susceptible species
- movement of consumer products
- facilitation of business continuity where possible, for example, declared areas should facilitate movement of essential equipment, personnel and product between farms or to processing plants.

Anthropogenic factors (recreational fishing)

- establish areas based on likely historical movement of fishers that may have transmitted infection
- structure areas to minimise disruption to recreational fishing (where possible)
- structure areas so that movement bans can be legally and practically enforced.

Practices that must be considered when implementing response options include:

- both freshwater and marine phases of salmonid production
- possibility of the existence and presence of a previously unknown susceptible species of wild fish that may act as an asymptomatic carrier
- other river user movements
- discharge of effluent from processors and farms
- disposal of dead fish and products
- decontamination.

Movement controls
The feasibility of movement restrictions and bans, and extent to which these are able to be enforced, will depend on the strain of the virus (for example, HPR0 vs. HPR-deleted), the location of infection, the location and type of enterprises affected, and the control response

option chosen (for example, whether the aim is to eradicate the disease agent or to control its spread). Depending on the circumstances, some restrictions may be impractical or unnecessary but others will be of critical importance to eradication or control. In all cases, the implementation of bans and restrictions will be a dynamic process.

Movement controls include:

- bans on the movement of live salmonids out of infected areas
- bans on the movement of live salmonids into disease-free areas
- restrictions or bans on releasing salmonids into river systems or marine locations
- restrictions or bans on the movement of salmonids between different river systems, between marine farm locations and between marine and freshwater farm locations
- restrictions or bans on the use and movement of equipment and personnel within and between river systems and marine farms
- restrictions on the movement of dead salmonids and salmonid processing waste out of infected areas into disease free areas.

Implementation of bans and restrictions will be a dynamic process, determined by the location and extent of the disease outbreak and whether the aim is to eradicate the disease agent or to control its spread. Some restrictions may be impractical or unnecessary but others will be of critical importance to eradication or control.

The feasibility of restrictions and bans and extent to which these are able to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

2.2.3 Zoning and compartmentalisation

It is sometimes possible to maintain a sub-population of salmonids with distinct aquatic animal health status (for example, infected or free of ISAV). This can be done on a geographical basis (referred to as zoning) or on a common biosecurity basis such as management practices (referred to as compartmentalisation).

Zoning

If ISAV was to become endemic in specific regions of Australia, a zoning policy specific for ISAV may be necessary to protect non-infected areas and to prevent further spread of infection. Zones would be based on the distribution of ISAV-susceptible species and of any vector species present (if appropriate), the geographical and hydrological characteristics of water bodies and landform, and predictions of the most likely method of spread of infection. Zoning may rely on the identification of biogeographic barriers. A corresponding surveillance and monitoring program for ISAV would be required to support the zoning policy. Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines (DAFF 2000) and in the OIE Aquatic Animal Health Code (OIE 2016a).

Compartmentalisation

A compartment means one or more aquaculture establishments under a common biosecurity management system containing an animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose international trade.

A compartment does not have to be contiguous facilities—it can apply to a series of farms over a large area, including over several jurisdictions. It must have in place a biosecurity management system that meets guidelines provided in Chapters 4.1 and 4.2 of the OIE Aquatic Animal Health

Code (OIE 2016a) and this system must have been documented by the competent authority (that is, the veterinary authority of the jurisdiction).

Disease management in aquatic environments
The establishment of Disease Management Area (DMA) boundaries during an Emergency Aquatic Animal Disease event presents particular difficulties requiring detailed consideration beyond that normally required for terrestrial animal disease control. Water movement through and around farms, within streams or rivers, and in the marine environment represents a substantial risk for spread of disease through transfer of infectious pathogens in the water column, movement of infected material (particularly on suspended organic and inorganic matter), and any infected wild organisms.

For example, although an infected area may be established around an individual land-based hatchery or farm, water bodies adjacent to the infected area as well as in the same catchment should be considered for monitoring and control measures. The establishment of DMA boundaries around marine farms or wild fisheries may need comparatively large areas that must take into consideration local currents, natural barriers and the normal range of susceptible wild species.

Establishment of the relevant DMA boundaries must take into account dispersal of water discharged from any infected semi-closed aquaculture systems, for example hatcheries or potentially infected processing facilities, and how this enters adjacent water. Similarly, outbreaks in semi-open systems (marine farms) require the consideration of all oceanographically connected areas and distribution of wild host or vector populations. Spread of infected material through scavenging by other species also needs to be considered.

Thus, rather than property boundaries, the geography, water flow, distance between farming areas and the range of susceptible species will define where DMA boundaries are placed.

Establishment of DMA boundaries and their classification must also take into account potential mechanisms by which disease may move beyond these boundaries. In most circumstances it is advisable to overestimate the size of DMAs and reduce their area as the response takes effect. In most cases, in the initial response, the DMA boundaries will need to include the whole of a catchment area in freshwater systems and complete bays or regions in marine environments.

2.2.4 Tracing

Tracing a disease outbreak is the process of retrospectively determining the method, route and pattern of disease spread. Tracing investigations are crucial in determining all confirmed and potential locations of the disease and its causative agent, as well as defining restricted and control areas. The information gathered from these investigations will assist in determining the most appropriate response action. The immediate steps required are to trace-back all contacts with infected fish, premises and sites (to establish the origin of the outbreak) and to trace-forward all contacts with infected fish, premises and sites (to establish the current location and potential spread of infection).

These items must be traced:

- salmonids—such as broodstock and smolts
- salmonid products—for human consumption, eggs, effluent and waste products from slaughter and processing
- water—input and output
- vehicles—salmonid transport vehicles, feed trucks, visitors' cars, boats
- materials—salmonids cages, nets, other floating installations, tools and instruments

- personnel—farm workers, sales and feed representatives, tradespeople, veterinarians, scientists, technicians and visitors
- shared jetty
- natural movements of wild or feral salmonids and water should be modelled and surveyed.

In Australia, it is essential during tracing to consider:

- Infection may have been present in a salmonid population for days or weeks before detection of clinical event, which may have been triggered by a stress event such as grading or freshwater bathing for amoebic gill disease (AGD).
- Salmonids, people, boats, motor vehicles/trucks and birds regularly move between the catchment areas on which salmonid farms are located. Tasmania has three main catchment areas and up to six trucks per day move salmonids between freshwater and seawater operations.

2.2.5 Surveillance

Surveillance, by screening for clinical signs and by laboratory testing of samples, is necessary for:

- ongoing for early detection
  - detect new outbreaks
- delimitation—both pre and post outbreak
  - define the extent of the infection
  - establish restricted and control areas to which quarantine and movement restrictions are applied
- establishing prevalence
  - further stock management
- post-response surveillance
  - establish infected and non-infected areas/zones for a ISAV zoning program
  - monitor the progress and success of an eradication strategy
  - assist demonstrating freedom from infection after an outbreak
  - detect new outbreaks.

As described in Section 1.4.3, examination of diseased fish for gross pathology and histological lesions can provide an initial diagnosis. Confirmatory tests include indirect fluorescent antibody test (IFAT) on kidney imprints, immunohistochemistry (IHC), real-time RT-PCR, RT-PCR followed by sequencing of the PCR product and virus isolation in cell culture (OIE 2016b). For detection of subclinical infections by HPR0, methods are limited as this strain has never been isolated in culture. RT-PCR and sequencing are the most important diagnostic methods. Other subclinical infections may be diagnosed with virus isolation and RT-PCR.

A number of factors require consideration when developing a surveillance scheme to demonstrate freedom from infection, some of which will depend on specific characteristics of the disease outbreak, such as time of year, stage of life cycle, location, production system and management practices. Suitable surveillance techniques exist including both passive and active surveillance.

Passive surveillance uses existing systems and processes to identify and notify any cases of disease. The absence of reports of disease can be important evidence that supposedly free areas—for example, zones or eradicated areas—are indeed free if a suitable passive system exists.

Active surveillance seeks to collect new information—for example, samples of fish or worms—to detect disease if it is present. Practical active sampling strategies exist to demonstrate freedom (Cameron 1999, 2002; Cameron & Baldock 1998a,b). For example, a sample size that is sufficient to detect infection if it was present at a certain unrealistically low prevalence can be designed. The absence of infection from such samples allows one to make probabilistic statements that disease is likely absent.

Newer approaches have been developed that allow a holistic analyses of disparate surveillance sources—for example, active and passive sources to determine the probability that disease freedom has been achieved in a farm, region or country (Cameron 2012; Martin et al. 2007).

These techniques are widely accepted internationally and often considered required information during international trade negotiations. They could be applied to ISAV to demonstrate freedom from disease.

Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2016c) and OIE Aquatic Animal Health Code (OIE 2016a).

2.2.6 Treatment of infected host species

No registered antiviral drug treatments or vaccines are available for ISA in Australia but several vaccines exist overseas (see 2.1. Treatment of affected hosts).

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered when determining the treatment/processing and destiny of salmonid products and by-products. ISAV does not pose any risk to human health.

Products for human consumption may transmit infection between salmonids unless certain steps are taken. Relatively high titres of virus are found in the viscera. Removal and inactivation of viscera at processing will reduce the viral load in products from infected fish. Cooking at high temperatures such as 50°C or higher for 2 minutes (Torgersen 1997) or 5 minutes incubation at 56°C (Falk et al. 1997) inactivates the virus.

Fish carcases can remain infective in natural conditions for some time. For example infectious virus particles could be obtained from the heart tissue of dead fish for 4–5 days post-mortem (Vike et al. 2014). Dixon et al. (2012) demonstrated that infected fish extracts treated with sodium hydroxide for 48 hours were no longer infective. Alkaline hydrolysis at ambient temperature therefore has potential as a disinfection method for fish by-products. See the AQUAVETPLAN Operational Procedures Manual—Decontamination (DAFF 2008) for details.

2.2.7 Destruction of hosts

Overseas experience has indicated that prompt destruction and disposal of diseased fish reduces the risk of disease spread. Diseased salmonids located in sea cages can be killed onsite. For more details on destruction of ISA-affected salmonid fish see the AQUAVETPLAN Operational Procedures Manual—Destruction (DAFF 2009a).

2.2.8 Disposal of hosts

Slaughtered animals need to be disposed of in a safe and effective manner. In Australia, best practice has been to bury slaughtered terrestrial animals onsite. Clearly, this is not possible for diseased salmonids. Firstly, culled fish need to be transported safely to the burial (or incineration) site without spreading disease to other sites. Operations will be supervised by state authorities and further details on the most appropriate methods of disposal of host

organisms can be found in the AQUAVETPLAN Operational Procedures Manual—Disposal (DAFF 2009b).

2.2.9 Decontamination

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, the state or territory chief veterinary officer (CVO) and/or director of fisheries. The protocol should also take into consideration the factors outlined in Section 1.6. These factors in particular should be considered:

- the source and location of infection
- the type of enterprise—for example, farm, processing plant, hatchery, grow-out cages, water source
- the construction materials of the buildings and other structures on the site
- the design of the site and its proximity to other waterways or buildings
- current disinfection protocols
- workplace safety concerns
- environmental impact of the disinfectant protocol
- legislative requirements (occupational health and safety, environmental protection, chemical use)
- availability of approved, appropriate and effective disinfectants.

See the AQUAVETPLAN Operational Procedures Manual—Decontamination (DAFF 2008) for details of decontamination methods.

2.2.10 Vaccination

Vaccination has been used in several overseas countries or regions including Chile, Faroe Islands and Canada (Falk 2014). Use in Australia would require registration or application for a permit, such as an emergency use permit from the Australian Pesticides and Veterinary Medicines Authority.

Immunity is short lived and variable and therefore regular re-vaccination is required. This is now theoretically practically possible with the development of an orally delivered vaccine (Tobar et al. 2015).

Some caution is required when using vaccines. Inactivated viral vaccines have not been shown to be 100% protective (Kibenge et al. 2003); immunised fish do not completely eliminate virus and may become carriers. In addition, the existence of different ISAV strains indicates that efficacy of any vaccine against local or introduced ISAV strains needs to be demonstrated.

See section 1.4.5 for more information.

2.2.11 Vector control

Other fish species susceptible to infection with ISAV have been documented in Section 1.2. Any of these species present in Australia should be considered potential carriers of infection and appropriate investigation should be carried out.

In addition, parasitic copepods have been implicated in the spread of ISAV and control of infestation with any such organism should be considered.

2.2.12 Bay area management

Transmission of ISAV between salmon farms and the maintenance of infection in a region for long time periods can lead to:

- prolonged epidemics (in the event of ISA)
- continual cycling of ISAV HPR0, thereby increasing the opportunity for the avirulent virus to mutate to a pathogenic strain (Christiansen et al. 2011).

Subsequently, many countries now institute bay area management approaches (see section 2.2.21). Here salmon populations that are connected hydrologically undergo coordinated production cycles (coordinated stocking, fallowing and depopulation). This facilitates a fallow period across the entire local production area that eliminates ISAV infection in a local population of farmed salmon (Jones et al. 2015; Murray et al. 2010; Olivares et al. 2015; Werkman et al. 2011). This is an important management approach to controlling ISAV and several other infections/infestations of Atlantic salmon.

2.3 Environmental considerations

Release of potentially infected effluent into the environment needs to be avoided. All potentially infected effluent from slaughter premises and processing plants must be inactivated. See the AQUAVETPLAN Operational Procedures Manual—Decontamination (DAFF 2008) for details. During decontamination operations, all legislation and regulations concerning the disposal or discharge of chemicals, for example disinfectants, and cleaning agents into the environment must be observed.

2.4 Sentinel animals and restocking measures

Restocking should only occur after all diseased and potentially exposed salmonids have been removed from the farm sites—either destroyed or processed. The presence of subclinical carrier populations of wild fish cannot be discounted. It is likely that, following a significant disease outbreak, it would take several weeks or months for any management program to be completed and for the industry to be in a position to consider restocking. The length of the fallow period would depend on a number of factors—the number of sites in which ISA has been confirmed, the extent of the outbreak and the characteristics of the affected sites (OIE 2016b). In Norway, the fallow period following an ISA outbreak is no shorter than three months (Anonymous 2015a). Susceptible sentinel fish (Atlantic salmon) could be re-stocked and observed for several weeks to be sure that ISA was eradicated before commercial re-stocking.

2.4.1 Public Awareness

A public awareness campaign emphasising education, surveillance and cooperation from industry and the community is essential. The public should be informed that:

- ISAV is not infective for humans
- eating fish that may have been exposed to ISAV is not considered a health risk
- fish that have died from infectious disease must not be used as bait
- the transport of water, fish and fish products and contaminated equipment from infected areas can transmit infection and is not permitted.

2.5 Control or eradication of ISAV in Australia

The feasibility of controlling an infection of ISAV depends on the nature and location of the infection—for example, an outbreak of ISA versus detection of HPR0—and the management strategy adopted. As outlined in Section 2.1, there are three response options:

- eradication
- containment and control
- control and mitigation.

Eradication is the preferred option for ISA (an outbreak of disease due to infection with a virulent form of ISAV). If epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the virus, an eradication strategy may be successful and should be attempted. Compared with the other two response options, eradication has the highest short-term economic costs. However, if ISA were successfully eradicated, long-term economic benefits would likely outweigh those short-term costs.

Industries preferred response option will be different for infection with HPR0 (avirulent ISAV). If the infection was isolated to a single freshwater premises, eradication may be attempted. However, if the infection was detected in grow-out cages, either containment and control or control and mitigation are likely to be the preferred options. Eradication would not generally be preferred because:

- infection may be widely dispersed at a low prevalence across many leases in a production area before detection
- provided appropriate management was instigated—for example, bay area management to reduce probability of mutation to virulence (see section 2.2.21)—the economic impact due to infection with HPR0 would likely be low
- eradication of HPR0 would be economically very damaging
- eradication of HPR0 would be difficult—for example, surveillance to even detect infected premises would be problematic as infection is subclinical, viral shedding is short lived or intermittent, and laboratory methods are limited (culture is unsuccessful).

2.5.1 Response option 1: eradication

Eradication would likely be pursued in cases of ISA and where infection with an avirulent strain was limited to a single freshwater premises.

In semi-open systems eradication is unlikely to be successful or feasible if epidemiological investigations determine that disease is widespread, has no point source, is unable to be contained or is present in wild fish species inhabiting the surrounding vicinity.

The known host range for ISAV is restricted to a relatively small number of fish species, none of which are endemic in Australia, and so eradication may generally be considered feasible. Thus, if it can be demonstrated that reservoirs of infection have not become established in farmed and wild fish populations, eradication may be considered feasible and the control measures described in this section should be put into place. Disease eradication is complicated by the presence of wild reservoirs of infected fish, but may still be possible—for example, through compartmentalisation, which occurred in Scotland's successful eradication of ISA.

Unexposed fish

If unexposed fish can be maintained without any risk of exposure, they can be grown up to harvest size and processed.

Exposed or potentially exposed, clinically normal fish
Immediate destruction of exposed fish prevents further virus replication and minimises further spread. Emergency harvest of commercial-sized fish, processing and sale for human consumption is an option but must not compromise eradication effort.

Clinically diseased fish
All diseased and dead fish need to be removed, destroyed and disposed of as soon as possible following their identification. These fish are the main source of ISAV in the environment. Sea cages with diseased fish need to be totally depopulated irrespective of the number of fish showing clinical disease.

2.5.2 Response option 2: containment and control

The relative host specificity of ISAV makes control and containment of the virus feasible. Knowledge of the strains present is required to determine the virulence range likely to be expressed by the virus population. The presence of avirulent virus in infected but clinically healthy fish complicates control and management procedures. For example, surveillance is more difficult than for ISA and the economic case for expensive control efforts is less clear.

Unexposed fish
If unexposed fish can be maintained without any risk of exposure they can be grown to harvest size and processed as for the eradication response option.

Exposed or potentially exposed, clinically normal fish
A successful zoning program for farmed fish will rely on movement restrictions of exposed, potentially exposed or subclinical HPR0 infected fish to prevent infection spreading to uninfected zones. The feasibility of implementing a zoning program will depend on farm management practices—for example, farm connectivity in terms of hydrodynamic or biosecurity linkages—the extent to which infection has already spread and the location of reservoirs of infection. If salmon are near harvestable weight it is likely they would be immediately harvested. If they are much smaller, attempts to grow them out for several months may be made if disease containment can be assured.

Clinically diseased fish
All diseased and dead fish need to be removed, destroyed and disposed of as soon as possible following their identification. These fish are the main source of ISAV in the environment. Sea cages with diseased fish also need to be totally depopulated, irrespective of the number of fish showing clinical disease, because while the virus may kill only a small portion of fish each day, the cumulative mortality over time is usually very high in a sea cage infected with virulent ISAV (HPR-deleted), and the sea cage will therefore act as a constant source of infection for nearby salmon.

2.5.3 Response Option 3: Control and mitigation

In a control and mitigation program, the aim may simply be to reduce the frequency of existing disease to biologically and/or economically acceptable levels. Critically, there may be a level of disease in the population below which the cost of further expenditure on control would be greater than the benefit. Additionally, infection with an avirulent strain (for example, HPR0) produces little economic impact. However, it is important to note that avirulent strains such as HPR0 may be capable of mutating to virulent strains, and some strains can interact with environmental and host factors to variably produce disease. Therefore the continued presence of avirulent strains pose a potential risk to producers in terms of future development of ISA. Proactive management is required to reduce this probability, and mainly entails bay area management strategies (see section 2.2.21) (Christiansen et al. 2011).

2.5.4 Trade and industry considerations

Trade regulations, market requirements and food safety standards must be considered as part of a response strategy. Permits may be required from the relevant authorities to allow products from declared areas to be released and sold for human consumption.

Export markets

Infection with HPR0 or HPR-deleted ISAV is listed by the OIE as a notifiable disease. Some countries may have import conditions in place related to the ISAV, such as requiring imports to be certified free of infection.

The Department of Agriculture is responsible for the health certification of all exports. Contact the department for further information.

Domestic markets

A cautious approach is required for the harvest of infected, exposed or partially exposed product for the domestic market to prevent the spread of infection. Decisions regarding the release of salmonids or salmonid products to the domestic market will depend on the response strategy implemented and will be made by the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD).

3 Preferred Australian response options

3.1 Overall policy for infectious salmon anaemia virus

Infection with HPR-deleted infectious salmon anaemia virus (ISAV) can produce a serious viral disease of Atlantic salmon (infectious salmon anaemia, ISA). Infection with HPR0 (the ancestral form of ISAV) is generally subclinical but presents a small risk of mutation to a virulent form of ISAV and therefore ISA. Both strains are exotic to Australia, and are listed as a notifiable disease in Australia's National List of Reportable Diseases of Aquatic Animals and by the OIE (World Organisation for Animal Health). Outbreaks of ISA overseas have been associated with high mortality rates ranging between 15% and 100%. Such a disease has the potential to devastate the Australian salmonid farming industry.

The policy for the response to an outbreak of ISA, or detection of ISAV in Australia depends upon both the nature of the outbreak and the management strategy adopted. The choice of response option will be decided by the director of fisheries and/or the chief veterinary officer of the state or territory in which the outbreak occurs, following epidemiological investigation. Any response would take into consideration the views of relevant industry groups (such as the Tasmanian Salmonid Growers Association) obtained during consultation at the time.

There are three possible response options for ISA in Australia:

- option 1—eradication of ISAV from Australia
- option 2—containment and control via zoning/compartmentalisation of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas
- option 3—control and mitigation by implementing management practices that decrease the incidence and severity of disease.

Each of these options involves the use of a combination of strategies, which may include:

- quarantine and movement controls on fish, fish products and things in declared areas to prevent spread of infection
- destruction of all clinically diseased or dead fish as soon as possible, to prevent further viral shedding
- decontamination of facilities, products and things to eliminate the virus on infected premises and to prevent spread of infection
- surveillance to determine the source and extent of infection and to provide proof of freedom from the disease
- zoning to define and maintain infected and disease-free zones
- bay area management to enable localised eradication of ISAV.

An uncontrolled outbreak of ISA due to infection with HPR-deleted ISAV could cause severe, long-term production losses with consequent dislocation and economic losses in the fish farming industry and associated production, sales and export industries. It will therefore be necessary to act immediately to eradicate or contain and control the disease. Infection with HPR0 will likely have little impact on the industry. In this instance, control and mitigation of the disease would most likely be warranted. However, Australia will lose its ISAV-free status.

The Director of Fisheries and/or the CVO in the state or territory in which the outbreak occurs will be responsible for developing an emergency animal disease response plan (EAD Response Plan). This plan will be submitted to the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD), who will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

Directors of fisheries and/or CVOs will implement the disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing

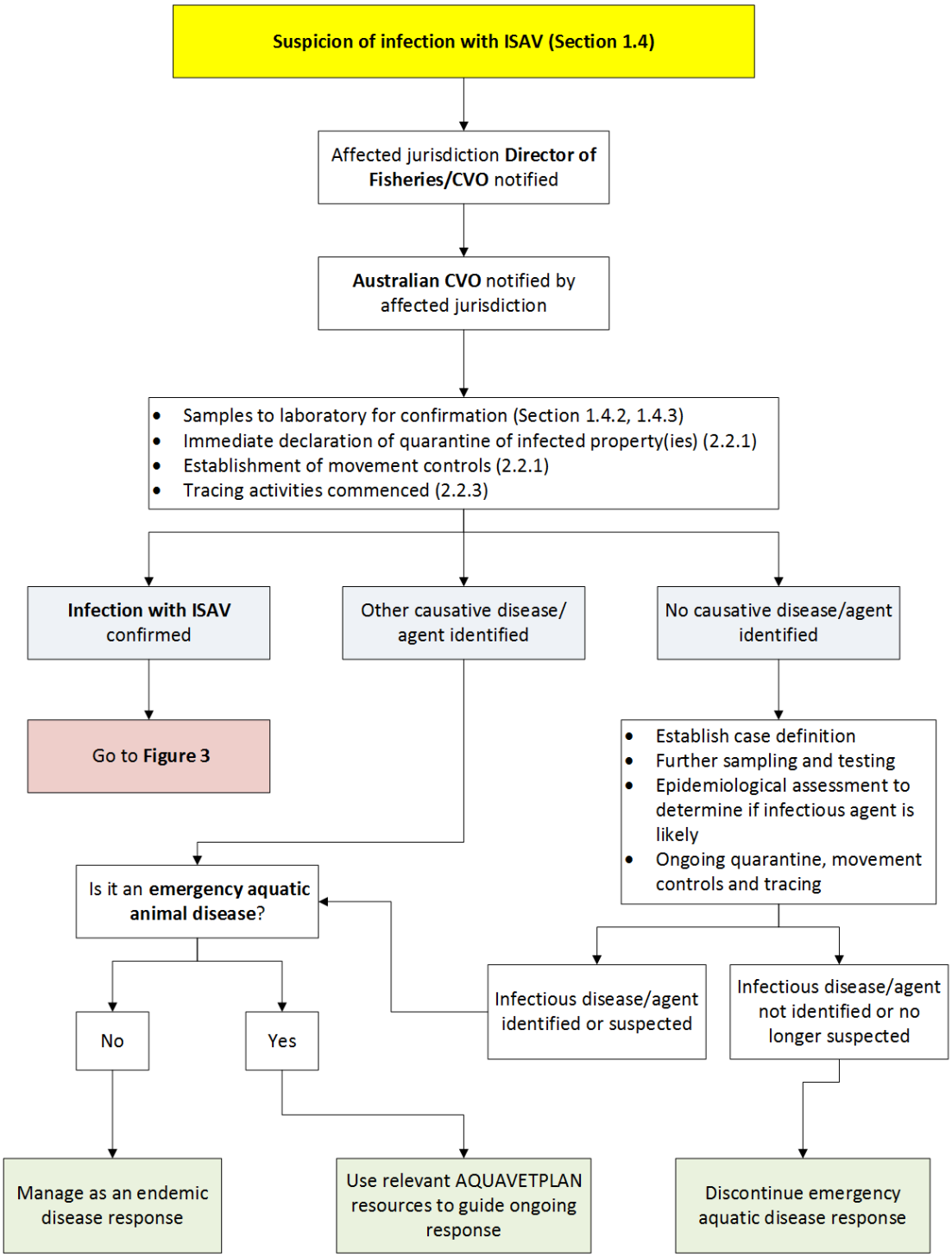
decisions on follow-up disease response measures in consultation with AqCCEAD. The detailed response measures adopted will be determined using the principles of disease eradication, containment, control and mitigation (see Section 2), depending on the strain of virus detected, epidemiological information about the outbreak and the financial and logistical feasibility of the selected option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres Management Manual (DAFF 2001).

3.2 Response options

The circumstances surrounding an outbreak of ISA will greatly influence selection of the most suitable response option. Figure 2 shows the actions that should occur on initial suspicion of the presence of ISAV. Once the presence of ISAV has been confirmed, it is appropriate to refer to Figure 3, which has been developed to help identify the most appropriate response option. These decision trees are flexible, depending on the specific situations experienced.

Figure 2 Decision flow chart for suspected infectious salmon anaemia virus infection



CVO Chief veterinary officer. **ISAV** Infectious salmon anaemia virus

Figure 3 Most appropriate response option to ISA outbreak or confirmed ISAV infection

3.2.1 Option 1: eradication

Eradication is the preferred option for an outbreak of ISA. It may also be the preferred option for a detection of infection due to non-pathogenic strains of ISAV (for example, HPR0) if the infection is contained to a small population of fish, such as in a single isolated premises such as a hatchery. Although it has the highest short-term economic costs, these costs could be outweighed by long-term economic benefits if ISAV is successfully eradicated.

However, infection with HPR0 is unlikely to cause serious economic damage to the salmon industry, and eradication may not be the most favourable option if HPR0 is widely disseminated when detected, or if the cost of an eradication response is estimated to be very high compared to the likely benefits.

In semi-open systems, the success of an eradication strategy would depend on the availability of resources for surveying and destocking farmed and wild hosts in the immediate area (although it should be noted that wild hosts may or may not sustain infection).

For an eradication plan to be successful:

- quarantine and movement controls must be declared immediately and stringently enforced on salmonids, salmonid products, water and if required or possible any vectors located in declared areas. Restrictions must apply to movement out of the infected area of anything capable of transmitting ISAV from infected to uninfected fish, and to aquaculture facilities or processing plants. Movement controls should be maintained until the agent is either eradicated or declared endemic
- surveillance and tracing must occur to determine where infection may have spread
- all diseased and dead fish must immediately be removed, destroyed and disposed of
- any incubating (exposed) or suspect (potentially exposed) salmonids must immediately be removed, destroyed and disposed of. Emergency harvest of market-size fish can be considered but this must not compromise the eradication effort
- any product from exposed or potentially exposed but clinically normal salmonids must immediately be disinfected for example heated and canned, or destroyed and disposed of
- all buildings, tanks, materials and equipment that may be contaminated—including nets, boats, vehicles, and personal equipment and clothing—must be decontaminated
- all infected salmonids, wastes, effluent and equipment that cannot be decontaminated effectively must immediately be disposed of safely
- effluent must be treated
- restocking with sentinel Atlantic salmon can occur only after the site has been thoroughly decontaminated and has remained fallow for a period specified by the state CVO in consultation with the Australian salmonid farming industry.

3.2.2 Option 2: containment and control

If reservoirs of infection became established in farmed or wild fish stocks or in a water system, eradication may not be feasible. In addition, eradication may not always be the most economically favourable option, for example if the virus is avirulent (for example, HPR0). In this case, control in infected areas and containment and prevention of further spread is the preferred response option in order to protect and maintain uninfected areas.

If it were possible to maintain uninfected areas free of ISA/ISAV, the implementation of a zoning or compartmentalisation program would be advantageous to the Australian Atlantic salmon

industry and to the protection of potentially susceptible wild fish species. Restrictions on the movement of fish and fish products, and a surveillance and monitoring program, would be necessary to support such a zoning/compartmentalisation program.

Farms in infected areas would need to consider management practices to reduce the severity and incidence of ISA outbreaks. Control measures are required to reduce the severity of disease in infected areas and to prevent transmission of infection to fish in uninfected areas. Management practices generally focus on good hygiene practices to reduce horizontal transmission and reduce infective pressure. In general this requires management or regulation of fish movements, health control, transport and slaughterhouse practices (Rimstad et al. 2011). More specifically, this could include:

- restrictions on affected, suspected and neighbouring farms—for example, careful and thorough cleaning and disinfection, control of movement of staff, equipment and fish
- sanitary slaughtering—culling infected/incubating/suspect fish, immediate removal of sick or dead fish (daily diving if necessary)
- hygienic fish slaughtering—containment, treatment and safe disposal of infected blood water and effluent from processing plants
- surveillance and monitoring: close observation of stock health—examination and fresh sampling of any morbidity or mortality event suspicious of ISA or not readily recognisable as any other endemic disease
- improved husbandry
  - low stress environment (low stocking densities, minimal handling)
  - bay area management (coordinated production in hydrologically related sites where bays are stocked with single-year classes and undergo coordinated harvesting and fallowing). This will prevent transmission between fish generations
- Vaccination and consideration of chemotherapeutics realising that neither of these tools are currently commercially available in Australia nor is vaccination perfectly efficacious.

3.2.3 Option 3: control and mitigation

In some circumstances, establishment of a zoning or compartmentalisation program for ISA in Australia may not be considered feasible. This would be the case if, for example:

- infection with ISAV became widespread or enzootic throughout large areas of Australia
- financial expenses associated with setting up and maintaining a zoning or compartmentalisation program (including movement restrictions and a targeted surveillance and monitoring program) were considered prohibitive
- infection was due to a non-pathogenic ISAV strain such as HPR0.

In this situation, control and mitigation of disease may be the only possible response option. Husbandry, management and hygiene practices should be implemented to decrease the incidence and severity of ISA outbreaks.

The options outlined in Section 3.2.2 (with the exception of the restrictions associated with zoning or compartmentalisation) may be implemented, with the aim of minimising the impact of disease through minimising the infectious load of farms, exposure of the fish to the virus and host compromise.

3.3 Criteria for proof of freedom

Proof of freedom from ISAV, which may be important for trade, can be demonstrated at the aquaculture establishment, zone and country level. Criteria for proof of freedom at each level are given in the OIE Aquatic Animal Health Code (OIE 2016a) (see Appendix 1).

A number of factors should be considered when developing a surveillance program to demonstrate freedom from infection. Some factors will be dependent on specific characteristics of the disease outbreak, such as time of year, location, production system and management practices. Suitable surveillance techniques exist including both passive and active surveillance.

Passive surveillance uses existing systems and processes to identify and notify any cases of disease. This is particularly useful to detect new cases of disease. The absence of reports of disease can be important evidence that supposedly free areas—for example, zones or eradicated areas—are indeed free if a suitable passive system exists.

Active surveillance seeks to collect new information, such as samples of fish to detect disease if it is present. Practical active sampling strategies exist to demonstrate freedom (Cameron 1999; 2002; Cameron & Baldock 1998a; 1998b). The standard approach is to design a survey using a sample size that is sufficient to detect infection if it was present at a certain unrealistically low prevalence. The absence of infection from such samples allows the assertion that disease is likely absent.

Newer approaches have been developed that allow a holistic analyses of disparate surveillance sources—for example, active and passive sources—to determine the probability that disease freedom has been achieved in a farm, region or country (Cameron 2012; Martin et al. 2007).

These techniques are widely accepted internationally and often considered required information during international trade negotiations. They could be applied to ISAV to demonstrate freedom from disease.

3.4 Funding and compensation

No national cost-sharing agreements are currently in place for emergency responses to ISA. The users of this publication are responsible for seeking advice about any relevant funding or compensation arrangements within the relevant jurisdiction.

3.5 Export markets

Some countries may have import conditions in place related to ISAV, such as requiring imports to be certified free of ISAV. The Department of Agriculture and Water Resources is responsible for the health certification of all exports and should be emailed for further information.

Appendix 1 OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals

OIE Aquatic Code

The objective of the OIE (World Organisation for Animal Health) *Aquatic Animal Health Code* (OIE 2016a) is to prevent the spread of aquatic animal diseases, while facilitating international trade in aquatic animals and aquatic animal products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade of aquatic animals and their products.

Chapter 10.4 of the 2016 OIE *Aquatic Animal Health Code* (19th edition) is relevant to this manual.

OIE Aquatic Manual

The purpose of the OIE Manual of Diagnostic Tests for Aquatic Animals is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases (OIE 2016c). Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the OIE Aquatic Manual was published in 2016 and is available on the OIE website at:

http://www.oie.int/en/international-standard-setting/aquatic-manual/

The chapter relevant to this manual is:

http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_isav.htm

Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at:

http://www.oie.int/international-standard-setting/overview/

Appendix 2 Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. In addition, an import permit is required from the Department of Agriculture and Water Resources if a product containing biological material is to be sourced from overseas.

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permits. Since the assessment process is so detailed, the evaluation may take some time to complete.

Registration

Registration is the default method for APVMA to allow the use of a veterinary chemical in Australia. Registration is time consuming and expensive and it may be necessary to apply for a minor or emergency use permit during an emergency.

Minor use permit system

The minor use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. The system was devised by the APVMA for Australia, and allows the restricted use of a limited amount of a drug or chemical in a specified species when inadequate data are available to satisfy APVMA requirements for registration. Conditions are applied to the permit, which often include the collection of data related to the use of the product. The MUP system aims to enable restricted use of a drug or chemical until sufficient data are available to enable full registration.

For example, the APVMA may set a temporary withholding period with a wide margin of safety for an MUP. This withholding period may have been extrapolated from data relating to the use of the product in other species. In such cases, a condition of the MUP will be the collection of residue testing data. Results from the data are assessed by the APVMA (usually after 12 months—the duration of most permits) and used to more accurately set a withholding period for the product.

Emergency use permits

The APVMA has a permit system for the emergency use of a product that is either unregistered in Australia or registered for use in a different species or for a different use pattern. The APVMA will verify with the appropriate state and territory coordinators that the emergency is genuine.

For further details or permit application forms, visit the APVMA website (APVMA 2015).

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